ELISE M. PHILIPS

Environmental Exposures and Maternal and Child Health

Focus on bisphenols, phthalates and smoking

Environmental Exposures and Maternal and Child Health

Focus on bisphenols, phthalates and smoking

Elise M. Philips

Acknowledgements

The work presented in this thesis was conducted within the Generation R Study Group. The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organisation for Health Research and Development (ZonMw), the Dutch Research Council (NWO), the Dutch Ministry of Health, Welfare and Sport and the Dutch Ministry of Youth and Families. The studies on bisphenols and phthalates were supported by the National Institutes of Health, USA. The funders had no role in design or conduct of the studies; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscripts described in this thesis.

The publication of this thesis was kindly supported by the Generation R Study Group and the Erasmus University Rotterdam. Financial support was also kindly provided by the SBOH, employer of GP trainees. Financial support by the Dutch Heart Foundation for the publication of this thesis is gratefully acknowledged.

Cover design James Jardine | jamesjardine.nl

Layout Ridderprint B.V. | ridderprint.nl

Printing Ridderprint B.V. | ridderprint.nl

ISBN 978-94-6416-196-0

Environmental Exposures and Maternal and Child Health

Focus on bisphenols, phthalates and smoking

Blootstelling aan omgevingsfactoren en de gezondheid van moeders en kinderen

Focus op bisfenolen, ftalaten en roken

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam

op gezag van de

rector magnificus

Prof.dr. F.A. van der Duijn Schouten

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

dinsdag 12 januari 2021 om 15.30 uur

door

Elise Margriet Philips

geboren te Diemen.

Erasmus University Rotterdam

Erasmus

Promotiecommissie:

Promotoren:	prof. dr. V.W.V. Jaddoe prof. dr. E.A.P. Steegers
Overige leden:	prof. dr. I.K.M. Reiss prof. dr. E.F.C. van Rossum prof. dr. M. Vrijheid
Copromotor:	dr. S. Santos

Paranimfen:	Jonneke Hollanders Marjolein Brekelmans

Contents

Manuscripts based on this thesis

Chapter 1.2

Philips EM, Jaddoe VWV, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2017 Mar;68:105-118.

Chapter 2.1

Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, Trasande L. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ Res. 2018 Feb;161:562-572.

Chapter 2.2

Philips EM, Kahn LG, Jaddoe VWV, Shao Y, Asimakopoulos AG, Kannan K, Steegers EAP, Trasande L. First trimester urinary bisphenol and phthalate concentrations and time to pregnancy: a population-based cohort analysis. J Clin Endocrinol Metab. 2018 Sept 1;103(9):3540-3547.

Chapter 2.3

Philips EM, Trasande L, Kahn LG, Gaillard R, Steegers EAP, Jaddoe VWV. Early pregnancy bisphenol and phthalate metabolite levels, maternal hemodynamics and gestational hypertensive disorders. Hum Reprod. 2019 Feb 1;34(2):365-373.

Chapter 2.4

Philips EM, Santos S, Steegers EAP, Asimakopoulos AG, Kannan K, Trasande L, Jaddoe VWV. Maternal bisphenol and phthalate urine concentrations and gestational weight gain. Environ Int. 2019 Dec 18;135:105342.

Chapter 2.5

Philips EM, Jaddoe VWV, Deierlein A, Asimakopoulos AG, Kannan K, Steegers EAP, Trasande L. Exposures to Phthalates and Bisphenols in Pregnancy and Postpartum Weight Gain in a Population-Based Longitudinal Birth Cohort. Environ Int. 2020 Jul 31;144:106002.

Chapter 3.1

Philips EM[*], Santos S[*], Trasande L, Aurrekoetxea JJ, Barros H, von Berg A, Bergström A, Bird PK, Brescianini S, Chaoimh CN, Charles MA, Chatzi L, Chevrier C, Chrousos GP, Costet N, Criswell R, Crozier S, Eggesbø M, Fantini MP, Farchi S, Forastiere F, van Gelder MMHJ, Georgiu V, Godfrey KM, Gori D, Hanke W, Heude B, Hryhorczuk D, Iñiguez C, Inskip H, Karvonen AM, Kenny LC, Kull I, Lawlor DA, Lehmann I, Magnus P, Manios Y, Melén E, Mommers M, Morgen CS, Moschonis G, Murray D, Nohr EA, Nybo Andersen AM, Oken E, Oostvogels AJJM, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Roeleveld N, Rusconi F, Santos AC, Sørensen TIA, Standl M, Stoltenberg C, Sunyer J, Tayler M, Thiering E, Thijs C, Torrent M, Vrijkotte TGM, Wright J, Zvinchuk O, Gaillard R, Jaddoe VWV. Changes in parental smoking during pregnancy and risks of adverse birth outcomes and childhood overweight: an individual participant data meta-analysis of 230,000 families. PLOS Medicine. 2020 Aug 18;17(8):e1003182.

1

CHAPTER

Introduction

1.1

CHAPTER

General Introduction

Background

Environmental exposures are experienced throughout the human lifespan and may have a vast influence on human health. Certain subgroups of the population might be more susceptible to adverse effects. The developmental origins of health and disease (DOHaD) hypothesis suggests that adverse exposures in early life might induce permanent developmental adaptations leading to increased risks of cardiometabolic disease in later life.[1,2] For women, pregnancy itself is also considered a period of increased susceptibility to potentially long-term physiological changes due to exposure to endocrine disrupting chemicals.[3]

Studies presented in this thesis were designed to investigate potential associations of environmental exposures during pregnancy with maternal and child health. The studies are particularly focused on exposures of the endocrine disrupting chemicals bisphenols and phthalates and parental smoking.

Bisphenols and phthalates

Bisphenols are used to produce polycarbonate plastics and epoxy resins which are used in various consumer products, including the lining of metal cans, toys and water pipes.[4] Phthalates are synthetic chemical esters of phthalic acid that are widely used to impart flexibility, pliability and elasticity to plastics.[5] Phthalates can be divided in low molecular weight (LMW) phthalates, which are frequently added to personal care products to impart flexibility or retain scent, and high molecular weight (HMW) phthalates, that are used as plasticizers to impart flexibility in vinyl plastics for diverse applications including flooring, medical devices and food packaging. [6,7] Both bisphenols and phthalates are at risk for leaching into the human environment.[4,6] Bisphenols and phthalates are lipophilic chemicals (phthalates > bisphenols), have short biological half-lives (<24h, bisphenols < phthalates) and undergo a first-pass effect when ingested orally before excretion in urine. [8-10] Bisphenols and phthalates have several potential mechanisms of effect, including endocrine disruption through estrogen and androgen receptor binding, activation of nuclear transcription factors leading to epigenetic changes, and induction of oxidative or nitrosative stress.[10-15]

As pregnancy and early life are periods with increased vulnerability to environmental exposures, exposure to bisphenols and phthalates during pregnancy may pose a risk for maternal and fetal health in the short and long term. As example, estimated health care costs of obesity and diabetes attributable to adult bisphenol and phthalate exposure in Europe is in the order of €17 billion annually.[16] Thus far, only few studies have been performed assessing the impact of bisphenols and phthalates on the course of pregnancy and maternal health. Recently, the European Union expanded its regulations concerning bisphenol A and several phthalates. [17,18] In the meantime, these embargoes stimulated the industries to progressively switch to synthetic bisphenol analogues and di-2-ethylhexylphthalate (DEHP) replacements.[19] Effects of these replacements have been studied scarcely. Further studies are needed to investigate effects of these substitute chemicals. Assessing the associations of bisphenols and phthalates with maternal and pregnancy related outcomes may give an insight in potential pathways in which bisphenols and phthalates contribute to maternal and also fetal complications.

Smoking

Since the first Surgeon General's report in 1964 more than 20 million premature adult deaths can be attributed to cigarette smoking and a wide range of studies have causally linked smoking to numerous diseases.[20] Although strategies to prevent smoking are globally implemented, Europe has the highest prevalence of adult tobacco smoking among the World Health Organization (WHO) regions. Total costs attributable to smoking were estimated in the order of €544 billion annually, about 4.6% of the EU27 combined gross domestic product.[21]

While tobacco smoking used to be mainly a male phenomenon, the gap in prevalence between male and female adults is now very small (<5%) in several European countries, including The Netherlands.[22] It has been estimated that one in five women of reproductive age worldwide are expected to be tobacco users by 2025.[23] Many studies have been conducted on the impact of maternal smoking during pregnancy on adverse birth outcomes showing causal pathways leading to congenital abnormalities, stillbirth, preterm birth and low birth weight and sudden infant death syndrome.[24-28] Maternal smoking during pregnancy has also been associated to increased risks of overweight in childhood.[29-31] The effects of changing maternal smoking habits during pregnancy and the share of paternal smoking in these associations remain inconclusive.[32-37] Further examination of these associations are needed to develop preventive strategies.

General aim

The general aims of this thesis was to investigate the associations of well-known adverse exposures, namely endocrine disruptors and parental smoking during pregnancy with maternal, fetal and childhood outcomes.

General design

The studies described in this thesis were embedded in the Generation R Study, a population-based prospective cohort study, and in an international consortium of collaborating pregnancy and birth cohort studies.

Generation R Study

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands.[38] The Generation R Study was designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment was aimed at early pregnancy, but was allowed until the birth of the child. At baseline 9,778 mothers were enrolled in the study, of whom 8,880 (91%) were included during pregnancy. The Generation R study is a multi-ethnic cohort. Participants of European origin constitute the largest ethnic group (58%), followed by Surinamese (9%), Turkish

(7%) and Moroccan (6%). Measurements were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18-25 weeks of gestation) and late pregnancy (≥25 weeks of gestation) and included parental physical examinations, biological samples (i.e. blood and urine), fetal ultrasound examinations, self-administered questionnaires and medical records completed by midwives and obstetricians. From child age 6 years onward, all children and mothers were invited to a dedicated research center in the Erasmus MC – Sophia's Children Hospital to participate in detailed body composition and cardiovascular follow-up measurements. Data collection at age 13 is currently ongoing.

Bisphenol and phthalate metabolite concentrations were measured among a subgroup of 1,405 women who delivered singletons, had at least one urine sample available for analysis and whose children also participated in postnatal studies at 6 years of age.

The LifeCycle Project – EU Child Cohort Network

As part of this thesis, we conducted an individual participant data meta-analysis on the associations of parental smoking with risks of adverse birth outcomes and childhood overweight. We used data of more than 220,000 parents and children from different cohorts that started during pregnancy or childhood working together in the EU Child Cohort Network established by the LifeCycle Project.[39]

Outline of this thesis

The general aim of this thesis is addressed in the several studies presented in this thesis. **Chapter 1.2** gives an introduction on bisphenols and phthalates, their potential routes of exposure, metabolism, mechanisms of effect and a narrative review of the literature. **Chapter 2** presents multiple studies that examine the associations of bisphenols and phthalates with maternal outcomes. In **Chapter 2.1** we studied the determinants of maternal bisphenol and phthalate concentrations among pregnant women. In **Chapter 2.2** we examined whether maternal bisphenol and phthalate concentrations in early pregnancy were associated with time to pregnancy. **Chapter 2.3** presents the associations of maternal bisphenol and phthalate concentrations in early pregnancy with maternal hemodynamics and the risks of gestational hypertensive complications. We assessed associations of maternal bisphenol and phthalate concentrations in early and mid-pregnancy with maternal gestational weight gain in **Chapter 2.4** and with maternal postpartum weight gain in **Chapter 2.5**. In **Chapter 3**, we examined whether changes in parental smoking during pregnancy affect the risks of adverse birth outcomes and childhood overweight. All findings are discussed in **Chapter 4** where we will place our results in a broader context. An overall summary of this thesis in both English and Dutch is provided in **Chapter 5**.

References

1. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet. 1993;341(8850):938-41.
2. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359(1):61-73.
3. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36(6):E1-E150.
4. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139-77.
5. Sathyanarayana S. Phthalates and children's health. Curr Probl Pediatr Adolesc Health Care. 2008;38(2):34-49.
6. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
7. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health. 2014;13(1):43.
8. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9; discussion 81-5.
9. Meeker JD, Calafat AM, Hauser R. Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women. J Expo Sci Environ Epidemiol. 2012;22(4):376-85.
10. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
11. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect. 1995;103(6):582-7.
12. Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. J Endocrinol. 1998;158(3):327-39.
13. Sarath Josh MK, Pradeep S, Vijayalekshmi Amma KS, Balachandran S, Abdul Jaleel UC, Doble M, et al. Phthalates efficiently bind to human peroxisome proliferator activated receptor and retinoid X receptor alpha, beta, gamma subtypes: an in silico approach. J Appl Toxicol. 2014;34(7):754-65.
14. Ferguson KK, Cantonwine DE, Rivera-Gonzalez LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, et al. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. Environ Sci Technol. 2014;48(12):7018-25.
15. Watkins DJ, Ferguson KK, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. Int J Hyg Environ Health. 2015;218(2):212-9.
16. Legler J, Fletcher T, Govarts E, Porta M, Blumberg B, Heindel JJ, et al. Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab. 2015;100(4):1278-88.
17. The European Commission. Commission Regulation (EU) 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials. Official Journal of the European Union. 2018.
18. The European Commission. Commission Regulation (EU) 2018/2005 of 17 December 2018 amending Annex XVII to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) as regards bis(2-ethylhexyl) phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP). Official Journal of the European Union. 2018.

19. Russo G, Barbato F, Mita DG, Grumetto L. Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Food Chem Toxicol. 2019;131:110575.
20. National Center for Chronic Disease P, Health Promotion Office on S, Health. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. 2014.
21. The European Commission. A study on liability and the health costs of smoking. GHK, 2012.
22. World Health Organization. WHO / Europe- Tobacco- Data and statistics [Available from: http://www.euro.who.int/en/health-topics/disease-prevention/tobacco/data-and-statistics.
23. Samet JM, Yoon SY. Women and the tabacco epidemic: challenges for the 21st century: WHO, Institute for Global Tabacco Control, Johns Hopkins School of Public Health; 2011.
24. Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update. 2011;17(5):589-604.
25. Marufu TC, Ahankari A, Coleman T, Lewis S. Maternal smoking and the risk of still birth: systematic review and meta-analysis. BMC Public Health. 2015;15:239.
26. Zhang K, Wang X. Maternal smoking and increased risk of sudden infant death syndrome: a meta-analysis. Leg Med (Tokyo). 2013;15(3):115-21.
27. Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol. 2000;182(2):465-72.
28. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65(5):663-737.
29. Rayfield S, Plugge E. Systematic review and meta-analysis of the association between maternal smoking in pregnancy and childhood overweight and obesity. J Epidemiol Community Health. 2016.
30. Durmus B, Heppe DH, Taal HR, Manniesing R, Raat H, Hofman A, et al. Parental smoking during pregnancy and total and abdominal fat distribution in school-age children: the Generation R Study. Int J Obes (Lond). 2014;38(7):966-72.
31. Albers L, Sobotzki C, Kuss O, Ajslev T, Batista RF, Bettiol H, et al. Maternal smoking during pregnancy and offspring overweight: is there a dose-response relationship? An individual patient data meta-analysis. Int J Obes (Lond). 2018;42(7):1249-64.
32. Raisanen S, Sankilampi U, Gissler M, Kramer MR, Hakulinen-Viitanen T, Saari J, et al. Smoking cessation in the first trimester reduces most obstetric risks, but not the risks of major congenital anomalies and admission to neonatal care: a population-based cohort study of 1,164,953 singleton pregnancies in Finland. J Epidemiol Community Health. 2014;68(2):159-64.
33. Blatt K, Moore E, Chen A, Van Hook J, DeFranco EA. Association of reported trimester-specific smoking cessation with fetal growth restriction. Obstet Gynecol. 2015;125(6):1452-9.
34. Durmus B, Kruithof CJ, Gillman MH, Willemsen SP, Hofman A, Raat H, et al. Parental smoking during pregnancy, early growth, and risk of obesity in preschool children: the Generation R Study. Am J Clin Nutr. 2011;94(1):164-71.
35. Grzeskowiak LE, Hodyl NA, Stark MJ, Morrison JL, Clifton VL. Association of early and late maternal smoking during pregnancy with offspring body mass index at 4 to 5 years of age. J Dev Orig Health Dis. 2015;6(6):485-92.
36. Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, et al. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. Paediatr Perinat Epidemiol. 2008;22(2):162-71.
37. Fasting MH, Oien T, Storro O, Nilsen TI, Johnsen R, Vik T. Maternal smoking cessation in early pregnancy and offspring weight status at four years of age. A prospective birth cohort study. Early Hum Dev. 2009;85(1):19-24.

38. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.

39. Jaddoe VWV, Felix JF, Andersen AN, Charles MA, Chatzi L, Corpeleijn E, et al. The LifeCycle Project-EU Child Cohort Network: a federated analysis infrastructure and harmonized data of more than 250,000 children and parents. Eur J Epidemiol. 2020;35(7):709-24.

1.2

CHAPTER

Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood

Reprod Toxicol. 2017 Mar;68:105-118.

Abstract

Pregnant women are exposed to various chemicals, including endocrine-disrupting chemicals (EDCs) such as phthalates and bisphenols. Increasing evidence suggests that early life exposures to phthalates and bisphenols may contribute to cardiometabolic risks. The aim of this narrative review was to summarize current knowledge of the effects of fetal and childhood exposure to phthalates and bisphenols on child growth and child cardiometabolic outcomes and the effects on maternal outcomes. In total, 54 studies were identified and included. The majority of studies found effects of phthalates and bisphenols on maternal, child growth, and cardiometabolic outcomes. Currently results suggest that early life exposure to phthalates and bisphenols may have a substantial influence on perinatal and postnatal cardiometabolic programming. In a large part of the investigated outcomes studies show contradictory results. However, the majority of the existing evidence is based on non-cohort studies with single samples neglecting time-variant effects and complicating conclusions regarding causal inference. More studies are needed investigating the mechanisms and its potential interactions.

Introduction

Pregnant women are exposed to a variety of chemicals,[1,2] including endocrine-disrupting chemicals (EDCs) such as phthalates and bisphenols.[3-6] Increasing evidence suggests that early life exposures to phthalates and bisphenols may contribute to the burden of cardiovascular and metabolic disease in western countries. Recent work suggests that these exposures may be costly. Health care costs of obesity and diabetes attributable to adult phthalate and bisphenol exposure in Europe is in the order of €17 billion annually.[7] Insofar as prenatal and childhood exposures may even be more impactful, the costs of cardiometabolic conditions due to these exposures may be higher.

In this narrative review, we summarize current knowledge of the effects of fetal and childhood exposure to phthalates and bisphenols on child growth and child cardiometabolic outcomes. Additionally, we summarize the effects of phthalate and bisphenol exposure on maternal outcomes.

Phthalates and bisphenols

Phthalates are synthetic chemical esters of phthalic acid that are widely used in a variety of consumer products to impart flexibility, pliability and elasticity to plastics and therefore known as “plasticizers”.[8] Phthalates can be classified in two groups. Low molecular weight (LMW) phthalates (e.g. di-methyl phthalate (DMP), di-ethyl phthalate (DEP), di-n-butyl phthalate (DBP)) are frequently added to personal care products as aerosol delivery agents, emollients, to impart flexibility in nail polishes, and to retain scent.[9] High molecular weight (HMW) phthalates (e.g. di-2-ethylhexylphthalate (DEHP), di-isononylphthalate (DiNP), di-isodecylphthalate (DiDP), di-n-octylphthalate (DnOP), butylbenzyl phthalate (BBzP)) are used as plasticizers to impart flexibility in vinyl plastics (e.g. polyvinyl chloride plastics (PVC)) for diverse applications including flooring, medical devices and food packaging.[10] In the category of HMW phthalates, di-2-ethylhexylphthalate (DEHP) is of particular interest, considering many food packaging methods include the use of plastics containing DEHP.[11] However, the last few years DiNP and DiDP have replaced DEHP to a great extent, mainly due to governmental embargoes.[12]

Bisphenol A (BPA) is used to produce polycarbonate plastics and epoxy resins used in various consumer products, including the lining of metal cans, toys and water pipes.[13] The last few years, bisphenol A has been substituted by synthetic bisphenol analogues like bisphenol F (BPF) and bisphenol S (BPS), which has been determined in various food items[14]. BPS has been found as well in paper and paper products, including currency bills.[15]

Routes of exposure and metabolism

Phthalates are non-covalently bound to many plastics, creating a large risk for release into the environment over time.[9] Phthalates are generally lipophilic[16] and have short biological half-lives (less than 24h), undergoing hydrolysis and sometimes oxidation before glucuronidation or sulfation before excretion into urine of feces, but it can be measured as well in blood and breast milk.[9] A portion of the unconjugated (free) monoester and/or its secondary metabolites may also be directly excreted in urine.[17] The primary routes of exposure to phthalates are ingestion, salivary absorption, inhalation,

intravenous, and transdermal. Depending on the route of exposure, the chemical is distributed into various body parts based on vascular blood supply and affinity, which in turn may lead to a difference in bioavailability. Ingested chemicals often undergo a first-pass effect, entering the liver through the hepatic portal system for metabolization, which reduces bioavailability. Following inhalation, salivary absorption, intravenous, and transdermal exposure this first-pass effect is initially bypassed, provoking a higher bioavailability.[18]

Population based studies often use urine as a measurement for exposure to phthalates because it is noninvasive and notwithstanding the short biological half-life it may reasonably reflect the exposure in the last several weeks or even months.[18,19] The majority of the population based studies using urinary phthalate concentrations measured the concentration of the free plus glucuronidated species of phthalate metabolites, together being the total concentration. However, the free metabolite concentrations are less stable over time than the total metabolite concentration, suggesting free metabolite concentrations are not a useful indicator of metabolic susceptibility. Time of collection is an important factor that must be taken into account, since concentrations of metabolites vary during the day as a result of timing of exposure.[17]

Various products containing polycarbonate plastics and epoxy resins have been studied to obtain more knowledge on bisphenol leaching. Regarding polycarbonate plastics, different results have been obtained on the effects of washing and heating on BPA leaching, although all studies found leaching. Several studies have been performed that found that heating temperature had a significant effect on BPA leaching from metallic coated food cans.[13]

Studies investigating the metabolism of BPS and BPF are lacking. Concerning BPA it is known that after ingestion BPA undergoes a first-pass metabolism in the gastrointestinal tract and liver consisting of glucuronidation and, to a lesser extent, sulfation metabolizing BPA to bisphenol A monoglucuronide (BPAG) and bisphenol A sulphate (BPAS) for approximately 98%. In plasma, more than 90% of BPA is bound, depending on the route of exposure. Exposure through inhalation and skin absorption have been reported as important routes of exposure, as unconjugated bisphenols might circulate longer in the plasma, while ingested bisphenols undergo the first-pass metabolism.[20,21] However, it has been reported that UDP-glucuronosyltransferase (UGT) enzymes found in the airways exhibit a high activity towards bisphenols.[21] Both BPAG and BPAS are excreted in urine within 5-7 hours after oral administration.[20,22] BPA penetrates and accumulates in the human placenta, with higher levels of BPA in the placenta compared to maternal and fetal plasma.[23] In a rat-study, BPF residues have been detected in the uterus, placenta, amniotic fluid, and fetuses, with comparable higher levels of BPF in the (intra) uterine compartment compared to maternal blood.[24]

Biomonitoring studies have observed high plasma concentrations not consistent with the observation of an extensive first-pass metabolism of oral BPA. However, concentrations of urinary BPA tend to be much higher than serum concentrations. It has been hypothesized that these relatively high concentrations both in plasma and urine could be explained by sublingual absorption, bypassing the first-pass metabolism.[25] While another study has suggested that this hypothesis does not hold, the contradictory study is beset by critical differences in site of blood collection and volume of urinary output that actually support sublingual absorption as a substantial contributor to exposure.[22,25]

A potentially important role in metabolism with a large effect on bioavailability is reserved for the human microbiome. The microbiome comprises the residential microbes humans are colonized by and there is a broad interindividual variation. Several bacterial species possess β-glucuronidases and β-glucuronides, enzymes involved in deconjugation and conjugation. Depending on the composition of the microbiome, phthalates and bisphenols could be conjugated or deconjugated after enterohepatic circulation, resulting in a smaller or larger exposure to unconjugated chemicals, respectively.[26,27]

Potential mechanisms of effect

Hydrolysed phthalate metabolites have been shown to penetrate the human placenta.[28] In vitro studies demonstrated that several commercially used phthalates may bind to estrogen receptor alpha (ERα), having a weak estrogenic activity,[29,30] and to androgen receptors (ARs), having a strong anti-androgen activity.[31,32]

Another potential mechanism is by activation of nuclear transcription factor peroxisome proliferator-activated receptors (PPARs). PPAR-gamma (PPARγ), 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.[33,34] However, despite potential benefits, PPARγ agonists have been shown to cause adverse effects regarding increased lipid accumulation and release of adipocyte-related hormones leading to an increased susceptibility for the development of obesity.[35] Several phthalates have been shown to be PPARγ activators, causing obesogenic effects.[34-37] PPARs form heterodimers with the retinoid X receptors (RXRs), binding together on the target DNA and thereby activating the expression of downstream genes. Therefore, RXRs have the same targets as PPARs. Many common phthalates have been shown to bind to RXRs.[37] Likewise, oxidative stress is a potential mechanism for phthalate effects. In a prospective cohort study of pregnant women all urinary phthalates were associated with increased oxidative stress markers.[38]

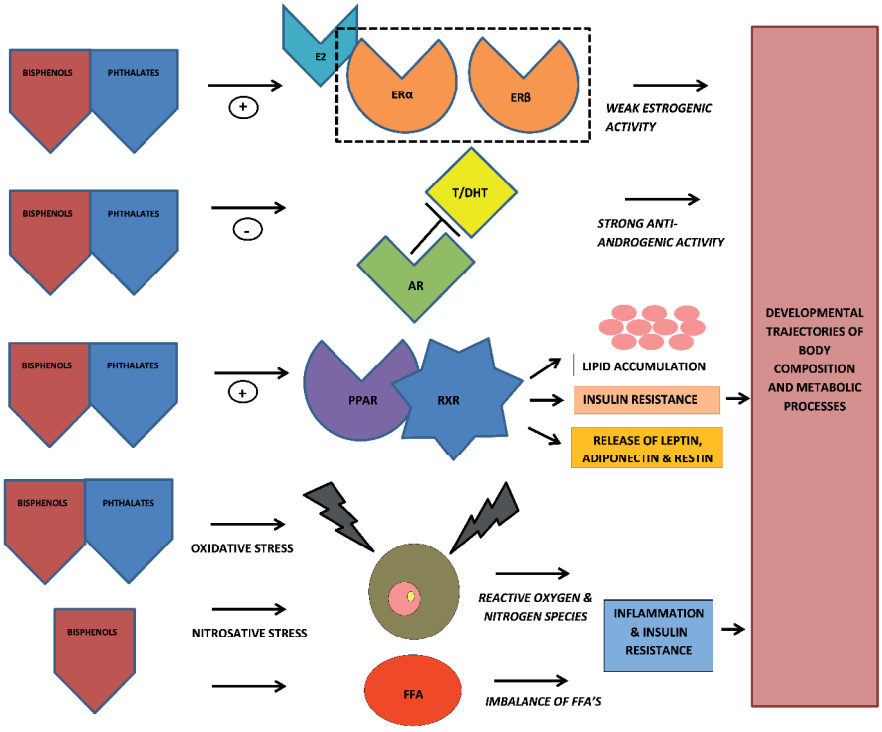
The potential mechanisms of action from bisphenols resemble those from phthalates to a great extent. Studies regarding the mechanism of action from BPF and BPS are scarce. Bisphenols are weak xenoestrogens binding to estrogen receptors (ER) and the G-protein-coupled receptor 30 (GPR30) in its unconjugated form, with greater binding affinity to ERβ compared to ERα, considering a 100 to 10.000-fold lower relative binding affinity of bisphenols to ERs compared to estradiol (E2).[20,39,40] However, findings suggest that BPA is equally potent as E2 and it is suggested that this results from actions through non-genomic pathways and disruption of steroidogenesis.[13,20,41] BPF and BPA have been reported to increase the level of 17β-estradiol. BPF appears to be even more potent than BPA, given the higher concentrations of 17β-estradiol after exposure of H295R human adrenocortical carcinoma cell line to BPF.

Like phthalates, bisphenols have anti-androgen capacities, binding to ARs.[31,39] A decrease in free testosterone level is reported when exposed to bisphenols, in the order BPF > BPS > BPA. Since the binding affinity of BPS to the AR is low, the testosterone effect of BPS seems to be androgen receptor independent.[39,42] Both BPS and BPF increased progesterone levels, BPA and BPS decreased cortisol levels, and BPF increased cortisol levels.[42]

Similar to phthalates, BPA appears to induce an activation of PPARα and possibly to a smaller extent a weak activation of PPARγ, causing obesogenic effects.[34,35,37] Besides the binding to PPARs, also BPA binds to RXRs with more affinity to RXRs.[37] Likewise, oxidative stress is a potential mechanism for BPA effects. The same Puerto Rican prospective cohort study describing the association between phthalates during pregnancy and oxidative stress markers found a significant association between urinary BPA and oxidative stress markers in pregnant women.[43] An in vitro study showed oxidative stress effects from BPS and BPF.[42] Furthermore, BPA has been reported to have nitrosative stressor effects and effects on free fatty acids (FFAs). Oxidative stress and imbalances in FFAs are known mediators of tissue specific insulin resistance and inflammation.[44]

Adverse metabolic outcomes could also be hypothesized by interaction of phthalates and bisphenols with the adipocytokines leptin and adiponectin. Adiponectin and leptin are adipocyte-produced hormones having a role in metabolic regulation and function. In adults, leptin inhibits appetite, stimulates thermogenesis, enhances fatty acid oxidation, decreases glucose, and reduces body weight and fat. Paradoxically, leptin levels increase in obesity.[45] Meanwhile, in adults low adiponectin levels have been associated with insulin resistance, dyslipidemia, atherosclerosis, and metabolic syndrome.[45,46] An overview of the potential mechanisms of effect is shown in Figure 1.

Figure 1. Potential mechanisms of effect



ERα: estrogen receptor alpha ; ERβ: estrogen receptor beta ; E2: estradiol ; AR : androgen receptor ; T/DHT: testosterone / dihydrotestosterone ; PPAR: peroxisome proliferator-activated receptors ; RXR: retinoid X receptors ; FFA: free fatty acids.

Maternal pregnancy outcomes

Two studies investigated the effect of phthalates and BPA on the time to pregnancy. A Canadian pregnancy cohort found no association between first trimester phthalates or BPA and recalled time to pregnancy.[47] Meanwhile, a preconceptional cohort study exploring the effects of phthalates and BPA on fertility in the both men and women in the United States found several phthalates to be associated with longer time to pregnancy in males, while other phthalates were associated with shorter time to pregnancy in females. Neither male nor female BPA concentration was associated with time to pregnancy.[48] Two human studies have pointed out that a higher level of BPA was associated with (recurrent) aneuploidy and euploid miscarriages.[49,50]

During pregnancy maternal glucose levels increase to provide adequate nutrition for fetal growth and development. Two studies investigated the effect of phthalates and BPA on maternal glucose levels and gestational diabetes mellitus (GMD).[51,52] Neither phthalates nor BPA was associated with increased maternal glucose levels or GMD. However, a small cohort study of pregnant women found that women in the highest tertile of urinary MiBP and MBzP had lower blood glucose levels at time of the routine GMD screening compared to women in the lowest concentration tertile.[52] A Japanese study investigated the effect of DEHP on maternal triglycerides and fatty acid levels, detecting an inverse association between maternal serum MEHP concentrations and triglyceride and fatty acid levels during pregnancy. These findings could have implications for fetal health. The growth and development of the fetus and its organs depend on a sufficient supply of nutrients including fatty acids and lipids crossing the placenta, determining birth outcomes. However, in this particular study no effect on fetal growth was observed.[53]

Only one study explored the association with preeclampsia, investigating differences in the distribution of BPA between maternal, placental and fetal compartments. This small case-control study reported that preeclamptic women have significantly higher concentrations of BPA in placental tissue compared to normotensive pregnant women, while concentrations of BPA in maternal serum and cord blood did not differ significantly. Accordingly, BPA was equally distributed between maternal, placental and fetal compartments in the normotensive group, while preeclamptic women showed an unequal distribution with a high level of BPA in the placental compartment.[54]

Overall, the amount of studies for the separate outcome measures is limited. However, the presented outcomes suggest potential adverse effects of phthalates and bisphenols on fecundity, miscarriages, and preeclampsia. There are no studies reporting on pregnancy induced hypertension or HELLP syndrome. An overview of the included studies including outcomes, strengths and weaknesses is given in Table 1. Study characteristics and results of all included studies separately are shown in Supplementary Table 1.

Table 1. Overview of the included studies per outcome

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
MATERNAL PREGNANCY OUTCOMES						
Time to Pregnancy[47-48]	Phthalates	2001[47] 501[48]	First trimester[47] Preconceptional inclusion[48]	Positive / negative / null	One pregnancy cohort found no association between first trimester phthalates and recalled time to pregnancy.[47] A preconceptional cohort found several phthalates to be associated with longer time to pregnancy in males, while other phthalates were associated with shorter time to pregnancy in females.[48]	S: 1 preconceptional cohort study, couples with infertility/sterility excluded, urinary samples adjusted for urinary dilution, the preconceptional study explored both males and females W: 1 retrospective analysis in a cohort study based on recalled data.
	BPA	2001[47] 501[48]	First trimester[47] Preconceptional inclusion[48]	Null	One pregnancy cohort found no association between first trimester BPA and recalled time to pregnancy.[47] A preconceptional cohort found no associations between BPA and time to pregnancy.[48]	S: 1 preconceptional cohort study, couples with infertility/sterility excluded, urinary samples adjusted for urinary dilution, the preconceptional study explored both males and females W: 1 retrospective analysis in a cohort study based on recalled data
Miscarriage risk[49-50]	BPA	115[49] 50[50]	First trimester[49-50]	Positive	Both studies found a positive association between the level of BPA and (recurrent) aneuploidy and euploid miscarriages.[49,50]	S: 1 cohort study, only unknown etiology of miscarriages included W: 1 case-control study, blood samples instead of urinary samples

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
Maternal blood glucose, triglycerides and fatty acid levels[51-53]	Phthalates	72[52] 318[53]	First trimester[52] Second trimester[53]	Negative	Women in the highest tertile of urinary MiBP and MBzP had lower blood glucose levels at time of routine GMD screening compared to women in the lowest tertile.[52] Maternal serum MEHP concentrations were associated with lower triglyceride and fatty acid levels during pregnancy.[53]	S: 2 cohort studies, quite accurate about the time of sampling W: Only one sample, 1 study with blood samples, regarding triglycerides and fatty acids only DEHP and MEHP measured
	BPA	94[51]	Third trimester[51]	Null	No association between BPA and gestational diabetes mellitus was found.[51]	S: Accurate about time of sampling W: Case-control study, only one urinary sample
Preeclampsia [54]	BPA	58	Before delivery and umbilical cord blood	Positive	Preeclamptic women have significantly higher concentrations of BPA in placental tissue compared to normotensive pregnant women, while concentrations in serum and cord blood did not differ significantly.[54]	S: Placental biopsies W: Case-control study, blood samples instead of urinary samples, only BPA measured

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
FETAL OUTCOMES						
Gestational age at birth[55-65]	Phthalates	283[55] 404[56] 86[57] 207[58] 482[59] 482[60] 84[61] 60[62] 72[63]	First trimester[55] Third trimester[56,62] Shortly post-partum or cord blood[57,58,61] Four samples over pregnancy[59-60] Term not specified[63]	Mainly negative	Six studies found a significant association between phthalate exposure and gestational age reduction or preterm delivery.[58-63] One study found no association [58] and two studies found a positive correlation between phthalate exposure and gestational age at delivery.[55, 56]	S: All (subsets from) cohort studies, mainly urinary samples used all corrected for dilution, the majority of studies reported information on gestational age estimation W: Only 2 studies multiple sampling, sampling often taken over broad time, sampling time in one study not specified, various outcome measures used
	BPA	404[56] 72[63] 567[64] 60[65]	Third trimester[56,65] Term not specified[63] Before delivery[64]	Mainly negative	Three studies found a significant association between BPA and gestational age reduction [63-65] of which in one this association only remained in male infants after stratification for gender.[63] One study found no associations.[56]	S: All cohort studies or subsets from cohort studies, mainly urinary samples used all corrected for dilution, the majority of studies reported information on gestational age estimation W: No multiple sampling, sample often taken over a broad period of time, sampling time in one study not specified, various outcome measures used

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
Body size measures at birth [53,56-58,64,66-73]	Phthalates	318[53] 404[56] 86[57] 207[58] 126[66] 119[67] 201[68]	Second trimester[53] Third trimester[56,66,67] Shortly postpartum or cord blood[57,58,68]	Mainly negative	Four studies found a negative effect of phthalates on body size measures at birth.[58,66-68] One study found no associations.[53] Two studies found positive associations between LMW phthalates and head circumference at birth.[56,57]	S: The majority of studies were cohort studies or subsets from cohort studies, based on urinary samples corrected for dilution W: No multiple sampling, sample often taken over a broad period of time
	BPA	404[56] 567[64] 520[69] 550[70] 97[71] 219[72] 737[73]	Second trimester[69,70] Third trimester[56, 73] Before delivery[64, 71] Cord blood[71] Three samples over pregnancy[72]	Mainly null, study with repeated measurements negative	Four studies found no associations.[56,64,69,70] Two studies, including a study with up to 3 measurements during pregnancy, found a negative association between BPA exposure and body size measures at birth.[71,72] One study found a positive association between BPA and birth weight in male neonates and ponderal index in female neonates.[73]	S: The majority of studies were cohort studies or subsets from cohort studies, based on urinary samples corrected for dilution. One study collected up to three samples W: The majority of studies collected only one sample, which was often taken over a broad period of time.

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
CHILDHOOD OUTCOMES						
Childhood growth, prenatal exposure [6,69,74-78]	Phthalates	89[74] 234[75] 391[76]	Cord blood[74] Third trimester[75] First & third trimester[76]	Boys: negative Girls: positive	All three studies found prenatal exposure to phthalates to be associated with reduced BMI in boys. Additionally, one of the studies found also a reduced fat mass and waist circumference in boys, not girls.[75] In one of the studies prenatal exposure to phthalates was associated with a higher BMI in girls.[76]	S: All studies were cohort studies, 1 study with repeated sampling, one of the studies collected additionally multiple childhood samples, urinary samples corrected for dilution W: Study with multiple urinary samples did not analyse the measurements separately but averaged the results, 1 smaller study with cord blood
	BPA	402[6] 520[69] 402[77] 297[78]	First & second trimester[6] Second trimester[69,78] First & third trimester[77]	Positive / negative	Two studies showed that prenatal BPA exposure had a negative effect on BMI in girls, not boys.[6, 78] After stratification for puberty stage in one of these studies, only in prepubertal girls prenatal BPA exposure remained negatively associated with BMI and waist circumference.[6] Two studies, of which one consisted only of boys, reported a positive effect of prenatal BPA exposure on several growth parameters, including height adjusted weight (not significant), waist circumference and BMI.[69,77]	S: All studies were cohort studies with urinary samples, 3 studies collected two pregnancy samples, two of the studies collected additionally multiple childhood samples, all except for one study corrected for urinary dilution, one study performed sensitivity analyses for BPA measurements by trimester W: Studies with multiple urinary samples did not analyse the measurements separately but averaged the results

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
Childhood growth, childhood exposure [6,75,78-93]	Phthalates	234[75] Not specified[79] 493[80] 259[81] 387[82] 2884[83] 845[84] 90[85] 76[86]	Toddlers till adolescence[86] Preschoolers[75] Preschool till mid childhood[84] Mid Childhood[80,82,85] Mid childhood till adolescence[79,81,83]	Mainly positive, most for boys	Five studies found a positive effect of childhood exposure to phthalates on childhood growth parameters, including BMI, subscapular skinfold thickness, hip- and waist circumference, predominantly found in boys.[79-83] Two studies found no associations.[85,86] Two studies found a negative association between childhood exposure to phthalates and mid-childhood growth, including, BMI, BSA, weight and height.[75,84]	S: All included studies collected urinary samples of which 1 study with multiple childhood samples, all samples were corrected for dilution
	BPA BPS	402[6] 297[78] 90[85] 76[86] 39[87] 80[88] 259[89] 2838[90] 3370[91] 2200[92] 1326[93]	Toddlers till adolescence[86] Preschool till mid childhood[87,93] Mid childhood[85,88] Mid childhood till adolescence[89-92]	Mainly positive, not enough evidence to draw conclusions for BPS	Six studies found a positive effect of childhood exposure to BPA on childhood growth parameters measured from preschool till adolescence, including BMI, waist-to-height ratio and hip circumference.[6,89-93] One study found that early childhood exposure was associated with a lower BMI at the age of two, but BMI slopes increased more rapidly between 2 and 5 years of age.[78] No clear difference between the sexes. Two studies found no associations.[87,88] Two studies found a negative association between childhood	S: All included studies collected urinary samples of which 2 studies with multiple childhood samples, all samples were corrected for dilution, one study also included the newer bisphenol BPS

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
					exposure to BPA and BMI.[85,86] One of these studies also investigated BPS, but no associations were found.[86]	
Cardiovascular risk, prenatal exposure, outcome measured during childhood[76]	Phthalates	391[76]	First & third trimester	Negative	In girls the molar sum of both HMW and LMW phthalates were significantly associated with lower systolic blood pressure z-score. No studies have been performed on bisphenols.	S: Cohort study with two maternal urinary samples, reasonable follow-up, samples corrected for urinary dilution W: Urinary samples were averaged in the analysis, only phthalates measured, only blood pressure as a cardiovascular risk outcome
Cardiovascular risk, childhood exposure and outcome[87,94-96]	Phthalates	2838[94] 667[96]	Mid childhood till adolescence[94,96]	Positive	Both studies reported a positive link between phthalate exposure during childhood and adverse cardiovascular outcomes, including systolic blood pressure z-score and increased albuminuria.[94,96]	S: Childhood urinary samples corrected for urinary dilution, 1 study on blood pressure, 1 on albuminuria W: 2 cross-sectional studies, no studies with multiple sampling
	BPA	39[87] 770[95]	Preschool till mid childhood[87] Mid childhood till adolescence[95]	Positive	Both studies showed also a positive association between childhood BPA exposure and adverse cardiovascular outcomes, including increased albuminuria and in boys an increased diastolic blood pressure.[87,95]	S: Childhood urinary samples corrected for urinary dilution, 1 study on blood pressure, 1 on albuminuria. W: 1 cross-sectional and one small case study, no studies with multiple sampling, one study measured also lipid levels but did not report on that outcome.

Outcome	Phthalates and/ or bisphenols	Sample size	Time of exposure	Associations	Outcome	Strenghts (S)/Weaknesses (W)
Metabolic risk, childhood exposure and outcome [87-88,91,97-99]	Phthalates	766[97] 356[98]	Adoles-cence[97,98]	Positive	Both studies found a positive asso-ciation between childhood HMW phthalate exposure, mainly DEHP, and the newer DINP and insulin resistance.[97,98]	S: Both studies based on urinary samples corrected for urinary dilution W: No multiple sampling, both cross-sectional studies
	BPA	39[87] 80[88] 3370[91] 188[99]	Preschool till mid childhood[87] Mid child-hood[88,99] Mid childhood till adolescence[91]	Mainly null	One study showed a negative association between childhood BPA exposure and fasting insulin and insulin resistance, unfortunately not adjusted for urinary dilution. [87] Three studies did not find any associations between BPA and childhood metabolic outcomes. [88,91,99]	S: All studies collected urinary sam-ples corrected for urinary dilution, except for one all studies used insulin resistance as an outcome measure W: No multiple sampling

Fetal outcomes

In addition, the same mechanisms that affect maternal outcomes may also affect fetal outcomes. Phthalates have been investigated more thoroughly for their effects on prematurity compared to bisphenols. Nine studies explored the relationship between prenatal phthalate exposure and prematurity. In contrast to our hypothesis, two of these studies found a positive association between phthalate exposure and gestational age at delivery, reporting an increase of only one or two days to the gestational age at birth due to higher levels of phthalates. Both studies measured phthalates in urinary samples in the beginning of the third trimester.[55,56] A French small prospective cohort study found no association of dibutylphthalate and its main metabolite monobutylphthalate in cord blood and breast milk with length of gestation.[57] The remaining six studies found a significant association between a higher exposure to phthalates and reduced gestational age at time of birth.[58-63] Two of these studies performed the measurements of exposure in cord blood samples.[58,61] Of the remaining four studies with phthalate measurements performed in urinary samples, one found a significant reduction in gestational age which only remained in male infants after analysis was stratified for gender. Unfortunately, the timing of urinary sample collection was not specified.[63] Two included studies consist of the same birth cohort with up to four urinary samples during pregnancy investigating the overall relationship with prematurity and windows of vulnerability for spontaneous and placental preterm birth, respectively. Both studies report that the odds of preterm birth compared to term birth are 1.3-1.5 times higher for children prenatally exposed to high levels of phthalates compared to children exposed to lower levels of phthalates.[59,60] One of these studies revealed that spontaneous and placental preterm birth, defined as delivery with presentation of preeclampsia or intrauterine growth restriction, had different sensitivity windows of exposure to phthalates during pregnancy. Spontaneous preterm birth, defined as delivery with presentation of spontaneous labor of preterm premature rupture of membranes, showed to be significantly associated with higher phthalate metabolite concentrations measured at the beginning of the third trimester, while placental preterm birth was associated with higher phthalate metabolite concentrations measured during the first trimester.[60]

Regarding bisphenols, all four studies presented in this review examined only BPA and its relationship with prematurity.[56,63-65] Three of these studies associated higher urinary BPA levels, mainly measured during third trimester, with a significant reduction in gestational age at time of birth of one to four days.[63-65] As for phthalates the gestational age reduction remained only significant in male infants in one of these pregnancy cohorts.[63] The remaining study on bisphenols found no relationship between BPA with gestational age at time of birth.[56]

The association of phthalates and bisphenols with fetal growth is explored with 7 studies reporting on phthalates and 7 studies on bisphenols, including the already presented results from the Japanese study investigating also fetal nutrients during pregnancy displaying no association between DEHP and fetal growth.[53] With respect to phthalates, of the remaining 6 studies, 4 all Chinese studies found that a higher exposure to phthalates was significantly related with reduced body size measures at birth.[58,66-68] A cohort study among 207 women reported a reduction in birth weight of 15-139 grams per natural log increase in phthalate concentrations.[58] One of these studies also displayed a negative association between phthalates and DNA methylation in the human placenta, suggesting placental methylation as a possible mediator of the relationship between phthalates and birth measurements.[67] Contrasting

with our hypothesis, two studies found positive associations between phthalates and fetal head circumference at time of birth.[56,57]

Of the studies on BPA, 4 studies found no association between BPA and body size measures at birth.[56,64,69,70] Two studies found higher BPA levels to be associated with decreased body size measures at birth.[71,72] In a subset of the Dutch Generation R study fetal weight and head circumference were significantly decreased per unit increase in creatinine corrected BPA among women with three urinary BPA measurements during pregnancy. Children born to women in second highest exposure group had an average decrease of 683 grams birth weight. This relationship progressively attenuated among women with fewer BPA measurements.[72] On the contrary, a Korean multi-center birth cohort study found a higher creatinine corrected urinary BPA levels during third trimester to be associated with and increased birth weight in male neonates and increased ponderal index in female neonates.[73]

Hence, the majority of studies show mostly negative effects of phthalates and negative or no effects of BPA on gestational age and body size measures at birth. No studies have explored the other bisphenols besides BPA. An overview of the included studies is given in Table 1. Details of all described studies are represented in Supplementary Table 1.

Childhood outcomes

Phthalates and bisphenols have been reported to have an influence on childhood growth. All three studies exploring the relationship between prenatal phthalates and childhood growth discovered different results in males and females, predominantly resulting in negative associations with growth in males.[74-76] An extensive Spanish birth cohort reported that the molar sum of first and third trimester urinary HMW phthalates was significantly associated with reduced weight gain z-score in the first 6 months in boys and nonsignificantly with lower BMI z-scores in boys at any age (measured until 7 years of age). Meanwhile, in girls the molar sum of first and third trimester HMW phthalates was nonsignificantly associated with higher BMI z-scores.[76]

Two out of 4 studies reported that a higher level of prenatal BPA exposure was associated with an increase in several childhood growth parameters in preschoolers, including BMI z-score, weight and waist circumference.[69,77] The remaining two studies showed prenatal BPA exposure to be related to a lower postnatal BMI in preschool till mid-childhood aged girls, not boys.[6,78] After subdivision in prepubertal and pubertal girls, only in prepubertal girls prenatal BPA exposure remained negatively associated with BMI z-score and waist circumference.[6]

In total, 18 studies investigated the influence of exposure to phthalates and bisphenols during childhood on childhood growth. Five out of nine studies found childhood exposure to phthalates to be associated with increased growth parameters in mid-childhood till adolescence, including obesity indices consisting of subscapular skinfold thickness, BMI, hip- and waist circumference.[79-83] Four of these studies reported different effects in males and females, suggesting sex to be an effect modifier.[79-82] Also ethnicity is a potential modifier of effects. A cross-sectional analysis of the 2003-2008 NHANES data showed an increase in overweight and obesity among non-Hispanic blacks related to childhood phthalate exposure, while among other ethnic groups no significant associations have been found.[83]

Two studies found childhood phthalate exposure to be associated with reduced mid-childhood growth measures, including BMI, fat mass, waist circumference, BSA, weight and height.[75,84] The two remaining studies found no relationship between childhood phthalate exposure and preschool till adolescent growth, defined as a BMI >85th and >95th percentile, respectively.[85,86]

Eleven studies examined the relationship between levels of BPA in childhood and growth, of which 2 studies did not find any relationship between BPA and preschool till mid-childhood growth measures, including weight, height, waist circumference and BMI.[87,88] The earlier mentioned HOME study found two-sided outcomes: exposure early in childhood to higher levels of BPA was associated with a lower BMI at the age of two. However, their BMI slopes increased more rapidly between 2 and 5 years of age.[78] Six studies showed that higher BPA levels in childhood were associated with increased growth parameters, including BMI, waist circumference, waist-to-height ratio, hip circumference and body fat.[6,89-93] Measurements are obtained over the whole age spectrum of childhood and adolescence. Three of these studies were based on subsamples from the National Health and Nutrition Examination Survey (NHANES) in the United States. Samples were not identical as a result of heterogeneous inclusion criteria and outcome measures differed to a certain extent. However, all examined the relationship with BMI.[90-92] Both sex and ethnicity were shown to be potential effect modifiers of the relation between childhood exposure to BPA and childhood growth parameters. In the NHANES data, childhood urinary BPA concentrations were significantly associated with increased risks for obesity and increased waist-to-height ratios among non-Hispanic white boys.[90-92] Contrastingly, a study of Chinese schoolchildren found a significant association between childhood BPA exposure and increased BMI and hip circumference in girls, but not in boys. However, it must be noted that BPA concentrations were not adjusted for urinary dilution.[93] Two earlier mentioned studies found childhood BPA exposure to be associated with reduced overweight and obesity, respectively.[85,86] One of these studies is the only study included in this review investigating not only BPA but also BPS. However, no associations have been found with BPS regarding growth measures.[86]

The aforementioned Spanish study showed that in girls the molar sum of prenatal exposure to both HMW and LMW phthalates was significantly associated with lower systolic blood pressure z-scores at 4 and 7 years of age. For both sexes there was a negative association with diastolic blood pressure, but none of the associations reached the level of significance.[76]

Four studies have examined childhood exposure to phthalates and bisphenols with respect to cardiovascular outcomes. All studies, including 2 studies published on phthalates and 2 studies on BPA, confirmed the hypothesis that childhood exposure to phthalates and bisphenols is associated with adverse cardiovascular outcomes in terms of increased blood pressure and low-grade albuminuria in preschool till adolescent age.[87,94-96] Details are shown in Supplementary Table 1.

The relationship between childhood exposure to phthalates and bisphenols and childhood metabolic outcomes has been explored in six studies. The majority of studies used the homeostatic model assessment of insulin resistance (HOMA-IR) to assess insulin resistance in which a HOMA-IR ≥ 4.39 was defined as insulin resistance.[87,88,91,97,98] The analysis of the 2003-2008 NHANES data showed HMW phthalate metabolites to be related to a higher HOMA-IR in adolescents, which was mainly DEHP dependent. Adding BPA to the model did not change the association.[97] A recent follow-up analysis

of the 2009-2012 NHANES data confirmed this association and explored whether the newer DINP, a replacement for DEHP, was also associated with increased insulin resistance. Also DINP showed to be associated with an increased insulin resistance.[98] Further evidence on the association with phthalates is lacking.

Three studies of BPA failed to find an association with childhood metabolic outcomes in mid-childhood till adolescent age, including adipokines, insulin resistance, blood lipids, insulin and glucose.[88,91,99] The earlier mentioned case study of obese children found a significant mainly negative non-monotonic exposure-response relationship between BPA and fasting insulin and HOMA-IR in a case study of obese or overweight children aged 3-8 years. Unfortunately, BPA was not adjusted for urinary dilution in this analysis, which made it difficult to estimate its adjusted effect.[87]

To conclude, the greater part of studies examined the association of phthalates and bisphenols with growth, giving contradictory results. Sex, stage of puberty and ethnicity are proposed as potential effect modifiers. An overview of the included studies is given in Table 1. Details of all described studies are represented in Supplementary Table 1.

Discussion

We identified many studies investigating potential effects of prenatal and childhood phthalate and bisphenol exposure on growth and cardiometabolic risks. As presented throughout the paragraphs, exposure to phthalates and bisphenols is believed to induce pathways towards several adverse health effects. This conceptual model of mechanisms is shown in Figure 2. In vitro studies showed that exposure to phthalates and bisphenols causes hormonal disruption, epigenetic changes, oxidative and nitrosative stress. The effects could be affected by the time of exposure, both during fetal life and childhood. Exposure during fetal life may induce prematurity and low birth weight. Aside from the many disadvantageous outcomes from premature birth, both premature birth and low birth weight have been associated with cardiometabolic dysfunction, including overweight in adolescence and adulthood, dyslipidemia, insulin resistance, endothelial and glomerular dysfunction.[100-103] As shown before, exposure during childhood is associated with aberrant growth patterns inducing overweight and obesity and early signs of cardiovascular and metabolic dysfunction, including glomerular and endothelial dysfunction, increased blood pressure and increased insulin resistance. Together with the earlier mentioned cellular changes both fetal and childhood exposure to phthalates and bisphenols may give rise to an increased risk for cardiovascular and metabolic disease in adulthood. As shown in the earlier paragraphs, effects could be affected by the time of exposure, both during fetal life and childhood, as well as by the sex and race of the child.

Little is known about the epigenetic changes phthalates and bisphenols can cause and what mechanism lies behind it. The epigenetic effects of fetal BPA exposure were explored in mouse models. This study showed that BPA induces DNA hypomethylation, while maternal nutritional supplementation of methyl donors like folate negated the BPA-induced DNA hypomethylation in the offspring.[104] This could be a major point of engagement, yet has not been examined in humans. An in vitro study of human adipocytes from prepubertal non-obese children investigated the effect of BPA on gene expression in

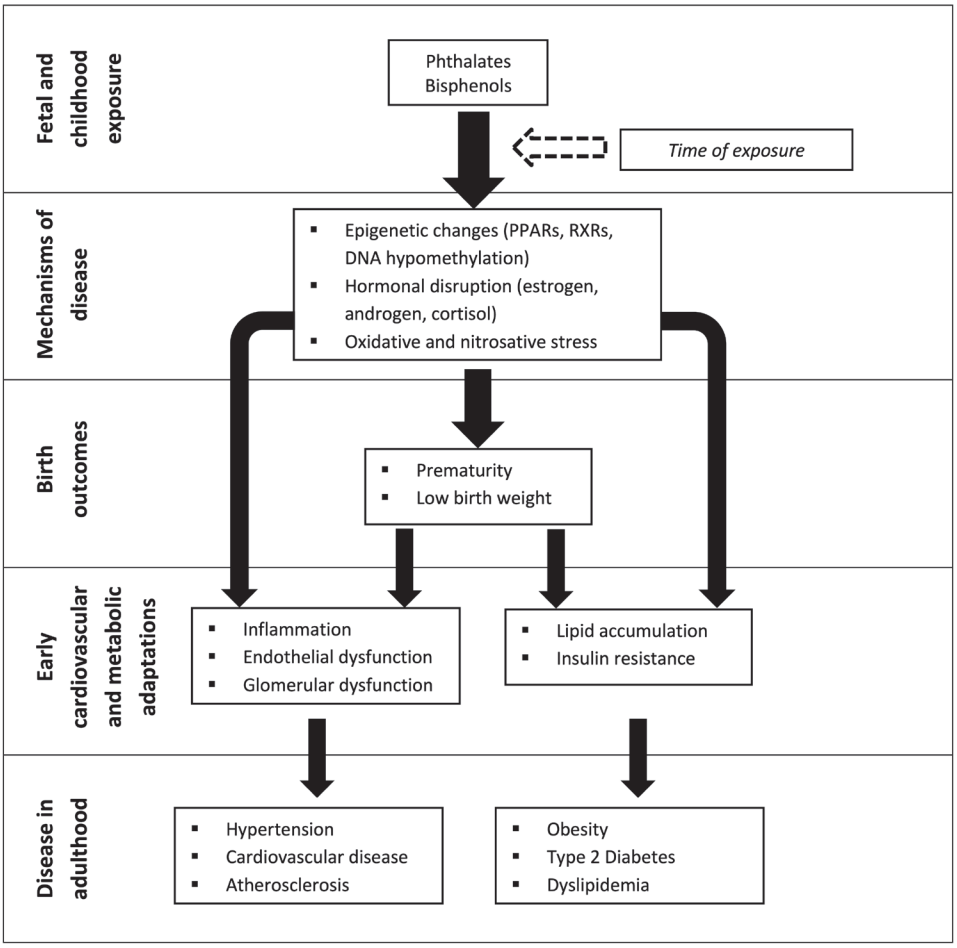
adipocytes, disclosing an increase in pro-inflammatory cytokines involved in the lipid metabolism and a decrease in a gene involved in insulin production.[105] As mentioned in the mechanisms paragraph phthalates and bisphenols are reported to interfere with PPARs and RXRs, potentially causing epigenetic changes. RXRs form heterodimers using its nuclear receptor with all members of the class 1 nuclear receptors, including PPARs, but also the vitamin D3 receptor and the thyroid hormone receptor. Several drugs and mechanisms are claimed to interfere with the nuclear receptor from RXRs.[106,107] However, little is known about the interaction mechanisms. Overall, very few studies have been investigating the mechanisms of phthalates and bisphenols combined with clinical outcomes in humans. More studies are needed investigating mechanisms and potential.

This review shows several limitations in the present literature, especially regarding time of exposure. One of the main limitations is the inconsistent exposure-response relationship in almost all studies performed so far, causing non-differential misclassification of exposure leading to underestimation of the effect. Only one study controlled for puberty, while the degree of puberty could be an important modifier of effects. Only one study described in this review investigated the effects of exposure to BPS, while all other studies only examined BPA. Exposure to BPF has not been studied at all. As shown in the paragraph on maternal pregnancy outcomes, studies on maternal pregnancy outcomes are scarce, in particular studies investigating the effect of phthalates. Studies investigating prenatal exposure to phthalates and bisphenols and metabolic outcomes are lacking, just as studies investigating prenatal exposure to bisphenols and childhood cardiovascular outcomes. The cardiovascular effects of prenatal phthalates have only been examined in one study; therefore it is difficult to draw conclusions. Adult studies on delayed effects of prenatal and childhood exposure to phthalates and bisphenols are lacking. Due to the long effect latency this might be harder to investigate.

In total, 54 studies are included in this review, of which the majority uses urinary samples to estimate phthalate or bisphenol exposure. Eight cohort studies collected more than one urine sample during pregnancy,[6,59,60,72,76-78,99] of which 3 studies collected additionally childhood urine samples.[6,78,99] Only three of these multi-sample studies during pregnancy did not take the average of measured concentrations, but analyzed the samples separately, trying to find windows of vulnerability.[6,60,99] An overview of the included studies and their strengths and weaknesses is presented in Table 1.

Concluding, the human evidence for effects of these chemicals is suggested but limited by methodological difficulties that complicate interpretation. However, an underestimation of effect is most likely. Future studies should focus on also the newer phthalates and bisphenols investigated in a distinct range of time with preferably multiple urine samples to reveal windows of sensitivity for the various biomarkers of effect. When windows of sensitivity during pregnancy would be uncovered, future parents could be informed through preconceptional consults or in early pregnancy. Moreover, to gain a better understanding in the effects, more studies are needed investigating the mechanisms and its potential interactions preferably combined with clinical outcomes. Furthermore, to provide more evidence on prolonged health effects epigenetics should be included in future studies in order to guide evidence-based prevention of harmful exposures.

Figure 2.



Exposure to phthalates and bisphenols is believed to induce pathways towards several adverse health effects, including epigenetic changes, hormonal disruption, oxidative and nitrosative stress. Effects could be affected by the time of exposure, both during fetal life and childhood. Fetal exposure has been linked with prematurity and low birth weight. Childhood exposure has been associated with early signs of cardiovascular and metabolic dysfunction. Both prematurity and low birth weight have also been associated with cardiometabolic dysfunction. Therefore, both fetal and childhood exposure may give rise to an increased risk of cardiovascular and metabolic disease in adulthood.

References

1. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect. 2011;119(6):878-85.
2. Ye X, Pierik FH, Hauser R, Duty S, Angerer J, Park MM, et al. Urinary metabolite concentrations of organophosphorous pesticides, bisphenol A, and phthalates among pregnant women in Rotterdam, the Netherlands: the Generation R study. Environ Res. 2008;108(2):260-7.
3. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, et al. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int. 2014;68:55-65.
4. Casas L, Fernandez MF, Llop S, Guxens M, Ballester F, Olea N, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011;37(5):858-66.
5. Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int. 2013;56:10-8.
6. Harley KG, Aguilar Schall R, Chevrier J, Tyler K, Aguirre H, Bradman A, et al. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. Environ Health Perspect. 2013;121(4):514-20.
7. Legler J, Fletcher T, Govarts E, Porta M, Blumberg B, Heindel JJ, et al. Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. J Clin Endocrinol Metab. 2015;100(4):1278-88.
8. Sathyanarayana S. Phthalates and children's health. Curr Probl Pediatr Adolesc Health Care. 2008;38(2):34-49.
9. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
10. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health. 2014;13(1):43.
11. Fromme H, Gruber L, Schlummer M, Wolz G, Bohmer S, Angerer J, et al. Intake of phthalates and di(2-ethylhexyl)adipate: results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. Environ Int. 2007;33(8):1012-20.
12. Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ Health Perspect. 2014;122(3):235-41.
13. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139-77.
14. Liao C, Kannan K. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2014;31(2):319-29.
15. Liao C, Liu F, Kannan K. Bisphenol s, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol a residues. Environ Sci Technol. 2012;46(12):6515-22.
16. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9; discussion 81-5.
17. Meeker JD, Calafat AM, Hauser R. Urinary phthalate metabolites and their biotransformation products: predictors and temporal variability among men and women. J Expo Sci Environ Epidemiol. 2012;22(4):376-85.
18. Needham LL, Barr DB, Calafat AM. Characterizing children's exposures: beyond NHANES. Neurotoxicology. 2005;26(4):547-53.
19. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.

20. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
21. Gramec Skledar D, Troberg J, Lavdas J, Peterlin Masic L, Finel M. Differences in the glucuronidation of bisphenols F and S between two homologous human UGT enzymes, 1A9 and 1A10. Xenobiotica. 2014:1-9.
22. Teeguarden JG, Twaddle N, Churchwell MI, Yang X, Fisher JW, Seryak LM, et al. 24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup. Toxicol Appl Pharmacol. 2015.
23. Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. 2002;110(11):A703-7.
24. Cabaton N, Chagnon MC, Lhuguenot JC, Cravedi JP, Zalko D. Disposition and metabolic profiling of bisphenol F in pregnant and nonpregnant rats. J Agric Food Chem. 2006;54(26):10307-14.
25. Gayrard V, Lacroix MZ, Collet SH, Viguie C, Bousquet-Melou A, Toutain PL, et al. High bioavailability of bisphenol A from sublingual exposure. Environ Health Perspect. 2013;121(8):951-6.
26. Dabek M, McCrae SI, Stevens VJ, Duncan SH, Louis P. Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria. FEMS Microbiol Ecol. 2008;66(3):487-95.
27. Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe. 2011;10(4):324-35.
28. Mose T, Mortensen GK, Hedegaard M, Knudsen LE. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod Toxicol. 2007;23(1):83-91.
29. Jobling S, Reynolds T, White R, Parker MG, Sumpter JP. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic. Environ Health Perspect. 1995;103(6):582-7.
30. Harris CA, Henttu P, Parker MG, Sumpter JP. The estrogenic activity of phthalate esters in vitro. Environ Health Perspect. 1997;105(8):802-11.
31. Sohoni P, Sumpter JP. Several environmental oestrogens are also anti-androgens. J Endocrinol. 1998;158(3):327-39.
32. Christen V, Crettaz P, Oberli-Schrammli A, Fent K. Some flame retardants and the antimicrobials triclosan and triclocarban enhance the androgenic activity in vitro. Chemosphere. 2010;81(10):1245-52.
33. Millar JS. Novel benefits of peroxisome proliferator-activated receptors on cardiovascular risk. Curr Opin Lipidol. 2013;24(3):233-8.
34. Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, et al. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One. 2013;8(10):e77481.
35. Taxvig C, Dreisig K, Boberg J, Nellemann C, Schelde AB, Pedersen D, et al. Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARgamma activation. Mol Cell Endocrinol. 2012;361(1-2):106-15.
36. Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. Toxicol Sci. 2003;74(2):297-308.
37. Sarath Josh MK, Pradeep S, Vijayalekshmi Amma KS, Balachandran S, Abdul Jaleel UC, Doble M, et al. Phthalates efficiently bind to human peroxisome proliferator activated receptor and retinoid X receptor alpha, beta, gamma subtypes: an in silico approach. J Appl Toxicol. 2014;34(7):754-65.
38. Ferguson KK, Cantonwine DE, Rivera-Gonzalez LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, et al. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. Environ Sci Technol. 2014;48(12):7018-25.

39. Goldinger DM, Demierre AL, Zoller O, Rupp H, Reinhard H, Magnin R, et al. Endocrine activity of alternatives to BPA found in thermal paper in Switzerland. Regul Toxicol Pharmacol. 2015;71(3):453-62.

40. Gutendorf B, Westendorf J. Comparison of an array of in vitro assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology. 2001;166(1-2):79-89.

41. Zhang X, Chang H, Wiseman S, He Y, Higley E, Jones P, et al. Bisphenol A disrupts steroidogenesis in human H295R cells. Toxicol Sci. 2011;121(2):320-7.

42. Rosenmai AK, Dybdahl M, Pedersen M, Alice van Vugt-Lussenburg BM, Wedebye EB, Taxvig C, et al. Are structural analogues to bisphenol a safe alternatives? Toxicol Sci. 2014;139(1):35-47.

43. Watkins DJ, Ferguson KK, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. Int J Hyg Environ Health. 2015;218(2):212-9.

44. Veiga-Lopez A, Pennathur S, Kannan K, Patisaul HB, Dolinoy DC, Zeng L, et al. Impact of gestational bisphenol a on oxidative stress and free Fatty acids: human association and interspecies animal testing studies. Endocrinology. 2015;156(3):911-22.

45. Yadav A, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin in insulin resistance. Clin Chim Acta. 2013;417:80-4.

46. Ashley-Martin J, Dodds L, Arbuckle TE, Ettinger AS, Shapiro GD, Fisher M, et al. A birth cohort study to investigate the association between prenatal phthalate and bisphenol A exposures and fetal markers of metabolic dysfunction. Environ Health. 2014;13:84.

47. Velez MP, Arbuckle TE, Fraser WD. Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. Fertil Steril. 2015;103(4):1011-20 e2.

48. Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. Fertil Steril. 2014;101(5):1359-66.

49. Lathi RB, Liebert CA, Brookfield KF, Taylor JA, vom Saal FS, Fujimoto VY, et al. Conjugated bisphenol A in maternal serum in relation to miscarriage risk. Fertil Steril. 2014;102(1):123-8.

50. Sugiura-Ogasawara M, Ozaki Y, Sonta S, Makino T, Suzumori K. Exposure to bisphenol A is associated with recurrent miscarriage. Hum Reprod. 2005;20(8):2325-9.

51. Robledo C, Peck JD, Stoner JA, Carabin H, Cowan L, Koch HM, et al. Is bisphenol-A exposure during pregnancy associated with blood glucose levels or diagnosis of gestational diabetes? J Toxicol Environ Health A. 2013;76(14):865-73.

52. Robledo CA, Peck JD, Stoner J, Calafat AM, Carabin H, Cowan L, et al. Urinary phthalate metabolite concentrations and blood glucose levels during pregnancy. Int J Hyg Environ Health. 2015;218(3):324-30.

53. Jia X, Harada Y, Tagawa M, Naito H, Hayashi Y, Yetti H, et al. Prenatal maternal blood triglyceride and fatty acid levels in relation to exposure to di(2-ethylhexyl)phthalate: a cross-sectional study. Environ Health Prev Med. 2015;20(3):168-78.

54. Leclerc F, Dubois MF, Aris A. Maternal, placental and fetal exposure to bisphenol A in women with and without preeclampsia. Hypertens Pregnancy. 2014;33(3):341-8.

55. Adibi JJ, Hauser R, Williams PL, Whyatt RM, Calafat AM, Nelson H, et al. Maternal urinary metabolites of Di-(2-Ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. Am J Epidemiol. 2009;169(8):1015-24.

56. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 2008;116(8):1092-7.

57. Brucker-Davis F, Wagner-Mahler K, Bornebusch L, Delattre I, Ferrari P, Gal J, et al. Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from Nice area (France). Chemosphere. 2010;81(2):169-76.
58. Huang Y, Li J, Garcia JM, Lin H, Wang Y, Yan P, et al. Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. PLoS One. 2014;9(2):e87430.
59. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 2014;168(1):61-7.
60. Ferguson KK, McElrath TF, Ko YA, Mukherjee B, Meeker JD. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ Int. 2014;70:118-24.
61. Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, et al. In utero exposure to di-(2-ethylhexyl) phthalate and duration of human pregnancy. Environ Health Perspect. 2003;111(14):1783-5.
62. Meeker JD, Hu H, Cantonwine DE, Lamadrid-Figueroa H, Calafat AM, Ettinger AS, et al. Urinary phthalate metabolites in relation to preterm birth in Mexico city. Environ Health Perspect. 2009;117(10):1587-92.
63. Weinberger B, Vetrano AM, Archer FE, Marcella SW, Buckley B, Wartenberg D, et al. Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age. J Matern Fetal Neonatal Med. 2014;27(4):323-7.
64. Tang R, Chen MJ, Ding GD, Chen XJ, Han XM, Zhou K, et al. Associations of prenatal exposure to phenols with birth outcomes. Environ Pollut. 2013;178:115-20.
65. Cantonwine D, Meeker JD, Hu H, Sanchez BN, Lamadrid-Figueroa H, Mercado-Garcia A, et al. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environ Health. 2010;9:62.
66. Zhao Y, Chen L, Li LX, Xie CM, Li D, Shi HJ, et al. Gender-specific relationship between prenatal exposure to phthalates and intrauterine growth restriction. Pediatr Res. 2014;76(4):401-8.
67. Zhao Y, Shi HJ, Xie CM, Chen J, Laue H, Zhang YH. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. Environ Mol Mutagen. 2015;56(3):286-92.
68. Zhang Y, Lin L, Cao Y, Chen B, Zheng L, Ge RS. Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. J Pediatr. 2009;155(4):500-4.
69. Philippat C, Botton J, Calafat AM, Ye X, Charles MA, Slama R, et al. Prenatal exposure to phenols and growth in boys. Epidemiology. 2014;25(5):625-35.
70. Burstyn I, Martin JW, Beesoon S, Bamforth F, Li Q, Yasui Y, et al. Maternal exposure to bisphenol-A and fetal growth restriction: a case-referent study. Int J Environ Res Public Health. 2013;10(12):7001-14.
71. Chou WC, Chen JL, Lin CF, Chen YC, Shih FC, Chuang CY. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health. 2011;10:94.
72. Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, et al. Fetal growth and prenatal exposure to bisphenol A: the generation R study. Environ Health Perspect. 2013;121(3):393-8.
73. Lee BE, Park H, Hong YC, Ha M, Kim Y, Chang N, et al. Prenatal bisphenol A and birth outcomes: MOCEH (Mothers and Children's Environmental Health) study. Int J Hyg Environ Health. 2014;217(2-3):328-34.
74. de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M. First year growth in relation to prenatal exposure to endocrine disruptors- a Dutch prospective cohort study. Int J Environ Res Public Health. 2014;11(7):7001-21.
75. Maresca MM, Hoepner LA, Hassoun A, Oberfield SE, Mooney SJ, Calafat AM, et al. Prenatal Exposure to Phthalates and Childhood Body Size in an Urban Cohort. Environ Health Perspect. 2015.

76. Valvi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, et al. Prenatal Phthalate Exposure and Childhood Growth and Blood Pressure: Evidence from the Spanish INMA-Sabadell Birth Cohort Study. Environ Health Perspect. 2015.
77. Valvi D, Casas M, Mendez MA, Ballesteros-Gomez A, Luque N, Rubio S, et al. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology. 2013;24(6):791-9.
78. Braun JM, Lanphear BP, Calafat AM, Deria S, Khoury J, Howe CJ, et al. Early-life bisphenol a exposure and child body mass index: a prospective cohort study. Environ Health Perspect. 2014;122(11):1239-45.
79. Buser MC, Murray HE, Scinicariello F. Age and sex differences in childhood and adulthood obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health. 2014;217(6):687-94.
80. Zhang Y, Meng X, Chen L, Li D, Zhao L, Zhao Y, et al. Age and sex-specific relationships between phthalate exposures and obesity in Chinese children at puberty. PLoS One. 2014;9(8):e104852.
81. Wang H, Zhou Y, Tang C, He Y, Wu J, Chen Y, et al. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One. 2013;8(2):e56800.
82. Teitelbaum SL, Mervish N, Moshier EL, Vangeepuram N, Galvez MP, Calafat AM, et al. Associations between phthalate metabolite urinary concentrations and body size measures in New York City children. Environ Res. 2012;112:186-93.
83. Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect. 2013;121(4):501-6.
84. Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010;118(10):1458-64.
85. Wolff MS, Teitelbaum SL, Windham G, Pinney SM, Britton JA, Chelimo C, et al. Pilot study of urinary biomarkers of phytoestrogens, phthalates, and phenols in girls. Environ Health Perspect. 2007;115(1):116-21.
86. Xue J, Wu Q, Sakthivel S, Pavithran PV, Vasukutty JR, Kannan K. Urinary levels of endocrine-disrupting chemicals, including bisphenols, bisphenol A diglycidyl ethers, benzophenones, parabens, and triclosan in obese and non-obese Indian children. Environ Res. 2015;137:120-8.
87. Khalil N, Ebert JR, Wang L, Belcher S, Lee M, Czerwinski SA, et al. Bisphenol A and cardiometabolic risk factors in obese children. Sci Total Environ. 2014;470-471:726-32.
88. Lee HA, Kim YJ, Lee H, Gwak HS, Park EA, Cho SJ, et al. Effect of urinary bisphenolA on androgenic hormones and insulin resistance in preadolescent girls: a pilot study from the Ewha Birth & Growth Cohort. Int J Environ Res Public Health. 2013;10(11):5737-49.
89. Wang HX, Zhou Y, Tang CX, Wu JG, Chen Y, Jiang QW. Association between bisphenol A exposure and body mass index in Chinese school children: a cross-sectional study. Environ Health. 2012;11:79.
90. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA. 2012;308(11):1113-21.
91. Eng DS, Lee JM, Gebremariam A, Meeker JD, Peterson K, Padmanabhan V. Bisphenol A and chronic disease risk factors in US children. Pediatrics. 2013;132(3):e637-45.
92. Bhandari R, Xiao J, Shankar A. Urinary bisphenol A and obesity in U.S. children. Am J Epidemiol. 2013;177(11):1263-70.
93. Li DK, Miao M, Zhou Z, Wu C, Shi H, Liu X, et al. Urine bisphenol-A level in relation to obesity and overweight in school-age children. PLoS One. 2013;8(6):e65399.
94. Trasande L, Sathyanarayana S, Spanier AJ, Trachtman H, Attina TM, Urbina EM. Urinary phthalates are associated with higher blood pressure in childhood. J Pediatr. 2013;163(3):747-53 e1.

95. Trasande L, Attina TM, Trachtman H. Bisphenol A exposure is associated with low-grade urinary albumin excretion in children of the United States. Kidney Int. 2013;83(4):741-8.

96. Trasande L, Sathyanarayana S, Trachtman H. Dietary phthalates and low-grade albuminuria in US children and adolescents. Clin J Am Soc Nephrol. 2014;9(1):100-9.

97. Trasande L, Spanier AJ, Sathyanarayana S, Attina TM, Blustein J. Urinary phthalates and increased insulin resistance in adolescents. Pediatrics. 2013;132(3):e646-55.

98. Trasande L, Attina TM. Association of Exposure to Di-2-Ethylhexylphthalate Replacements With Increased Insulin Resistance in Adolescents From NHANES 2009-2012. J Clin Endocrinol Metab. 2015;100(7):2640-50.

99. Volberg V, Harley K, Calafat AM, Dave V, McFadden J, Eskenazi B, et al. Maternal bisphenol a exposure during pregnancy and its association with adipokines in Mexican-American children. Environ Mol Mutagen. 2013;54(8):621-8.

100. Sipola-Leppanen M, Vaarasmaki M, Tikanmaki M, Matinolli HM, Miettola S, Hovi P, et al. Cardiometabolic risk factors in young adults who were born preterm. Am J Epidemiol. 2015;181(11):861-73.

101. Lewandowski AJ, Davis EF, Yu G, Digby JE, Boardman H, Whitworth P, et al. Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. Hypertension. 2015;65(3):607-14.

102. Juonala M, Cheung MM, Sabin MA, Burgner D, Skilton MR, Kahonen M, et al. Effect of birth weight on life-course blood pressure levels among children born premature: the Cardiovascular Risk in Young Finns Study. J Hypertens. 2015;33(8):1542-8.

103. Hussain SM, Kahonen M, Raitakari OT, Skilton MR, Witt N, Chaturvedi N, et al. Impact of fetal growth and preterm birth on the retinal microvasculature in mid-adulthood. Microcirculation. 2015;22(4):285-93.

104. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104(32):13056-61.

105. Menale C, Piccolo MT, Cirillo G, Calogero RA, Papparella A, Mita L, et al. Bisphenol A effects on gene expression in adipocytes from children: association with metabolic disorders. J Mol Endocrinol. 2015;54(3):289-303.

106. Gronemeyer H, Gustafsson JA, Laudet V. Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discov. 2004;3(11):950-64.

107. Ghosh JC, Yang X, Zhang A, Lambert MH, Li H, Xu HE, et al. Interactions that determine the assembly of a retinoid X receptor/corepressor complex. Proc Natl Acad Sci U S A. 2002;99(9):5842-7.

Supplementary table 1. The included studies

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Velez (2015) [47]	Time to pregnancy	Phthalates & BPA	Not applicable	2001 women participating the MIREC pregnancy cohort study in Canada. Recruited during first prenatal visit (<14 weeks of gestation). To determine time to pregnancy, women were asked how many months it took to get pregnant and about the type of birth control the couple had used. Women with egg donation or reported male infertility were excluded, as well as pregnancies as a result of birth control failure. 1742 women were included in the BPA analysis, and 1597 in the phthalates analysis.	One spot urine sample was collected during the first trimester visit for the analysis of BPA and 11 phthalate metabolites. To adjust for urinary dilution specific gravity as included as a covariate in the regression model.	BPA concentrations were not significantly associated with diminished fecundity either in crude or adjusted models. All phthalate metabolites showed the similar suggestion of a shorter time to pregnancy, however all were not statistically significant.
Buck Louis (2014) [48]	Time to pregnancy	Phthalates & BPA	FOR: Fecundability odds ratio; estimates the odds of becoming pregnant each cycle.	501 couples from the LIFE Study, discontinuing contraception and attempting to become pregnant in Michigan and Texas, USA. Women with injectable hormonal contraceptives in the past year, currently lactating or couples with infertility/sterility were excluded. Baseline interviews and anthropometric measurements menstruation and home preg-	Urinary samples were collected at baseline for the measurement of total BPA and 14 phthalate metabolites. To adjust for urinary volume, all models were adjusted for creatinine.	Neither female nor male BPA concentration was associated with time to pregnancy. Men's urinary concentrations of mMP (monomethyl), mBP (mono-n-butyl), and mBzP (monobenzyl phthalates) were associated with a significantly longer time to pregnancy (FOR 0.80, 95% CI 0.70 to 0.93; 0.82, 0.70 to 0.97; and 0.77, 0.65 to 0.92, respectively). Women's MCPP (mono (3-carboxypropyl) phthalate) was significantly associated with shorter time

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
				nancy tests, while men did on lifestyle. Women used the Clearblue Easy Fertility Monitor to display the LH rise.		to pregnancy (FOR 1.20, 95% CI 1.00 to 1.43). When couples were analyzed together, mMP and mBzP showed significantly associated with longer time to pregnancy in males, while mCPP and mOP (monooctyl phthalate) were significantly associated with shorter time to pregnancy in females.
Lathi (2014) [49]	Miscarriage risk	BPA	Miscarriage: loss of a clinical pregnancy after a gestational sac was confirmed on ultrasound.	Cohort study of 115 women, including 68 first-trimester spontaneous miscarriages and 47 live births from women who sought treatment for infertility or recurrent pregnancy loss at Stanford Fertility and Reproductive Medicine Clinic. Intrauterine pregnancy was confirmed by serum β human chorionic gonadotrophin (β-hCG) and ultrasound. Miscarriages were only included if a karyotype was performed. Women with known etiology for miscarriage were excluded.	Blood samples around the gestational age of approximately 4 weeks for conjugated BPA measurement. For the majority of women a second blood sample was collected 2-15 days after diagnosis of pregnancy.	In a logistic regression model a statistically significant positive association between serum conjugated BPA level quartile and miscarriage was observed. Women in the fourth quartile had a significantly increased risk of miscarriage, both aneuploid and eupoid (RR 1.83, 95% CI 1.14 to 2.96). Intrauterine insemination (IUI) and in vitro fertilization (IVF) were protective relative to natural conception.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Sugiura-Ogasawara (2005) [50]	Recurrent miscarriages	BPA	Consecutive miscarriages: three or more miscarriages	45 patients with consecutive first-trimester miscarriages. Cases with uterine anomaly or chromosome abnormality were excluded. 32 healthy non-pregnant women with no history of live birth, infertility and miscarriage were examined as controls. All women lived in Nagoya City and surrounding neighbourhood.	Blood samples were taken 5-9 days after ovulation in at least two cycles for serum BPA levels.	Patients had significantly higher mean ± SD values for BPA than control women (2.59 ± 5.23 ng/ml vs. 0.77 ± 0.38 ng/ml). Women who subsequently miscarried again, had higher nonsignificant BPA levels than women whose subsequent pregnancy was successful. Patients who miscarried with abnormal embryonal karyotype had a higher BPA than patients miscarrying with a normal embryonal karyotype.
Robledo (2013) [51]	Blood glucose levels and gestational diabetes mellitus	BPA	Gestational diabetes mellitus (GDM): standard oral glucose tolerance test (OGTT) ≥200mg/dL or 3-hour OGTT ≥2 times over threshold.	22 cases of gestational diabetes mellitus and 72 control pregnant women from the University of Oklahoma Medical Center Women's Clinic.	Maternal urinary samples were obtained upon enrollment, around 30 gestational weeks, for the measurement of BPA. To adjust for dilution, samples were adjusted for specific gravity.	No significant associations were observed between BPA exposure and GDM diagnosis.
Robledo (2015) [52]	Blood glucose levels and gestational diabetes mellitus	Phthalates	Gestational diabetes mellitus (GDM): standard oral glucose tolerance test (OGTT) ≥200mg/dL or 3-hour OGTT ≥2 times over threshold.	72 pregnant women from the University of Oklahoma Medical Center Women's Clinic, all with no history of adverse pregnancy outcomes.	Maternal urinary samples were obtained upon enrollment, at a mean of 12.8 gestational weeks, for the measurement of phthalate metabolites and creatinine to adjust for dilution. Midpregnancy patients had a routine	Compared to pregnant women in the lowest concentration tertile, women with the highest urinary concentrations of MiBP and MBzP had lower blood glucose levels at time of GDM screening after adjustment for urinary creatinine and demographic covariates.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
					oral glucose tolerance test to test for gestational diabetes mellitus.	
Jia (2015) [53]	Maternal triglycerides and fatty acid levels and fetal growth	DEHP	Not applicable	318 mother-infant pairs in Sapporo, Japan, with no serious illnesses or history of adverse pregnancy outcomes. Maternal and infant medical information were obtained from medical records.	Maternal blood samples were taken preferably at the next prenatal visit after enrollment (after 23-25 weeks of gestational age) for the measurement of MEHP, triglyceride and fatty acids levels.	Maternal MEHP concentrations were inversely associated with triglyceride and fatty acids levels during pregnancy. No relationship with fetal growth has been found.
Leclerc (2014) [54]	Preeclamp-sia	BPA	Not applicable	Case control study of 23 women with preeclampsia and 35 normotensive pregnant women at the Centre Hospitalier Universitaire de Sherbrooke. All women had normal delivery and no other adverse perinatal outcomes than preeclampsia.	Maternal blood samples were obtained before delivery and umbilical cord blood sampling after birth for measurement of BPA. Three placental biopsies were collected from all women after birth to assess for BPA accumulation.	Maternal and fetal serum concentrations of BPA were not significantly different in preeclamptic women in comparison with normotensive pregnant women. In placental tissue, concentrations of BPA were higher in preeclamptic women compaired to normotensive women (median 9.40 (0.40-101) vs. 3.00 (0.30-36.1) ng/ml, respectively, p=0.04). The comparative distribution of bioaccumulated BPA between maternal, placental and fetal compartments showed normal distribution in the normotensive group, while a high level of BPA in the placental compartment was found in the preeclamptic group.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Adibi (2009) [55]	Gestational length at time of delivery	DEHP	Not applicable	Pregnancy cohort study, the Study for Future Families, consisting of 283 women in 4 US states (California, Iowa, Minnesota, and Missouri). Gestational age at delivery was clinically estimated with ultrasound data, examination of the newborn, and dates reported by the mother. This was rounded up to the next-highest week.	1 urinary sample at time of recruitment, on average 12.2 weeks before delivery (SD 7.6 weeks) or the beginning of third trimester measuring DEHP metabolites corrected for creatinine.	After covariate adjustment, women at the 75th percentile of DEHP metabolite concentrations had a 2-day-longer mean length of gestation than women at the 25th percentile (95% CI 1.4-3.3). Log-unit increases in mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP) and mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP) concentrations were significantly associated with reduced odds of preterm delivery (respectively 50%, 60% and 50% decreased odds).
Wolff (2008) [56]	Gestational age at time of delivery & body size measures at birth	Phthalates & phenols, including BPA	Not applicable	New York prospective multiethnic birth cohort, The Children's Environmental Health Study, containing 404 mother-infant pairs. Gestational age was assigned using reported date of last menstrual period. Maternal characteristics were collected by interview, and birth outcomes were obtained from a computerized perinatal database within the Department of Obstetrics at Mount Sinai Hospital.	1 urinary sample per woman. Samples obtained between 25 and 40 gestational weeks (mostly third trimester) for analyzation of 5 phenol metabolites, including BPA, and 10 phthalate metabolites, corrected for creatinine.	Among phenols no associations with birth outcomes. ∑LMW phthalates is significantly positively associated gestational age at birth (β=0.14 weeks (95% CI 0.01-0.27)) and head circumference at birth (β=0.13 cm (95% CI 0.01-0.24)). MEHP is significantly positively associated with gestational age (β=0.15 weeks (95% CI0.02-0.29)), MBzP with birth length (β=0.20 cm (95% CI 0.00-0.40)) and MEP with head circumference (β=0.12 cm (95% CI 0.01-0.23)). All with a p-value <0.05.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Brucker-Davis (2010) [57]	Delivery and neonatal outcomes	Dibutyl-phthalate and its main metabolite monobutyl-phthalate	Not applicable	86 mother-healthy infant boy pairs, all included as controls in a prospective cohort study on cryptorchism at Nice University Hospital and Grasse General Hospital, France. All infants were born after a gestational age of 34 weeks.	20 mL of cord blood and 10 mL of maternal milk, collected between day 2 and 5 postpartum, were obtained in phthalate free containers for the measurement of various xenobiotics, including dibutylphthalate (DiBP) and its main metabolite monobutylphthalate (mBP).	Infant head circumference was positively associated with mBP in cord blood. There were no associations between mBP and birth weight, birth length, and length of gestation.
Huang (2014) [58]	Prematurity & fetal growth	Phthalates	Preterm birth: delivery before 37 weeks of gestational age.	207 consecutive pregnant women who delivered at Southwest Hospital in Chongqing, China, were recruited when going into labor. 33 of the women had a preterm delivery. Delivery characteristics and fetal growth parameters were obtained from the perinatal database of Southwest Hospital. After labor a questionnaire was administered to obtain information on socio-demographic characteristics, medical history and lifestyle factors.	Cord blood sample for the identification of 15 phthalates.	Exposure to all analyzed phthalates except dicyclohexyl phthalate (DCHP) was associated with gestational age reduction and preterm delivery ($p<0.01$). There were associations between phthalates and fetal growth parameters. However, many of which disappeared when analyses were adjusted for gestational age, especially in male infants. In female infants several phthalates remained significantly associated with decreased fetal growth parameters.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Ferguson (2014) [59]	Prematurity	Phthalates	Preterm birth: delivery before 37 weeks of gestational age. Spontaneous preterm birth: delivery with presentation of spontaneous labor of preterm premature rupture of membranes (PPROM).	Subset of women from a prospective, longitudinal birth cohort at the Brigham and Women's Hospital in Boston, Massachusetts. This subset included 130 women who delivered preterm and 352 randomly selected controls. Gestational age was calculated based on last menstrual period and confirmed by first trimester ultrasound. All women were followed during pregnancy and birth outcome characteristics were recorded at delivery.	During pregnancy, women provided up to 4 urine samples for the measurement of nine phthalate metabolites. Visit 1 urine samples were taken at median 9.71 weeks of gestation, visit 2 at median 17.9 weeks, visit 3 at median 26.0 weeks, and visit 4 at median 35.1 weeks. To adjust for urinary dilution, corrections have been made for specific gravity.	In adjusted models, MEHP, MECPP, and ∑DEHP metabolites were associated with significantly increased odds of preterm birth (respectively aORs of 1.34 (95% CI 1.07-1.68), 1.40 (95% CI 1.13-1.74), and 1.33 (95% CI 1.04-1.70)). MEHP, MEOHP, MECPP, ∑DEHP, MBP, and MCPP were all associated with significantly elevated odds of spontaneous preterm birth (for numbers see original study).
Ferguson (2014) [60]	Windows of vulnerability for prematurity during pregnancy	Phthalates	Preterm birth: delivery before 37 weeks of gestational age. Spontaneous preterm birth: delivery with presentation of spontaneous labor of preterm premature rupture of membranes (PPROM).	Subset of women from a prospective, longitudinal birth cohort at the Brigham and Women's Hospital in Boston, Massachusetts. This subset included 130 women who delivered preterm and 352 randomly selected controls. Gestational age was calculated based on last menstrual period and confirmed by first trimester ultrasound. All women were followed during pregnancy and birth outcome	During pregnancy, women provided up to 4 urine samples for the measurement of nine phthalate metabolites. Visit 1 urine samples were taken at median 9.71 weeks of gestation, visit 2 at median 17.9 weeks, visit 3 at median 26.0 weeks, and visit 4 at median 35.1 weeks. To adjust for urinary dilution, correc-	Adjusted odds ratios (aOR) for spontaneous preterm birth were strongest in association with phthalate metabolite concentrations measured at the beginning of the third trimester (aOR for ∑DEHP 1.33 (95% CI 1.02-1.73), from which aOR for MECPP 1.33 (95% CI 1.04-1.70), for MbzP 1.43 (95% CI 1.05-1.95), and for MBP 1.45 (95% CI 1.08-1.96)). aORs for placental preterm birth were slightly elevated in the first trimester for DEHP metabolites (aOR for ∑DEHP 1.33 (95% CI 0.99-1.78)) and elevated for the

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			Placental preterm birth: preterm birth following preeclampsia or intra uterine growth retardation (IUGR).	characteristics were recorded at delivery.	tions have been made for specific gravity.	DEHP metabolite MECPP (aOR 1.46 (95% CI 1.10-1.95).
Latini (2003) [61]	Prematurity	DEHP	Definition of preterm delivery not described.	84 consecutive newborns, born at the general-practice Brindisi Hospital, Italy. Eleven of 84 infants were preterm, only three had very low birth weight, and four were small for gestational age (SGA). No data available on the exact time of inclusion and measurement or calculation of gestational age.	Cord blood samples were collected for the identification of DEHP and its main metabolite MEHP.	MEHP-positive newborns showed a significantly lower mean gestational age compared with MEHP-negative infants (p=0.033), with a positive correlation between the absence of MEHP and gestational age at delivery (OR 1.50, 95% CI 1.013-2.21)).
Meeker (2009) [62]	Prematurity	Phthalates	Not applicable	Subset of a Mexican birth cohort. Gestational length based on maternal recall of last menstrual period. Possible confounding information was collected through questionnaire. Randomly selected cases (= 30) with premature delivery (< 37 weeks of gestation) and controls (= 30) with delivery at term (≥ 37 weeks of gestation).	Measurement of urinary phthalate metabolite concentrations in 1 sample during third trimester, corrected for urine dilution by specific gravity and by creatinine.	Preterm birth cases had elevated odds for nearly all metabolites unadjusted for dilution. After adjustment for specific gravity odds remained elevated for MECPP (3.4 (95% CI 1.0-12.0)), MBP (4.5 (95% CI 1.2-16.6)) and MCPP (3.2 (95% CI 1.0-9.8)). After adjustment for creatinine odds remained elevated for MBP (5.4 (95% CI 1.5-19.3)) and ∑DEHP

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
				No significant differences between groups.		(4.1 (95% CI 1.0-17.5)).
Weinberger (2014) [63]	Gestational age	BPA and Phthalates	Not applicable	72 pregnant women from the High Risk Obstetric Clinic at Robert Wood Johnson University Hospital, USA. Gestational age was determined based on medical record, using ultrasound or date of implantation. Infants were examined <72 hours after birth in the nursery. Infant birth characteristics were obtained from the medical chart. Maternal medical and demographic details were obtained during first prenatal visit.	One urine sample was obtained for the measurement of phthalates and BPA, corrected for urinary dilution by specific gravity, at which term was not specified.	After adjusting for parity and maternal race, each interquartile change in urinary MEHHP and free + glucuronide BPA concentration was associated with a significant reduction of 4.2 and 1.1 days of gestation, respectively. After stratification for gender, this association only remained in male infants.
Tang (2013) [64]	Birth outcomes including birth weight, length, and gestational age	Phenols, including BPA	Preterm birth: delivery before 37 weeks of gestational age. Low birth weight: <2500g.	567 pregnant women from hospitals affiliated to Nanjing Medical University, China, without medical complications and newborn infants with severe neonatal illness. Gestational age was estimated based on the onset of the last menstrual period and a clinical estimate was made by ultrasound. If there was a significant discordance between these two, the first clinical estimation	Urine samples were collected during hospital admission for delivery for analyzation of 4 phenols, including BPA. Total BPA was adjusted for creatinine to correct for urine dilution.	After adjustment for maternal age, BMI in late pregnancy, parity and creatinine the middle exposure group to BPA was negatively associated with gestational age compared to the low exposure group (β adjusted =-0.48 week (95% CI-0.91 to -0.05)). No associations between BPA and fetal growth.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
				was used. Infant sex, birth date, parity, weight, and crown- heel length were obtained from medical records.		
Cantonwine (2010) [65]	Prematurity	BPA	Preterm birth: delivery before 37 weeks of gestational age.	Subset of a Mexican birth cohort. Gestational length based on maternal recall of last menstrual period. Possible confounding information was collected through questionnaire. Randomly selected cases (= 30) who delivered < 38 weeks of gestation at time of delivery and controls (= 30) who completed ≥ 38 weeks of gestation at time of delivery. Among the group the cases were 12 women who delivered prior to 37 weeks. No significant differences between groups.	Measurement of the total urinary concentration of BPA (free plus conjugated species) in 1 urinary sample during third trimester, corrected for urine dilution by specific gravity and by creatinine.	In an unadjusted analysis specific gravity adjusted BPA is significantly associated with preterm delivery (OR 2.50 (95% CI 1.05-5.96)). No adjusted analysis performed.
Zhao (2014) [66]	Intrauterine growth retardation	Phthalates	IUGR: estimated fetal weight below the 10th centile for gestational age. Quetelet's index: weight in kg / height^2 in meters	Case-control study of 126 mother-newborn pairs, including 42 IUGR cases and 84 healthy controls, in the Second Affiliated Hospital of Wenzhou Medical College, China.	One maternal spot urine sample during third trimester was collected to measure five phthalate metabolites, adjusted for specific gravity to correct for urinary dilution.	After adjusting for potential confounders, MEHHP and MEOHP remained significant inversely associated with birth weight and Quetelet's index (per log-unit increase 0.213 (β=-0.2013 (95% CI-0.401 to -0.025)) and 0.233 (β=-0.233 (95% CI -0.411 to 0.056) kg decrease in birthweight, respectively; per log-unit increase 0.656 (β=-0.656 (95% CI-1.307 to-0.005)

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
						and 0.816 (β=-0.816 (95% CI-1.426 to -0.206) kg/m^2 decrease, respectively). When mothers were stratified by infant sex, MEHHP and MEOHP concentrations were significantly inversely associated with birth weight and Quetelet's index in male infants, while no significant association was observed in females.
Zhao (2015) [67]	Fetal growth & DNA methylation in human placenta	Phthalates	Intrauterine growth restriction (IUGR): estimated fetal weight below the 10th centile for gestational age. Low birth weight (LBW): fetal birth weight <2500g at gestational age ≥37 weeks. Fetal growth restriction (FGR): all cases of IUGR or LBW.	Case-control study of 119 mother-newborn pairs, including 55 FGR cases and 64 healthy controls, in the Second Affiliated Hospital of Wenzhou Medical College, China.	One maternal first morning urine sample during third trimester was collected to measure five phthalate metabolites, adjusted for specific gravity to correct for urine dilution. After delivery, eight biopsies of the maternal side of the placenta were collected immediately after delivery for genomic DNA extraction. Placental LINE-1 methylation was measured by PCR-pyrosequencing of the bisulfite-treated DNA.	DEHP metabolites were significantly higher in FGR cases than in controls (for MEHHP, MEOHP, and SumDEHP p= 0.002, 0.003, and 0.002, respectively). When preterm births were stratified out, adjusted odds ratios for the same DEHP metabolites were statistically significant in the subset of non-preterm births. LINE-1 methylation was positively associated with birth weight SDS, a stronger association was found among women with fetal growth restriction. Urinary concentrations of MEHHP (p=0. 39) and SumDEHP (p=0.038) were significantly inversely associated with placental LINE-1 methylation in all subjects. Among FGR cases, MEHHP (p = 0.004), MEOHP (p = 0.039), and SumDEHP (p = 0.005) were negatively associated with placental LINE-1 methylation, suggesting LINE-1

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
						methylation as a possible mediator of the association between phthalates and fetal weight.
Zhang (2009) [68]	Low birth weight	Phthalates	LBW: fetal birth weight <2500g at gestational age ≥37 weeks.	Case-control study of 201 mother-newborn pairs, including 88 LBW cases and 113 control term newborns. All infants were born at Shanghai Medical Center for Maternal and Child Health. Body size measures at birth were obtained from medical records.	Maternal blood samples and umbilical vein blood was obtained after delivery. Meconium of the infant was collected from every diaper during the first 48 hours after delivery, and all these samples were pooled into one sample. Three common phthalates and two of their metabolites were analyzed.	LBW infants had significantly higher levels of DBP and MEHP in cord blood samples (p= 0.02 and 0.000, respectively), and MBP and MEHP in meconium samples (p= 0.003 and 0.000, respectively). After controlling for potential covariates DBP exposure was significantly associated with LBW (p=0.01), and DEHP exposure with reduced birth length (DEHP p=0.05 and MEHP, its main metabolite, p=0.001 in cord blood and 0.000 in meconium). Adjusted odds ratios between LBW and phthalates showed dose response relationships for DBP (p=0.008) and MEHP (p=0.05) in cord blood and for both MBP (p=0.000) and MEHP (p=0.04) in meconium.
Philippat (2014) [69]	Prenatal and postnatal growth in boys until the age of 3	BPA	Not applicable	A subgroup of 520 mother-child pairs of the French Étude des Déterminants pré et postnatals du développement et de la santé e l'ENfant mother-child cohort consisting of boys, with at least 1 maternal urine sample and complete data on growth. Growth	Urine samples were collected between 22 and 29 gestational weeks, preferably first morning urine.	No clear associations have been observed between BPA and prenatal or postnatal measurements. After adjustment for height, BPA was not associated with weight at birth or at 6 months, but tended to be positively associated with weight at 12, 24, and 36 months. Adjustment for child caloric intake did not change this effect.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
				was measured by ultrasound. Fetal weight was estimated using the Hadlock formula. Weight and length at birth were extracted from hospital records. Children were weighed and measured at 1 and 3 years. Additionally, at 4, 8, 12, 24, and 36 months, mother mailed questionnaires with the boys' weight and height measures. Head circumference was assessed 4 days after birth and at 3 years, abdominal circumference was measured at 3 years. Both were done in duplicate.		
Burstyn (2013) [70]	Fetal growth restriction	BPA	Fetal growth restriction: full term (≥37 weeks of gestation) infants with birth weight below the 10th percentile, normalized for sex and gestational age.	550 matched case-referent pairs from a nested cohort study of pregnant women in and around Edmonton, Alberta, Canada. Cases and referents were pooled for analyses.	Maternal serum was collected at 15-16 weeks of gestation for the measurement of BPA.	No associations have been found between early pregnancy BPA and fetal growth restriction. Stratification by sex and control for confounding did not suggest BPA increased fetal growth restriction.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Chou (2011) [71]	Birth outcomes and adipokine expression	BPA	LBW: newborn's birth weight less than the 10th percentile (<2600 g). SGA: birth weight less than the 10th percentile, adjusted for gestational age and sex in Taiwan. High leptin: level of leptin more than 90th percentile (>9.56ng/ml) in cord blood samples. Low adiponectin: level of adiponectin less than 10th percentile (<10.32 µg/ml)	97 mother-newborn pairs from an obstetrics and gynecology clinic in Hsinchu County, Taiwan.	Maternal blood and umbilical cord blood were collected at delivery for determination of BPA, adiponectin and leptin concentrations.	Overall, maternal BPA had a significant negative association with birth weight ($p<0.05$). After adjustment for covariates, maternal BPA exposure was significantly associated with LBW and SGA in male infants (Adjusted OR 2.12 (95% CI 1.05 to 2.38) and 1.34 (1.13 to 2.83), respectively), while these associations were not observed in female infants.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Snijder (2013) [72]	Fetal growth	BPA	Not applicable	Random sample of 219 women from the Generation R study, a population-based prospective cohort study in Rotterdam, the Netherlands. Growth characteristics were measured to the nearest millimeter using standardized ultrasound procedures in the second and third trimesters. First trimester measurements are used to establish gestational age. Information on gestational age, sex, weight, length, and head circumference at birth was obtained from medical records.	Urine samples were collected during early pregnancy (<18 weeks), mid-pregnancy (18-25 weeks), and late pregnancy (>25 weeks). 99 women had one measurement, 40 had two measurements, and 80 had three measurements. In general, the creatinine-based BPA concentrations have been used.	Among women with three BPA measurements, fetal growth and fetal head circumference were significantly decreased per unit creatinine-based total BPA concentration (resp. β-0.017 (95% CI-0.033 to-0.001) and β-0.018 (95% CI-0.037 to 0.000)). Among women with fewer measurements, this relationship was progressively attenuated and nonsignificant. The effect estimates of univariable and multivariable analyses in the restricted sample were comparable, suggesting little influence of the potential confounders.
Lee (2014) [73]	Anthropometric measures at birth	BPA	Ponderal index: the ratio of birth weight in grams to length in centimeters cubed.	All 757 women with BPA levels measured during pregnancy and recordings on birth outcomes from the Mothers and Children's Environmental Health study, a multi-center birth cohort in Korea. Gestational age was estimated based on the onset of the last menstrual period, or the first ultrasonoraphic estimation in case the last menstrual period was unreliable of there was a significant discordance.	One spot urinary sample collected during late pregnancy (28-42 gestational weeks) to estimate the level of BPA exposure. BPA concentrations were adjusted for the urinary creatinine concentration to correct for the urine volume.	In unadjusted analysis birth weight and ponderal index were positively related to BPA levels corrected for creatinine. In adjusted analysis birth weight was significantly increased in the second tertile of maternal creatinine adjusted BPA level compared to first tertile (p=0.04), which remained in male neonates (p=0.04), while no significant results were found in female neonates (p=0.22). Regarding birth length, only in male neonates a significant relationship with BPA level was found (second vs. first tertile p=0.01).

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
						In case of the ponderal index, the positive association was found mainly in female neonates (in adjusted analysis second vs. first tertile p=0.003).
De Cock (2014) [74]	First year growth	Phthalates	BMI: weight in kilograms / height^2 in meters.	89 mother child-pairs from six midwifery clinics in the area of Zwolle, the Netherlands. Women were invited to participate during the first antenatal visit to the midwife (between 10 and 12 weeks of pregnancy). Gestational age was determined by early ultrasound. On average each child is seen six times during the first year of life by youth care at 1, 2, 4, 6, 9, and 11 months of age.	Cord blood was collected immediately after birth and breast milk was collected in the second month after birth with a minimum of 100mL total for determination of markers of early exposure to several endocrine disrupting chemicals.	For MEOHP, boys in the first quartile had a higher BMI than higher exposed boys from the age of 3 months on (p=0.029). Boys with high MECPP exposure had a greater head circumference than other boys (p=0.047), as did girls in the second quartile of MEHHP (p=0.018).
Maresca (2015) [75]	BMI	Phthalates	Obese: BMI z-score ≥95th percentile.	234 mother-child pairs from the CCCEH longitudinal birth cohort study's Obesity Project in New York, the United States. Prenatal recruitment. Infant's birth weight and sex were obtained from medical records. At 5 and 7 years of age anthropometric measurements including height, weight and waist circumference.	Spot urine samples were collected during third trimester and at 3 and 5 years of age for the measurement of phthalate metabolites. To adjust for urinary dilution, samples were adjusted for specific gravity.	In boys, but not in girls, prenatal non-DEHP was significantly associated with a lower BMI at age 5 and 7, fat mass and waist circumference. Also non-DEHP at age 5 was significantly associated with a lower BMI at age 5 and 7 in boys, but not in girls.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Valvi (2015) [76]	Childhood growth and blood pressure	Phthalates	Weight gain: difference in age- and sex-specific Z-scores for weight between 6 months and birth using the WHO referent. Rapid growers: children with a Z-score difference >0.67SD. BMI: weight in kg / height squared in meters. BMI Z-scores: based on the WHO referent. Overweight: BMI Z-score ≥85th percentile. Waist-to-height ratio: waist circumference / height. Central obesity: waist-to-height ratio >0.50	391 mother-child pairs with available phthalate and creatinine determinations from the Spanish population-based birth cohort study INMA ("Infancia y Medio Ambiente"). Weight measurements from birth to 6 months of age were extracted from medical records. Child weight and height were measured at 1, 4 and 7 years of age using standard protocols. Additionally, at age 4 and 7 waist circumference was measured. Systolic and diastolic blood pressure were measured at 4 and 7 years of age.	Two maternal spot-urine samples collected in the first and third pregnancy trimesters for the measurement of 8 phthalate metabolites adjusted for urine creatinine to correct for urine dilution. All phthalate metabolite concentrations had reproducibility between the two pregnancy trimesters. Therefore, the average concentration is used.	The molar sum of HMW phthalates was significantly associated with reduced weight gain z-score in the first 6 months of age in boys (adjusted β =-0.41 (95% CI -0.75 to-0.06) and nonsignificantly associated with lower BMI Z-scores in boys at any age and higher BMI Z-scores in girls. In girls the molar sum of HMW phthalates was significantly associated with lower systolic blood pressure Z-score for all ages combined, but no associations have been found for boys. All significant negative associations were mainly driven by the molar sum of DEHP. In girls the molar sum of LMW phthalates was significantly associated with lower systolic blood pressure Z-scores, but not in boys. This trend showed mainly to be driven by MEP. For both sexes there were negative associations with diastolic blood pressure Z-scores, but none reached the level of statistical significance.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			High systolic and diastolic BP: BP Z-score ≥90th percentile.			
Valvi (2013) [77]	Rapid growth and overweight risk in childhood	BPA	Rapid growth from birth to 6 months: a z score weight gain greater than 0.67 SD. Slow/average growth: z score weight gain equal to or below 0.67 SD. Overweight: BMI z score ≥ 85th percentile.	402 children from the population-based birth cohort study INfancia y Medio Ambiente (INMA) in the Spanish region of Sabadell, all with two maternal spot urine samples during pregnancy, growth data, and born at term. Repeated weight measures from birth to 6 months of age were extracted from medical records. For children without weight measurement within ± 14 days of their 6-month anniversary, sex-specific growth models have been used to predict the weight at 6 months. Child weight, length, and waist circumference were measured at 14 months and 4 years of age.	Total BPA was measured in two maternal spot-urine samples collected in the 1st and 3rd trimester of gestation. BPA was adjusted for creatinine. As main exposure variable the average of the creatinine-adjusted BPA concentrations measured in the 1st and 3rd trimester was used.	At 4 years of age, after excluding outliers, the average creatinine-corrected BPA concentration was associated with both waist circumference and BMI Z scores (adjusted β per log_{10} ug BPA/g creatinine = 0.35 (95% CI 0.04-0.66) and 0.41 (0.03-0.79) respectively) and risk of overweight (adjusted RR = 1.70 (95% CI 0.90-3.45)). In sensitivity analyses separately with BPA concentrations measured during the 1st and 3rd trimester, there was no indication that 1st- or 3rd-trimester BPA concentrations were more strongly associated with the obesity-related outcomes

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Harley (2013) [6]	Body mass index in childhood	BPA	Body mass index: weight (in kilograms) divided by height squared (in meters). Overweight: ≥ 85th but <95th percentile. Obese: >95th percentile.	402 children of mothers included in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) cohort study in California, the United States, with all at least one BMI measurement between 2 and 9 years of age. At age 2, 3.5, 5, 7, and 9 years children were weighed and measured. Started at 5 years of age, the waist circumference has been measured at each visit in triplicate and averaged. BMI was compared with sex-specific BMI-for-age percentile data.	Spot urine samples were collected from mothers at two time points during pregnancy and from children at 5 and 9 years of age to measure total urinary BPA, corrected for specific gravity to normalize for urinary dilution. Samples during pregnancy were collected near the end of first and second trimester.	No significant associations between BPA during pregnancy and any body size measures at the age of 9 have been found looking at boys and girls combined. However, the highest (vs. lowest) tertile of exposure was negatively associated in girls but not boys with BMI z-score (p=0.05) at the age of 9. After subdivision in prepubertal and pubertal girls, only in prepubertal girls prenatal BPA was negatively associated with BMI z-score and waist circumference (β =-0.58 (95% CI -1.15 to-0.01) and -6.16 (-12.15 to-0.18) respectively). BPA concentrations at age 9 were associated with increased BMI z-score, waist circumference, and body fat and increased odds of obesity/overweight at age 9 after controlling for confounders (all p<0.05).
Braun (2014) [78]	Body mass index in childhood	BPA	BMI z-scores: based on U.S. references available from the National Center for Health Statistics.	297 mother-child pairs from the Health Outcomes and Measures of the Environment (HOME) study, a prospective cohort study in the Cincinatti area, United States. Pregnant women were recruited early in pregnancy. Anthropometric measurements, including weight, height, and waist circumference were	Two maternal urine samples around 16 and 26 weeks of gestational age and two child urine samples around 1 and 2 years of age for measurement of BPA, adjusted for urinary creatinine to correct for urinary dilution.	After confounder adjustment, each 10-fold increase in prenatal or early-childhood BPA concentrations was associated with a modest and nonsignificant reduction in child BMI. Inverse associations were stronger in girls. Children in the third tertile of early-childhood BPA concentrations had a lower BMI at 2 years of age compared to children in the first tertile (BMI difference-0.3, 95% CI-0.6

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
				measured in triplicate and averaged at each annual visit until the age of 5.		to 0.0) and their BMI slopes increased more rapidly between 2 and 5 years (BMI increase per year 0.12, 95% CI 0.07 to 0.18, interaction p-value 0.14).
Buser (2014) [79]	Age and sex differences in childhood and adulthood obesity	Phthalates	BMI: weight in kg / height squared in meters. BMI z-score: number of standard. deviations by which a child differs from the mean BMI of children of the same age and sex. Obesity: BMI z-score >95th percentile. Overweight: BMI z-score between 85th and less than 95th percentile	Participants aged 6-19 years from the National Health and Nutrition Examination Survey (NHANES) 2007-2010, a cross-sectional, nationally representative survey of the population of the United States, who had measurements for urinary phthalate metabolites.	Spot urine samples were collected and analyzed for several phthalate metabolites. To correct for dilution, urinary creatinine was entered in the analyses as an independent variable.	In multinomial logistic regressions a statistically significant positive association was found between urinary concentrations of LMW phthalate metabolites and obesity in children and adolescents (compared to the lowest LMW phthalate quartile, adjusted ORs for the second, third and highest quartile for obesity were 2.96 (95% CI 1.66 to 5.30), 2.80 (1.60 to 4.90), and 2.84 (1.40 to 5.78), respectively. After stratification for sex, this association was maintained in males. In individual analyses, the highest quartile of MEP was significantly associated with obesity in all children and adolescents and in males and MiBP in males. No association was found with urinary HMW phthalate metabolites or urinary DEHP metabolite concentrations and obesity.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Zhang (2014) [80]	Obesity	Phthalates	BMI: weight in kg / height squared in meters. Pubertal onset: first appearance of testicular volume ≥4mL in boys and breast buds in girls. Overweight: between 80th and 90th percentile of age- and gender-specific weight distribution. Obesity: ≥90th percentile of age- and gender-specific weight distribution.	493 children age 8 to 13 years from the national Puberty Timing and Health Effects in Chinese Children (PTHEC) study. Anthropometric measurements, including body weight, waist circumference, hip circumference, triceps and subscapular skinfold thickness were measured twice. Body fat proportion was calculated with Yao's formula, BSA was calculated with the Haycock formula. Breast stages, pubic hair stages, and testicular volume were assessed.	Morning spot urine samples were obtained for the analysis of six phthalate monoesters, adjusted for specific gravity to correct for urinary dilution.	In boys age 8-10 years, the highest quartile of ∑All phthalates was significantly positively associated with all measured obesity indices compared to the lowest quartile. For MEHHP, the highest quartile was positively associated with subscapular skinfold thickness and BMI, and all quartiles were positively associated with hip circumference compared to the first quartile. In boys age 11-13 years, ∑LMW phthalates level was positively associated with all measured obesity indices. Of the LMW phthalates, MBP was the main contributive phthalate. After adjusting for confounders MBP and ∑LMW phthalate concentrations in the highest quartile were associated with boys' obesity in a concentration-effect manner. In girls, negatively significant associations were found between urinary MEHP, MEHP and ∑MEHP levels and obesity.
Wang (2013) [81]	BMI and waist circum-ference	Phthalates	BMI: weight in kg / height squared in meters. Normal weight, overweight, and obesity were identified	Subset of a cross-sectional study, conducted in the Changning District of Shanghai City of China, comprising 259 randomly chosen children, based on BMI-based age- and sex-specific criteria, age 8-15 years of which 124 with	First morning urine was collected for measurement of fourteen phthalate metabolites (free and conjugated), adjusted for specific gravity to correct for urinary dilution.	After adjustment for age and sex, seven of eleven metabolites and five sums were found to have a significant positive association with BMI, but after additional adjustment for urine phthalate metabolites, only MEHP and MEP remained significant. For waist circumference, there

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			according to the criteria proposed by the Working Group on Obesity in China (WGOC).	normal weight, 53 overweight, and 82 obese. Body weight, height and waist circumference were measured.		was a similar result, with 5 of eleven metabolites and five sums significantly positive associated, but after additional adjustment, only MEHP and MEP remained significant. The associations between urinary phthalates and BMI tended to be stronger in younger children than in older ones and in males than in females.
Teitelbaum (2012) [82]	Body size measures	Phthalates	BMI: weight in kg / height squared in meters. Overweight/ obese: ≥85th BMI percentile.	387 children, of which 307 girls and 80 boys, between age 6 and 8 at time of enrollment in the Growing Up Healthy prospective cohort study at the Mount Sinai Medical Center Pediatric Clinic in New York City, United States. One year after baseline, anthropometric measurements were obtained, including weight, standing height, waist circumference, hip circumference and body composition using standard protocols adapted from NHANES.	At baseline a casual spot urine sample was provided by the children to measure nine phthalate metabolites. Creatinine concentration was used as a covariate to normalize for urine dilution.	Continuously, phthalate metabolite concentrations did not have a significant association with any of the anthropometric measurements. Sex-stratification did not reveal any association as well. Among girls, there was a significant interaction of BMI percentile (< and ≥85th) with MEP and LMW phthalates for adjusted mean BMI and waist circumference. Among overweight/obese girls, MEP and LMW phthalates exhibited a positive dose response relationship with BMI and waist circumference. Among boys, associations could not be examined due to small sample size.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Trasande (2013) [83]	Body size measures	Phthalates	BMI: weight in kg / height squared in meters. BMI z-scores: derived from 2000 CDC reference growth curves. Overweight: BMI z-score ≥85th percentile. Obese: BMI z-score ≥95th percentile.	Cross-sectional analysis of 2884 participants 6-19 years of age with urinary phthalate measurements from the NHANES 2003-2008 in the United States. Data from questionnaire, laboratory, diet, and physical examination have been used in the analysis.	Phthalate metabolites were measured in a spot urine sample. To adjust for urinary dilution, urinary creatinine was included as a covariate. Urinary biomarkers were grouped for exposure according to their use in product categories: molar sums were calculated for LMW phthalate, HMW phthalate, and DEHP metabolites.	In stratified, multivariable models, each log unit increase in LMW metabolites was associated with 21% and 22% increase in odds (95% CI 1.05 to 1.39 and 1.07 to 1.39, respectively) of overweight and obesity, and a 0.090-SD unit increase in BMI z-score (95% CI 0.003 to 0.18), among non-Hispanic blacks. Analysis of individual metabolites showed that this effect was mainly based on MEP. Among other ethnic subgroups no significant associations with phthalates have been found.
Boas (2010) [84]	Thyroid function, Insulin-like growth factor I, and growth	Phthalates	Body Surface Area (BSA): calculated using DuBois formula	845 children 4-9 years of age, who had all participated in a longitudinal cohort study at the three university hospitals in Copenhagen, Denmark. The present study comprised measurements of height, weight, clinical assessment of pubertal stage (Tanner), ultrasound of the thyroid gland, including gland volume, blood samples, and spot urine samples.	Spot urine samples were collected for the measurement of 12 phthalate metabolites, and urinary iodine. In samples of 100 randomly selected children, the content of both free and total (sum of free and conjugated) phthalate metabolites was determined. Phthalate concentrations were adjusted for creatinine to correct for urinary	After adjustment for creatinine, several phthalate metabolites were significantly negatively associated with current growth measures, including height, weight, BMI, and BSA, and with IGF-1 in both sexes. However, after stratification for sex the association with IGF-1 remained only in boys.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
					dilution. Nonfasting peripheral venous blood samples were drawn between midmorning and late afternoon for the measurement of TSH, thyroid hormones, IGF-I, and insulin-like growth factor binding protein 3.	
Wolff (2007) [85]	To detect relevant, prevalent exposures for a study of female pubertal development	Phthalates, phenols, including BPA, and phytoestrogens	BMI: weight in kg / height squared in meters.	Pilot study of 90 girls 6-8 years of age from the Breast Cancer and the Environment Research Centers (BCERC) in New York City, New York, Cincinnati, Ohio, and northern California. Height and weight were uniformly measured. BMI was classified as <85th national percentile, age- and sex-specific, or ≥85th percentile.	Urine specimens were collected at the time of baseline visit or in a 6-month follow-up visit (Cincinatti) for measurement of phthalate metabolites, phenols, including BPA, phytoestrogens, and creatinine to normalize for urine dilution.	Multivariate adjusted analysis of creatinine-corrected biomarkers showed that BPA was significantly higher in girls with a BMI <85th percentile than in those with BMI ≥85th percentile. Regarding phthalates, no significant relationships have been found.
Xue (2015) [86]	Obesity	Bisphenols, including BPA and BPS, & phthalates	Overweight: ≥ 85th but <95th percentile. Obese: >95th percentile.	Case-control study of 49 obese and 27 non-obese children age 2-14 years old from the Endocrinology Outpatient Department from Amrita Institute of Medical Sciences, Kochi, India. Study participants underwent a detailed history, anthropometry, and clinical examination.	Spot urine samples were obtained at the time of visit. Results were examined based on both the creatinine- unadjusted and-adjusted concentrations.	Creatinine adjusted mean urinary BPA concentration in non-obese children was significantly higher than in obese children (p<0.010). The other bisphenols and phthalates showed no associations.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Khalil (2013) [87]	Growth and cardio-metabolic risk factors	BPA	Overweight: BMI z-score ≥85th percentile. Obese: BMI z-score ≥95th percentile. HOMA-IR: multiplying fasting glucose in mmol/L by fasting insulin in µU/mL and dividing by 22.5.	Case study of 39 obese or overweight (38 obese, 1 overweight) children aged 3-8 years from the Lipid Clinic at Children's Medical Center of Dayton Ohio. Children with thyroid disease, diabetes or other chronic diseases were excluded. Age and ethnicity were self-reported by parents. Anthropometric data including weight, stature, and waist circumference were measured following standardized protocol. Age- and sex-specific BMI z-scores were calculated. Seated systolic (SBP) and diastolic blood pressure (DBP) were obtained using 2 cuff sizes, based on the age of the child.	Spot sample of urine was collected for the measurement of BPA, adjusted for urinary creatinine to correct for urinary dilution. Blood samples were obtained after an overnight fast for the measurement of fasting insulin (FI), glucose, glycated hemoglobin (HbA1C), low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), thyroid stimulating hormone (TSH), and free thyroxine (FT4).	In simple linear regression was found that overall one log unit increase in BPA, creatinine adjusted, was significantly positively associated with FT4 (p=0.028). After stratification for sex, in females BPA did not show any significant associations. However, in males, BPA, adjusted for creatinine, was a negative predictor of age (p=0.029), positive predictor of serum AST (p=0.032) and DBP (p=0.011). After adjustment for age and ethnicity, BPA, adjusted for creat inine, in males was significantly positively associated with DBP (p=0.014). BPA, not adjusted for creatinine, showed to be a significant negative predictor for FI and HOMA-IR (p=0.0.22 and 0.006, respectively). All relations with anthropometric measurements were nonsignificant.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Lee (2013) [88]	Growth and insulin resistance	BPA	Onset of puberty: ≥Tanner stage 2 HOMA-IR: multiplying fasting glucose in mmol/L by fasting insulin in µU/mL and dividing by 22.5.	80 preadolescent 7 year old girls from the Ewha Birth & Growth Cohort, a longitudinal birth cohort study at Mokdong Hospital, Ewha Woman's University, Seoul, South Korea. At follow-up, one year later, 60% of the girls were examined again. Anthropometric data, including height, weight, and waist circumference were measured. BMI was transferred to an age- and gender-specific z-score from the Korean Children and Adolescents Growth Standards. Pubertal development was determined based on clinician-reported Tanner stage assessments.	Urine samples were collected in the morning for BPA measurement, adjusted for urinary creatinine in multivariate analysis. Venous blood was obtained after at least 8-12 hours of fasting for the measurement of luteinizing hormone (LH), adrostenedione, free testosterone, free estradiol, serum insulin, glucose and insulin resistance using HOMA-IR.	After adjustment for age, household income, urinary creatinine level, and pubertal level BPA was significantly associated with estradiol and androstenedione one year later. In adjusted analysis, no significant relationships have been revealed regarding insulin resistance. No relationship has been discovered on BPA and BMI z-score either.
Wang (2012) [89]	BMI	BPA	BMI: weight in kg / height squared in meters. Normal weight, overweight, and obesity were identified according to the criteria proposed	Subset of a cross-sectional study, conducted in the Changning District of Shanghai City of China, comprising 259 randomly chosen children, based on BMI-based age- and sex-specific criteria, age 8-15 years of which 124 with normal weight, 53 overweight, and 82 obese. Body weight, height	First morning urine was collected for measurement of total BPA (free and conjugated), adjusted for specific gravity to correct for urinary dilution.	After adjustment for age, sex, and specific gravity, BMI was significantly associated with an increase in BMI (β=0.017, 95% CI 0.002 to 0.032).

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			by the Working Group on Obesity in China (WGOC).	and waist circumference were measured.		
Trasande (2012) [90]	Obesity prevalence	BPA	BMI: weight in kg / height squared in meters. Overweight: BMI ≥85th percentile. Obese: BMI ≥95th percentile	Cross-sectional analysis of a subsample of 2838 children, age 6-19 years, of the 2003-2008 National Health and Nutrition Examination Survey (NHANES) in the United States. Body size measures were assessed following standardized procedures. BMI z-scores were used to adjust BMI for age and sex.	1 spot urine sample was collected to analyze BPA. To adjust for urinary dilution, urinary creatinine was included as a covariate.	Controlling for race/ethnicity, age, caregiver education, poverty to income ratio, sex, serum cotinine level, caloric intake, television watching, and urinary creatinine level, children and adolescents in the lowest urinary BPA quartile had a significantly lower estimated prevalence of obesity than children in the higher quartiles. This relationship was not seen in overweight children and adolescents. In the whole group, per log unit BPA an increment of 0.06 (95% CI 0.001 to 0.11) in BMI z-score was identified ($p<0.05$). Further stratified analyses showed significant associations between urinary BPA concentrations and obesity among whites ($p<0.001$), but not among blacks or Hispanics.
Eng (2013) [91]	Obesity and waist circum-ference	BPA	Overweight: BMI ≥85th percentile. Obese: BMI ≥95th percentile. Abnormal WC:	Cross-sectional analysis of a subsample of 3370 children, age 6-18 years, of the 2003-2010 NHANES in the United States, who all had urinary BPA levels, anthropometric measurements, total choles-	1 urine sample was collected for the measurement of BPA. Urinary creatinine was used as a covariate to correct for urinary dilution. Choles-terol, TG and	Both unadjusted and adjusted analyses for age, gender, race/ethnicity, urine creatinine, poverty-to-income ratio, serum cotinine, and soda consumption found a significant positive association between urinary BPA and obesity (quartile 2, 3, and

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			≥75th percentile and ≥90th percentile. Abnormal WC-to-height ratio: ≥0.5 Abnormal body fat percentage: ≥85th percentile. Abnormal cholesterol levels: based on the National Cholesterol Education Program guidelines. HOMA-IR: multiplying fasting glucose in mmol/L by fasting insulin in µU/mL and dividing by 22.5. Insulin	terol (TC), and high-density lipoprotein (HDL) levels. A subset of 12-18 year old adolescents with fasting low-density lipoprotein (LDL), triglycerides (TG), and glucose and insulin measurements was evaluated. Body fat was measured with whole body dual-energy radiograph absorptiometry (DXA) for individuals aged ≥8 years during 2003-2006. Weight, length, waist circumference (WC) were measured.	HDL cholesterol were measured in serum. LDL cholesterol level was calculated from measured values of TC, TG, and HDL cholesterol based on the Friedewald equation. Insulin and glucose were measured in blood.	4 vs. quartile 1 in adjusted analysis OR 1.73 (95% CI 1.16 to 2.58), 1.63 (1.08 to 2.46), and 2.05 (1.38 to 3.04), respectively). Additionally, increasing with the quartile a positive association between urinary BPA and waist-to-height ratio was found (quartile 2, 3, and 4 vs. quartile 1 in adjusted analysis OR 1.37 (95% CI 0.97 to 1.92), 1.41 (1.07 to 1.87), and 1.56 (1.11 to 2.17), respectively). No significant associations have been found with abnormal body fat or cardiovascular and diabetes measures.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			resistance: HOMA-IR ≥4.39. Abnormal fasting glucose: ≥100mg/dL			
Bhandari (2012) [92]	Obesity	BPA	BMI: weight in kg / height squared in meters. Obesity: age- and sex-specific BMI ≥95th percentile.	Cross-sectional analysis of a subsample of 2200 children, age 6-18 years, of the 2003-2008 National Health and Nutrition Examination Survey (NHANES) in the United States, who all had urinary BPA levels.	One urine samples was collected for the measurement of BPA concentration, including BPA parent compound and conjugated metabolites. Urinary creatinine concentrations were used as a measure of urinary dilution.	Overall, there was a positive association between increasing urinary BPA levels and BMI in both the age- and sex-adjusted model and the multivariable-adjusted model. Per log unit increase in BPA, BMI increased with 0.30 (95% CI 0.05 to 0.55) in the multivariable-adjusted model (adjusted for sex, race/ethnicity, education, moderate activity, urinary creatinine, and serum cotinine). Also, a significant positive association was found between urinary BPA and obesity in both models. After stratification for gender, this relationship was stronger and statistically significant in boys, but weak and statistically nonsignificant in girls. Additionally, the relationship between urinary BPA and obesity was only significant among non-Hispanic whites. In combined analysis was observed that the positive association between BPA and obesity was predominantly present among non-Hispanic white boys (per log unit BPA increase of 1.70 (95% CI 1.30 to 2.21)).

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Li (2013) [93]	Obesity	BPA	Obesity: BMI ≥ 90th percentile.	1326 children from grade 4-12 from 3 schools in Shanghai, China. Anthropometric measures were taken by research staff.	One urinary sample was collected for the measurement of total BPA concentration.	After adjustment for potential confounders, girls aged 9-12 with a higher BPA level (≥ 2 ug/l) had over 2 times more chance of having a BMI ≥ 90th percentile (adjusted OR 2.32 (95% CI 1.15-4.65)). This was not seen in boys. In girls there was correspondingly a positive significant association with hip circumference.
Trasande (2013) [94]	Blood pressure and lipid levels	Phthalates	Prehypertension: blood pressure ≥90th percentile for age/height z-score/sex. Overweight: BMI z-score ≥1.036 Obese: BMI z-score ≥1.64 Low HDL: <40mg/dL High triglyceride: ≥100mg/dL	Cross-sectional analysis of a subsample of 2838 children, age 6-19 years, of the 2003-2008 National Health and Nutrition Examination Survey (NHANES) in the United States. Data from questionnaire, laboratory, diet, and physical examination have been used in the analysis. Blood pressure was measured 3 consecutive times in all children aged 8-19 years who had been sitting quietly for 5 minutes. Z-scores for blood pressure were calculated to adjust for age, sex, and height.	Phthalate metabolites were measured in a spot urine sample. To adjust for urinary dilution, urinary creatinine was included as a covariate. Urinary biomarkers were grouped for exposure according to their use in product categories: molar sums were calculated for LMW phthalate, HMW phthalate, and DEHP metabolites. High-density lipoprotein (HDL) level and triglyceride levels were measured in blood.	In multivariate analysis increases in systolic blood pressure (SBP) z-score emerged in association with urinary DEHP metabolite levels. For each log unit increase in DEHP metabolite levels, a 0.041 SD unit increase in SBP z-score as identified (p= 0.047). In regression analysis of individual metabolites significant associations were found between SBP z-score with DEHP metabolites MEHP, ME HHP, and MEOHP (p=0.014, 0.022, and 0.021 respectively) as with MBP (p=0.046). MEP showed an association with prehypertension (p=0.038). After stratification, the effect of urinary phthalates with SBP z-score only remained significant in males, younger children and the non-overweight subpopulation.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Trasande (2013) [95]	Albuminuria	BPA	ACR: albumin/ creatinine ratio	Cross-sectional analysis of a sub-sample of 710 children, age 6-19 years, of the 2009-2010 NHANES in the United States. Data from questionnaire, laboratory, diet, and physical examination have been used in the analysis.	One first morning urinary sample was obtained for the measurement of BPA, urinary albumin and creatinine.	In a multivariable model with continuous measures of BPA, a significant 0.28mg/g albumin-to-creatinine ratio increase was identified for each log unit increase in urinary BPA.
Trasande (2014) [96]	Albuminuria	Phthalates	ACR: albumin/ creatinine ratio	Cross-sectional analysis of sub-sample of 667 children, age 6-19 years, of the 2009-2010 NHANES in the United States. Data from questionnaire, laboratory, diet, and physical examination have been used in the analysis.	One first morning urinary sample was obtained for the measurement of phthalates, urinary albumin and creatinine.	In multivariable analysis each log unit (roughly 3-fold) increase in DEHP metabolites was associated with 0,55mg/g increase in ACR (p=0.02). No significant associations were identified with LMW phthalates.
Trasande (2013) [97]	Insulin resistance	Phthalates	Homeostatic model assessment of insulin resistance (HOMA-IR): multiplying fasting glucose in mmol/L by fasting insulin in µU/mL and dividing by 22.5. Overweight: BMI z-score ≥1.036 Obese: BMI	Cross-sectional analysis of 766 children aged 12-19 years, of the 2003-2008 NHANES in the United States. Data from questionnaire, laboratory, diet, and physical examination components were used in the analysis.	1 spot urine sample was collected to analyze phthalate metabolites. To adjust for urinary dilution, urinary creatinine was included as a covariate. Urinary biomarkers were grouped for exposure according to their use in product categories: molar sums were calculated for LMW phthalate, HMW phthalate, and DEHP metabolites. Insulin	Controlling for demographic and behavioral factors, diet, continuous age, BMI category, and urinary creatinine, for each log increase in DEHP metabolites, a 0.27 increase (95% CI 0.14 to 0.40, p<0.001) in HOMA-IR was identified. Also for each log increase in HMW phthalate metabolites a significant increase in HOMA-IR was found (0.26 (95% CI 0.13 to 0.40). After categorizing the population in tertiles, adolescents with the highest HMW and DEHP had higher HOMA-IR prevalence than the others (21.3% vs. 14.3% and 21.6% vs. 14.5%, respectively). Analysis of

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
			z-score ≥1.64 Insulin resistance: HOMA-IR >4.39		resistance was calculated with fasting glucose and insulin (see 'definitions').	individual metabolites showed that the association with HMW phthalate metabolites is mainly DEHP dependent. When quartiled urinary BPA was added to regression models, associations of HMW and DEHP with HOMA-IR remained unchanged, and urinary BPA quartiles were not associated with HOMA-IR, although the highest BPA quartile was associated with insulin resistance.
Trasande (2015) [98]	Insulin resistance	Phthalates	Homeostatic model assessment of insulin resistance (HOMA-IR): multiplying fasting glucose in mmol/L by fasting insulin in µU/mL and dividing by 22.5. Insulin resistance: HOMA-IR >4.39	Cross-sectional analysis of 356 fasting 12-19 year olds of the 2009-2012 NHANES in the United States. Data from questionnaire, laboratory, diet, and physical examination components were used in the analysis.	Phthalates were measured in 1 spot urinary sample. To adjust for urinary dilution, urinary creatinine was included as a covariate. Fasting (>9 hours) insulin and glucose have been measured.	Urinary DINP metabolites, a replacement for DEHP, were associated with increased insulin resistance. The previously identified association of DEHP with insulin resistance was also confirmed.

	Outcome measures	Analyte	Definitions	Study population	Samples	Main outcome(s)
Volberg (2013) [99]	Key metabolism related hormones, including adipokines	BPA	BMI: weight in kg / height squared in meters. Overweight children: BMI ≥85th percentile. Obesity children: BMI ≥95th percentile. Overweight mothers: BMI 25-29.9 kg/m^2. Obesity mothers: BMI ≥30 kg/m^2.	Subsample of 188 mother-child pairs, all with adiponectin and leptin measured at 9 years of age and anthropometric and demographic data from the CHAMACOS prospective birth cohort in Salinas Valley, California. Mothers were included during early pregnancy (<20 weeks of gestational age). At birth, 6 months, and 1, 2, 3 ½, 5, 7 and 9 years of age anthropometric measurements were performed.	Maternal urine samples were collected in the first half and second half of the pregnancy for measurement of BPA. Similarly, one urine sample of the child was obtained at the age 9. To account for urinary dilution, maternal BPA concentrations were adjusted for specific gravity. Child 9-year BPA concentrations were corrected using urinary creatinine. Nonfasting blood samples were measured at the age of 9 for adiponectin and leptin levels.	After adjustment for maternal pre-pregnancy BMI, pregnancy soda consumption and smoking, years in US prior to pregnancy, maternal education, household poverty status, child 9-year BMI and child soda, fast food and sweet snack consumption at 9 years of age among boys, late pregnancy urinary BPA concentrations were positively associated with 9-year leptin levels (β=0.06, 95% CI 0.01 to 0.11, p=0.01) and among girls early pregnancy urinary BPA concentrations were positively associated with adiponectin levels (β=3.71, 95% CI 0.38 to 7.04, p=0.03). Measures of urinary BPA at 9 years of age were not associated with adipokine levels.

Abbreviations: ACR: albumin/creatinine ratio, ALT: alanine aminotransferase, aOR: adjusted odds ratio, AST: aspartate aminotransferase, BCERC: Breast Cancer and the Environment Research Centers, β-hCG: β human chorionic gonadotrophin, BMI: body mass index, BPA: bisphenol A, BSA: body surface area, CCCEH: Columbia Center of Children's Environmental Health, CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas, CI: confidence interval, DEHP: di(2-ethylhexyl)phthalate, DiBP: dibutylphthalate, DBP: diastolic blood pressure, DCHP: dicyclohexyl phthalate, DINP: di-isononlphthalate, DXA: dual-energy radiograph absorptiometry, FGR: fetal growth restriction, FI: fastin insulin, FOR: fecundability odds ratio, FT4: free thyroxine, GDM: gestational diabetes mellitus, HMW: high molecular weight, HOMA-IR: homestatic model assessment of insulin resistance, INMA: Infancia y Medio Ambiente, HDL: high-density lipoprotein, HOME: Health Outcomes and Measures of the Environment, IGF-1: insulin like growth factor 1, IVF: in vitro fertilization, IUGR: intrauterine growth retardation, LBW: low birth weight, LDL: low-density lipoprotein, LH: luteinizing hormone, LIFE Study: Longitudinal Investigation of Fertility and the Environment Study, LMW: low molecular weight, mBP: mono-n-butylphthalate, mBzP: monobenzylphthalate, mCPP: mono-(3-carboxypropyl) phthalate, MEHP: mono-2-ethylhexyl phthalate, MEHHP: mono-2-ethyl-5-hydroxyhexyl phthalate, MEP: mono-ethyl phthalate, MiBP: mono-isobutyl phthalate, MIREC: Maternal-Infant Research on Environmental Chemicals Study, mMP: monomethylphthalate, MEOHP: mono-2-ethyl-5-oxyhexyl phthalate, mOP: monooctylphthalate, NHANES: National Health and Nutrition Examination Survey, OGTT: oral glucose tolerance test, OR: odds ratio, PPROM: preterm premature rupture of membranes, PTHEC: Puberty Timing and Health Effects in Chinese Children, SBP: systolic blood pressure, SD: standard deviation, SGA: small for gestational age, TC: total cholesterol, TG: triglycerides, TSH: thyroid stimulating hormone, WC: waist circumference, WGOC: Working Group on Obesity, WHO: World Health Organization

2

CHAPTER

Bisphenol and phthalate concentrations during pregnancy

2.1

CHAPTER

Bisphenol and phthalate concentrations and its determinants among pregnant women

Environ Res. 2018 Feb;161:562-572.

Abstract

Background: Exposure to bisphenols and phthalates in pregnancy may lead to adverse health effects in women themselves and their offspring.

Objective: To describe first trimester bisphenol and phthalate urine concentrations, including bisphenol and phthalate replacements, and determine nutritional, socio-demographic and lifestyle related determinants.

Methods: In a population-based prospective cohort of 1,396 mothers, we measured first trimester bisphenol, phthalate and creatinine urine concentrations (samples collected in 2004-2005, median gestational age 12.9 weeks [inter-quartile range (IQR) 12.1-14.4]). We examined associations of potential determinants with log-transformed bisphenol and phthalate concentrations. Outcomes were back-transformed. Nutritional analyses were performed in a subgroup of 642 Dutch participants only, as the Food Frequency Questionnaire was aimed at Dutch food patterns.

Results: Bisphenol A, bisphenol S, and bisphenol F were detected in 79.2%, 67.8% and 40.2% of the population, respectively. Mono-n-butylphthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate and monobenzylphthalate were detected in >90% of the population. Nutritional intake was not associated with bisphenol and phthalate concentrations after correction for multiple testing was applied. Obesity was associated with higher high-molecular-weight phthalate concentrations and the lack of folic acid supplement use with higher di-n-octylphthalate concentrations (respective mean differences were 46.73 nmol/l [95% CI 14.56-93.72] and 1.03 nmol/l [0.31-2.06]).

Conclusion: Bisphenol S and F exposure was highly prevalent in pregnant women in the Netherlands as early as 2004-5. Although associations of dietary and other key factors with bisphenol and phthalate concentrations were limited, adverse lifestyle factors including obesity and the lack of folic acid supplement use seem to be associated with higher phthalate concentrations in pregnant women. The major limitation was the availability of only one urine sample per participant. However, since phthalates are reported to be quite stable over time, results concerning determinants of phthalate concentrations are expected to be robust.

Introduction

Bisphenols are used to produce polycarbonate plastics and epoxy resins used in various consumer products, including the lining of metal cans, toys, water pipes and paper products.[1-3] Phthalates are frequently added to personal care products and vinyl plastics to impart flexibility, pliability and elasticity.[4-6] When ingested, both bisphenols and phthalates undergo a first-pass metabolism consisting of glucuronidation or sulfation and these chemicals have been shown to cross the placenta-blood barrier.[5,7-9]

During the last few decades, concerns over human exposure and potential health effects from bisphenol A (BPA) and several phthalates including di-2-ethylhexylphthalate (DEHP) have led to regulations on its production and usage in North America and the European Union. However, these governmental embargoes apply mainly to toys and childcare products for oral exposure. In the meantime, this stimulated the use of synthetic bisphenol analogues and DEHP replacements. A shift in phthalate metabolite concentrations has been observed in the first decade of this century.[10] The European Chemicals Agency (ECHA) reported that the share of phthalate replacements, such as di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP), in total phthalate sales in Europe has increased with over 40% in the years between 2001 and 2010 with concurrently a decline in the share of DEHP.[11] DEHP replacements have been introduced in the mid-20th century and have first been identified in human urinary samples from 1988 .[12] Several studies reported the presence of bisphenol analogues in environmental compartments, foods and consumer products in the last decade.[13] However, bisphenol S (BPS) has not been reported in a human biomonitoring study before 2010.[14] Quantification of bisphenol analogues in those specimens that were collected before the governmental regulations were effective is lacking. Human biomonitoring and association studies have rarely focused on bisphenol analogues and were performed in non-pregnant subjects.

An increasing body of evidence suggests that early life exposure to bisphenols and phthalates may lead to several adverse short and long term health effects.[15] Diet is considered an important source of bisphenol and phthalate exposure.[16-18] Certain food groups such as canned food, fish, meat and poultry have been associated with bisphenol and phthalate levels.[19-22] Previous studies among pregnant women generally reported higher levels of bisphenols and phthalates to be associated with lower socio-economic status, younger maternal age and smoking,[23-26] but results are inconsistent.[24,27] Overweight has also been suggested as a determinant of bisphenol and phthalate levels.[23] Detailed information on nutritional, socio-demographic and lifestyle related determinants of bisphenol and phthalate concentrations in pregnant women might improve identification of women at risk for higher exposure to these chemicals.

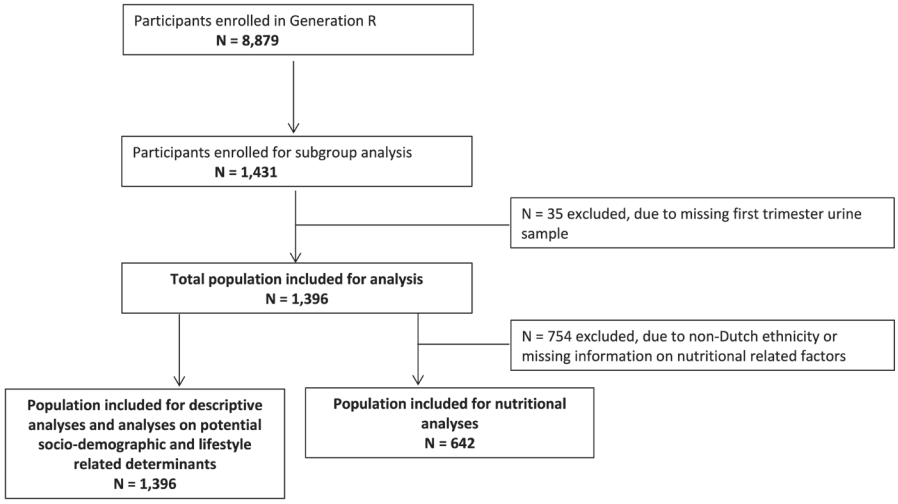
We performed a population-based prospective cohort study among 1,396 pregnant women in 2004-2005 to describe first trimester bisphenol and phthalate urinary concentrations and determine nutritional, socio-demographic and lifestyle related determinants.

Methods

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 onwards.[28] In total, 8,879 women were enrolled in pregnancy, of which 76% before a gestational age of 18 weeks. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam. Written consent was obtained from all participating women.[29] Bisphenol and phthalate concentrations were measured in a subgroup study among 1,431 mothers whose children also participated in postnatal studies. This subgroup included singleton pregnancies only. Thirty-five women without a first trimester urinary sample were excluded, which led to 1,396 women included in the analysis. Dietary intake assessment in the Generation R study was aimed at Dutch dietary intake patterns. Therefore, information on maternal dietary intake was only included for Dutch participants, leading to 642 women included in the analysis for nutrition related factors (Flow chart is given in Figure 1).

Figure 1. Flowchart



Bisphenol and phthalate measurements in urine

Bisphenol and phthalate concentrations were measured in a spot urine sample obtained from each subject during the first trimester measurement (median gestational age 12.9 weeks, inter-quartile range 12.1-14.4 weeks). All urine samples were collected between February 2004 and July 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.[30] Assay precision is improved by incorporating $^{13}C_4$- or $^{2}D_4$-isotopically-labeled internal standards for each of the phthalate metabolites. This selective method allows for rapid detection of eighteen metabolites of phthalates with the majority of limits of detection (LOD) in the range of 0.008-0.3 ng/ml.

Quantitative detection of bisphenols was achieved utilizing a liquid-liquid extraction (LLE) method followed by enzymatic deconjugation of the glucuronidated bisphenols coupled with high performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS). With the use of $^{13}C_{12}$-BPA and $^{13}C_{12}$-BPS isotopically labeled internal standards, eight bisphenols were quantified with limits of detection in the range of 0.03 and 0.18 ng/ml, except for bisphenol AF with an LOD of 0.79 ng/ml.

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. The regression coefficients of the calibration curves were >98%. For each batch of 25 samples, one procedural blank was analyzed. Throughout the analysis, 1 pre-extraction matrix spike sample was prepared for every 25 samples analyzed by spiking known concentrations (40 ng mL^{-1}) of target analytes and passing them through the entire analytical procedure. In addition, the Standard Reference Materials 3672 (Organic Contaminants in Smokers' Urine) and 3673 (Organic Contaminants in Non-Smokers' Urine) from the National Institute of Standards & Technology (NIST), which contain certified values for 11 phthalate metabolites and BPA, were analyzed with every 50 samples.[31] Our results for NIST SRMs were within ±15% of the certified values. A calibration check standard was performed, and methanol was injected after every 25 samples as a check for drift in instrumental sensitivity and carry-over between samples, respectively.

Recoveries of target chemicals passed through the entire analytical procedure ranged between 84.4 and 112.0%, except for mono-(8-methyl-1-nonyl)phthalate (mIDP), phthalic acid (PA), monooctylphthalate (mOP) and monoisononylphthalate (mINP). Relative recoveries for mINP, PA and mIDP were 77-125% after correction with isotope labeled analogues. 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. All identified bisphenol and phthalate urinary biomarkers, their values, detection rates, limits of detection and quantification, and recoveries are shown in Supplementary Table S1. Detailed description of the analytical procedure is shown in Supplementary Material S2.

We grouped urinary biomarkers for exposure to phthalates according to their origin and use in product categories. Metabolites were included in the metabolite groups if >20% of metabolites were above the limit of detection (LOD). We calculated the weighted molar sums for total bisphenols, low molecular weight (LMW) phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate (DEHP), and di-n-octylphthalate (DNOP) metabolites by using the formula: *((concentration in ng/ml) * (1 / molecular weight) * (1 / 10^-3)) + ((concentration in ng/ml) * (1 / molecular weight) * (1 / 10^-3)) + etc*. By using this formula, weighted molar sums are presented in nmol/L. Phthalate groups were constructed based on molecular weight and parent phthalates. LMW phthalate concentration was calculated as the

weighted sum of molar concentrations of mono-methyl phthalate (mMP), mono-ethyl phthalate (mEP), mono-n-butyl phthalate (mBP), and mono-isobutyl phthalate (mIBP); HMW phthalate concentration as the sum of mono-(2-ethyl-5-carboxypentyl) phthalate (mECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (mEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (mEOHP), mono-[(2-carboxymethyl)hexyl] phthalate (mCMHP), mono(3-carboxypropyl) phthalate (mCPP), monobenzyl phthalate (mBzP), mono-hexylphthalate (mHxP), and mono-2-heptylphthalate (mHpP). DEHP concentration was calculated by adding the molarities of mECPP, mEHHP, mEOHP, and mCMHP. DNOP concentrations was calculated as the molarity of mono(3-carboxypropyl)phthalate (mCPP). Phthalic acid (PA) was analyzed separately as a proxy for total phthalate exposure. Bisphenols with >50% of the samples above the LOD were analyzed separately. 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.[32]

Maternal nutrition, socio-demographic, and lifestyle related factors

Nutrition related factors: Dietary determinants included maternal daily caloric intake and 23 food groups. 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.*[33] The FFQ covered the average dietary intake over the previous three months, covering the dietary intake in the first trimester of pregnancy.[34] The Dutch food composition table 2006 was used for calculating daily intake of nutritional values.[35] Maternal daily caloric intake was categorized in four groups (<1600 kcal, 1600-1999 kcal, 2000-2399 kcal, and ≥2400 kcal).

Socio-demographic related factors: Information on maternal age at enrollment (<25 years, 25-29.9 years, 30-34.9 years, and ≥35 years), parity (nulliparity/multiparity), educational level (low/high) and maternal ethnicity (Dutch or European/Non-European) was obtained from the first questionnaire at enrollment. Low educational level was defined as no education, or finished primary or secondary education. High educational level was defined as higher education finished.

Lifestyle related factors: Information on pre-pregnancy weight (kg) and use of folic acid supplementation (yes/no) was obtained from the first questionnaire at enrollment. Maternal height (cm) was measured at enrollment and used to calculate pre-pregnancy body mass index (BMI) (<20 kg/m^2, 20-24.9 kg/m^2, 25-29.9 kg/m^2, and ≥30 kg/m^2). Information on smoking (yes/no) was assessed by questionnaires in each trimester.

Statistical analysis

First, we performed descriptive statistics to examine all identified bisphenol and phthalate urinary biomarkers, including their values and detection rates, and general subject characteristics.

Second, we examined correlations between first trimester bisphenol and phthalate urine concentrations using Spearman's correlation coefficients, taking the skewed distribution of bisphenols and phthalates into account.

Third, we investigated the associations of maternal nutrition, socio-demographic and lifestyle related determinants with differences in maternal first trimester bisphenol and phthalate urine concentrations

using basic and multivariable linear regression models. For all regression models, all bisphenol and phthalate urinary metabolite concentrations were log-transformed to account for right skewness in the distribution and to prevent for negative numbers. Outcomes were back-transformed by using the formula: $(e^{constant} + e^{coef}) - (e^{constant})$.

We examined the associations of dietary food groups and daily dietary caloric intake in Dutch women with differences in bisphenol and phthalate urinary concentrations. Non-Dutch women were excluded from nutritional analyses, since dietary intake assessment was aimed at Dutch dietary intake patterns. Food groups were dichotomized for the amount of consumption by using the 90th percentile as a cutoff. Daily dietary caloric intake was categorized to account for potential non-linear effects, with the largest group as the reference group.

We examined socio-demographic and lifestyle related factors in the larger, entire study population with differences in bisphenol and phthalate urinary concentrations. To account for non-linear effects of potential determinants and confounders, all were included as categorical variables. The largest group was used as the reference.

Sub-analyses of individual bisphenols or phthalate metabolites were performed for all significant models to determine which metabolites were driving the association. All basic models were adjusted for urinary creatinine to adjust for dilution. Multivariable models were additionally adjusted for maternal age, parity, educational level, maternal ethnicity, pre-pregnancy BMI, smoking and use of folic acid supplementation. Multivariable models investigating nutrition related determinants were additionally adjusted for daily dietary caloric intake. Owing to the fact that nutrition related determinants were derived from a food frequency questionnaire (FFQ) aimed at Dutch food consumption patterns, nutrition related determinants were not imputed. Missing data of the potential socio-demographic and lifestyle related determinants and covariates were imputed using multiple imputation. Five imputed data sets were created and pooled for analyses. Imputed socio-demographic and lifestyle related determinants were used for both the univariate and multivariable models to prevent exclusion of incomplete cases. The percentage of missing values within the population for analysis was ≤15%, except for folic acid supplement use (20.1%). We used the Bonferroni correction to account for multiple testing at $P<0.05/k$ (k: the number of hypotheses by means of the number of potential determinants tested summed by the two main metabolite groups of bisphenols and phthalates). All analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Participant characteristics and correlations

Table 1 shows the subject characteristics of included women. Table 2 shows all the metabolites that were included in all separate groups as abovementioned, their values and detection rates in urine. Supplementary Table S1 shows all identified bisphenol and phthalate urinary biomarkers.

Table 1. Subject characteristics (n=1,396)[1]

	Value
Maternal age (years)	30.6 (4.8)
Pre-pregnancy body mass index (kg/m²)[2]	22.7 (18.5, 35.0)
Parity (% nulliparous)	61.0
Child sex (% males)	50.5
Maternal ethnicity (%)	
Dutch/European	61.9
Non-European	38.1
Highest completed education (%)	
Low education	49.6
High education	50.4
Daily dietary caloric intake (kcal)	2077 (509)
Maternal smoking during pregnancy (%)	
No	75.2
Yes	24.8
Folic acid supplement use (%)	
No	19.4
Yes	80.6
Creatinine (µg/mL)[2]	1011 (153, 3435)

[1]Values represent means (standard deviation) or valid percentages
[2]Median (95% range)

Measurable urinary concentrations of BPA, bisphenol S (BPS), and bisphenol F (BPF) were found in 79.2%, 67.8% and 40.2% of the samples, respectively. Bisphenol Z (BPZ), bisphenol B (BPB), bisphenol AP (BPAP) and bisphenol P (BPP) were detected in <15% of samples. We did not detect BPAF in any sample. In the detected samples, median concentrations of BPA, BPS and BPF were 1.66 ng/mL (IQR 0.72-3.56), 0.36 ng/mL (IQR 0.17-1.08), and 0.57 (IQR 0.30, 1.29), respectively. LMW phthalate, and DEHP metabolites were detected in >98% of the population. Mono-hexylphthalate (mHxP) and mono-2-heptylphthalate (mHpP) were detected in a considerable number of samples; however a significant part of values was detected in the range between the limit of detection (LOD) and the limit of quantification (LOQ). DEHP replacements including di-isononylphthalate (DINP) and di-isodecylphthalate (DIDP) had detection rates below 15%.

Table 2. Bisphenol and phthalate urinary concentrations (n=1,396)

	Median (IQR) (nmol/L)[1]	Median (IQR) (ng/mL)[2]	Percentage of values below the limit of detection (LOD)	Percentage of values above the limit of quantification (LOQ)
Total bisphenols	9.24 (3.54, 20.25)			
Bisphenol A (BPA)		1.66 (0.72, 3.56)	20.8	66.0
Bisphenol S (BPS)		0.36 (0.17, 1.08)	32.2	52.7
Bisphenol F (BPF)		0.57 (0.30, 1.29)	59.8	19.6
Phthalic acid (PA) metabolites		57.08 (30.77, 121.91)	0.3	99.1
Low molecular weight (LMW) metabolites	1087.27 (429.24, 2934.47)			
Monomethylphthalate (mMP)		5.43 (2.75, 9.88)	0.1	99.9
Monoethylphthalate (mEP)		138.03 (41.22, 486.71)	0.1	99.9
Mono-isobutylphthalate (mIBP)		21.55 (9.55, 45.91)	0.1	99.8
Mono-n-butylphthalate (mBP)		16.21 (7.01, 31.22)	0.7	98.9
High molecular weight (HMW) metabolites	221.94 (112.98, 407.84)			
Di-2-ethylhexylphthalate (DEHP) metabolites	174.28 (89.26, 325.95)			
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)		16.43 (8.26, 31.82)	0.1	99.3
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)		12.02 (5.83, 23.21)	0.1	99.6
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)		7.81 (3.53, 15.48)	0.1	99.1
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)		14.17 (7.60, 26.69)	0.1	99.9
Di-n-octylphthalate (DNOP) metabolites	5.81 (3.11, 11.07)			
Mono(3-carboxypropyl)phthalate (mCPP)		1.46 (0.78, 2.78)	0.2	99.8
Other high molecular weight metabolites				
Monobenzylphthalate (mBzP)		6.59 (3.07, 12.91)	8.2	90.4
Mono-hexylphthalate (mHxP)		0.33 (0.16, 0.63)	23.6	53.9
Mono-2-heptylphthalate (mHpP)		1.10 (0.60, 2.34)	35.5	35.2

[1]Weighted molar sums of total bisphenols, low molecular weight (LMW) phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate (DEHP), and di-n-octylphthalate (DNOP) metabolites in nmol/L with non-detectable levels of separate compounds imputed as LOD/sqr(2). Separate metabolites are included only if more than 20% of values was above the LOD.
[2]Values represent the median (IQR) of detected values.

Table 3 shows Spearman's correlation coefficients of bisphenol and phthalate concentrations, including separately BPA, BPS and PA. Bisphenol and phthalate concentrations were strongly correlated (Spearman's correlation coefficient for the correlation between total bisphenols and PA metabolites 0.37, *P value* <0.001). Phthalate groups were strongly correlated, ranging from 0.46 for the correlation between LMW phthalate and DNOP metabolites to 0.98 for the correlation between HMW phthalate and DEHP metabolites (all have a *P value* <0.001). Among the separate bisphenols, except for correlations of BPA with BPS , BPF and BPAP, bisphenols are not correlated (Supplementary Table S3). For the separate phthalate metabolites, PA is strongly correlated with all other phthalates with >20% of samples above LOD (Spearman's correlation coefficient ranging from 0.34 to 0.69 (*P value* <0.001) (Supplementary Table S4). All in the analyses included phthalate metabolites with >20% of samples above LOD are strongly correlated (*P value* <0.001). Among the DEHP metabolites correlation is very high, ranging from 0.77 to 0.98 (*P value* <0.001).

Maternal dietary determinants

Urinary bisphenol and phthalate concentrations did not vary by intake frequencies of examined food groups or daily dietary caloric intake after correction for multiple testing, both in basic and multivariable models. Basic models showed high consumption of grains, cakes, coffee, soft drinks, alcoholic drinks, soups and bouillon and daily dietary caloric intake to be associated with differences in bisphenol or phthalate urine concentrations (Supplementary Table S5).

Table 4 shows multivariable models for nutritional related factors with a nominal p-value <0.05. At nominal level, pregnant women in the upper 10% of consumption of vegetables had higher HMW phthalate, DEHP and DNOP metabolite concentrations (respective mean differences 38.17 nmol/l (95% CI 7.18, 90.7), 31.4 nmol/l (95% CI 5.16, 77.3) and 1.14 nmol/l (95% CI 0.12, 2.98). In a subanalysis of individual phthalate metabolites, the association between vegetable intake and HMW phthalates was mainly driven by DEHP metabolites, however associations did not remain after correction for multiple testing (Supplementary Table S6). We observed lower concentrations of bisphenol S (BPS) and PA in pregnant women who had eaten a large quantity of grains in the past three months, while for women with a high consumption of fish and shellfish higher total bisphenol or bisphenol A (BPA) concentrations were found. Women who consumed a large amount of soft drinks had higher LMW phthalate concentrations, while women with a frequent intake of soups and bouillon had lower LMW phthalate concentrations. Both associations were mainly driven by mono-ethylphthalate (mEP) metabolites, but attenuated to non-significance after Bonferroni correction (Supplementary Table S6). As compared to pregnant women who consumed 2000-2399 kcal per day, women who consumed somewhat less or more kcal per day had lower BPS concentrations.

Table 3. Spearman's correlation coefficients of bisphenols and phthalates (N = 1,396)

	Bisphenols			Phthalates				
	Total bisphenols	**Bisphenol A (BPA)**	**Bisphenol S (BPS)**	**Phthalic acid (PA) metabolites**	**Low molecular weight (LMW) phthalate metabolites**	**High molecular weight (HMW) phthalate metabolites**	**Di-2-ethylhexyl phthalate (DEHP) metabolites**	**Di-n-octylphthalate (DNOP) metabolites**
Total bisphenols	1.00	-	-		-	-	-	-
BPA	0.87*	1.00	-		-	-	-	-
BPS	0.42*	0.16*	1.00		-	-	-	-
PA metabolites	0.37*	0.37*	0.15*	1.00	-	-	-	-
LMW phthalate metabolites	0.26*	0.27*	0.12*	0.75*	1.00	-	-	-
HMW phthalate metabolites	0.35*	0.37*	0.16*	0.60*	0.50*	1.00	-	-
DEHP metabolites	0.35*	0.37*	0.16*	0.58*	0.47*	0.98*	1.00	-
DNOP metabolites	0.33*	0.34*	0.18*	0.57*	0.46*	0.77*	0.74*	1.00

Spearman's correlation coefficients of bisphenol and phthalate groups, included separately BPA, BPS, PA and mCPP. For both the groups and separate components, values below the limit of detection (LOD) are imputed by LOD/sqr(2).
*P-value < 0.001.

Table 4. Multivariable associations between nutritional related factors and urinary bisphenol and phthalate concentrations in Dutch women (N = 642)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenol (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphthalate (DEHP) (nmol/l) [95% CI]**	**Di-n-octylpht-halate (DNOP) (nmol/l) [95% CI]**
Food groups[a]								
Vegetables	-	-	-	-	-	38.07 [7.18, 90.72]*	31.40 [5.16, 77.28)*]	1.14 [0.12, 2.98]*
Grains	-	-	-0.07 [-0.08,-0.01]*	-8.47 [-11.50,-0.24]*	-	-	-	-
Fish and shellfish	2.02 [0.08, 5.99]*	0.23 [0.02, 0.74]*	-	-	-	-	-	-
Soft drinks	-	-	-	-	211.65 [6.58, 659.22]*	-	-	-
Soups and bouillon	-	-	-	-	-159.60 [-196.07,-25.59]*	-	-	-
Daily dietary caloric intake[b]								
<1600 kcal *(n=85)*	-	-	-0.05 [-0.07, 0.03]	-	-	-	-	-
1600-1999 kcal *(n=180)*	-	-	-0.06 [-0.07,-0.01]*	-	-	-	-	-
2000-2399 kcal *(n=199)*	-	-	Reference	-	-	-	-	-
≥2400 kcal *(n=178)*	-	-	-0.06 [-0.07,-0.01]*	-	-	-	-	-

[a] Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect the difference in bisphenol and phthalate urine concentrations in ng/ml or nmol/l in women in the upper 10% of consumption of that particular food group or dietary pattern compared to the first 90%.
[b] Values are regression coefficients (95% confidence intervals) from linear regression models that reflect the difference in bisphenol and phthalate concentrations in ng/ml or nmol/l compared with the reference.
Significance levels are accounted for multiple testing using Bonferroni correction. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, daily dietary caloric intake, maternal highest finished education, smoking, folic acid supplement use and creatinine.
*P-value<0.05 †Significant using Bonferroni.

Maternal socio-demographic and lifestyle determinants

After correction for multiple testing was applied, all investigated determinants were associated with differences in bisphenol or phthalate urine concentrations in basic models (Supplementary Table S7). Table 5 shows multivariable associations of socio-demographic and lifestyle related factors with urinary bisphenol and phthalate concentrations. After Bonferroni correction, pre-pregnancy obesity was associated with higher HMW phthalate concentrations (mean difference 46.73 nmol/l (95% CI 14.56, 93.72) and the lack of folic acid supplement use with higher DNOP concentrations (mean difference 1.03 nmol/l (95% CI 0.31, 2.06). In a subanalysis of individual HMW phthalate metabolites, the association of pre-pregnancy BMI was driven by mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP), monobenzylphthalate (mBzP) and mono-2-heptylphthalate (mHpP) metabolites (Supplementary Table S8).

Maternal age was not associated with bisphenol or phthalate concentrations. At nominal level, multiparous women had higher DNOP concentrations (mean difference 0.58 ng/ml [95% CI 0.11, 1.24]). Pregnant women with a low educational level had higher PA and LMW phthalate concentrations. Among pregnant women with non-European descent, we observed lower urinary BPS concentrations, but higher HMW phthalate concentrations which was driven by mBzP metabolites. The association with mBzP metabolites remained significant after correction for multiple testing. Pregnant women with a higher pre-pregnancy BMI, who smoked during pregnancy and did not use folic acid supplementation had higher first trimester bisphenol and phthalate urine concentrations.

Table 5. Multivariable associations of socio-demographic and lifestyle related factors with urinary bisphenol and phthalate concentrations (N = 1,396)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenols (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphthalate (DEHP) (nmol/l) [95% CI]**	**Di-n-oc-tylphthalate (DNOP) (nmol/l) [95% CI]**
Maternal age								
<25 years *(n=190)*	0.44 [-0.68, 2.26]	0.07 [-0.05, 0.29]	0.02 [-0.03, 0.11]	2.14 [-4.06, 11.80]	98.91 [-45.28, 344.55]	16.56 [-6.04, 49.79]	11.37 [-6.85, 38.59]	0.31 (-0.32, 1.26)
25-29.9 years *(n=374)*	0.15 [0.61, 1.33]	-0.01 [-0.08, 0.11]	0.00 [-0.03, 0.05]	0.61 [-3.65, 6.93]	47.71 [-49.27, 202.18]	1.21 [-12.56, 20.82]	-0.57 [-11.66, 15.47]	0.02 (-0.39, 0.60)
30-34.9 years *(n=598)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
≥35 years *(n=234)*	-0.07 [-0.86, 1.19]	0.03 [-0.06, 0.18]	0.02 [-0.02, 0.09]	2.00 [-3.12, 9.69]	2.73 [-90.23, 158.95]	0.62 [-14.68, 22.80]	0.68 [-12.07, 19.40]	0.48 (-0.08, 1.29)
Parity								
Primiparous *(n=849)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Multiparous *(n=547)*	0.49 [-0.27, 1.62]	0.02 [-0.05, 0.14]	0.00 [-0.03, 0.05]	-3.98 [-6.70, 0.15]	-41.66 [-102.77, 59.17]	8.52 [-5.02, 27.25]	8.14 [-3.37, 24.24]	0.58 (0.11, 1.24)*
Educational level								
Low *(n=709)*	0.50 [-0.34, 1.77]	0.09 [-0.01, 0.25]	-0.01 [-0.03, 0.04]	5.83 [0.57, 13.37]*	132.74 [17.43, 311.57]*	8.67 [-6.17, 29.46]	4.25 [-7.38, 20.80]	-0.08 (-0.46, 0.60)
High *(n=687)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Maternal ethnicity								
Dutch/European *(n=860)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Non-European *(n=536)*	-0.73 [-1.23, 0.09]	-0.05 [-0.10, 0.05]	-0.04 [-0.05,-0.00]*	-0.35 [-4.24, 5.42]	75.51 [-24.74, 233.95]	18.45 [1.65, 41.70]*	12.95 [-0.63, 32.01]	0.03 (-0.36, 0.60)
Pre-pregnancy body mass index (kg/m²)								
< 20 kg/m² *(n=203)*	0.05 [-0.80, 1.41]	-0.01 [-0.09, 0.14]	-0.02 [-0.05, 0.04]	0.03 [-4.72, 7.27]	-62.33 [139.45, 73.68]	-1.20 [-17.37, 22.62]	0.52 [-13.53, 21.52]	-0.23 (-0.66, 0.42)
20-24.9 kg/m² *(n=785)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
25-29.9 kg/m² *(n=290)*	0.77 [-0.30, 2.45]	0.07 [-0.05, 0.28]	0.01 [-0.02, 0.07]	7.64 [0.88, 17.63]*	58.78 [-44.09, 225, 04]	7.46 [-9.00, 30.99]	5.63 [-7.88, 25.20]	-0.02 (-0.49, 0.68)

	Difference in bisphenol concentrations				Difference in phthalate concentrations			
	Total Bisphenols (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphthalate (DEHP) (nmol/l) [95% CI]**	**Di-n-oc-tylphthalate (DNOP) (nmol/l) [95% CI]**
≥30 kg/m² *(n=118)*	1.88 [0.13, 4.78]*	0.17 [-0.00, 0.49]	0.10 [0.01, 0.27]*	13.16 [2.51, 29.86]*	195.31 [-12.50, 564.77]	46.73 [14.56, 93.72]*†	32.34 [6.90, 70.25]*	0.79 (-0.07, 2.12)
Smoking during pregnancy								
No *(n=1051)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Yes *(n=345)*	1.27 [0.20, 2.89]*	0.15 [0.03, 0.33]*	0.01 [-0.02, 0.07]	5.32 [0.21, 12.66]*	90.37 [-12.13, 250.89]	6.80 [-7.70, 27.18]	6.45 [-5.67, 23.64]	0.07 (-0.34, 0.65)
Folic acid supplement use								
No *(n=290)*	1.04 [-0.05, 2.74]	0.16 [0.03, 0.38]*	0.03 [-0.01, 0.11]	2.34 [-3.27, 10.86]	63.92 [-52.75, 257.51]	27.90 [6.29, 59.28]*	19.75 [2.53, 44.31]*	1.03 (0.31, 2.06)*†
Yes *(n=1106)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]

Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect differences in urinary concentrations of bisphenol and phthalate concentrations, compared with the reference. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, maternal ethnicity, maternal highest finished education, smoking, folic acid supplement use and creatinine.
*P-value<0.05 †Significant using Bonferroni. Numbers are after multiple imputation.

Discussion

Main findings

One of the major findings of this study is that bisphenol exposures other than BPA were widely prevalent in pregnant women in a population-based cohort in the Netherlands between 2004 and 2005. The second major finding was that first trimester bisphenol and phthalate urine concentrations were mainly influenced by lifestyle related factors such as high pre-pregnancy BMI and the lack of folic acid supplement use. Nutritional factors were not associated with bisphenol and phthalate concentrations when correction for multiple testing was applied.

Interpretation of findings

To our knowledge, this is the first study to describe BPS and BPF in samples collected as early as 2004-2005. Generally, comparisons of values need to be restricted to the time and place of sampling, because bisphenol and phthalate use changes over the years due to governmental embargoes.[10] Urinary concentrations of BPA and phthalate metabolites found in our study population were generally somewhat lower than concentrations in other Western studies performed in the same time period. Detection rates were comparable or slightly lower.[26,36-39]

Except substitution of BPA by BPS and BPF in man-made consumer products as an explanation for our findings, we cannot exclude BPS and BPF exposure through natural sources. A recent study found BPF in mustard made from seeds of Sinapis alba.[40] Contamination was ruled out. The authors explain this finding as reaction product of a breakdown pathway. This finding suggest potential other natural sources that could explain BPF and also BPS exposure.

Since the year 1999 certain phthalates, including DEHP, dibutylphthalate (DBP), butylbenzylphthalate (BBP), DINP, DIDP and DNOP, have been banned from toys and childcare products in the European Union.[41] We observed high detection rates for DEHP metabolites, metabolites of DBP and BBP (respectively mBP and mBzP) and mCPP, which is a DNOP metabolite. DINP and DIDP metabolites were scarcely detected in our study population. A recent re-evaluation concluded that there was no evidence for further exposure bans on DINP and DIDP. However, the other banned phthalates have not been included in this assessment.[11] Although, since February 2015 (REACH sunset date), several phthalates, including DEHP, BBP, DBP and diisobutylphthalate (DIBP), have been listed on the Authorisation List of the ECHA.[42] Our findings suggest that DEHP, DBP, BBP and DNOP were widely used in the early 2000s. Due to recent regulations DEHP, DBP and BBP exposure should be limited nowadays. DNOP has not been included in these regulations and is therefore a cause for concern.

In the general population, diet has been considered the major source of phthalate exposure.[16,17] DEHP seems to be of major concern, which is widespread in food packaging.[6] Also bisphenols are potentially transferred into food items from food packaging, including the lining of metal cans.[15,43] In previous studies, high consumption of canned vegetables and canned fish was associated with higher BPA concentrations in pregnant women.[22,26] For phthalates, frequent consumption of poultry, meat and fish

were associated with higher DEHP and HMW phthalate concentrations, while soy consumption was inversely associated with DEHP concentrations.[20,21]

In contrast to our hypothesis and previous findings, none of the nutrition related factors was associated with bisphenol and phthalate urine concentrations when corrections for multiple testing were applied. The absence of multiple testing correction and the use of short-term diet recall data (24/48h) in previous studies might be responsible for this discrepancy. In contrast to previous studies, we have used a FFQ for the average intake in the past three months. Both bisphenols and phthalates have short biological half-lives (less than 24h).[5,7] The trends we have observed for high consumption of certain food groups, though not significant, may therefore rather be a proxy for healthy or unhealthy food consumption patterns of lifestyle than that those food groups actually contain these compounds. However, we cannot explain why women with a high intake of vegetables have higher concentrations of HMW phthalate metabolites.

Several studies have examined potential determinants of BPA and phthalate concentrations during pregnancy using various methodologies, which complicates comparisons. In general, previous studies identified maternal younger age, lower education, overweight and smoking as determinants of higher BPA and LMW phthalate concentrations during pregnancy.[20,23-26] However, for HMW phthalates maternal older age, higher education and nonsmoking have been associated with higher concentrations.[20,24] In our study, maternal age was not associated with bisphenol or phthalate concentrations. Correction for multiple testing has not been applied in previous studies. In agreement with previous studies, we found an association at nominal level of lower education with higher LMW phthalate concentrations. In contrast to our finding that multiparity was associated with higher DNOP metabolite concentrations at nominal level, previous studies did not find consistent associations between parity and phthalate concentrations.[20,24] Nulliparity, however, has been identified as a determinant of higher BPA concentrations in a Spanish birth cohort study.[26]

To some extent in line with our findings, a cohort study in 350 pregnant women reported that non-Hispanic black and Hispanic women had higher concentrations of LMW phthalate metabolites, mBzP and mono-(2-ethylhexyl)phthalate (mEHP) metabolites in early pregnancy than other ethnic groups.[44] Although, the role of ethnicity as a determinant of bisphenol and phthalate concentrations has been scarcely studied, several association studies have identified race/ethnicity-specific associations of bisphenols and phthalates with childhood BMI and diabetes risks.[45-47] Consequently, determinants underlying these ethnic differences need to be further studied.

Previous studies have shown associations of higher maternal BMI and smoking during pregnancy with higher BPA and LMW phthalate concentrations, whereas inversed associations or no associations were found for HMW phthalate concentrations.[20,23,24,48] Conversely, in our study we found strong associations between pre-pregnancy BMI in the obesity range and higher HMW phthalate concentrations. Phthalates, and bisphenols to some extent, are lipophilic chemicals.[15,49] Even though we cannot rule out that obese women are more exposed to HMW phthalates, it seems metabolically feasible that obese women have greater adipose stores of lipophilic chemicals. In line with previous studies we found higher total bisphenol, PA and LMW phthalate concentrations in smokers than in non-smokers, driven by BPA and mEP.[23,24] Although the associations with smoking might reflect lifestyle habits, in- and

exhaled tobacco smoke may contain BPA and PA because BPA and di-2-methoxyethyl phthalate are used in some cigarette filters.[50]

To our knowledge, this is the first study to examine folic acid supplement use as a determinant of bisphenol and phthalate concentrations. In our study, we found the lack of folic acid supplement use to be associated with higher DNOP metabolite concentrations. Although epigenetic effects of bisphenols and phthalates are not yet clear, methyl donors like folate have been proposed as a potential point of engagement to prevent potential epigenetic effects.[51] Effects of folic acid supplement use on epigenetic changes due to bisphenol and phthalate exposure need to be further investigated.

In line with our hypothesis, in this population-based sample in the Netherlands adverse lifestyle related factors seem to be associated with higher bisphenol and phthalate urine concentrations rather than socio-demographic factors. Many research groups have raised their concerns about confounding effects on bisphenol and phthalate concentrations from nutrition related factors. As a sensitivity analysis, we included maternal daily dietary caloric intake as a covariate in models for potential socio-demographic and lifestyle related determinants. In these analysis, maternal daily dietary caloric intake has been included as a categorical variable with a separate missing group to prevent exclusion of incomplete cases. Since maternal daily dietary caloric intake was only included for Dutch mothers and the missing category was highly correlated with non-European descent, effects on ethnicity estimates could not be compared. Conclusions for all other socio-demographic and lifestyle related determinants did not change.

All adverse lifestyle related factors assessed in this study were associated with higher bisphenol and phthalate urine concentrations at nominal level. However, despite of the scarce associations found with certain food groups in Dutch mothers, we cannot rule out that lifestyle related factors are a proxy for unmeasured food consumption patterns. Several studies have found associations of phthalate concentrations with the use of personal care products, household products and materials used for flooring and walls.[23,52] The adverse lifestyle related factors identified in this study might therefore be a proxy for harmful product exposure. Altogether, we cannot exclude that the associations we have observed are rather a proxy for unhealthy lifestyle habits in general with consequently higher exposure levels than that these factors actually increase exposure or urinary excretion.

Strengths and limitations

Strengths of this study were the prospective data collection from early pregnancy onwards in a multi-ethnic population, the relatively large sample size, and large amount of bisphenols and phthalate metabolites analyzed. Analyses for the current study are performed in a subgroup from the Generation R Study. Non-response analyses showed similar distributions and values of potential socio-demographic and lifestyle determinants (Supplementary Table S9). The response rate at baseline was 61%.[28] It seems unlikely that this level of non-response would lead to biased effect estimates, since selection bias in large cohort studies arises mainly from loss to follow up rather than from a non-response at baseline.[53]

For this study, a large amount of bisphenols and phthalate metabolites have been analyzed. The analytical techniques as used for this study allowed for detection of both unconjugated (free) compounds, as well

as glucuronidated and sulfated bisphenol and phthalate conjugates. Assay precision has been improved by incorporating isotopically-labeled internal standards and confirmed with regularly proficiency testing and reference maternal analysis.

Urinary concentrations of bisphenols and phthalates were based on a single spot urine in the first trimester of pregnancy. Both bisphenols and phthalates have short biological half-lives (less than 24h).[5,7] Despite of the reported short biological half-lives, it has been suggested that one single urine sample for phthalate concentrations reasonably reflects exposure for up to three months or even longer.[54,55] Within-person variability of BPA urinary concentrations is reported to be high, constraining the value of one single urine sample for bisphenol measurement.[56] We can therefore not exclude that the results of our study with regards to the bisphenols would be different when multiple samples were included. However, for the phthalates, we expect the results to be robust.

Samples have been stored at-20 °C for approximately 10 years. Therefore, we cannot exclude that there has been some biological activity during the storage period. It has been suggested that -80 °C would be a more optimal storage temperature. A study investigating effects of storage time and temperature on bisphenol and phthalate levels found that samples stored at room temperature (20 °C) had lower concentrations of phthalate metabolites and BPA than samples stored at-80 °C after a time period of 8 weeks.[57] Studies investigating effects of storage temperature over a longer period are lacking.

Some studies measure mEHP concentrations as a metabolite of DEHP. Based on literature, we decided not to measure mEHP concentrations. MEHP only represents a small percentage (<1%) of DEHP metabolites and is not considered a reliable biomarker for human DEHP exposure.[58] MEHP occurs naturally from the hydrolysis of DEHP and is ubiquitous in the environment, explaining the high background that tempers our analysis. The other DEHP metabolites, such as mECPP, mEHHP, mCMHP and mEOHP are oxidative metabolites that are produced only in vivo and therefore reliable biomarkers.[59]

The FFQ as used in this study was aimed at Dutch dietary intake patterns, making this assessment less reliable for non-Dutch participants. We therefore excluded non-Dutch participants from this analysis. As mentioned before, ethnicity might play a key factor in bisphenol and phthalate metabolism and effects. Therefore, the lack of ethnic variance results in non-generalizable effect estimates of dietary determinants. As abovementioned, we have used a FFQ for the average intake in the past three months instead of the last 24 hours while both bisphenols and phthalates have short biological half-lives, making it difficult to draw firm conclusions about food groups as sources of exposure.

In our study, information on nutritional and several lifestyle related determinants was self-reported, in which underreport may have led to misclassification and consequently underestimation of effects. However, the prevalence of lifestyle related factors corresponds with national figures for that time period.[60,61] Previous studies reported time of sampling, fasting time and seasonality as potential determinants for bisphenol and phthalate urine concentrations.[62,63] As mentioned above, personal care products and household products have been identified as determinants of phthalate concentrations.[23,52] In our study, this information was not available.

Conclusion

BPS and BPF exposure was highly prevalent in pregnant women in the Netherlands as early as 2004-2005. This finding questions the assumption that BPS and BPF exposure was limited in the early 2000s. Associations attributed to BPA in this time-period may be therefore confounded. DEHP replacements were scarcely detected in our population. However, high detection rates were found of DEHP itself and other phthalates that already were banned from toys childcare products, suggesting continued exposure through consumer products. We identified limited associations of dietary and other key determinants with bisphenol and phthalate concentrations. Adverse lifestyle factors including obesity and the lack of folic acid supplement use seem to be associated with higher phthalate concentrations in pregnant women. Notwithstanding the associations found between obesity and higher HMW phthalate concentrations, associations were limited suggesting a lower likelihood of confounding of phthalate body mass contaminant relationships which we plan to examine in forthcoming work.

References

1. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139-77.
2. Liao C, Kannan K. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2014;31(2):319-29.
3. Liao C, Liu F, Kannan K. Bisphenol s, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol a residues. Environ Sci Technol. 2012;46(12):6515-22.
4. Sathyanarayana S. Phthalates and children's health. Curr Probl Pediatr Adolesc Health Care. 2008;38(2):34-49.
5. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
6. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health. 2014;13(1):43.
7. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
8. Schonfelder G, Wittfoht W, Hopp H, Talsness CE, Paul M, Chahoud I. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. 2002;110(11):A703-7.
9. Silva MJ, Reidy JA, Herbert AR, Preau JL, Jr., Needham LL, Calafat AM. Detection of phthalate metabolites in human amniotic fluid. Bull Environ Contam Toxicol. 2004;72(6):1226-31.
10. Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ Health Perspect. 2014;122(3):235-41.
11. European Chemicals Agency (ECHA). Evaluation of new scientific evidence concerning DINP and DIDP in relation to entry 52 of Annex XVII to REACH Regulation (EC) No 1907/2006. Helsinki, Finland2013.
12. Wittassek M, Wiesmuller GA, Koch HM, Eckard R, Dobler L, Muller J, et al. Internal phthalate exposure over the last two decades--a retrospective human biomonitoring study. Int J Hyg Environ Health. 2007;210(3-4):319-33.
13. Chen D, Kannan K, Tan H, Zheng Z, Feng YL, Wu Y, et al. Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review. Environ Sci Technol. 2016;50(11):5438-53.
14. Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon HB, et al. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ Sci Technol. 2012;46(12):6860-6.
15. Philips EM, Jaddoe VW, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2016.
16. Schecter A, Lorber M, Guo Y, Wu Q, Yun SH, Kannan K, et al. Phthalate concentrations and dietary exposure from food purchased in New York State. Environ Health Perspect. 2013;121(4):473-94.
17. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9; discussion 81-5.
18. Lorber M, Schecter A, Paepke O, Shropshire W, Christensen K, Birnbaum L. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ Int. 2015;77:55-62.
19. Watkins DJ, Eliot M, Sathyanarayana S, Calafat AM, Yolton K, Lanphear BP, et al. Variability and predictors of urinary concentrations of phthalate metabolites during early childhood. Environ Sci Technol. 2014;48(15):8881-90.
20. Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, et al. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environ Int. 2014;62:1-11.

21. Trasande L, Sathyanarayana S, Jo Messito M, R SG, Attina TM, Mendelsohn AL. Phthalates and the diets of U.S. children and adolescents. Environ Res. 2013;126:84-90.
22. Braun JM, Kalkbrenner AE, Calafat AM, Bernert JT, Ye X, Silva MJ, et al. Variability and predictors of urinary bisphenol A concentrations during pregnancy. Environ Health Perspect. 2011;119(1):131-7.
23. Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, et al. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int J Hyg Environ Health. 2015;218(2):220-31.
24. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, et al. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int. 2014;68:55-65.
25. Arbuckle TE, Marro L, Davis K, Fisher M, Ayotte P, Belanger P, et al. Exposure to free and conjugated forms of bisphenol A and triclosan among pregnant women in the MIREC cohort. Environ Health Perspect. 2015;123(4):277-84.
26. Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int. 2013;56:10-8.
27. Berman T, Goldsmith R, Goen T, Spungen J, Novack L, Levine H, et al. Demographic and dietary predictors of urinary bisphenol A concentrations in adults in Israel. Int J Hyg Environ Health. 2014;217(6):638-44.
28. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
29. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
30. Asimakopoulos AG, Xue J, De Carvalho BP, Iyer A, Abualnaja KO, Yaghmoor SS, et al. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. Environ Res. 2016;150:573-81.
31. Schantz MM, Benner BA, Jr., Heckert NA, Sander LC, Sharpless KE, Vander Pol SS, et al. Development of urine standard reference materials for metabolites of organic chemicals including polycyclic aromatic hydrocarbons, phthalates, phenols, parabens, and volatile organic compounds. Anal Bioanal Chem. 2015;407(11):2945-54.
32. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.
33. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, Geleijnse JM, Hofman A, Grobbee DE, et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr. 1998;52(8):588-96.
34. Tielemans MJ, Steegers EA, Voortman T, Jaddoe VW, Rivadeneira F, Franco OH, et al. Protein intake during pregnancy and offspring body composition at 6 years: the Generation R Study. Eur J Nutr. 2016.
35. Netherlands-Nutrition-Centre. Nevo: Dutch food composition database 2006. The Hague: Hoontetijl; 2006.
36. Philippat C, Botton J, Calafat AM, Ye X, Charles MA, Slama R, et al. Prenatal exposure to phenols and growth in boys. Epidemiology. 2014;25(5):625-35.
37. Valvi D, Casas M, Mendez MA, Ballesteros-Gomez A, Luque N, Rubio S, et al. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology. 2013;24(6):791-9.
38. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect. 2011;119(6):878-85.
39. Harley KG, Berger K, Rauch S, Kogut K, Claus Henn B, Calafat AM, et al. Association of prenatal urinary phthalate metabolite concentrations and childhood BMI and obesity. Pediatr Res. 2017;82(3):405-15.
40. Zoller O, Bruschweiler BJ, Magnin R, Reinhard H, Rhyn P, Rupp H, et al. Natural occurrence of bisphenol F in mustard. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2016;33(1):137-46.

41. Byrne D. Commision Decision 1999/815/EC. Brussels: Official Journal of the European Communities; 1999. p. 46-9.
42. European Chemicals Agency (ECHA). Authorisation List 2017 [updated 16 June 2017. Available from: https://echa.europa.eu/authorisation-list.
43. Liao C, Kannan K. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J Agric Food Chem. 2013;61(19):4655-62.
44. James-Todd TM, Meeker JD, Huang T, Hauser R, Seely EW, Ferguson KK, et al. Racial and ethnic variations in phthalate metabolite concentration changes across full-term pregnancies. J Expo Sci Environ Epidemiol. 2017;27(2):160-6.
45. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA. 2012;308(11):1113-21.
46. Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect. 2013;121(4):501-6.
47. Huang T, Saxena AR, Isganaitis E, James-Todd T. Gender and racial/ethnic differences in the associations of urinary phthalate metabolites with markers of diabetes risk: National Health and Nutrition Examination Survey 2001-2008. Environ Health. 2014;13(1):6.
48. Lewin A, Arbuckle TE, Fisher M, Liang CL, Marro L, Davis K, et al. Univariate predictors of maternal concentrations of environmental chemicals: The MIREC study. Int J Hyg Environ Health. 2017.
49. Wang L, Asimakopoulos AG, Kannan K. Accumulation of 19 environmental phenolic and xenobiotic heterocyclic aromatic compounds in human adipose tissue. Environ Int. 2015;78:45-50.
50. Jackson WJ, Darnell WR, inventorsProcess for foaming cellulose acetate rod1985 Mar 26, 1985.
51. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104(32):13056-61.
52. Buckley JP, Palmieri RT, Matuszewski JM, Herring AH, Baird DD, Hartmann KE, et al. Consumer product exposures associated with urinary phthalate levels in pregnant women. J Expo Sci Environ Epidemiol. 2012;22(5):468-75.
53. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? Epidemiology. 2006;17(4):413-8.
54. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.
55. Townsend MK, Franke AA, Li X, Hu FB, Eliassen AH. Within-person reproducibility of urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in the Nurses' Health Studies: a prospective cohort study. Environ Health. 2013;12(1):80.
56. Pollack AZ, Perkins NJ, Sjaarda L, Mumford SL, Kannan K, Philippat C, et al. Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. Environ Res. 2016;151:513-20.
57. Guo Y, Wang L, Kannan K. Effect of storage time and temperature on levels of phthalate metabolites and bisphenol A in urine. Advances in Environmental Research. 2013;2(1):9-17.
58. Silva MJ, Reidy JA, Preau JL, Jr., Samandar E, Needham LL, Calafat AM. Measurement of eight urinary metabolites of di(2-ethylhexyl) phthalate as biomarkers for human exposure assessment. Biomarkers. 2006;11(1):1-13.
59. Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure--an update and latest results. Int J Androl. 2006;29(1):155-65; discussion 81-5.

60. de Walle HEK, de Jong-van den Berg LTW. Onvoldoende foliumzuurinname rond de conceptie, vooral onder lager opgeleide vrouwen. Ned Tijdschr Geneeskunde. 2002;146:1990-3.

61. Lanting CI, Wouwe K, van Dommelen P, van der Pal-de Bruin KM, Josselin-de Jong S, Kleinjan M, et al. Roken tijdens de zwangerschap: percentages over de periode 2001-2015. Leiden, the Netherlands: 2015.

62. Aylward LL, Lorber M, Hays SM. Urinary DEHP metabolites and fasting time in NHANES. J Expo Sci Environ Epidemiol. 2011;21(6):615-24.

63. Hoepner LA, Whyatt RM, Just AC, Calafat AM, Perera FP, Rundle AG. Urinary concentrations of bisphenol A in an urban minority birth cohort in New York City, prenatal through age 7 years. Environ Res. 2013;122:38-44.

Supplementary Table S1. Bisphenol and phthalate urinary concentrations (N = 1,396)

	Median (IQR) (ng/mL)	Percentage of values below the limit of detection (LOD)	Percentage of values above the limit of quantification (LOQ)	Limit of detection (LOD) / limit of quantification (LOQ) (ng/mL)	Percentage absolute mean recoveries (relative SD)
Total bisphenols					
Bisphenol A (BPA)	1.66 (0.72, 3.56)	20.8	66.0	0.15 / 0.50	95.6 (13.1)
Bisphenol S (BPS)	0.36 (0.17, 1.08)	32.2	52.7	0.05 / 0.15	102 (16.9)
Bisphenol F (BPF)	0.57 (0.30, 1.29)	59.8	19.6	0.18 / 0.59	103 (13.7)
Bisphenol Z (BPZ)	0.17 (0.14, 0.25)	88.0	1.8	0.12 / 0.41	98.8 (16.7)
Bisphenol B (BPB)	0.17 (0.08, 0.29)	90.4	6.4	0.03 / 0.10	99.5 (19.0)
Bisphenol AP (BPAP)	0.24 (0.13, 0.42)	92.4	3.9	0.07 / 0.24	112 (17.2)
Bisphenol P (BPP)[1]	0.18 (0.14, 0.35)	98.4	0.3	0.11 / 0.38	-
Bisphenol AF (BPAF)	-	100.0	-	0.79 / 2.61	109 (11.8)
Phthalic Acid (PA)	57.08 (30.77, 121.91)	0.3	99.1	1.11 / 3.67	42.5 (18.3)
Low molecular weight (LMW) phthalates					
Monomethylphthalate (mMP)	5.43 (2.75, 9.88)	0.1	99.9	0.06 / 0.19	94.9 (14.8)
Monoethylphthalate (mEP)	138.03 (41.22, 486.71)	0.1	99.9	0.06 / 0.19	98.8 (14.8)
Mono-isobutylphthalate (mIBP)	21.55 (9.55, 45.91)	0.1	99.8	0.09 / 0.30	95.0 (13.2)
Mono-n-butylphthalate (mBP)	16.21 (7.01, 31.22)	0.7	98.9	0.14 / 0.46	94.7 (13.2)
High molecular weight (HMW) phthalates					
Di-2-ethylhexylphthalates (DEHP)					
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)	16.43 (8.26, 31.82)	0.1	99.3	0.29 / 0.97	90.8 (15.7)
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)	12.02 (5.83, 23.21)	0.1	99.6	0.08 / 0.25	91.4 (15.6)
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)	7.81 (3.53, 15.48)	0.1	99.1	0.04 / 0.12	90.8 (18.2)

	Median (IQR) (ng/mL)	Percentage of values below the limit of detection (LOD)	Percentage of values above the limit of quantification (LOQ)	Limit of detecti-on (LOD) / limit of quantification (LOQ) (ng/mL)	Percentage absolute mean recoveries (relative SD)
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)	14.17 (7.60, 26.69)	0.1	99.9	0.04 / 0.13	85.3 (16.1)
Di-isononylphthalate (DINP)					
Monoisononylphthalate (mINP)	0.81 (0.39, 1.69)	86.1	8.4	0.18 / 0.58	65.7 (15.4)
Di-isodecylphthalate (DIDP)					
Mono-(8-methyl-1-nonyl)phthalate (MIDP)	1.90 (1.30, 2.72)	92.8	1.6	0.89 / 2.93	15.3 (23.6)
Di-n-octylphthalate (DNOP)					
Mono(3-carboxypropyl)phthalate (mCPP)	1.46 (0.78, 2.78)	0.2	99.8	0.008 / 0.03	95.0 (14.5)
Monooctylphthalate (mOP)	0.46 (0.33, 0.80)	90.3	2.4	0.25 / 0.81	64.8 (12.9)
Mono-(7-carboxy-n-heptyl)phthalate (mCHpP)	0.10 (0.08, 0.13)	99.2	99.2	0.06 / 0.20	85.8 (16.9)
Other high molecular weight phthalates					
Monobenzylphthalate (mBzP)	6.59 (3.07, 12.91)	8.2	90.4	0.15 / 0.50	90.5 (15.5)
Mono-hexylphthalate (mHxP)	0.33 (0.16, 0.63)	23.6	53.9	0.06/ 0.19	87.2 (18.4)
Mono-2-heptylphthalate (mHpP)	1.10 (0.60, 2.34)	35.5	35.2	0.30 / 0.99	84.4 (20.5)
Monocyclohexylphthalate (mCHP)	0.16 (0.08, 0.40)	80.5	11.2	0.04 / 0.12	95.7 (20.1)

[1]Recoveries of BPP have not been calculated, due to the low detection rate

Supplementary Material S2 can be found online

Supplementary Table S3. Spearman correlation coefficients of separate bisphenols (N = 1,396)

	Bisphenol A	Bisphenol S	Bisphenol Z	Bisphenol B	Bisphenol F	Bisphenol AP	Bisphenol P
Bisphenol A	1.00	-	-	-	-	-	-
Bisphenol S	0.21**	1.00	-	-	-	-	-
	(n = 765)						
Bisphenol Z	0.05	0.08	1.00	-	-	-	-
	(n = 163)	(n = 138)					
Bisphenol B	-0.01	-0.11	0.44	1.00	-	-	-
	(n = 114)	(n = 102)	(n = 25)				
Bisphenol F	0.16**	0.07	0.17	-0.04	1.00	-	-
	(n = 504)	(n = 392)	(n = 85)	(n = 51)			
Bisphenol AP	0.26*	0.15	0.03	-0.21	-0.11	1.00	-
	(n = 103)	(n = 72)	(n = 17)	(n = 32)	(n = 61)		
Bisphenol P	-0.12	-0.22	0.50	-0.09	-0.14	0.03	1.00
	(n = 22)	(n = 14)	(n = 3)	(n = 6)	(n = 11)	(n = 15)	

Spearman's correlation coefficients of separate bisphenols for detected values.
*P-value < 0.05. **P-value < 0.001.

Supplementary Table S4. Spearman correlation coefficients of separate phthalate metabolites (N = 1,396)

	LMW phthalate metabolites					HMW phthalate metabolites												
						DEHP				DINP	DIDP	DNOP		Other HMW phthalates				
	mMP	mEP	mIBP	mBP	PA	mECPP	mCMHP	mEHHP	mEOHP	mINP	mIDP	mCPP	mOP	mCHpP	mBzP	mHxP	mHpP	mCHP
mMP	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mEP	0.48** (n = 1393)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mIBP	0.45** (n = 1392)	0.32** (n = 1394)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mBP	0.49** (n = 1384)	0.38** (n = 1386)	0.78** (n = 1385)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PA	0.62** (n = 1390)	0.69** (n = 1392)	0.44** (n = 1391)	0.51** (n = 1383)	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-
mECPP	0.56** (n = 1393)	0.35** (n = 1394)	0.61** (n = 1393)	0.67** (n = 1385)	0.59** (n = 1391)	1.00	-	-	-	-	-	-	-	-	-	-	-	-
mEHHP	0.47** (n = 1392)	0.27** (n = 1394)	0.66** (n = 1393)	0.73** (n = 1385)	0.47** (n = 1391)	0.88** (n = 1393)	1.00	-	-	-	-	-	-	-	-	-	-	-
mCMHP	0.57** (n = 1393)	0.41** (n = 1395)	0.60** (n = 1394)	0.66** (n = 1386)	0.61** (n = 1392)	0.85** (n = 1394)	0.77** (n = 1394)	1.00	-	-	-	-	-	-	-	-	-	-
mEOHP	0.45** (n = 1394)	0.28** (n = 1395)	0.68** (n = 1394)	0.76** (n = 1386)	0.47** (n = 1392)	0.87** (n = 1394)	0.98** (n = 1394)	0.77** (n = 1395)	1.00	-	-	-	-	-	-	-	-	-
mINP	0.16* (n = 194)	0.15* (n = 195)	0.06 (n = 195)	0.12 (n = 194)	0.10 (n = 194)	0.22* (n = 194)	0.25** (n = 195)	0.10 (n = 195)	0.20* (n = 195)	1.00	-	-	-	-	-	-	-	-

	LMW phthalate metabolites					HMW phthalate metabolites												
						DEHP				DINP	DIDP	DNOP		Other HMW phthalates				
	mMP	mEP	mIBP	mBP	PA	mECPP	mCMHP	mEHHP	mEOHP	mINP	mIDP	mCPP	mOP	mCHpP	mBzP	mHxP	mHpP	mCHP
mIDP	0.09 (n = 101)	-0.03 (n = 101)	0.21* (n = 101)	0.22* (n = 101)	0.01 (n = 100)	0.22 (n = 101)	0.31* (n = 101)	0.18 (n = 101)	0.32* (n = 101)	0.38* (n = 38)	1.00	-	-	-	-	-	-	-
mCPP	0.57** (n = 1394)	0.36** (n = 1395)	0.60** (n = 1394)	0.69** (n = 1386)	0.57** (n = 1392)	0.74** (n = 1394)	0.69** (n = 1394)	0.70** (n = 1395)	0.68** (n = 1396)	0.22* (n = 195)	0.15 (n = 101)	1.00	-	-	-	-	-	-
mOP	0.20* (n = 136)	0.03 (n = 136)	0.15 (n = 136)	0.20* (n = 134)	0.08 (n = 136)	0.19* (n = 136)	0.28* (n = 1394)	0.19* (n = 136)	0.28* (n = 136)	-0.20 (n = 30)	0.32 (n = 25)	0.30** (n = 136)	1.00	-	-	-	-	-
mCHpP	0.36 (n = 11)	0.54 (n = 11)	-0.14 (n = 11)	-0.07 (n = 11)	0.56 (n = 11)	0.44 (n = 11)	0.35 (n = 11)	0.40 (n = 11)	0.39 (n = 11)	- (n = 0)	-1.00 (n = 2)	0.28 (n = 11)	- (n = 1)	1.00	-	-	-	-
mBzP	0.39** (n = 1280)	0.33** (n = 1282)	0.59** (n = 1281)	0.65** (n = 1273)	0.42** (n = 1281)	0.50** (n = 1281)	0.54** (n = 1281)	0.52** (n = 1282)	0.55** (n = 1282)	0.11 (n = 189)	0.20* (n = 100)	0.50** (n = 1282)	0.09 (n = 123)	-0.09 (n = 11)	1.00	-	-	-
mHxP	0.39** (n = 1071)	0.27** (n = 1070)	0.44** (n = 1069)	0.50** (n = 1063)	0.36** (n = 1069)	0.47** (n = 1070)	0.53** (n = 1069)	0.44** (n = 1070)	0.54** (n = 1071)	0.27* (n = 150)	0.12 (n = 65)	0.59** (n = 1071)	0.49** (n = 127)	0.50 (n = 3)	0.34** (n = 992)	1.00	-	-
mHpP	0.36** (n = 905)	0.25** (n = 905)	0.50** (n = 904)	0.43** (n = 900)	0.34** (n = 904)	0.44** (n = 905)	0.39** (n = 905)	0.43** (n = 905)	0.39** (n = 905)	0.29** (n = 138)	0.15 (n = 59)	0.45** (n = 905)	-0.00 (n = 107)	0.50 (n = 3)	0.41** (n = 856)	0.41** (n = 816)	1.00	-
mCHP	0.18* (n = 273)	0.13* (n = 273)	0.17* (n = 273)	0.25** (n = 272)	0.12 (n = 273)	0.21** (n = 273)	0.25** (n = 273)	0.16* (n = 273)	0.27** (n = 273)	0.43* (n = 60)	0.31 (n = 18)	0.18* (n = 273)	0.05 (n = 44)	- (n = 1)	0.27** (n = 262)	0.30** (n = 243)	0.20* (n = 203)	1.00

Spearman's correlation coefficients of separate phthalate metabolites for detected values.
*P-value < 0.05. **P-value < 0.001.

Supplementary Table S5. Associations between maternal dietary intake and urinary bisphenol and phthalate concentrations in Dutch women (N=642)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenol (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphtha-late (DEHP) (nmol/l) [95% CI]**	**Di-n-oc-tylphthalate (DNOP) (nmol/l) [95% CI]**
Food groups[a]								
Vegetables	0.11	0.08	-0.01	-1.06	-18.91	31.46	26.50	0.88
	(-1.37, 2.80)	(-0.10, 0.45)	(-0.05, 0.10)	(-7.12, 15.71)	(-157.02, 262.86)	(-0.57, 80.69)	(-0.46, 68.78)	(-0.09, 2.44)
Fruit	-0.12	-0.01	0.02	3.97	23.55	5.87	1.68	0.45
	(-1.52, 2.55)	(-0.15, 0.30)	(-0.04, 0.14)	(-5.17, 20.16)	(-130.78, 333.48)	(-19.28, 45.60)	(-18.31, 34.17)	(-0.39, 1.83)
Potatoes	-0.05	-0.05	-0.00	4.88	-170.39	25.70	22.37	0.56
	(-1.48, 2.64)	(-0.17, 0.23)	(-0.05, 0.11)	(-4.63, 21.52)	(-250.85, 13.80)	(-5.04, 73.02)	(-3.66, 63.23)	(-0.31, 1.99)
Legumes	0.80	0.11	0.00	-8.37	-27.15	7.35	5.02	0.18
	(-0.91, 3.93)	(-0.09, 0.50)	(-0.04, 0.11)	(-13.40, 1.40)	(-69.81, 41.17)	(-18.00, 47.16)	(-15.75, 38.43)	(-0.57, 1.43)
Grains	-0.03	-0.01	-0.06	-13.40	-237.06	-23.44	-17.37	-0.15
	(-1.46, 2.66)	(-0.15, 0.29)	(-0.08,-0.00)*	(-16.84, -6.06)*	(-293.54,-92.26)*	(-40.61, 4.98)	(-31.95, 7.29)	(-0.80, 0.96)
Cakes	1.59	0.09	0.03	-2.40	-191.05	-4.87	-0.72	0.17
	(-0.41, 5.19)	(-0.09, 0.48)	(-0.03, 0.16)	(-9.42, 10.43)	(-263.69,-20.30)*	(-27.05, 30.51)	(-19.99, 30.58)	(-0.58, 1.42)
Sugar and confectionary	1.56	0.02	0.06	4.15	-28.96	7.60	7.99	0.19
	(-0.44, 5.13)	(-0.13, 0.35)	(-0.02, 0.21)	(-5.07, 20.34)	(-163.20, 244.90)	(-18.01, 47.76)	(-13.78, 42.82)	(-0.57, 1.45)
Vegetable oil	0.51	0.11	-0.04	4.49	93.91	7.68	8.52	-0.60
	(-1.11, 3.50)	(-0.08, 0.50)	(-0.07, 0.03)	(-4.79, 21.01)	(-87.87, 446.95)	(-17.96, 47.89)	(-13.42, 45.58)	(-1.11, 0.31)
Margarines	-1.09	-0.15	-0.02	-6.54	-58.82	-25.03	-15.75	0.70
	(-2.15, 1.00)	(-0.23, 0.05)	(-0.06, 0.07)	(-12.18, 4.15)	(-181.61, 195.89)	(-41.69, 2.57)	(-30.73, 9.40)	(-1.18, 0.17)
Butter	0.79	0.09	0.01	3.53	-0.40	11.42	7.81	0.63
	(-0.93, 3.93)	(-0.09, 0.46)	(-0.04, 0.13)	(-5.46, 19.43)	(-145.52, 292.98)	(-15.19, 53.11)	(-13.88, 42.63)	(-0.26, 2.08)
Milk	1.17	0.06	0.03	2.37	-19.63	-10.61	-10.21	-0.40
	(-0.69, 4.52)	(-0.11, 0.42)	(-0.03, 0.17)	(-6.25, 17.65)	(-157.45, 260.24)	(-31.21, 22.56)	(-26.77, 17.21)	(-0.98, 0.60)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenol (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphtha-late (DEHP) (nmol/l) [95% CI]**	**Di-n-oc-tylphthalate (DNOP) (nmol/l) [95% CI]**
Dairy products	-0.18	-0.03	-0.03	-0.74	-99.75	-1.09	-4.15	-0.05
	(-1.56, 2.42)	(-0.16, 0.25)	(-0.06, 0.05)	(-8.32, 12.94)	(-206.97, 129.15)	(-24.30, 35.74)	(-22.44, 25.76)	(-0.73, 1.10)
Fresh meat	-0.58	-0.08	-0.04	3.94	17.20	4.60	0.60	0.30
	(-1.81, 1.80)	(-0.19, 0.18)	(-0.07, 0.03)	(-5.23, 20.06)	(-135.09, 320.92)	(-20.23, 43.65)	(-19.08, 32.45)	(-0.49, 1.61)
Processed meat	1.29	0.15	-0.01	4.06	121.32	4.52	4.49	-0.20
	(-0.61, 4.72)	(-0.06, 0.58)	(-0.05, 0.10)	(-5.14, 20.22)	(-71.18, 492.15)	(-20.26, 43.52)	(-16.30, 37.92)	(-0.84, 0.89)
Eggs	-0.03	0.04	-0.02	-2.17	47.19	-0.90	-2.86	-0.53
	(-1.33, 2.33)	(-0.10, 0.35)	(-0.05, 0.06)	(-8.59, 9.18)	(-101.99, 330.85)	(-21.92, 31.66)	(-19.71, 23.94)	(1.01, 0.29)
Fish and shellfish	1.91	0.24	-0.02	5.54	-17.70	-0.89	-0.39	-0.48
	(-0.21, 5.69)	(-0.00, 0.73)	(-0.06, 0.07)	(-4.14, 22.45)	(-156.25, 263.42)	(-24.16, 36.01)	(-19.76, 31.03)	(-1.03, 0.48)
Sauces	0.31	-0.05	0.00	8.05	173.16	15.06	9.57	0.73
	(-1.24, 3.19)	(-0.17, 0.23)	(-0.05, 0.11)	(-2.45, 26.23)	(-38.26, 576.55)	(-12.54, 58.10)	(-12.62, 45.07)	(-0.19, 2.23)
Tea	-0.67	-0.12	-0.03	-7.57	-68.63	10.29	7.82	0.53
	(-1.78, 1.43)	(-0.20, 0.09)	(-0.06, 0.05)	(-12.44, 1.62)	(-178.37, 155.26)	(-13.97, 47.59)	(-12.19, 39.29)	(-0.27, 1.80)
Coffee	2.46	0.15	0.07	-0.43	113.27	-10.58	-4.52	-0.09
	(0.17, 6.50)*	(-0.05, 0.57)	(-0.01, 0.24)	(-8.06, 13.34)	(-74.20, 475.69)	(-31.09, 22.47)	(-22.61, 25.07)	(-0.75, 1.04)
Soft drinks	1.20	0.12	0.03	10.91	333.46	18.55	7.56	-0.06
	(-0.66, 4.55)	(-0.07, 0.52)	(-0.03, 0.16)	(-0.55, 30.47)	(59.69, 837.45)*	(-9.95, 62.66)	(-14.00, 42.00)	(-0.73, 1.08)
Fruit and vegeta-ble juices	-1.60	-0.15	-0.03	-0.37	66.97	-3.65	-1.84	-0.36
	(-2.48, 0.21)	(-0.28, 0.06)	(-0.07, 0.05)	(-8.07, 13.50)	(-104.16, 402.44)	(-26.17, 32.19)	(-20.80, 28.99)	(-0.95, 0.65)
Alcoholic drinks	1.88	0.24	0.01	4.81	67.01	1.87	3.28	-0.17
	(-0.18, 5.52)	(0.00, 0.71)*	(-0.04, 0.13)	(-4.40, 20.86)	(-100.11, 392.61)	(-21.58, 38.78)	(-16.65, 35.28)	(-0.80, 0.89)
Soups and bouillon	0.75	0.02	0.02	-1.76	-212.34	5.32	2.77	-0.45
	(-0.96, 3.87)	(-0.13, 0.35)	(-0.03, 0.15)	(-8.99, 11.41)	(-277.14,-54.61)*	(-19.65, 44.61)	(-17.49, 35.49)	(-1.01, 0.53)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenol (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphtha-late (DEHP) (nmol/l) [95% CI]**	**Di-n-oc-tylphthalate (DNOP) (nmol/l) [95% CI]**
Daily dietary caloric intake[b]								
<1600 kcal	-0.13	0.03	-0.05	4.56	203.28	8.81	5.76	-0.59
(n=85)	(-1.43, 2.60)	(-0.12, 0.37)	(-0.08, 0.05)	(-4.69, 22.18)	(-15.30, 651, 55)	(-15.59, 49.71)	(-14.10, 40.02)	(-1.09, 0.39)
1600-1999 kcal	-0.05	0.03	-0.06	2.17	95.67	16.41	12.13	-0.04
(n=180)	(-1.14, 2.07)	(-0.09, 0.30)	(-0.08,-0.01)*	(-4.71, 14.68)	(-48.70, 380.53)	(-5.79, 51.43)	(-6.05, 41.55)	(-0.60, 0.94)
2000-2399 kcal	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
(n=199)								
≥2400 kcal	0.04	0.01	-0.06	-7.22	-99.52	-7.27	-5.77	-0.54
(n=178)	(-1.09, 2.23)	(-0.10, 0.26)	(-0.08,-0.00)*	(10.87, 0.39)	(-166.87, 58.12)	(-22.81, 18.48)	(-18.70, 16.21)	(-0.94, 0.22)

[a] Values are regression coefficients (95% confidence intervals) from linear regression models that reflect the difference in bisphenol and phthalate urine concentrations in ng/ml or nmol/l in women in the upper 10% of consumption of that particular food group or dietary pattern compared to the first 90%.
[b] Values are regression coefficients (95% confidence intervals) from linear regression models that reflect the difference in bisphenol and phthalate urine concentrations in ng/ml or nmol/l compared with the reference.
Significance levels are accounted for multiple testing using Bonferroni correction. Models are adjusted for creatinine.
*P-value<0.05 †Significant using Bonferroni

Supplementary Table S6. Multivariable associations of food groups with individual bisphenol and phthalate urine concentrations (n=642)

	Bisphenols	Low molecular weight phthalate metabolites				High molecular weight phthalate metabolites							
						DEHP metabolites				*DNOP metabolites*	*Other HMW phthalate metabolites*		
	BPF (ng/ml) [95% CI]	**mMP (ng/ml) [95% CI]**	**mEP (ng/ml) [95% CI]**	**mIBP (ng/ml) [95% CI]**	**mBP (ng/ml) [95% CI]**	**mECPP (ng/ml) [95% CI]**	**mEHHP (ng/ml) [95% CI]**	**mEOHP (ng/ml) [95% CI]**	**mCMHP (ng/ml) [95% CI]**	**mCPP (ng/ml) [95% CI]**	**mBzP (ng/ml) [95% CI]**	**mHxP (ng/ml) [95% CI]**	**mHpP (ng/ml) [95% CI]**
Food groups													
Vegetables (01)	-	-	-	-	-	2.45 (0.15, 6.62)*	2.62 (0.45, 6.69)*	1.87 (0.35, 4.82)*	2.34 (0.37, 5.69)*	0.29 (0.03, 0.75)*	0.43 (-0.19, 2.04)	0.04 (-0.00, 0.14)	0.11 (0.01, 0.27)*
Fish and shellfish (12)	0.05 (-0.02, 0.19)	-	-	-	-	-	-	-	-	-	-	-	-
Soft drinks (14c)	-	-0.08 (-0.60, 0.95)	32.35 (0.44, 114.24)*	2.52 (-0.26, 8.03)	0.35 (-1.28, 3.86)	-	-	-	-	-	-	-	-
Soups and bouillon (16)	-	-0.33 (-0.75, 0.56)	-24.75 (-26.81, -6.84)*	0.18 (-1.62, 3.99)	0.64 (-1.06, 4.23)	-	-	-	-	-	-	-	-

Values are regression coefficients (95% confidence intervals) from multivariable linear regression models in individual chemicals from associated bisphenol and phthalate groups that reflect the difference in individual bisphenol and phthalate urine concentrations in ng/ml in women in the upper 10% of consumption of that particular food group or dietary pattern compared to the first 90%. Significance levels are accounted for multiple testing using Bonferroni correction. Models are adjusted for maternal age, pre-pregnancy body mass index, parity, daily dietary caloric intake, maternal highest finished education, smoking, folic acid supplement use and creatinine. *P-value<0.05 †Significant using Bonferroni.

Supplementary Table S7. Univariate associations of socio-demographic and lifestyle related factors with urinary bisphenol and phthalate concentrations (N = 1,396)

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenols (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphthalate (DEHP) (nmol/l) [95% CI]**	**Di-n-octylphthalate (DNOP) (nmol/l) [95% CI]**
Maternal age								
<25 years *(n=190)*	0.84 (-0.40, 2.71)	0.20 (0.03, 0.48)*	0.01 (-0.03, 0.07)	9.31 (1.46, 20.55)*	320.90 (111.40, 640.71)*†	46.16 (16.61, 86.61)*†	29.91 (7.26, 61.38)*	0.473 (-0.18, 1.39)
25-29.9 years *(n=374)*	0.23 (-0.64, 1.51)	0.02 (-0.07, 0.17)	-0.01 (-0.03, 0.04)	3.37 (-1.83, 10.65)	126.51 (0.70, 314.44)*	8.99 (-8.25, 32.33)	3.86 (-9.61, 22.36)	0.00 (-0.43, 0.60)
30-34.9 years *(n=598)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
≥35 years *(n=234)*	0.03 (-0.92, 1.46)	0.04 (-0.07, 0.23)	0.02 (-0.02, 0.08)	1.90 (-3.71, 9.99)	13.14 (-97.43, 188.08)	5.69 (-13.42, 32.21)	4.68 (-11.02, 26.68)	0.67 (0.02, 1.57)*
Parity								
Primiparous *(n=849)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Multiparous *(n=547)*	0.69 (-0.14, 1.83)	0.06 (-0.03, 0.19)	0.01 (-0.02, 0.05)	-3.00 (-6.53, 1.89)	-22.92 (-105.04, 98.91)	18.40 (2.02, 39.67)*	15.15 (1.78, 32.65)*	0.86 (0.36, 1.52)*†
Educational level								
Low *(n=709)*	1.16 (0.27, 2.41)*	0.20 (0.08, 0.37)*†	0.00 (-0.02, 0.04)	9.80 (4.40, 16.95)*†	278.63 (142.14, 469.03)*†	38.31 (19.28, 62.82)*†	24.67 (10.10, 43.66)*†	0.38 (-0.06, 0.96)
High *(n=687)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Maternal ethnicity								
Dutch/European *(n=860)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Non-European *(n=536)*	0.11 (-0.62, 1.14)	0.08 (-0.01, 0.22)	-0.02 (-0.04, 0.01)	4.70 (-0.07, 11.09)	228.52 (97.47, 412.22)*†	45.46 (25.22, 71.27)*†	30.76 (15.07, 50.99)*†	0.53 (0.06, 1.13)*

	Difference in bisphenol concentrations			Difference in phthalate concentrations				
	Total Bisphenols (nmol/l) [95% CI]	**Bisphenol A (BPA) (ng/ml) [95% CI]**	**Bisphenol S (BPS) (ng/ml) [95% CI]**	**Phthalic acid (PA) (ng/ml) [95% CI]**	**Low Molecular Weight (LMW) Phthalate (nmol/l) [95% CI]**	**High Molecular Weight (HMW) Phthalate (nmol/l) [95% CI]**	**Di-2-ethyl-hexylphthalate (DEHP) (nmol/l) [95% CI]**	**Di-n-octylphthalate (DNOP) (nmol/l) [95% CI]**
Pre-pregnancy body mass index (kg/m²)								
< 20 kg/m² *(n=203)*	0.09	-0.01	-0.02	0.17	-72.65	-1.41	0.46	-0.25
	(-0.88, 1.55)	(-0.10, 0.16)	(-0.05, 0.04)	(-5.10, 7.81)	(-170.38, 88.16)	(-21.01, 26.34)	(-16.21, 24.35)	(-0.77, 0.50)
20-24.9 kg/m²	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
(n=785)								
25-29.9 kg/m²	1.05	0.12	0.01	9.55	124.29	18.02	12.79	0.11
(n=290)	(-0.14, 2.82)	(-0.03, 0.35)	(-0.03, 0.06)	(2.15, 19.95)*	(-12.36, 330.49)	(-2.89, 46.42)	(-3.91, 35.72)	(-0.44, 0.87)
≥30 kg/m² *(n=118)*	2.47	0.27	0.09	16.32	317.35	69.94	46.94	1.14
	(0.48, 5.57)*	(0.05, 0.66)*	(0.01, 0.23)*	(4.83, 33.36)*†	(50.04, 759.09)*	(29.67, 126.30)*†	(16.13, 90.73)*†	(0.01, 1.49)*
Smoking during pregnancy								
No *(n=1051)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]
Yes *(n=345)*	1.60	0.22	0.01	8.63	182.45	17.12	13.14	0.14
	(0.42, 3.26)*†	(0.08, 0.43)*†	(-0.02, 0.06)	(2.50, 16.84)*†	(45.02, 378.99)*†	(-1.41, 41.41)	(-1.74, 32.81)	(-0.32, 0.76)
Folic acid supplement use								
No *(n=290)*	1.43	0.26	0.02	5.85	228.05	61.09	41.80	1.38
	(0.28, 3.04)*	(0.10, 0.49)*†	(-0.02, 0.07)	(-0.48, 14.53)	(66.13, 462.68)*†	(33.98, 96.07)*†	(20.98, 68.96)*†	(0.64, 2.35)*†
Yes *(n=1106)*	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]	[Reference]

Values are regression coefficients (95% confidence intervals) from univariate linear regression models that reflect differences in urinary concentrations of bisphenol and phthalate concentrations, compared with the reference. Performed in multiply imputed data. Models are adjusted for creatinine.
*P-value<0.05 †Significant using Bonferroni. Numbers are after multiple imputation.

Supplementary Table S8. Multivariable associations of socio-demographic and lifestyle factors with individual bisphenol and phthalate urine concentrations (n=1,396)

	Bisphenols	**Low molecular weight phthalate metabolites**				**High molecular weight phthalate metabolites**							
						DEHP metabolites				***DNOP metabolites***	***Other HMW metabolites***		
	BPF (ng/ml) [95% CI]	**mMP (ng/ml) [95% CI]**	**mEP (ng/ml) [95% CI]**	**mIBP (ng/ml) [95% CI]**	**mBP (ng/ml) [95% CI]**	**mECPP (ng/ml) [95% CI]**	**mEHHP (ng/ml) [95% CI]**	**mEOHP (ng/ml) [95% CI]**	**mCMHP (ng/ml) [95% CI]**	**mCPP (ng/ml) [95% CI]**	**mBzP (ng/ml) [95% CI]**	**mHxP (ng/ml) [95% CI]**	**mHpP (ng/ml) [95% CI]**
Educational level													
Low *(n=709)*	-	0.29 [-0.16, 0.95]	19.44 [1.81, 49.45]*	1.59 [-0.06, 4.06]	0.40 [-0.65, 1.99]	-	-	-	-	-	-	-	-
High *(n=687)*	-	[Ref]	[Ref]	[Ref]	[Ref]	-	-	-	-	-	-	-	-
Maternal ethnicity													
Dutch/European *(n=860)*	-	-	-	-	-	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]
Non-European *(n=536)*	-	-	-	-	-	1.40 [0.09, 3.26]*	0.96 [-0.09, 2.49]	0.39 [-0.24, 1.21]	1.08 [0.01, 2.56]*	0.01 [-0.09, 0.15]	0.67 [0.16, 1.50]*†	-0.02 [-0.04, 0.01]	0.05 [-0.01, 0.12]
Pre-pregnancy body mass index (kg/m²)													
< 20 kg/m² *(n=203)*	-0.01 (-0.04, 0.04)	-	-	-	-	0.10 (-1.92, 2.06)	0.20 [-0.91, 1.95]	0.18 [-0.57, 1.35]	-0.26 [-1.27, 1.23]	-0.06 [-0.17, 0.11]	-0.09 [-0.45, 0.57]	0.00 [-0.03, 0.05]	-0.00 [-0.05, 0.07]
20-24.9 kg/m² *(n=785)*	[Ref]	-	-	-	-	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]

	Bisphenols	Low molecular weight phthalate metabolites				High molecular weight phthalate metabolites							
						DEHP metabolites				*DNOP metabolites*	*Other HMW metabolites*		
	BPF (ng/ml) [95% CI]	**mMP (ng/ml) [95% CI]**	**mEP (ng/ml) [95% CI]**	**mIBP (ng/ml) [95% CI]**	**mBP (ng/ml) [95% CI]**	**mECPP (ng/ml) [95% CI]**	**mEHHP (ng/ml) [95% CI]**	**mEOHP (ng/ml) [95% CI]**	**mCMHP (ng/ml) [95% CI]**	**mCPP (ng/ml) [95% CI]**	**mBzP (ng/ml) [95% CI]**	**mHxP (ng/ml) [95% CI]**	**mHpP (ng/ml) [95% CI]**
25-29.9 kg/m² *(n=290)*	0.02 (-0.02, 0.07)	-	-	-	-	0.82 (-0.54, 2.84)	0.46 [-0.61, 2.10]	0.29 [-0.41, 1.38]	0.18 [-0.80, 1.58]	-0.01 [-0.12, 0.17]	0.15 [-0.25, 0.86]	0.01 [-0.02, 0.06]	0.01 [-0.04, 0.09]
≥30 kg/m² *(n=118)*	0.03 (-0.02, 0.12)	-	-	-	-	3.48 (0.96, 7.29)*†	2.52 [-0.48, 5.72]*	1.49 [0.18, 3.59]*	1.75 [0.02, 4.31]*	0.20 [-0.02, 0.53]	1.60 [0.54, 3.47]*†	0.04 [-0.01, 0.13]	0.17 [0.05, 0.36]*†
Smoking during pregnancy													
No *(n=1051)*	[Ref]	-	-	-	-	-	-	-	-	-	-	-	-
Yes *(n=345)*	0.01 (-0.02, 0.06)	-	-	-	-	-	-	-	-	-	-	-	-
Folic acid supplement use													
No *(n=290)*	-	-	-	-	-	2.00 (0.343, 4.41)*	1.14 [-0.10, 3.10]	0.82 [-0.03, 2.10]	1.57 [0.18, 3.54]*	0.26 [-0.08, 0.52]	0.58 [-0.02, 1.63]	0.04 [0.00, 0.11]*	0.08 [0.01, 0.20]*
Yes *(n=1106)*	-	-	-	-	-	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]	[Ref]

Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect differences in individual chemicals from associated bisphenol and phthalate groups that reflect the difference in individual bisphenol and phthalate urine concentrations in ng/ml, compared with the reference.
Models are adjusted for maternal age, pre-pregnancy body mass index, parity, maternal ethnicity, maternal highest finished education, smoking, folic acid supplement use and creatinine.
*P-value<0.05 †Significant using Bonferroni.
Numbers are after multiple imputation.

Supplementary Table S9. Nonresponse analysis[1]

	Sample n = 1396	**Generation R n = 4847 (inclusion <18w + visit F@5)**
Maternal age	30.6 (4.8)	30.4 (4.9)
Nulliparous (%)	61.0	58.8
Low educational level (%)	49.6	51.8
Dutch/European (%)	61.9	63.7
Maternal pre-pregnancy BMI[2]	22.7 (20.8, 25.3)	22.6 (20.8, 25.3)
Maternal non-smoking (%)	75.2	73.1
Folic acid supplement use (%)	90.6	88.0

[1]Values represent means (standard deviation) or valid percentages
[2]Median (IQR)

2.2

CHAPTER

First trimester urinary bisphenol and phthalate concentrations and time to pregnancy

J Clin Endocrinol Metab.
2018 Sept 1;103(9):3540-3547.

Abstract

Background: Increasing evidence suggests that exposure to synthetic chemicals such as bisphenols and phthalates can influence fecundability. The current study describes associations of first trimester urinary concentrations of bisphenol A (BPA), BPA analogues and phthalate metabolites with time to pregnancy (TTP).

Methods: Among 877 participants in the population-based Generation R pregnancy cohort, we measured first trimester urinary concentrations of bisphenols and phthalates (median gestational age 12.9 weeks [inter-quartile range 12.1-14.4 weeks]). We used fitted covariate-adjusted Cox proportional hazard models to examine associations of bisphenol and phthalate concentrations with TTP. Participants who conceived using infertility treatment were censored at 12 months. Biologically plausible effect measure modification by folic acid supplement use was tested.

Results: In the main models, bisphenol and phthalate compounds were not associated with fecundability. In stratified models, total bisphenols and phthalic acid were associated with longer TTP among women who did not use folic acid supplements preconceptionally (respective fecundability ratios per each natural log increase were 0.90 [95% Confidence Interval (CI) 0.81, 1.00] and 0.88 [95% CI 0.79, 0.99]). Using an interaction term for the exposure and folic acid supplement use showed additional effect measure modification by folic acid supplement use for high molecular weight phthalate metabolites.

Conclusions: We found no associations of bisphenols and phthalates with fecundability. Preconception folic acid supplementation seems to modify effects of bisphenols and phthalates on fecundability. Folic acid supplements may protect against reduced fecundability among women exposed to these chemicals. Further studies are needed to replicate these findings and investigate potential mechanisms.

Introduction

Increasing evidence suggests that synthetic chemicals can influence both male and female health and fecundability, the biological ability to conceive a pregnancy. Persistent organic pollutants have been linked to a host of reproductive disorders in both men and women,[1,2] including prolonged time to pregnancy (TTP), a marker of couple fecundability. The literature on nonpersistent chemicals is more limited. Prior studies of female urinary bisphenols and TTP have not found an association, but have relied exclusively on linear models.[3-5] Results of studies investigating female phthalate metabolites and TTP have been inconsistent.[3-8] Differences among these studies' conclusions may stem from variations in study size and population, as well as design, as they did not all measure the same metabolites and some measured TTP prospectively while others relied on retrospective recall of TTP from the first trimester of pregnancy.

DNA methylation is a potential mechanism through which bisphenols and phthalates may be related to female reproductive disorders.[9] Bisphenol A (BPA), in particular, has been shown to target reproductive tissues and affect reproductive outcomes in numerous animal studies.[10] In a now classic experiment, mice exposed to BPA during pregnancy were more likely to give birth to offspring with yellow coats as a result of decreased methylation upstream of the Agouti gene. The effect was negated when BPA-exposed dams were supplemented with folic acid, a methyl donor.[11] Folate depletion has been associated with global hypomethylation, but also with targeted hypermethylation (reviewed in [12]). A recent study among women undergoing infertility treatment reported that high urinary BPA concentrations were associated with lower probabilities of implantation, clinical pregnancy, and live birth, but only among women who consumed <400 µg/day of dietary folate; there were no associations among women who consumed ≥400 µg/day.[13]

Phthalates also affect reproductive outcomes in animals and humans [10] and, depending on the metabolite, have been shown to be associated with either DNA hypermethylation or hypomethylation. Among 336 Mexican-American newborns, phthalate metabolites—in particular, metabolites of di-2-ethylhexylphthalate (DEHP)--in maternal urine samples collected during pregnancy were associated with increased DNA methylation in cord blood.[14] By contrast, among 181 mother-newborn pairs in China, maternal third trimester urinary mono-(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl)phthalate (MEOHP) were associated with decreased DNA methylation in placental tissue.[15]

In the current study, we evaluate associations of urinary bisphenols and phthalate metabolites with TTP among 877 women participating in a large population-based pregnancy cohort. Because both bisphenols and phthalates affect DNA methylation, we assess potential effect measure modification by preconception folic acid supplementation, which prior studies have not explored. Our analysis expands upon earlier studies in three additional ways. 1) Where prior studies focused exclusively on BPA, we also investigate eight BPA analogues, chemical replacements that are commonly found in "BPA-free" products and have yet to be reported on in human fertility research. 2) In addition to measuring associations between molecular weight groupings of urinary phthalate metabolites and TTP, we also examine TTP in relation to phthalic acid (PA). As the final common metabolic form of all phthalates, PA represents a proxy for total phthalate exposure and captures exposure to phthalates for which we

do not have individual metabolite measures. 3) As contrasted to prior studies, which are restricted to women who have conceived naturally, we include women who conceived using some form of infertility treatment, permitting the investigation of potential associations of bisphenol and phthalate exposure with longer TTP.

Methods

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 that was approved by the Medical Ethical Committee of the Erasmus Medical Centre in Rotterdam.[16] In total, 8879 women were enrolled, 76% before gestational age of 18 weeks. Written consent was obtained from all participants.[17]

Bisphenol and phthalate concentrations were measured in first trimester urine samples among a subgroup of 1396 women with singleton pregnancies whose children also participated in postnatal studies at child age 5 years. Of these, 519 participants were excluded due to missing data on TTP and whether they had used infertility treatment, yielding an analytic sample of 877 women.

Urinary bisphenol and phthalate measurements

Bisphenol and phthalate concentrations were measured in a spot urine sample obtained from each participant during the first trimester visit (median gestational age 12.9 weeks, inter-quartile range 12.1-14.4 weeks). All urine samples were collected between February 2004 and July 2005. Details on collection, transportation, and analysis methodology are provided elsewhere.[18]

For analysis, we grouped bisphenols together and grouped phthalate metabolites according to their molecular weight categories and parent compounds. Phthalate metabolites and bisphenols were only included in groupings if <80% of the sample concentrations were below the limit of detection (LOD). Additionally, individual bisphenols were analyzed separately if <50% of the sample concentrations were below the LOD. We calculated the weighted molar sums for groups representing total bisphenols, low molecular weight (LMW) phthalates, high molecular weight (HMW) phthalates, and two subgroups of HMW phthalates, total di-2-ethylhexyl phthalate (DEHP) and di-n-octylphthalate (DNOP) metabolites, using the formula: *((concentration in ng/mL) * (1 / molecular weight) * (1 / 10^-3)) + ((concentration in ng/mL) * (1 / molecular weight) * (1 / 10^-3)) + etc*. PA was analyzed separately as a proxy for total phthalate exposure. For bisphenol and phthalate concentrations <LOD, we substituted LOD/√2.[19] Table 1 shows the concentrations and detection rates of all of the bisphenols and phthalate metabolites we analyzed, both individually and in groups.

Table 1. Urinary bisphenol and phthalate concentrations (n=877)

	Median (IQR) (nmol/L)[1]	Median (IQR) (ng/mL)	Percentage of values below the limit of detection (LOD)	Limit of detection (LOD) (ng/mL)
Total bisphenols	8.97 (3.49, 19.39)			
Bisphenol A (BPA)		1.65 (0.69, 3.42)	22.9	0.15
Bisphenol S (BPS)		0.35 (0.17, 1.03)	29.5	0.05
Bisphenol F (BPF)		0.58 (0.29, 1.30)	58.8	0.18
Bisphenol Z (BPZ)		0.17 (0.14, 0.27)	86.9*	0.12
Bisphenol B (BPB)		0.17 (0.08, 0.29)	90.8*	0.03
Bisphenol AP (BPAP)		0.25 (0.14, 0.45)	92.0*	0.07
Bisphenol P (BPP)		0.16 (0.13, 0.28)	98.3*	0.11
Bisphenol AF (BPAF)		-	100.0*	0.79
Phthalic acid (PA)		55.59 (29.71, 118.08)	0.5	1.11
Low molecular weight (LMW) phthalate metabolites	1018.81 (386.01, 2740.43)			
Monomethylphthalate (mMP)		5.07 (2.60, 9.31)	0.1	0.06
Monoethylphthalate (mEP)		130.99 (39.68, 438.52)	0.1	0.06
Mono-isobutylphthalate (mIBP)		20.28 (9.14, 42.51)	0.2	0.09
Mono-n-butylphthalate (mBP)		15.31 (6.73, 30.26)	1.0	0.14
High molecular weight (HMW) phthalate metabolites	208.11 (106.93, 397.30)			
Di-2-ethylhexylphthalate (DEHP) metabolites	168.53 (86.49, 322.20)			
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)		15.86 (8.00, 31.88)	0.1	0.29
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)		11.40 (5.67, 23.22)	0.2	0.08
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)		7.63 (3.43, 15.54)	0.0	0.04
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)		13.65 (7.23, 25.78)	0.1	0.04

	Median (IQR) (nmol/L)[1]	Median (IQR) (ng/mL)	Percentage of values below the limit of detection (LOD)	Limit of detection (LOD) (ng/mL)
Di-isononylphthalate (DINP)				
Monoisononylphthalate (mINP)		0.78 (0.33, 2.13)	86.1*	0.18
Di-isodecylphthalate (DIDP)				
Mono-(8-methyl-1-nonyl)phthalate (MIDP)		1.78 (1.27, 2.70)	92.4*	0.89
Di-n-octylphthalate (DNOP)	5.61 (3.00, 10.74)			
Mono(3-carboxypropyl)phthalate (mCPP)		1.41 (0.75, 2.70)	0.0	0.008
Monooctylphthalate (mOP)		0.47 (0.33, 0.78)	90.1*	0.25
Mono-(7-carboxy-n-heptyl)phthalate (mCHpP)		0.09 (0.08, 0.14)	99.3*	0.06
Other high molecular weight phthalate metabolites				
Monobenzylphthalate (mBzP)		6.19 (2.85, 11.92)	8.7	0.15
Mono-hexylphthalate (mHxP)		0.33 (0.16, 0.63)	23.6	0.06
Mono-2-heptylphthalate (mHpP)		1.04 (0.56, 2.10)	37.7	0.30
Monocyclohexylphthalate (mCHP)		0.16 (0.08, 0.41)	81.8*	0.04

[1]Individual metabolites have been included in the molar sums if the metabolite was detected in >20% of the samples. For the calculation of the molar sums, non-detectable levels of individual compounds with a detection rate of >20% were imputed as LOD/sqr(2) .

*Compound detected in <20% of the samples. Compound is not included in the molar sums.

TTP and infertility treatment

Information on TTP (months) and whether pregnancy was the result of any kind of infertility treatment (ovulation induction, surgery, artificial insemination, or in vitro fertilization) was obtained from the first questionnaire at enrollment. When we modeled TTP as a continuous outcome, we assigned 12 months as the TTP for all participants who received infertility treatment, reflecting the clinical definition of infertility,[20] regardless of when they commenced intervention, to avoid bias.

Covariates

Potential covariates were identified via causal diagram and a review of the literature. Information on maternal and paternal age (years), parity (nulliparous/multiparous), maternal educational level (low/high), ethnicity (Dutch or European/Non-European), pre-pregnancy weight (kg), and preconception folic acid supplementation (Y/N) was obtained from the first questionnaire at enrollment. Maternal height (cm) was measured at enrollment without shoes or heavy clothing. Paternal weight and height were measured once during pregnancy. Maternal pre-pregnancy and paternal body mass index (BMI) were calculated according to the standard formula (kg/m^2). Information on maternal smoking and alcohol consumption (Y/N) was assessed by questionnaires in each trimester.

Statistical analysis

Descriptive statistics were performed to assess participant characteristics. Depending on the distribution of the variables, Pearson or Spearman correlations were used to test for collinearity among potential covariates. Missing data for covariates were imputed using multiple imputation. Five imputed datasets were created and pooled for analyses. The percentage of missing values for any given covariate within the analytic sample was ≤10% except for folic acid supplementation (13.3%). For the main regression analyses, all bisphenol and phthalate urinary metabolite concentrations were natural log-transformed to reduce variability and all models were adjusted for urinary creatinine concentration.

To examine associations of first trimester urinary bisphenol and phthalate concentrations with TTP modeled continuously, we used discrete Cox proportional hazard models. Participants who conceived by use of infertility treatment were censored at 12 months. Proportional hazards assumptions for covariates were checked using plots of Schoenfeld residuals for continuous variables and log-minus-log plots for categorical variables. The resulting fecundability ratios (FR) represent the probability of becoming pregnant in a single menstrual cycle per log unit increase in bisphenol or phthalate metabolite/group (note: FR>1 indicates shorter TTP, while FR<1 indicates longer TTP). To select potential covariates for inclusion in the regression models, Cox proportional hazards models were manually fitted using log-likelihood ratio test based backward selection. Non-linearity of exposure variables in the Cox proportional hazards model was tested using quintiles.

Sensitivity analysis was performed excluding participants who used infertility treatment. Given the biological plausibility that folic acid may influence the mechanism by which bisphenol and phthalate compounds affect female reproductive function, we stratified on preconception folic acid supplement use and performed a test for interaction among participants with non-missing values of folic acid supplementation. Interaction on the multiplicative scale was considered significant for p-value <0.1.

For all models with statistically significant results, subanalyses of individual bisphenols or phthalate metabolites were performed to determine which compounds were driving the association.

Cox proportional hazard models were performed using R statistical software version 3.3.2 for Windows (package *survival* and *msm*). All other analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Participant characteristics

Table 1 shows first trimester urinary bisphenol and phthalate concentrations of participating women. Due to low detection rates, not all bisphenols and phthalates were included in subsequent analyses.

Maternal participants were mean age 31.2 years (standard deviation (SD) 4.4) and had a median BMI of 22.7 kg/m^2 (inter-quartile range (IQR) 20.9, 25.2). The majority were Dutch or European (68.9%), nulliparous (62.7%), and non-smokers (73.9%). The median TTP was 3 months (range 1-85), and 52 pregnancies (5.9%) resulted from any form of infertility treatment (Table 2).

We noted strong correlations between maternal and paternal age (Pearson correlation coefficient 0.6) and weak correlations between maternal and paternal BMI (Spearman's rho 0.2) (*data not shown*). To avoid multicollinearity, only maternal covariates were used in regression analyses.

Generation R participants who met the eligibility criteria for our study (enrollment <18 weeks gestation, information on TTP and infertility treatment use, singleton live born children, 5-year postpartum visit, n=2835) had generally similar population characteristics to those who additionally had first trimester urine samples and were included in our analysis (n=877) (Supplementary Table S1).

Table 2. Participant characteristics[1]

	Total
	n=877
Maternal age (years)	31.2 (4.4)
Paternal age (years)	33.7 (5.2)
Maternal pre-pregnancy BMI (kg/m²)*	22.7 (20.9, 25.2)
Paternal BMI (kg/m²)	25.3 (3.3)
Educational level	
Low	376 (42.9)
High	482 (55.0)
Missing	219 (2.2)
Ethnicity	
Dutch/European	604 (68.9)
Non-European	271 (30.9)
Missing	2 (0.2)
Parity	
Nulliparous	550 (62.7)
Multiparous	327 (37.3)
Creatinine first trimester (µg/mL)*	969 (479, 1565)
Smoking during pregnancy	
No	648 (73.9)
Yes	172 (19.6)
Missing	657 (6.5)
Alcohol consumption during pregnancy	
No	345 (39.3)
Yes	474 (54.0)
Missing	58 (6.6)
Preconception folic acid supplementation	
No	302 (34.4)
Yes	458 (52.2)
Missing	117 (13.3)
Time to pregnancy (months) †	3 (1, 85)
Use of infertility treatment	
Conceived naturally	825 (94.1)
Conceived using infertility treatment	52 (5.9)

[1]Values for continuous variables reported as mean (standard deviation); values for categorical variables reported as number of participants (percent)
*Median (IQR range)
†Median (minimum, maximum)

Associations with time to pregnancy

Schoenfeld residuals of all continuous covariates were evenly distributed using the Kaplan Meier time scale, indicating that the proportional hazards assumption was met (Supplementary Table S2a). In log-minus-log plots, lines for parity crossed and had a p-value of 0.055 for non-proportionality (Supplementary Table S2b). Therefore, all Cox proportional hazards models were stratified for parity. Although the p-value for non-proportionality for folic acid supplement use was 0.058, proportionality was assumed because the lines did not cross.

None of the first trimester urinary bisphenol or phthalate groups or compounds was associated with fecundability (Table 3). Modeling quintiles of exposures did not reveal any non-linear associations (*data not shown*). Sensitivity analysis, using only participants who conceived naturally, yielded similar fecundability ratios (Supplementary Table S3).

Table 3. Covariate-adjusted fecundability ratios (FR) for maternal first trimester urinary bisphenol and phthalate concentrations (n=877)

	FR (95% CI)
Total bisphenols	0.98 (0.92, 1.04)
Bisphenol A	0.99 (0.95, 1.04)
Bisphenol S	0.98 (0.94, 1.02)
Phthalic acid	0.96 (0.90, 1.02)
LMW phthalate metabolites	0.96 (0.92, 1.02)
HMW phthalate metabolites	0.98 (0.91, 1.05)
DEHP metabolites	0.99 (0.92, 1.06)
DNOP metabolites	0.96 (0.90, 1.03)

Data analyzed using a Cox proportional hazards model (R v3.3.2).
Increases are per natural log increase in first trimester urinary total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations. Models are fitted. All models are adjusted for maternal age, education, parity, folic acid supplement use, and urinary creatinine concentration.
*p-value <0.05.

Effect measure modification by preconception folic acid supplement use

Among women who did not use folic acid supplements preconceptionally, each log unit increase in total bisphenols and PA decreased fecundability (FR=0.90 [95% Confidence Interval (CI) 0.81, 1.00] and 0.88 [95% CI 0.79, 0.99], respectively). Slightly weaker associations were detected for BPA and bisphenol S (BPS) (FR=0.93 [95% CI 0.86, 1.01] and 0.94 [95% CI 0.87, 1.01], respectively). Correlations of first trimester BPA and BPS are weak.[18] BPA and BPS were assessed in a simplified multipollutant model by adding both in the same model. This yielded exactly the same estimates. Among preconception folic acid supplement users, bisphenol and phthalate compounds were not associated with fecundability. Tests for interaction indicated effect measure modification by folic acid supplement use for associations of total bisphenols, BPA, BPS, PA, HMW phthalates, and DNOP metabolites with fecundability (*p-values* <0.1). In all cases, fecundability was lower in women without preconception folic acid supplement use, indicating longer TTP (Table 4).

Table 4. Covariate-adjusted fecundability ratios (FR) for maternal first trimester urinary bisphenol and phthalate concentrations, stratified by folic acid supplement use (n=760; none or postconception (inadequate) n=302, preconception (adequate) n=458)

	FR (95% CI)
Total bisphenols†	
Inadequate folic acid supplement use	0.90 (0.81, 1.00)*
Folic acid supplement use	1.00 (0.92, 1.09)
Bisphenol A†	
Inadequate folic acid supplement use	0.93 (0.86, 1.01)**
Folic acid supplement use	1.00 (0.94, 1.07)
Bisphenol S†	
Inadequate folic acid supplement use	0.94 (0.87, 1.01)**
Folic acid supplement use	1.02 (0.96, 1.09)
Phthalic acid†	
Inadequate folic acid supplement use	0.88 (0.79, 0.99)*
Folic acid supplement use	0.99 (0.91, 1.08)
LMW phthalate metabolites	
Inadequate folic acid supplement use	0.94 (0.85, 1.03)
Folic acid supplement use	0.98 (0.91, 1.05)
HMW phthalate metabolites†	
Inadequate folic acid supplement use	0.93 (0.82, 1.04)
Folic acid supplement use	1.03 (0.93, 1.14)
DEHP metabolites	
Inadequate folic acid supplement use	0.94 (0.83, 1.05)
Folic acid supplement use	1.03 (0.93, 1.13)
DNOP metabolites†	
Inadequate folic acid supplement use	0.90 (0.80, 1.02)
Folic acid supplement use	1.02 (0.93, 1.11)

Data analyzed using a Cox proportional hazards model (R v3.3.2).
Increases are per natural log increase in first trimester urinary total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations. All models are adjusted for maternal age, education, parity, and urinary creatinine concentration.
*p-value <0.05 **p-value 0.05-0.1 †interaction term p-value <0.1.

Subanalysis of bisphenol F (BPF) did not reveal further associations (Supplementary Table S4). Subanalysis of individual HMW phthalate metabolites showed effect measure modification by folic acid supplement use for mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP), mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP), mono(3-carboxypropyl)phthalate (mCPP) and monobenzylphthalate (mBzP) in models that included interaction terms (*p-values* <0.1). Modeling quintiles of bisphenol and phthalate compounds did not show any non-linear associations (*data not shown*).

Discussion

In this population-based birth cohort study with recalled information on TTP, we found total bisphenols and PA in the first trimester of pregnancy to be associated with decreased fecundability among women without preconception folic acid supplementation. We found no associations of first trimester urinary concentrations of bisphenols and phthalates with fecundability in unstratified models. The addition of an interaction term suggested effect measure modification by folic acid use of the association of total bisphenols, BPA, BPS, PA, HMW phthalate metabolites and DNOP metabolites with TTP. Among those with preconception folic acid supplementation, bisphenols and phthalates were not associated with TTP.

We did not find maternal BPA to be associated with TTP, in line with previous studies.[3-5] Both animal and human studies, however, have suggested a role for BPA in the pathogenesis of several known causes of female infecundability, including endometriosis and polycystic ovarian syndrome (reviewed in [21,22]).

Results from previous studies examining associations between phthalates and TTP have been inconsistent.[3-5,7,8] Among prospective cohorts of couples discontinuing contraception, the North Carolina Early Pregnancy Study reported no associations between female urinary phthalates and TTP,[4] the Danish First Pregnancy Planner Study reported female urinary monoethylphthalate (mEP) to be associated with a longer TTP,[7],while the Longitudinal Investigation of Fertility and the Environment study observed an association between female urinary mCPP and shorter TTP.[3] Among pregnancy studies that used recalled TTP, as we did, the Canadian Maternal-Infant Research on Environmental Chemicals study reported no associations between first trimester urinary phthalate concentrations and TTP,[5] while the multi-site European INUENDO study reported a shorter TTP per log unit increase in maternal serum DEHP concentrations during pregnancy.[8]

Our findings of effect measure modification by folic acid supplementation of the associations of total bisphenols, BPA, BPS, PA, HMW phthalates and DNOP metabolites with TTP, and of reduced FRs for those without preconception folic acid supplementation support our hypothesis that bisphenols and phthalates may influence fecundability by inducing changes in DNA methylation. This is the first study to report this for phthalates and is in line with a recent study among women undergoing infertility treatment that reported that high urinary BPA concentrations were associated with lower probabilities of implantation, clinical pregnancy, and live birth, among women who consumed <400 µg/day of dietary folate.[13] Diet interactions might explain the null findings reported in previous studies. DNA methylation has been identified as a potential mechanism in the association of persistent organic pollutants and reduced fecundability. A recent study in European adult males observed a weak

association between exposure to persistent organic pollutants and global DNA methylation in sperm.[23] In rodents, polychlorinated biphenyls have been identified to impair endometrial receptivity and cause failure of embryo implantation by disturbing the methylation level of the implantation-associated gene Homeobox A10 (HOXA10).[24] However, no studies have investigated the role of preconception folic acid supplementation in this association. Further studies, ideally with maternal preconception serum folate concentrations rather than retrospective self-reported supplement use, are needed to confirm these results.

Strengths and limitations

Strengths of this study include the relatively large sample size of 877 participants with information on the use of infertility treatment and TTP. Participants were recruited in early pregnancy and asked to recall TTP in the first questionnaire at enrollment. Although prospectively measured TTP would have been preferable, the only published validation study comparing prospectively measured TTP to TTP recalled during the first trimester of pregnancy reported perfect agreement between measures among 53% of their sample. Recall error was on average small (only 12% had a discrepancy of ≥2 months), with a median of 0 and a mean of-0.11 months, indicating reasonable validity.[25]

Detailed information on a large number of potential confounding factors was available, although we lacked information on several potential covariates predicting TTP such as timing/frequency of sexual intercourse and medical history, which may have introduced residual confounding. Most covariates used for this analysis were self-reported, which may have led to misclassification and consequently underestimation of effects. In the stratified and interaction models, we adjusted for maternal age, education, and parity, which are all associated with self-reported preconception folic acid supplementation in this cohort,[26] but the possibility of residual confounding by other sociodemographic factors remains.

This is the first study to assess associations of BPA analogues such as BPS, which recent studies have shown to have adverse effects on the reproductive neuroendocrine system,[27-30] and of PA, a proxy for total phthalate exposure, with TTP. Unfortunately, our exposure measures were based on a single spot urine in the first trimester of pregnancy and may not accurately reflect preconception levels. Both bisphenols and phthalates are reported to have short biological half-lives (<24 h),[31,32] although it has been suggested that a single urine sample for phthalate concentrations reasonably reflects exposure for up to three months.[33] Studies investigating variability of bisphenols and phthalates before and during pregnancy are scarce. Variability has been reported to be biomarker specific, with reasonable correlations for BPA and DEHP metabolites and stronger correlations for LWM phthalate metabolites and mBzP.[34,35] Cross-sectional studies are at risk for reversed causation. In the current study, the observed interaction with folic acid supplementation use negates potential reversed causation induced by preconception behavioral changes in women taking longer to conceive. The absence of paternal exposure measurements may have introduced residual confounding, as several recent epidemiologic studies suggest that BPA and phthalates may diminish semen quality.[36-40]

Our main analyses violated the assumption of non-informative censoring for Cox proportional hazards models, as those who were censored because they used infertility treatment likely had a lower probability

of natural conception.[41] To overcome this limitation, we performed a sensitivity analysis including only participants who conceived naturally and found comparable results (Supplementary Table S3) despite differences among those with TTP <12 months vs. TTP ≥12 months in maternal age, daily dietary caloric intake, ethnicity, education and folic acid supplementation (Supplementary Table S5).

Finally, while there was reasonable similarity between the subgroup of the Generation R cohort in which we performed these analyses and the entire sample (Supplementary Table S1), the response rate at baseline for the Generation R study was 61%.[16] We therefore cannot rule out selection toward a relatively healthy population that may limit the generalizability of our results. As a population-based pregnancy cohort, all women in this study successfully conceived, with or without infertility treatment. Therefore, this study is less generalizable for the general population of couples discontinuing contraception, were 15-30% of couples are estimated to suffer from unexplained subfertility.[42]

Conclusion

Although we found no associations of first trimester bisphenol and phthalate urinary concentrations with fecundability in full-sample analyses, we detected evidence of effect measure modification by folic acid supplement use. Among women without preconception folic acid supplementation, increased bisphenol and phthalate concentrations were associated with reduced fecundability, suggesting that the mechanism by which these chemicals impair reproductive potential may involve changes in DNA methylation. Our findings add to the already substantial evidence of the benefits of preconception folic acid supplementation to reproductive health. Further studies are needed to replicate these findings and investigate potential mechanisms.

References

1. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Human reproduction (Oxford, England). 2001;16(5):972-8.
2. Buck Louis G, Cooney M, Peterson C. The ovarian dysgenesis syndrome. J Dev Orig Health Dis. 2011;2(1):25-35.
3. Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. Fertility and sterility. 2014;101(5):1359-66.
4. Jukic AM, Calafat AM, McConnaughey DR, Longnecker MP, Hoppin JA, Weinberg CR, et al. Urinary Concentrations of Phthalate Metabolites and Bisphenol A and Associations with Follicular-Phase Length, Luteal-Phase Length, Fecundability, and Early Pregnancy Loss. Environ Health Perspect. 2016;124(3):321-8.
5. Velez M, Arbuckle T, Fraser W. Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. 2015;103(4):1011–20.e2.
6. La Rocca C, Tait S, Guerranti C, Busani L, Ciardo F, Bergamasco B, et al. Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas. International journal of environmental research and public health. 2014;11(10):10146-64.
7. Thomsen AM, Riis AH, Olsen J, Jonsson BA, Lindh CH, Hjollund NH, et al. Female exposure to phthalates and time to pregnancy: a first pregnancy planner study. Human reproduction (Oxford, England). 2017;32(1):232-8.
8. Specht IO, Bonde JP, Toft G, Lindh CH, Jonsson BA, Jorgensen KT. Serum phthalate levels and time to pregnancy in couples from Greenland, Poland and Ukraine. PloS one. 2015;10(3):e0120070.
9. Menezo YJ, Silvestris E, Dale B, Elder K. Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. Reproductive biomedicine online. 2016;33(6):668-83.
10. Shelby MD. NTP-CERHR monograph on the potential human reproductive and developmental effects of bisphenol A. NTP CERHR MON. 2008(22):v, vii-ix, 1-64 passim.
11. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104(32):13056-61.
12. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr. 2012;3(1):21-38.
13. Minguez-Alarcon L, Gaskins AJ, Chiu YH, Souter I, Williams PL, Calafat AM, et al. Dietary folate intake and modification of the association of urinary bisphenol A concentrations with in vitro fertilization outcomes among women from a fertility clinic. Reprod Toxicol. 2016;65:104-12.
14. Solomon O, Yousefi P, Huen K, Gunier RB, Escudero-Fung M, Barcellos LF, et al. Prenatal phthalate exposure and altered patterns of DNA methylation in cord blood. Environ Mol Mutagen. 2017;58(6):398-410.
15. Zhao Y, Chen J, Wang X, Song Q, Xu HH, Zhang YH. Third trimester phthalate exposure is associated with DNA methylation of growth-related genes in human placenta. Scientific reports. 2016;6:33449.
16. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
17. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
18. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environmental research. 2018;161:562-72.

19. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.
20. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Human reproduction (Oxford, England). 2009;24(11):2683-7.
21. Ziv-Gal A, Flaws JA. Evidence for bisphenol A-induced female infertility: a review (2007-2016). Fertility and sterility. 2016;106(4):827-56.
22. Huo X, Chen D, He Y, Zhu W, Zhou W, Zhang J. Bisphenol-A and Female Infertility: A Possible Role of Gene-Environment Interactions. International journal of environmental research and public health. 2015;12(9):11101-16.
23. Consales C, Toft G, Leter G, Bonde JP, Uccelli R, Pacchierotti F, et al. Exposure to persistent organic pollutants and sperm DNA methylation changes in Arctic and European populations. Environ Mol Mutagen. 2016;57(3):200-9.
24. Qu XL, Ming Z, Yuan F, Wang H, Zhang YZ. Effect of 2,3',4,4',5-Pentachlorobiphenyl Exposure on Endometrial Receptivity and the Methylation of HOXA10. Reprod Sci. 2018;25(2):256-68.
25. Radin RG, Rothman KJ, Hatch EE, Mikkelsen EM, Sorensen HT, Riis AH, et al. Maternal Recall Error in Retrospectively Reported Time-to-Pregnancy: an Assessment and Bias Analysis. Paediatric and perinatal epidemiology. 2015;29(6):576-88.
26. Timmermans S, Jaddoe VW, Mackenbach JP, Hofman A, Steegers-Theunissen RP, Steegers EA. Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in The Netherlands: the Generation R study. Preventive medicine. 2008;47(4):427-32.
27. Eladak S, Grisin T, Moison D, Guerquin MJ, N'Tumba-Byn T, Pozzi-Gaudin S, et al. A new chapter in the bisphenol A story: bisphenol S and bisphenol F are not safe alternatives to this compound. Fertility and sterility. 2015;103(1):11-21.
28. Mersha MD, Patel BM, Patel D, Richardson BN, Dhillon HS. Effects of BPA and BPS exposure limited to early embryogenesis persist to impair non-associative learning in adults. Behavioral and brain functions : BBF. 2015;11:27.
29. Qiu W, Zhao Y, Yang M, Farajzadeh M, Pan C, Wayne NL. Actions of Bisphenol A and Bisphenol S on the Reproductive Neuroendocrine System During Early Development in Zebrafish. Endocrinology. 2016;157(2):636-47.
30. Zalmanova T, Hoskova K, Nevoral J, Adamkova K, Kott T, Sulc M, et al. Bisphenol S negatively affects the meotic maturation of pig oocytes. Scientific reports. 2017;7(1):485.
31. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
32. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
33. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.
34. Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect. 2012;120(5):739-45.
35. Mahalingaiah S, Meeker JD, Pearson KR, Calafat AM, Ye X, Petrozza J, et al. Temporal variability and predictors of urinary bisphenol A concentrations in men and women. Environ Health Perspect. 2008;116(2):173-8.

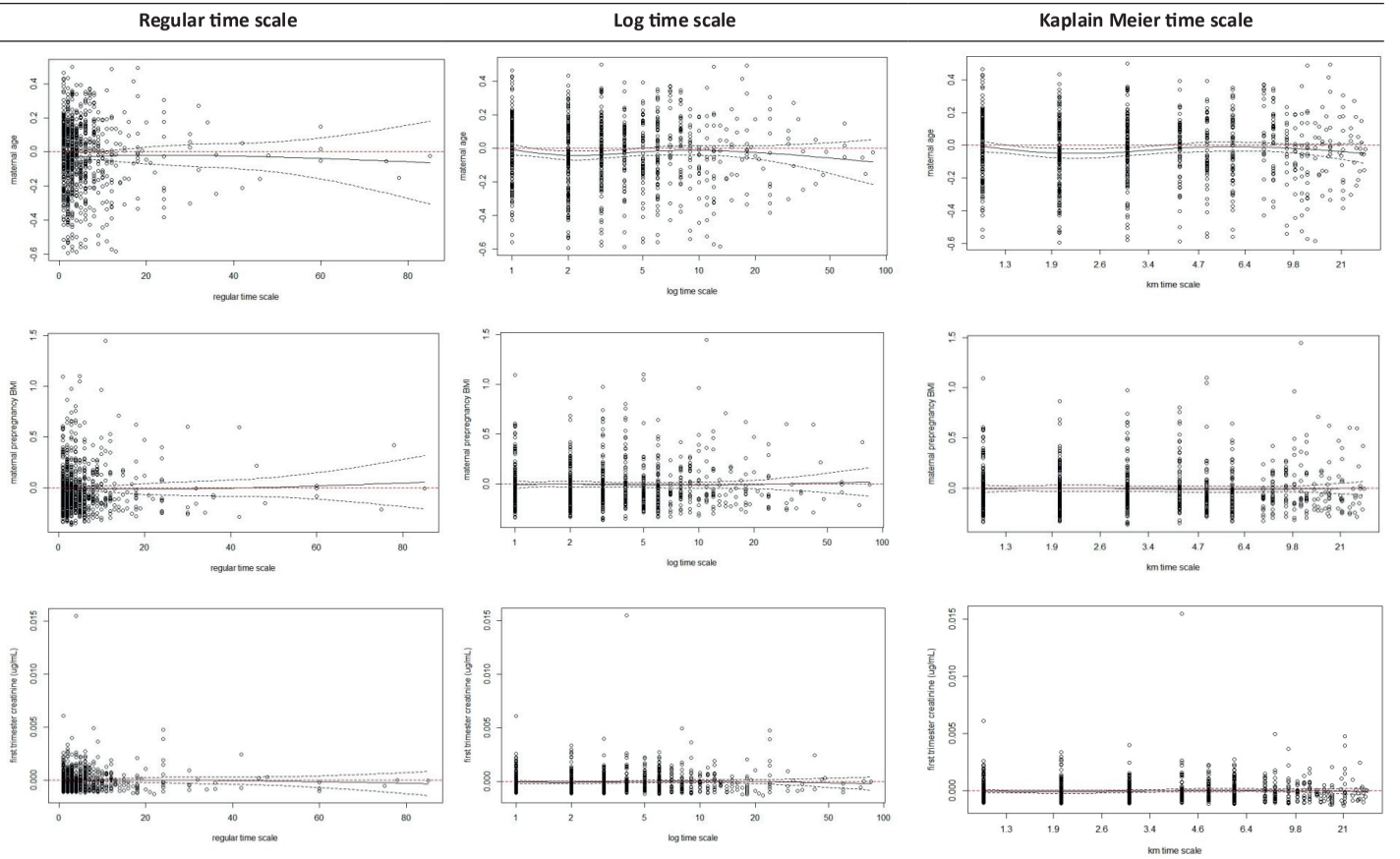
36. Knez J, Kranvogl R, Breznik BP, Voncina E, Vlaisavljevic V. Are urinary bisphenol A levels in men related to semen quality and embryo development after medically assisted reproduction? Fertility and sterility. 2014;101(1):215-21 e5.
37. Li DK, Zhou Z, Miao M, He Y, Wang J, Ferber J, et al. Urine bisphenol-A (BPA) level in relation to semen quality. Fertility and sterility. 2011;95(2):625-30 e1-4.
38. Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Joensen UN, Main KM, et al. Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. Environ Health Perspect. 2014;122(5):478-84.
39. Cai H, Zheng W, Zheng P, Wang S, Tan H, He G, et al. Human urinary/seminal phthalates or their metabolite levels and semen quality: A meta-analysis. Environmental research. 2015;142:486-94.
40. Wang C, Yang L, Wang S, Zhang Z, Yu Y, Wang M, et al. The classic EDCs, phthalate esters and organochlorines, in relation to abnormal sperm quality: a systematic review with meta-analysis. Scientific reports. 2016;6:19982.
41. Wiersma T. Landelijke Netwerkrichtlijn Subfertiliteit. 2010.
42. Gelbaya TA, Potdar N, Jeve YB, Nardo LG. Definition and epidemiology of unexplained infertility. Obstet Gynecol Surv. 2014;69(2):109-15.

Supplementary Table S1. Nonresponse analysis[a]

	Sample n = 877†	Eligible Generation R n = 2835††
Time to pregnancy (months)[b]	3.0 (1.0, 85.0)	3.0 (1.0, 120.0)
Maternal age (years)[c]	31.2 (4.4)	31.0 (4.5)
Infertility treatment use (yes)	52 (5.9)	148 (5.2)
Education (high education)	482 (56.2)	1482 (53.3)
Parity (nulliparous)	550 (62.7)	1746 (61.6)
Folic acid supplement use (yes, periconceptional)	458 (60.3)	1403 (57.0)

[a]Except where noted, values are reported as number of participants (valid percentages)
[b]Median (min, max)
[c]Mean (standard deviation)
† Singleton live born children + inclusion <18w + visit 5 years postpartum + information on time to pregnancy and the use of infertility treatment + first trimester urine sample
†† Singleton live born children + inclusion <18w + visit 5 years postpartum + information on time to pregnancy and the use of infertility treatment

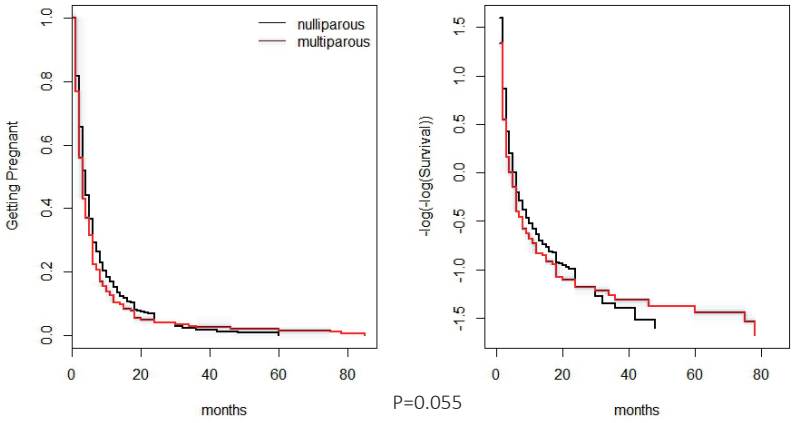
Supplementary Material S2a. Testing proportional hazards assumptions of continuous covariates



Plots of Schoenfeld residuals for all continuous variables (maternal age, maternal prepregnancy BMI, and first trimester urinary creatinine) are shown.For all continuous covariates, the spread of the residuals is most even in the Kaplan-Meier time scale. All p-values were nearly 1. Therefore, we assume the proportional hazards assumption not violated.

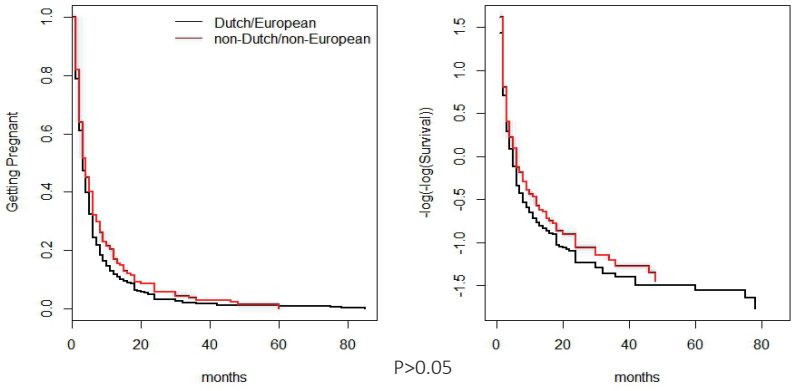
Supplementary Material S2b. Testing proportional hazards assumptions of categorical covariates

Parity



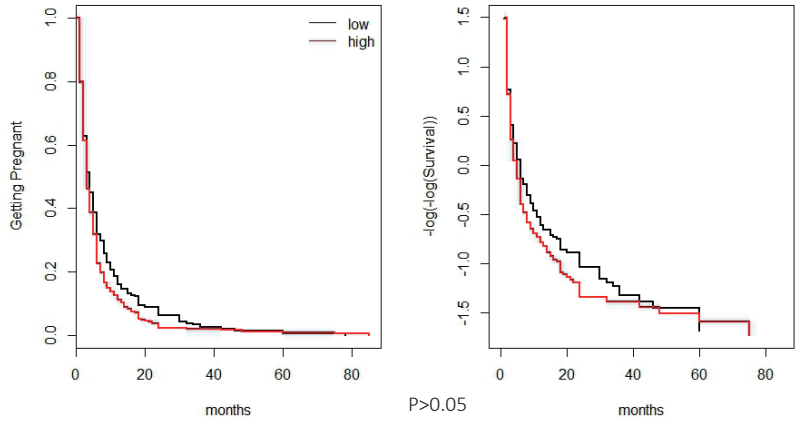
P=0.055

Ethnicity

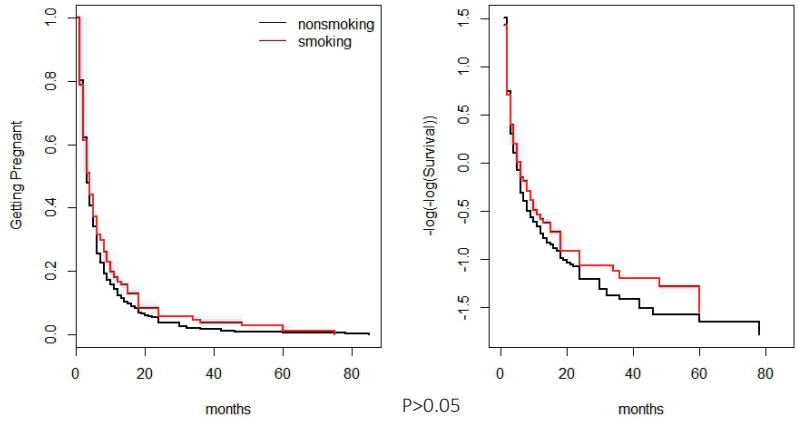


P>0.05

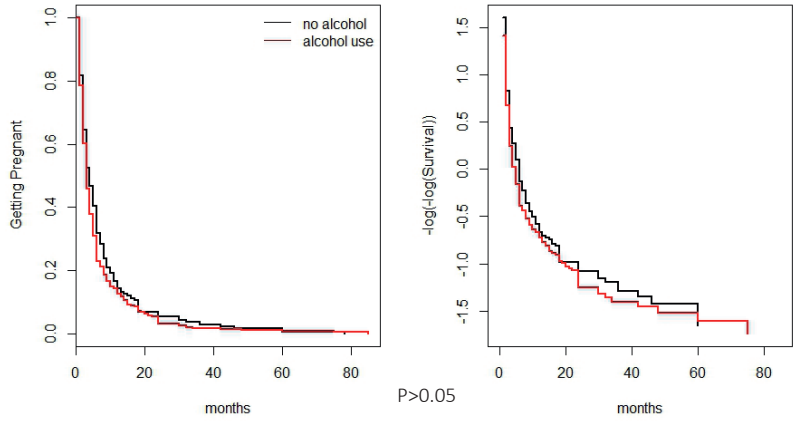
Education



Smoking

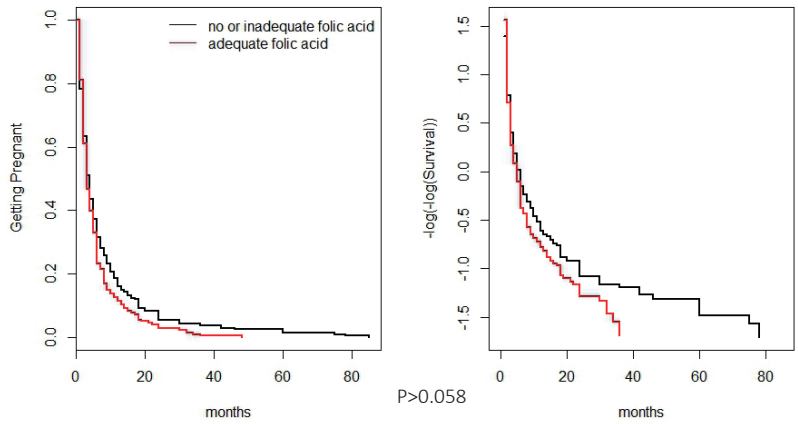


Alcohol use



P>0.05

Folic acid supplement use



P>0.058

Kaplan-Meier and –log(-log(Survival) plots are shown for all categorical covariates. If lines cross or the p-value <0.05, the proportional hazards assumption is not met. The proportional hazards assumption holds for all the above covariates except for parity. Therefore, stratification for parity was used to overcome this issue.

Supplementary Table S3. Sensitivity analysis; without participants who conceived through infertility treatment (n=825)

	FR (95% CI)[a]
Total bisphenols	1.00 (0.94, 1.06)
Bisphenol A	1.00 (0.95, 1.05)
Bisphenol S	0.99 (0.95, 1.04)
Phthalic acid	0.98 (0.92, 1.05)
LMW phthalate metabolites	0.97 (0.92, 1.03)
HMW phthalate metabolites	1.01 (0.94, 1.09)
DEHP metabolites	1.02 (0.96, 1.10)
DNOP metabolites	0.99 (0.93, 1.06)

Data analyzed using a Cox proportional hazards model (R v3.3.2).
Increases are per natural log increase in first trimester urinary total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations. Models are fitted.
[a]All models are adjusted for maternal age, education, parity, folic acid supplement use, and urinary creatinine concentration.
*p-value <0.05.

Supplementary Table S4. Covariate-adjusted[a] fecundability ratios (FR) for maternal first trimester urinary bisphenol and phthalate metabolite concentrations, stratified by folic acid supplement use (n=760; none or postconception (inadequate) n=302, preconception (adequate) n=458)

	FR (95% CI)
Total bisphenols†	
Inadequate folic acid supplement use	0.90 (0.81, 1.00)*
Folic acid supplement use	1.00 (0.92, 1.09)
Bisphenol A†	
Inadequate folic acid supplement use	0.93 (0.86, 1.01)**
Folic acid supplement use	1.00 (0.94, 1.07)
Bisphenol S†	
Inadequate folic acid supplement use	0.94 (0.87, 1.01)**
Folic acid supplement use	1.02 (0.96, 1.09)
Bisphenol F	
Inadequate folic acid supplement use	1.02 (0.91, 1.15)
Folic acid supplement use	1.00 (0.91, 1.10)
HMW phthalate metabolites†	
Inadequate folic acid supplement use	0.93 (0.82, 1.04)
Folic acid supplement use	1.02 (0.93, 1.14)
mECPP metabolites†	
Inadequate folic acid supplement use	0.94 (0.84, 1.04)
Folic acid supplement use	1.04 (0.95, 1.14)
mEHHP metabolites	
Inadequate folic acid supplement use	0.98 (0.89, 1.09)
Folic acid supplement use	1.01 (0.93, 1.10)
mEOHP metabolites	
Inadequate folic acid supplement use	0.98 (0.89, 1.09)
Folic acid supplement use	1.01 (0.93, 1.10)
mCMHP metabolites†	
Inadequate folic acid supplement use	0.91 (0.81, 1.03)
Folic acid supplement use	1.03 (0.93, 1.14)
mCPP metabolites†	
Inadequate folic acid supplement use	0.90 (0.80, 1.02)
Folic acid supplement use	1.02 (0.93, 1.11)
mBzP metabolites†	
Inadequate folic acid supplement use	0.95 (0.88, 1.03)
Folic acid supplement use	1.02 (0.96, 1.09)
mHxP metabolites	
Inadequate folic acid supplement use	0.99 (0.90, 1.08)
Folic acid supplement use	0.98 (0.90, 1.06)
mHpP metabolites	
Inadequate folic acid supplement use	1.01 (0.92, 1.11)
Folic acid supplement use	0.94 (0.85, 1.04)

Data analyzed using a Cox proportional hazards model (R v3.3.2).
Increases are per natural log increase in first trimester urinary bisphenol or phthalate metabolite concentrations.
[a]All models are adjusted for maternal age, education, parity, and urinary creatinine concentration.
*p-value <0.05 **p-value 0.05-0.1 †interaction term p-value <0.1.

Supplementary Table S5. Distributions of covariates in participants with TTP < 12 months and TTP ≥ 12 months (with cases of infertility treatment included as TTP=12)

	TTP <12m N=744	**TTP ≥12m N=133**	**P-value[a]**
Maternal age (y)	31.0 (4.2)	32.1 (5.0)	0.017*
Maternal BMI (kg/m2)*	22.7 (21.0, 25.1)	23.0 (20.8, 25.5)	0.418
Daily dietary caloric intake (kcal)	2100 (492)	1985 (511)	0.030*
Creatinine (ug/ml)*	976 (479, 1565)	912 (476, 1573)	0.908
Nulliparous (%)	61.6	69.2	0.094
Dutch/European (%)	70.9	58.3	0.004*
Low educated (%)	42.3	52.8	0.028*
Smokers (%)	20.1	26.0	0.136
Alcohol users (%)	58.6	53.7	0.316
Adequate folic acid supplement use (%)	62.0	50.9	0.025*

Values are presented as means (SD) or valid percentage.
*median (IQR)
[a]Differences between groups of TTP < 12 months and TTP ≥ 12 months were assessed using independent t-tests for continuous variables, Mann-Whitney tests for non-normally distributed continuous variables and chi-square tests for proportions.

2.3

CHAPTER

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

Hum Reprod. 2019 Feb 1;34(2):365-373.

Abstract

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

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

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

STUDY DESIGN, SIZE, DURATION: In a population-based prospective cohort study, bisphenol and phthalate metabolite concentrations were measured in a spot urine sample in early pregnancy among 1,396 women whose children participated in postnatal follow-up measurements.

PARTICIPANTS/MATERIALS, SETTING, METHODS: After exclusion of women without any BP measurement or with pre-existing hypertension, 1,233 women were included in the analysis. Urinary bisphenol and phthalate metabolite concentrations were measured in early-pregnancy [median gestational age 13.1 weeks, inter-quartile range 12.1–14.5]. Molar sums of total bisphenols and of low molecular weight phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate, and di-n-octylphthalate metabolites were calculated. Placental angiogenic markers (placental growth factor (PlGF), soluble fms-like tyrosine kinase (sFlt)-1), placental hemodynamic function measures (umbilical artery pulsatility index (PI), uterine artery resistance index (RI), notching, and placental weight), and maternal BP were measured in different trimesters. Information on gestational hypertensive disorders was obtained from medical records.

MAIN RESULTS AND THE ROLE OF CHANCE: Each log unit increase in HMW phthalate metabolites was associated with a 141.72 (95% CI 29.13, 373.21) higher early pregnancy sFlt-1/PlGF ratio (range in total sample 9-900). This association was driven by mono-[(2-carboxymethyl)hexyl]phthalate. In the repeated measurements regression models, each log unit increase in bisphenol A was associated with a 0.15 SD (95% CI 0.03, 0.26) higher intercept and -0.01 SD (95% CI -0.01, -0.00) decreasing slope of the umbilical artery PI Z-score and a -1.28 SD (95% CI -2.24, -0.33) lower intercept and 0.06 SD (95% CI 0.02, 0.11) increasing slope of the uterine artery RI Z-score. These associations remained significant after Bonferroni correction. Early-pregnancy bisphenols or phthalate metabolites showed no consistent associations with any other outcome.

LIMITATIONS, REASONS FOR CAUTION: Information on a large number of potential confounders was available but was partly self-reported. Bisphenols and phthalate metabolites, which typically have a half-life of 24-48 hours, were measured via single spot urine samples in early-pregnancy. In addition, at the current sample size, the study was powered to detect an odds ratio of 1.57 for gestational

hypertension and 1.78 for pre-eclampsia, but was underpowered to perform multivariable analyses for these outcomes. Further studies combining data from different cohorts may be necessary to increase power. These limitations are possible sources of non-differential misclassification leading to bias toward the null.

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

Introduction

Gestational hypertension and pre-eclampsia complicate 4-10% of all pregnancies and are major causes of morbidity and mortality.[1] They appear to originate in early-pregnancy.[2] In normal pregnancy, a balance of pro-angiogenic and anti-angiogenic factors, such as placental growth factor (PlGF) and soluble fms-tyrosine kinase (sFlt)-1, respectively, is established in the developing placenta.[3] An imbalance in these factors is associated with impaired vascular proliferation, which may result in placental dysfunction and increased risk of gestational hypertensive disorders.[4] Pregnant women are exposed to numerous chemicals, including bisphenols and phthalates.[5-7] Phthalates can be classified as low molecular weight (LMW) or high molecular weight (HMW). LMW phthalates are frequently added to personal care products, while HMW phthalates are used to impart flexibility to vinyl and plastic products.[8] Among HMW phthalates, di-2-ethylhexylphthalate (DEHP) is of particular interest because of its widespread use in food packaging.[9] Di-n-octylphthalate (DNOP) is also of concern because, although banned from use in the European Union since 2005, its primary metabolite, mono(3-carboxypropyl)phthalate (mCPP), is still detectable in biosamples.[10,11] Bisphenols and phthalate metabolites may disrupt early placental development.[12-15] Urinary bisphenol A (BPA) and DEHP concentrations have been associated with altered placental angiogenic markers.[14] Three previous studies on the associations of bisphenols and phthalate metabolites with gestational hypertensive disorders [16-18] showed inconsistent results.

Among 1,233 women participating in a population-based prospective cohort study, we examined associations of early-pregnancy urinary bisphenol and phthalate metabolite concentrations with placental angiogenic and hemodynamic function measures, blood pressure (BP) trajectories, and risk of gestational hypertensive disorders.

Materials and Methods

Study design and population for analysis

This study was embedded in a population-based prospective cohort study that enrolled 8,879 women from early-pregnancy onwards [19] and was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants. Bisphenol and phthalate metabolite concentrations were measured in 1,396 participants with singleton pregnancies, an available early-pregnancy urine sample, and whose children participated in postnatal studies. After excluding women without any BP measurement or with pre-existing hypertension, 1,233 women remained in the analysis (Supplementary Fig. S1).

Early-pregnancy urinary bisphenol and phthalate metabolite concentrations

Bisphenol, phthalate metabolite, and creatinine concentrations were measured in a spot urine sample obtained from participants in early-pregnancy (median gestational age 13.1 weeks, inter-quartile range (IQR) 12.1-14.5) between February 2004 and July 2005. Samples were collected between 8am and 8pm in 100-mL polypropylene urine collection containers, refrigerated, aliquoted, and frozen at -20

°C within 24 hr. Frozen samples were shipped to the Wadsworth Center, New York State Department of Health (Albany, NY, USA) for high-performance liquid chromatography-tandem mass spectroscopy analysis. Eight bisphenols and 18 phthalate metabolites were measured, including phthalic acid (PA), a common endpoint of phthalate metabolism, which was used as a proxy of total phthalate exposure [20]. Details have been described previously.[10,21]

We grouped phthalate metabolites according to molecular weight, reflecting their use in product categories. The inclusion criteria for chemicals and the formulae by which we calculated the weighted molar sums for total bisphenols and phthalate metabolite groupings are shown in Supplementary Data.[22] Supplementary Table S1 shows the bisphenols and phthalate metabolites in each group, with their concentrations and detection rates.

Placental angiogenic markers, hemodynamic function, and weight

sFlt-1 and PlGF concentrations were measured in early and mid-pregnancy blood samples using an immune-electrochemoluminence assay.[23] The sFlt1/PlGF ratio was calculated. Mid- and late-pregnancy placental vascular resistance was evaluated with flow velocity waveforms from the uterine and umbilical arteries.[24] Umbilical artery pulsatility index (PI) was measured in a free-floating loop of the umbilical cord. Uterine artery resistance index (RI) was measured in the uterine arteries near the crossover with the external iliac artery. Increased uterine artery RI and umbilical artery PI indicate elevated placental vascular resistance and are linked with gestational hypertensive disorders. We assessed the presence of uterine artery notching, which reflects increased resistance to blood flowing into the placenta and is used to identify high-risk pregnancies.[25] Placental weight was obtained from medical records and measured according to standard protocols.

Blood pressure and gestational hypertensive disorders

BP was measured at each visit (median gestational age 13.1 weeks, IQR 12.1-14.5; 20.4 weeks, IQR 19.9-20.9; and 30.2 weeks, IQR 29.9-30.6) using an Omron 907 automated digital oscillometer sphyghmanometer (OMRON Healthcare Europe, Hoofddorp, the Netherlands).[26] The mean value of two BP readings over a 60-s interval was documented for each participant.[25] Information on gestational hypertensive disorders was obtained from medical records.[27,28]

Covariates

Potential covariates were selected via causal diagram, literature review, and results from our previous study.[10] Maternal age at enrollment, education level, ethnicity, parity, pre-pregnancy weight, and folic acid supplementation were obtained from the enrollment questionnaire. Gestational age was established during the first ultrasound visit. Height (cm) was measured at enrollment without shoes and pre-pregnancy BMI (kg/m²) was calculated. Information on smoking and alcohol consumption was assessed by questionnaires in each trimester.

Statistical analysis

Descriptive statistics were performed to assess participant characteristics. Missing covariate data were imputed using multiple imputation and p-values were adjusted for multiple testing using the Bonferroni correction. Bisphenol and phthalate metabolite concentrations were natural log-transformed to reduce variability and account for right skewness in the distribution. To adjust for dilution, urinary creatinine was included as a covariate.[29]

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

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

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

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

Results

Subject characteristics

Maternal characteristics and investigated outcomes were similarly distributed between participants and non-participants (Supplementary Table S2). Women who developed gestational hypertension had higher pre-pregnancy BMI, were more often nulliparous, and were more likely to take folic acid supplements (Table 1). Compared to women who developed gestational hypertension, women with pre-eclampsia more often had a low education level and were less likely to take folic acid supplements.

Table 1. Subject characteristics according to gestational hypertensive disorders

	Total	**Non-hypertensive complicated pregnancy**	**Gestational hypertension**	**Pre-eclampsia**
	n = 1233	**n = 1155**	**n = 40**	**n = 24**
Maternal age (years)	30.5 (4.8)	30.5 (4.8)	30.8 (5.0)	31.4 (4.7)
Pre-pregnancy BMI (kg/m²)*	22.6 (20.8, 25.1)	22.6 (20.8, 25.0)	24.1 (23.0, 29.0)	22.5 (21.6, 26.7)
Educational level				
Low	595 (48.3)	560 (48.5)	17 (42.5)	15 (62.5)
High	618 (50.1)	577 (50.0)	21 (52.5)	9 (37.5)
Missing	20 (1.6)	18 (1.6)	2 (5.0)	-
Ethnicity				
Dutch/European	766 (62.1)	709 (61.4)	30 (75.0)	17 (70.8)
Non-European	465 (37.7)	444 (38.4)	10 (25.0)	7 (29.2)
Missing	2 (0.2)	2 (0.2)	-	-
Parity				
Nulliparous	767 (62.2)	712 (61.6)	34 (85.0)	17 (70.8)
Multiparous	466 (37.8)	443 (38.4)	6 (15.0)	7 (29.2)
Missing	-	-	-	-
Creatinine (µg/mL)*	1013 (491, 1655)	1032 (503, 1653)	945 (426, 1660)	1003 (462, 2018)
Smoking				
No	920 (74.6)	864 (74.8)	28 (70.0)	17 (70.8)
Yes	288 (23.4)	267 (23.1)	11 (27.5)	7 (29.2)
Missing	25 (2.0)	24 (2.1)	1 (2.5)	-
Alcohol consumption				
No	515 (41.8)	486 (42.1)	16 (40.0)	9 (37.5)
Yes	703 (57.0)	654 (56.6)	24 (60)	15 (62.5)
Missing	15 (1.2)	15 (1.3)	-	-
Folic acid supplementation				
No	211 (17.1)	200 (17.3)	2 (5.0)	7 (29.2)
Yes	873 (70.8)	815 (70.6)	33 (82.5)	15 (62.5)
Missing	149 (12.1)	140 (12.1)	5 (12.5)	2 (8.3)

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

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

Bisphenol concentrations were not associated with placental angiogenic markers (Table 2). Each log unit increase in HMW phthalate metabolites was associated with 0.20 ng/ml (95% CI 0.02, 0.56) higher sFlt-1 concentration and 141.72 (95% CI 29.13, 373.21) higher sFlt-1/PlGF ratio in early pregnancy (range sFlt-1/PlGF ratio in total sample 9-900): after Bonferroni correction, only the latter remained significant. Among individual HMW phthalate metabolites, each log unit increase in the four DEHP metabolites, especially mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP), was associated with higher sFlt-1/PlGF ratio in early pregnancy (Supplementary Table S3). In addition, each log unit increase in mCMHP concentration was associated with lower PlGF and higher sFlt-1 concentrations in early pregnancy. Each log unit increase in LMW phthalate metabolites was associated with 0.18 pg/ml (95% CI 0.02, 0.70) higher PlGF concentration in mid-pregnancy, which remained significant after Bonferroni correction (Table 2). In subanalyses, only monoethylphthalate (mEP) was associated with PlGF in mid-pregnancy, but the results were not significant after Bonferroni correction. Additional analyses also showed an association of higher mono-benzylphthalate (mBzP) concentration with higher sFlt-1 in early and mid-pregnancy. Early pregnancy bisphenols and phthalate metabolites were not associated with longitudinally modeled placental angiogenic markers (Supplementary Table S4).

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

Each log unit increase in total bisphenols was associated with 0.05 SD (95% CI 0.001, 0.10) higher umbilical artery PI in mid-pregnancy, while each log unit increase in DEHP metabolites was associated with -0.06 SD (95% CI -0.12, -0.001) lower umbilical artery PI and 0.08 SD (95% CI 0.00, 0.15) higher uterine artery RI in late pregnancy. Each log unit increase in DNOP metabolites was associated with 24% decreased odds of notching (Odds Ratio (OR) 0.76 [95% CI 0.60, 0.97]), but did not remain significant after Bonferroni correction (Table 3). Additional analysis of individual phthalate metabolites showed that each log unit increase in mono-isobutylphthalate (mIBP) concentration was associated with 24% lower odds of notching (OR 0.76 [95% CI 0.62, 0.93]), remaining significant after Bonferroni correction (Supplementary Table S5). PA was borderline associated with lower placental weight (per log unit increase:-8 grams [95% CI-17, 0]).

In the repeated measures models, each log unit increase in BPA was associated with 0.15 SD (95% CI 0.03, 0.26) higher intercept and -0.01 SD (95% CI -0.01, -0.00) lower slope of the umbilical artery PI Z-score over time and -1.28 SD (95% CI -2.24, -0.33) lower intercept and 0.06 SD (95% CI 0.02, 0.11) higher slope of the uterine artery RI Z-score over time (Supplementary Table S6 and Supplementary Fig. S2). These associations remained significant after Bonferroni correction. Higher concentrations of HMW phthalate metabolites were associated with a lower slope of the umbilical artery PI Z-score over time, but did not remain significant after Bonferroni correction.

Table 2. Associations of early pregnancy bisphenol and phthalate urine concentrations with placental angiogenic markers

	Placental Growth Factor (PlGF) <18 weeks (pg/ml), β (95% CI) (n=1,143)	Soluble fms-like tyrosine kinase (sFlt)-1 <18 weeks (ng/ml), β (95% CI) (n=1,143)	sFlt-1 : PlGF ratio <18 weeks, β (95% CI) (n=1,143)	Placental Growth Factor (PlGF) 18-25 weeks (pg/ml), β (95% CI) (n=1,173)	Soluble fms-like tyrosine kinase (sFlt)-1 18-25 weeks (ng/ml), β (95% CI) (n=1,173)	sFlt-1 : PlGF ratio 18-25 weeks, β (95% CI) (n=1,173)
Total bisphenols	0.02	-0.02	-51.83	0.04	-0.04	-11.14
	(-0.01, 0.08)	(-0.12, 0.18)	(-93.64, 60.01)	(-0.07, 0.42)	(-0.12, 0.45)	(-17.64, 69.12)
Bisphenol A	0.02	-0.01	-34.26	0.07	-0.06	-17.02
	(-0.01, 0.06)	(-0.09, 0.15)	(-69.14, 56.16)	(-0.03, 0.42)	(-0.10, 0.29)	(-16.66, 29.94)
Bisphenol S	0.00	-0.05	-27.77	-0.06	-0.09	-2.75
	(-0.02, 0.04)	(-0.11, 0.08)	(-60.49, 55.90)	(-0.09, 0.11)	(-0.11, 0.16)	(10.76, 64.30)
Phthalic acid	-0.03	0.05	74.40	0.02	0.13	14.71
	(-0.06, 0.02)	(-0.07, 0.32)	(-14.24, 263.58)	(-0.09, 0.40)	(-0.06, 0.88)	(-9.56, 150.84)
LMW phthalate metabolites	0.01	0.09	32.51	0.18	0.16	-7.14
	(-0.02, 0.06)	(-0.03, 0.31)	(-28.47, 171.90)	(0.02, 0.63)*†	(-0.01, 0.82)	(-15.29, 75.40)
HMW phthalate metabolites	-0.04	0.19	141.72	0.07	0.17	13.37
	(-0.06, 0.02)	(0.02, 0.54)*	(29.13, 373.21)*†	(-0.07, 0.56)	(-0.04, 1.05)	(-11.62, 162.29)
DEHP metabolites	-0.04	0.15	131.87	0.04	0.10	8.45
	(-0.07, 0.01)	(-0.01, 0.47)	(23.82, 354.10)*†	(-0.09, 0.48)	(-0.07, 0.88)	(-13.15, 145.77)
DNOP metabolites	-0.01	0.13	79.94	0.04	0.09	6.43
	(-0.04, 0.05)	(-0.03, 0.45)	(-19.05, 289.65)	(-0.08, 0.49)	(-0.08, 0.87)	(-14.16, 139.72)

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

Table 3. Associations of early pregnancy bisphenol and phthalate urine concentrations with placental hemodynamic function and weight

	Umbilical artery pulsatility index 18-25 weeks, SD (95% CI) (n=1,184)	**Uterine artery resistance index 18-25 weeks, SD (95% CI) (n=1,019)**	**Umbilical artery pulsatility index >25 weeks, SD (95% CI) (n=1,186)**	**Uterine artery resistance index >25 weeks, SD (95% CI) (n=755)**	**Notching, OR (95% CI) (n=83/793)**	**Placental weight, g (95% CI) (n=930)**
Total Bisphenols	0.05	-0.02	-0.03	-0.00	0.97	1
	(0.00, 0.10)*	(-0.07, 0.03)	(-0.08, 0.02)	(-0.06, 0.06)	(0.80, 1.18)	(-7, 8)
Bisphenol A	0.04	0.01	-0.03	0.02	0.95	0
	(-0.00, 0.08)	(-0.04, 0.05)	(-0.07, 0.01)	(-0.03, 0.07)	(0.81, 1.10)	(-6, 6)
Bisphenol S	-0.00	-0.01	-0.03	-0.00	0.99	2
	(-0.03, 0.03)	(-0.04, 0.03)	(-0.06, 0.01)	(-0.05, 0.04)	(0.86, 1.14)	(-4, 7)
Phthalic acid	0.02	0.03	-0.01	0.04	1.07	-8
	(-0.03, 0.07)	(-0.03, 0.09)	(-0.06, 0.04)	(-0.03, 0.11)	(0.86, 1.33)	(-17, 0)
LMW phthalate meta-	0.02	0.02	0.01	0.03	0.86	-4
bolites	(-0.03, 0.06)	(-0.02, 0.07)	(-0.03, 0.06)	(-0.03, 0.08)	(0.72, 1.03)	(-11, 3)
HMW phthalate meta-	0.01	0.05	-0.06	0.07	0.80	-3
bolites	(-0.05, 0.07)	(-0.02, 0.11)	(-0.12,-0.00)*	(-0.01, 0.15)	(0.61, 1.04)	(-13, 7)
DEHP metabolites	0.01	0.04	-0.06	0.08	0.85	-2
	(-0.05, 0.07)	(-0.02, 0.11)	(-0.12,-0.00)*	(0.00, 0.15)*	(0.67, 1.09)	(-12, 7)
DNOP metabolites	0.03	0.03	-0.02	0.05	0.76	-3
	(-0.03, 0.09)	(-0.03, 0.09)	(-0.08, 0.04)	(-0.02, 0.13)	(0.60, 0.97)*	(-12, 7)

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

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

Modeled continuously, bisphenol and phthalate metabolite concentrations showed no associations for the intercept or slope of systolic BP during pregnancy (Supplementary Table S7). In adjusted analysis, each log unit increase in DNOP metabolites was associated with lower diastolic BP from early pregnancy onward (-0.80 mmHg (95% CI-1.52,-0.07)) and a borderline significant increase of 0.02 mmHg (95% CI -0.00, 0.05) per week gestational age. Models gave no indication of a non-linear relationship between chemicals and BP change across pregnancy (Supplementary Table S8). Supplementary Fig. S3 shows a nonsignificant trend toward higher systolic and diastolic BP from early pregnancy onward among women in the highest tertile of PA exposure. Covariate adjustment did not change our conclusions. No interaction was observed between chemical concentrations and maternal pre-pregnancy BMI.

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

Bisphenol and phthalate metabolite concentrations were not associated with gestational hypertensive disorders (Table 4). Also, sub-analysis of individual phthalate metabolite concentrations did not show any associations with gestational hypertensive disorders (Supplementary Table S9).

Table 4. Associations of early pregnancy bisphenol and phthalate urine concentrations with gestational hypertensive disorders (N = 1,219)

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

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

Discussion

Main findings

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

Strengths and limitations

This analysis benefited from the size, prospective data collection, and availability of a wide range of covariates. BP, placental angiogenic markers, and hemodynamic function measures were assessed at multiple time points during pregnancy. Participants in our analysis were similar to non-participants, enhancing the generalizability of our results to the underlying Generation R cohort. However, compared to other cohorts, ours was a low-risk population with relatively few cases of gestational hypertension and pre-eclampsia, potentially limiting the generalizability of our results to other populations. At our current sample size, we were powered to detect an OR of 1.57 for gestational hypertension and 1.78 for pre-eclampsia, but we were underpowered to perform multivariable analyses for these outcomes. Further studies combining data from different cohorts may be necessary to increase power.

Information on many covariates in this study was self-reported. BP and uteroplacental vascular resistance measures are known to fluctuate diurnally and information on time of day when measurements were performed was not available. Bisphenols and phthalate metabolites, which typically have half-lives of 24-48 hours,[8,30] were measured via single spot urine samples in early pregnancy. It has been suggested that a single urine sample for phthalate metabolite concentrations reasonably reflects exposure for up to 3 months.[31] All of these limitations are possible sources of non-differential misclassification leading to bias toward the null.

A common method to account for dilution of urinary chemical concentrations is via creatinine adjustment.[32] Endogenous creatinine clearance, measured by 24-hr urine collection, remains the most precise estimation of the glomerular filtration rate in pregnant women.[33] It has been suggested that specific gravity adjustment is a better correction method in pregnant women.[34] Unfortunately, specific gravity measurements were not available. Additional analysis of models without creatinine adjustment yielded comparable results.

To adjust for multiple testing in this exploratory analysis, we have used an adjusted Bonferroni correction, correcting for the number of hypotheses tested per analysis rather than the number of models run. Bisphenols share some of their potential mechanisms of effect with phthalates.[35] In additional analyses we therefore tested for interaction between concentrations of total bisphenols and PA. Evident interaction at p-value <0.1 was observed for late pregnancy umbilical artery PI. A partial regression plot is given in Supplementary Fig. S4. This finding should be considered hypothesis generating and supports the use of mixture models in future studies.

Interpretation of main findings

Early-pregnancy exposure to bisphenols and phthalates may lead to early placental maladaptations and subsequent increased risks of higher BP in pregnancy and gestational hypertensive disorders. Several potential biological mechanisms have been proposed to support this hypothesis. Higher bisphenol and phthalate metabolite concentrations have been associated with increased oxidative stress,[36,37] which plays a role in the onset of pre-eclampsia, potentially through the release of anti-angiogenic factors.[2,38] Results have been inconsistent for associations between oxidative stress and placental angiogenic factors,[39,40] but one group observed a positive correlation between oxidative stress markers and BP during pregnancy.[41] In addition, BPA has been reported to have antiproliferative and pro-apoptotic effects on human trophoblastic cells, potentially through estrogen-related receptor γ and tumor necrosis factor α,[13,42] and phthalates have been shown to inhibit extravillous trophoblast invasion through the peroxisome proliferator-activated receptor γ.[43]

Our primary finding was a positive association between early-pregnancy HMW phthalate metabolite concentration and the sFlt-1/PlGF ratio in early pregnancy, driven by the DEHP metabolite mCMHP. In line with our results, a nested case-control study among 130 mothers who delivered preterm and 352 who delivered at term with four measurements of prentatal BPA, phthalate metabolites, and placental markers, also found a positive association between DEHP metabolites and sFlt-1/PlGF ratio.[14] In the Ferguson *et al.* (2015) study, mCMHP was not measured and the association seemed to be dependent on a decrease in PlGF rather than an increase in sFlt-1, as we observed. The Ferguson *et al.* (2015) study also observed an association of BPA with a higher sFlt-1and sFlt-1/PlGF ratio. For comparison, we performed additional analyses focused on individual phthalate metabolites with a detection level of >50%. We observed an association of mBzP with higher sFlt-1 concentrations in early-pregnancy. The relatively large proportion of preterm deliveries in the Ferguson *et al.* (2015) study and the repeated prenatal measurements of bisphenols and phthalate metabolites may have given rise to differences between our results. In our study, only early-pregnancy bisphenol and phthalate metabolite concentrations were included. Further studies are needed to explore associations of bisphenol and phthalate metabolite concentrations in different periods of pregnancy with hemodynamic adaptations during pregnancy.

To our knowledge, this is the first study to assess effects of prenatal bisphenol and phthalate metabolite concentrations on placental hemodynamic function measures and placental weight. In repeated measurements regression models we observed contradictory associations of BPA with umbilical artery PI Z-score and uterine artery RI Z-score even though both measurements represent placental resistance. In Generation R, both placental indices are associated with higher odds of pre-eclampsia, small size for gestational age at birth and preterm birth, and higher estimates were observed for uterine artery RI than for umbilical artery PI.[24] However, it has been suggested that the predictive value of Doppler indices in the low-risk population is low and should not be used in the clinical setting.[44] We cannot fully explain our findings and it is debatable whether this increase in uterine artery RI is clinically relevant.

Several studies have reported associations of BPA and phthalate metabolite concentrations with higher BP in both adults and children.[45-48] The only previous paper that focused on the associations of maternal phthalate concentrations with BP during pregnancy was a prospective cohort study of 369 women:[16]

this study reported that a higher urinary mBzP concentration at 16 weeks of gestation was associated with higher maternal diastolic BP before 20 weeks of gestation. No associations were found for BP values after 20 weeks of gestation. This previous study included women with higher BMI levels than in our cohort and women using medication for high BP. MBzP concentrations and the prevalence of gestational hypertensive disorders were higher than in our study population.

The associations of bisphenol and phthalate metabolite concentrations with gestational hypertensive disorders have been scarcely examined. Recently, a nested case-control study comprising 50 women with, and 432 women without, pre-eclampsia observed positive associations of BPA and mEP concentrations at 10 weeks of gestation with pre-eclampsia.[18] In a case-control study of 58 women, 23 with pre-eclampsia, higher concentrations of BPA were detected in placental tissue of pre-eclamptic women compared to normotensive pregnant women.[17]

The differences between our and other study populations may explain dissimilar results. In low-risk populations, bisphenol and phthalate metabolite concentrations might have limited effects on risks of hemodynamic adaptations. This might be different in high-risk populations. It has been debated whether gestational hypertension and pre-eclampsia are on the same spectrum of disease or whether they are two distinct entities.[49] An imbalance in pro- and anti-angiogenic markers has been attributed to pre-eclampsia but not to gestational hypertension.[50] Despite our low-risk population, we observed associations linking HMW phthalate metabolites to a higher sFlt-1/PlGF ratio in early pregnancy and BPA to an increasing slope in uterine artery RI Z-score. Our results are therefore more supportive of an association of early pregnancy bisphenols and phthalate metabolites with risk for pre-eclampsia than with gestational hypertension.

Conclusion

Bisphenols and phthalate metabolites were not associated with longitudinal changes in BP. Phthalate exposure may elevate subclinical associations with the sFlt-1/PlGF ratio while BPA was observed to increase the uterine artery RI Z-score. These effects may contribute to adverse pregnancy outcomes in the context of other environmental exposures.

References

1. Roberts CL, Ford JB, Algert CS, Antonsen S, Chalmers J, Cnattingius S, et al. Population-based trends in pregnancy hypertension and pre-eclampsia: an international comparative study. BMJ Open. 2011;1(1):e000101.
2. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631-44.
3. Llurba E, Crispi F, Verlohren S. Update on the pathophysiological implications and clinical role of angiogenic factors in pregnancy. Fetal Diagn Ther. 2015;37(2):81-92.
4. Saito S, Nakashima A. A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling. J Reprod Immunol. 2014;101-102:80-8.
5. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect. 2011;119(6):878-85.
6. Liao C, Liu F, Kannan K. Bisphenol s, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol a residues. Environ Sci Technol. 2012;46(12):6515-22.
7. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139-77.
8. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
9. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health. 2014;13(1):43.
10. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ Res. 2018;161:562-72.
11. Casas L, Fernandez MF, Llop S, Guxens M, Ballester F, Olea N, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011;37(5):858-66.
12. Takeda Y, Liu X, Sumiyoshi M, Matsushima A, Shimohigashi M, Shimohigashi Y. Placenta expressing the greatest quantity of bisphenol A receptor ERR{gamma} among the human reproductive tissues: Predominant expression of type-1 ERRgamma isoform. J Biochem. 2009;146(1):113-22.
13. Morice L, Benaitreau D, Dieudonne MN, Morvan C, Serazin V, de Mazancourt P, et al. Antiproliferative and proapoptotic effects of bisphenol A on human trophoblastic JEG-3 cells. Reprod Toxicol. 2011;32(1):69-76.
14. Ferguson KK, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. Phthalate metabolites and bisphenol-A in association with circulating angiogenic biomarkers across pregnancy. Placenta. 2015;36(6):699-703.
15. Meruvu S, Zhang J, Choudhury M. Mono-(2-ethylhexyl) Phthalate Increases Oxidative Stress Responsive miRNAs in First Trimester Placental Cell Line HTR8/SVneo. Chem Res Toxicol. 2016;29(3):430-5.
16. Werner EF, Braun JM, Yolton K, Khoury JC, Lanphear BP. The association between maternal urinary phthalate concentrations and blood pressure in pregnancy: The HOME Study. Environ Health. 2015;14:75.
17. Leclerc F, Dubois MF, Aris A. Maternal, placental and fetal exposure to bisphenol A in women with and without preeclampsia. Hypertens Pregnancy. 2014;33(3):341-8.
18. Cantonwine DE, Meeker JD, Ferguson KK, Mukherjee B, Hauser R, McElrath TF. Urinary Concentrations of Bisphenol A and Phthalate Metabolites Measured during Pregnancy and Risk of Preeclampsia. Environ Health Perspect. 2016.
19. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
20. Bang du Y, Lee IK, Lee BM. Toxicological characterization of phthalic Acid. Toxicol Res. 2011;27(4):191-203.
21. Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary levels of seven phthalate

metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environ Health Perspect. 2004;112(3):331-8.

22. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.
23. Coolman M, Timmermans S, de Groot CJ, Russcher H, Lindemans J, Hofman A, et al. Angiogenic and fibrinolytic factors in blood during the first half of pregnancy and adverse pregnancy outcomes. Obstet Gynecol. 2012;119(6):1190-200.
24. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.
25. Gaillard R, Eilers PH, Yassine S, Hofman A, Steegers EA, Jaddoe VW. Risk factors and consequences of maternal anaemia and elevated haemoglobin levels during pregnancy: a population-based prospective cohort study. Paediatr Perinat Epidemiol. 2014;28(3):213-26.
26. El Assaad MA, Topouchian JA, Darne BM, Asmar RG. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7(4):237-41.
27. Coolman M, de Groot CJ, Jaddoe VW, Hofman A, Raat H, Steegers EA. Medical record validation of maternally reported history of preeclampsia. J Clin Epidemiol. 2010;63(8):932-7.
28. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: statement from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertens Pregnancy. 2001;20(1):IX-XIV.
29. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005;113(2):192-200.
30. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
31. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.
32. O'Brien KM, Upson K, Cook NR, Weinberg CR. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. Environ Health Perspect. 2016;124(2):220-7.
33. Ahmed SB, Bentley-Lewis R, Hollenberg NK, Graves SW, Seely EW. A comparison of prediction equations for estimating glomerular filtration rate in pregnancy. Hypertens Pregnancy. 2009;28(3):243-55.
34. MacPherson S, Arbuckle TE, Fisher M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J Expo Sci Environ Epidemiol. 2018.
35. Philips EM, Jaddoe VWV, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2017;68:105-18.
36. Ferguson KK, Cantonwine DE, Rivera-Gonzalez LO, Loch-Caruso R, Mukherjee B, Anzalota Del Toro LV, et al. Urinary phthalate metabolite associations with biomarkers of inflammation and oxidative stress across pregnancy in Puerto Rico. Environ Sci Technol. 2014;48(12):7018-25.
37. Watkins DJ, Ferguson KK, Anzalota Del Toro LV, Alshawabkeh AN, Cordero JF, Meeker JD. Associations between urinary phenol and paraben concentrations and markers of oxidative stress and inflammation among pregnant women in Puerto Rico. Int J Hyg Environ Health. 2015;218(2):212-9.
38. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta. 2009;30 Suppl A:S43-8.

39. Li H, Gu B, Zhang Y, Lewis DF, Wang Y. Hypoxia-induced increase in soluble Flt-1 production correlates with enhanced oxidative stress in trophoblast cells from the human placenta. Placenta. 2005;26(2-3):210-7.

40. Ouyang YQ, Li SJ, Zhang Q, Xiang WP, Shen HL, Chen HP, et al. Plasma sFlt-1-to-PlGF ratio is correlated with inflammatory but not with oxidative stress in Chinese preeclamptic women. Arch Gynecol Obstet. 2009;280(1):91-7.

41. Draganovic D, Lucic N, Jojic D. Oxidative Stress Marker and Pregnancy Induced Hypertension. Med Arch. 2016;70(6):437-40.

42. Benachour N, Aris A. Toxic effects of low doses of Bisphenol-A on human placental cells. Toxicol Appl Pharmacol. 2009;241(3):322-8.

43. Gao F, Hu W, Li Y, Shen H, Hu J. Mono-2-ethylhexyl phthalate inhibits human extravillous trophoblast invasion via the PPARgamma pathway. Toxicol Appl Pharmacol. 2017;327:23-9.

44. North RA, McCowan LM, Dekker GA, Poston L, Chan EH, Stewart AW, et al. Clinical risk prediction for pre-eclampsia in nulliparous women: development of model in international prospective cohort. BMJ. 2011;342:d1875.

45. Khalil N, Ebert JR, Wang L, Belcher S, Lee M, Czerwinski SA, et al. Bisphenol A and cardiometabolic risk factors in obese children. Sci Total Environ. 2014;470-471:726-32.

46. Trasande L, Sathyanarayana S, Spanier AJ, Trachtman H, Attina TM, Urbina EM. Urinary phthalates are associated with higher blood pressure in childhood. J Pediatr. 2013;163(3):747-53 e1.

47. Shankar A, Teppala S. Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health. 2012;2012:481641.

48. Shiue I, Hristova K. Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3-19% of the population attributable risk for high blood pressure: US NHANES, 2009-2012. Hypertens Res. 2014;37(12):1075-81.

49. Melamed N, Ray JG, Hladunewich M, Cox B, Kingdom JC. Gestational hypertension and preeclampsia: are they the same disease? J Obstet Gynaecol Can. 2014;36(7):642-7.

50. Noori M, Donald AE, Angelakopoulou A, Hingorani AD, Williams DJ. Prospective study of placental angiogenic factors and maternal vascular function before and after preeclampsia and gestational hypertension. Circulation. 2010;122(5):478-87.

Supplementary Data. Weighted molar sums: inclusion and formulae

Individual compounds were included in groups if they were detected in ≥20% of the samples, so as not to bias the resulting molar sums by including metabolite measures based on a preponderance of imputed data. Bisphenols that were detected in ≥50% of the samples were analyzed separately. Because machine values were not available, bisphenol and phthalate metabolite concentrations below the level of detection (LOD) were substituted by LOD/√2, as routinely performed in bisphenol and phthalate analyses.[1]

Phthalic acid (PA) was used separately as a proxy of total phthalate exposure.[2]

Formula for weighted molar sums in nmol per liter:

*((concentration compound in ng/ml) * (1 / molecular weight in g/mol) * (1 / 10^-3)) + ((concentration compound in ng/ml) * (1 / molecular weight in g/mol) * (1 / 10^-3)) + etc.*

Formula for creatinine adjusted compounds in µg per gram creatinine:

*((concentration compound in ng/ml) / (concentration urinary creatinine in µg/ml)) * (1 / 10^-3)*

Formula for creatinine adjusted weighted molar sums in µmol per gram creatinine:

((concentration in µg/g creatinine) / (molecular weight in g/mol)) + ((concentration in µg/g creatinine) / (molecular weight in g/mol)) + etc.

References

1. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.
2. Bang du Y, Lee IK, Lee BM. Toxicological characterization of phthalic Acid. Toxicol Res. 2011;27(4):191-203.

Supplementary Table S1. Bisphenol and phthalate urinary concentrations (n=1,233)

	Median (IQR) (ng/mL)	Percentage of values below the limit of detection (LOD)
Total bisphenols[1]	9.22 (3.51, 20.28)	
Bisphenol A (BPA)	1.65 (0.72, 3.58)	21.7
Bisphenol S (BPS)	0.35 (0.17, 1.03)	31.5
Bisphenol F (BPF)	0.57 (0.30, 1.32)	60.0
Phthalic acid (PA)	56.99 (30.62, 124.05)	0.3
Low molecular weight (LMW) metabolites[1]	1087.42 (423.91, 2925.28)	
Monomethylphthalate (mMP)	5.43 (2.75, 9.75)	0.2
Monoethylphthalate (mEP)	137.85 (41.59, 484.50)	0.1
Mono-isobutylphthalate (mIBP)	20.69 (9.44, 45.33)	0.2
Mono-n-butylphthalate (mBP)	15.58 (6.89, 31.00)	0.7
High molecular weight (HMW) metabolites[1]	219.66 (110.84, 405.51)	
Di-2-ethylhexylphthalate (DEHP) metabolites[1]	171.33 (87.61, 323.30)	
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)	15.96 (8.08, 31.60)	0.2
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)	11.76 (5.67, 22.92)	0.2
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)	7.66 (3.46, 15,48)	-
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)	13.90 (7.55, 26.26)	0.1
Di-n-octylphthalate (DNOP)	5.72 (3.10, 11.03)	
Mono(3-carboxypropyl)phthalate (mCPP)	1.44 (0.78, 2.77)	-
Other high molecular weight metabolites		
Monobenzylphthalate (mBzP)	6.55 (3.06, 12.81)	8.3
Mono-hexylphthalate (mHxP)	0.33 (0.16, 0.63)	24.0
Mono-2-heptylphthalate (mHpP)	1.09 (0.60, 2.29)	36.7

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

Supplementary Table S2. Maternal characteristics of participants and eligible non-participants

	Sample n = 1,233 (at least one blood pressure measurement in pregnancy and no pre-existing hypertension)	**Generation R n = 4,056 (singleton live born children with inclusion <18w + visit F@5 + at least one blood pressure measurement in pregnancy and no pre-existing hypertension)**
Pre-eclampsia (%)	2.0	1.9
Gestational hypertension (%)	3.3	4.5
Systolic blood pressure trimester 1	115.7 (11.6)	115.6 (11.9)
Diastolic blood pressure trimester 1	67.9 (9.3)	68.1 (9.3)
Systolic blood pressure trimester 2	115.9 (11.7)	117.1 (11.9)
Diastolic blood pressure trimester 2	66.8 (9.8)	67.2 (9.3)
Systolic blood pressure trimester 3	117.2 (11.4)	118.7 (11.8)
Diastolic blood pressure trimester 3	68.8 (9.0)	69.2 (9.1)
Maternal pre-pregnancy BMI*	22.6 (20.8, 25.1)	22.6 (20.8, 25.2)

Values represent mean (SD) or valid percentages
*Median (IQR)
BMI: body mass index.

Supplementary Table S3. Associations of individual early pregnancy phthalate urine concentrations with placental angiogenic markers

	Placental Growth Factor (PlGF) <18 weeks (pg/ml), β (95% CI) (n=1,143)	Soluble fms-like tyrosine kinase (sFlt)-1 <18 weeks (ng/ml), β (95% CI) (n=1,143)	sFlt-1 : PlGF ratio <18 weeks, β (95% CI) (n=1,143)	Placental Growth Factor (PlGF) 18-25 weeks (pg/ml), β (95% CI) (n=1,173)	Soluble fms-like tyrosine kinase (sFlt)-1 18-25 weeks (ng/ml), β (95% CI) (n=1,173)	sFlt-1 : PlGF ratio 18-25 weeks, β (95% CI) (n=1,173)
LMW phthalate metabolites						
mMP metabolites	0.00	0.04	18.33	-0.05	0.02	9.75
	(-0.03, 0.06)	(-0.09, 0.31)	(-55.72, 187.59)	(-0.12, 0.28)	(-0.11, 0.65)	(-11.64, 139.11)
mEP metabolites	0.01	0.07	24.39	0.15	0.16	-0.80
	(-0.02, 0.05)	(-0.03, 0.25)	(-26.76, 137.35)	(0.01, 0.52)*	(-0.00, 0.74)	(-10.53, 72.71)
mIBP metabolites	0.02	0.06	-0.02	0.16	0.18	-0.06
	(0.01, 0.08)	(-0.06, 0.31)	(-61.08, 143.62)	(-0.00, 0.65)	(-0.02, 0.95)	(-14.04, 103.14)
mBP metabolites	-0.03	0.13	109.93	0.06	0.11	4.78
	(-0.05, 0.01)	(-0.02, 0.40)	(12.26, 307.42)*	(-0.06, 0.46)	(-0.06, 0.78)	(-12.10, 114.88)
HMW phthalate metabolites						
mECPP metabolites	-0.03	0.14	118.28	0.04	0.08	4.40
	(-0.06, 0.02)	(-0.02, 0.44)	(10.24, 338.48)*	(-0.08, 0.47)	(-0.09, 0.80)	(-14.28, 129.49)
mEHHP metabolites	-0.03	0.09	97.48	0.02	0.08	7.40
	(-0.06, 0.01)	(-0.05, 0.36)	(1.65, 294.51)*	(-0.08, 0.40)	(-0.08, 0.75)	(-11.98, 128.43)
mEOHP metabolites	-0.03	0.12	105.12	0.04	0.11	8.16
	(-0.05, 0.02)	(-0.02, 0.40)	(7.80, 302.98)*	(-0.07, 0.42)	(-0.06, 0.80)	(-11.18, 126.44)
mCMHP metabolites	-0.06	0.24	216.96	0.00	0.15	19.71
	(-0.08,-0.02)*	(0.04, 0.62)*†	(70.25, 500.36)*†	(-0.11, 0.44)	(-0.06, 1.03)	(-10.11, 182.53)
mCPP metabolites	-0.01	0.13	82.83	0.04	0.10	6.49
	(-0.04, 0.05)	(-0.04, 0.46)	(-19.72, 300.46)	(-0.09, 0.49)	(-0.08, 0.89)	(-14.24, 141.22)
mBzP metabolites	0.01	0.17	69.03	0.12	0.22	12.57
	(-0.02, 0.06)	(0.03, 0.42)*†	(-3.06, 217.28)	(-0.01, 0.49)	(0.02, 0.92)*	(-6.23, 113.49)
mHxP metabolites	0.01	0.03	-0.38	0.06	0.04	-2.91
	(-0.02, 0.06)	(-0.08, 0.25)	(-54.90, 127.80)	(-0.05, 0.42)	(-0.08, 0.58)	(-13.36, 82.73)
mHpP metabolites	0.01	0.08	28.30	-0.12	0.11	33.65
	(-0.02, 0.07)	(-0.06, 0.35)	(-47.90, 199.26)	(-0.15, 0.13)	(-0.07, 0.86)	(-3.40, 207.80)

Values are regression coefficients (95% confidence intervals) from multivariable linear regression models that reflect the difference in placental angiogenic markers per log unit increase in urinary phthalate metabolite concentrations.
Models are adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation, gestational age at time of measurement and creatinine.*p-value<0.05. † significant with Bonferroni correction.

Supplementary Table S4. Longitudinal associations of bisphenol and phthalate concentrations and placental angiogenic markers.[a]

	Placental Growth Factor (PlGF) (pg/ml), β (95% CI) (n=1218)		Soluble fms-like tyrosine kinase (sFlt)-1 (ng/ml), β (95% CI) (n=1218)		sFlt-1 : PlGF ratio, β (95% CI) (n=1218)	
	Intercept[b]	**Slope[c]**	**Intercept[b]**	**Slope[c]**	**Intercept[b]**	**Slope[c]**
Total bisphenols	0.11	-0.01	0.05	-0.01	-108.41	4.68
	(-0.00, 0.29)	(-0.01, 0.00)	(-0.49, 1.83)	(-0.05, 0.11)	(-206.14, 124.00)	(-4.06, 21.47)
Bisphenol A	0.09	-0.00	-0.02	0.00	-78.00	2.44
	(-0.01, 0.24)	(-0.01, 0.00)	(-0.52, 1.14)	(-0.00, 0.01)	(-154.48, 89.66)	(-3.72, 13.88)
Bisphenol S	0.05	-0.00	-0.05	0.01	-44.66	2.18
	(-0.04, 0.18)	(-0.01, 0.00)	(-0.50, 1.12)	(-0.04, 0.08)	(-125.31, 128.28)	(-3.63, 13.14)
Phthalic acid	-0.02	0.00	0.39	-0.03	34.63	1.34
	(-0.11, 0.17)	(-0.01, 0.01)	(-0.16, 3.10)	(-0.04, 0.08)	(-101.84, 362.36)	(-5.88, 17.80)
LMW phthalate metabolites	-0.01	0.00	0.23	-0.01	34.32	-1.48
	(-0.08, 0.16)	(-0.00, 0.01)	(-0.12, 2.89)	(-0.02, 0.10)	(-68.19, 315.03)	(-5.68, 10.74)
HMW phthalate metabolites	-0.05	0.00	0.34	-0.01	134.98	-3.71
	(-0.15, 0.17)	(-0.01, 0.02)	(-0.14, 4.61)	(-0.02, 0.17)	(-23.62, 547.22)	(-7.56, 9.05)
DEHP metabolites	-0.08	0.00	0.36	-0.02	138.94	-4.50
	(-0.17, 0.13)	(-0.00, 0.02)	(-0.14, 4.31)	(-0.03, 0.14)	(-18.38, 529.70)	(-8.04, 7.07)
DNOP metabolites	0.05	-0.00	0.61	-0.04	34.97	0.18
	(-0.08, 0.25)	(-0.01, 0.01)	(-0.24, 3.07)	(-0.07, 0.07)	(-124.42, 370.19)	(-8.03, 16.57)

[a]Change in PlGF, sFlt-1 and sFlt:PlGF ratio per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations based on repeated measurement analysis (angiogenic marker value = $\beta_0 + \beta_1$ * log unit compound + β_2 * gestational age + β_3 * log unit compound * gestational age (+ β_x * additional covariates)). [b]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in angiogenic marker per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations (β_2). [c]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in angiogenic marker per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gestational age in weeks (β_4). Models were adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation and urinary creatinine.

Supplementary Table S5. Associations of individual early pregnancy phthalate urine concentrations with placental hemodynamic function and weight

	Umbilical artery pulsatility index 18-25 weeks, SD (95% CI) (n=1,184)	Uterine artery resistance index 18-25 weeks, SD (95% CI) (n=1,019)	Umbilical artery pulsatility index >25 weeks, SD (95% CI) (n=1,186)	Uterine artery resistance index >25 weeks, SD (95% CI) (n=755)	Notching, OR (95% CI) (n=83/793)	Placental weight, g (95% CI) (n=930)
LMW phthalate metabolites						
mMP metabolites	0.01	0.01	-0.05	0.03	0.85	-0.79
	(-0.05, 0.06)	(-0.05, 0.07)	(-0.10, 0.01)	(-0.04, 0.10)	(0.69, 1.05)	(-9.23, 7.64)
mEP metabolites	0.02	0.01	0.01	0.01	0.90	-2.77
	(-0.02, 0.05)	(-0.03, 0.05)	(-0.02, 0.05)	(-0.03, 0.06)	(0.78, 1.04)	(-8.42, 2.88)
mIBP metabolites	0.00	0.02	-0.03	0.05	0.76	-2.58
	(-0.05, 0.05)	(-0.03, 0.08)	(-0.07, 0.02)	(-0.01, 0.11)	(0.62, 0.93)*†	(-10.46, 5.30)
mBP metabolites	-0.01	0.03	-0.02	0.08	0.79	-1.73
	(-0.06, 0.04)	(-0.02, 0.09)	(-0.07, 0.03)	(0.02, 0.15)*	(0.65, 0.96)*	(-9.41, 5.95)
HMW phthalate metabolites						
mECPP metabolites	0.03	0.05	-0.03	0.08	0.92	-2.44
	(-0.03, 0.08)	(-0.02, 0.11)	(-0.09, 0.02)	(0.00, 0.15)*	(0.73, 1.16)	(-11.39, 6.52)
mEHHP metabolites	0.03	0.04	-0.05	0.06	0.85	-1.71
	(-0.02, 0.08)	(-0.02, 0.10)	(-0.10, 0.01)	(-0.01, 0.13)	(0.68, 1.05)	(-10.05, 6.63)
mEOHP metabolites	0.01	0.05	-0.05	0.06	0.86	-0.81
	(-0.04, 0.06)	(-0.01, 0.10)	(-0.10, 0.00)	(-0.01, 0.12)	(0.70, 1.06)	(-8.86, 7.24)
mCMHP metabolites	-0.00	0.03	-0.07	0.06	0.80	-3.60
	(-0.06, 0.06)	(-0.04, 0.09)	(-0.13,-0.01)*	(-0.02, 0.14)	(0.62, 1.03)	(-13.23, 6.02)
mCPP metabolites	0.03	0.03	-0.02	0.05	0.76	-2.53
	(-0.03, 0.09)	(-0.03, 0.09)	(-0.08, 0.04)	(-0.02, 0.13)	(0.60, 0.97)*	(-11.68, 6.62)
mBzP metabolites	-0.01	0.02	-0.03	0.00	0.85	-1.81
	(-0.05, 0.03)	(-0.02, 0.06)	(-0.07, 0.01)	(-0.05, 0.05)	(0.73, 0.99)*	(-7.91, 4.29)
mHxP metabolites	0.03	0.04	-0.02	0.04	0.84	-0.49
	(-0.02, 0.07)	(-0.01, 0.08)	(-0.07, 0.02)	(-0.02, 0.09)	(0.70, 1.01)	(-7.64, 6.67)
mHpP metabolites	0.06	0.02	-0.02	0.02	0.88	-10.02
	(0.01, 0.11)*	(-0.03, 0.08)	(-0.07, 0.04)	(-0.05, 0.09)	(0.70, 1.10)	(-18.45,-1.59)*

Values are based on multivariable linear and logistic regression models that reflect differences or odds ratios and 95% confidence intervals in placental hemodynamic function measures and weight per log unit increase in urinary phthalate metabolite concentrations.
Models are adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation, gestational age at time of measurement and creatinine. *p-value<0.05. † significant with Bonferroni correction.

Supplementary Table S6. Longitudinal associations of bisphenol and phthalate concentrations and placental hemodynamic function markers[a]

	Umbilical artery pulsatility index, SD (95% CI) (n=1217)		**Uterine artery resistance index, SD (95% CI) (n=1134)**	
	Intercept[b]	**Slope[c]**	**Intercept[b]**	**Slope[c]**
Total bisphenols	0.16	-0.01	-1.77	0.09
	(0.02, 0.31)*	(-0.01,-0.00)*	(-2.93,-0.60)*†	(0.03, 0.14)*†
Bisphenol A	0.15	-0.01	-1.28	0.06
	(0.03, 0.26)*†	(-0.01,-0.00)*†	(-2.24,-0.33)*†	(0.02, 0.11)*†
Bisphenol S	0.04	-0.00	-0.62	0.03
	(-0.07, 0.15)	(-0.01, 0.00)	(-1.50, 0.26)	(-0.01, 0.07)
Phthalic acid	0.08	-0.00	-0.49	0.03
	(-0.08, 0.24)	(-0.01, 0.00)	(-1.89, 0.91)	(-0.04, 0.09)
LMW phthalate metabo-	0.04	-0.00	0.04	-0.00
lites	(-0.09, 0.17)	(-0.01, 0.00)	(-1.10, 1.19)	(-0.06, 0.06)
HMW phthalate metabo-	0.16	-0.01	0.30	-0.01
lites	(-0.02, 0.34)	(-0.01,-0.01)*	(-1.04, 1.63)	(-0.08, 0.05)
DEHP metabolites	0.15	-0.01	0.31	-0.01
	(-0.03, 0.32)	(-0.01,-0.00)*	(-0.98, 1.59)	(-0.08, 0.05)
DNOP metabolites	0.10	-0.00	0.32	-0.01
	(-0.07, 0.27)	(-0.01, 0.00)	(-1.06, 1.70)	(-0.08, 0.05)

[a]Change in umbilical artery pulsatility index and uterine artery resistance index per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations based on repeated measurement analysis (hemodynamic function marker Z-score = $\beta_0 + \beta_1$ * log unit compound + β_2 * gestational age + β_3 * log unit compound * gestational age (+ β_x * additional covariates)).

[b]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in hemodynamic function marker per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations (β_2).

[c]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in hemodynamic function marker in mmHg per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gestational age in weeks (β_4).

Models were adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol, folic acid supplementation and urinary creatinine.

*P-value<0.05. **†** significant with Bonferroni correction.

Supplementary Table S7. Longitudinal associations of bisphenol and phthalate concentrations and blood pressure

	Systolic blood pressure (mmHg), β (95% CI)[a]				Diastolic blood pressure (mmHg), β (95% CI)[b]			
	Intercept[c]		Slope[d]		Intercept[c]		Slope[d]	
	Basic model	Adjusted model	Basic model	Adjusted model	Basic model	Adjusted model	Basic model	Adjusted model
Total bisphenols	-0.16	-0.31	-0.01	0.01	-0.22	-0.36	0.01	0.01
	(-0.94, 0.61)	(-1.07, 0.35)	(-0.02, 0.04)	(-0.02, 0.04)	(-0.85, 0.41)	(-0.97, 0.26)	(-0.01, 0.03)	(-0.01, 0.04)
Bisphenol A	-0.09	-0.19	0.01	0.01	-0.11	-0.23	0.01	0.01
	(-0.71, 0.52)	(-0.79, 0.41)	(-0.02, 0.03)	(-0.02, 0.03)	(-0.61, 0.39)	(-0.72, 0.26)	(-0.01, 0.02)	(-0.01, 0.03)
Bisphenol S	-0.18	-0.30	0.01	0.01	-0.10	-0.18	0.01	0.01
	(-0.76, 0.40)	(-0.86, 0.27)	(-0.01, 0.03)	(-0.01, 0.03)	(-0.58, 0.37)	(-0.64, 0.28)	(-0.01, 0.03)	(-0.01, 0.03)
Phthalic acid	0.52	0.18	0.00	0.00	0.23	-0.13	-0.01	0.01
	(-0.33, 1.37)	(-0.65, 1.01)	(-0.03, 0.03)	(-0.03, 0.03)	(-0.47, 0.92)	(-0.80, 0.55)	(-0.02, 0.03)	(-0.02, 0.04)
LMW phthalate	0.30	0.14	-0.00	-0.01	0.33	0.11	0.00	-0.00
metabolites	(-0.40, 1.00)	(-0.53, 0.82)	(-0.03, 0.02)	(-0.03, 0.02)	(-0.23, 0.89)	(-0.44, 0.65)	(-0.02, 0.02)	(-0.02, 0.02)
HMW phthalate	-0.09	-0.15	-0.00	-0.00	-0.30	-0.56	0.02	0.02
metabolites	(-1.06, 0.87)	(-1.09, 0.79)	(-0.04, 0.04)	(-0.04, 0.04)	(-1.08, 0.49)	(-1.33, 0.20)	(-0.01, 0.05)	(-0.01, 0.05)
DEHP metabo-	-0.04	-0.05	-0.00	-0.00	-0.28	-0.46	0.01	0.01
lites	(-0.98, 0.89)	(-0.96, 0.86)	(-0.04, 0.03)	(-0.04, 0.03)	(-1.05, 0.48)	(-1.20, 0.28)	(-0.02, 0.04)	(-0.02, 0.04)
DNOP metabo-	-0.66	-0.62	0.01	0.02	-0.71	-0.80	0.02	0.02
lites	(-1.58, 0.26)	(-1.52, 0.27)	(-0.02, 0.05)	(-0.02, 0.05)	(-1.45, 0.04)	(-1.52,-0.07)*	(-0.01, 0.05)	(-0.00, 0.05)

[a]Change in systolic blood pressure in mmHg per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$ * log unit compound + β_2 * gestational age + β_3 * gestational age^{-2} + β_4 * log unit compound * gestational age (+ β_x * additional covariates)).

[b]Change in diastolic blood pressure in mmHg per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations based on repeated measurement analysis (diastolic blood pressure = $\beta_0 + \beta_1$ * log unit compound + β_2 * gestational age + β_3 * gestational age$^{0.5}$ + β_4 * log unit compound * gestational age (+ β_x * additional covariates)).).

[c]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in blood pressure in mmHg per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations (β_2).

[d]Values are regression coefficients (95% confidence intervals) from multivariable unbalanced repeated measurement regression models that reflect the change in blood pressure in mmHg per natural log unit increase of Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gestational age in weeks (β_4).

Basic models were adjusted for urinary creatinine. Adjusted models were additionally adjusted for maternal age, maternal pre-pregnancy BMI, parity, ethnicity, education, maternal smoking, maternal alcohol and folic acid supplementation.

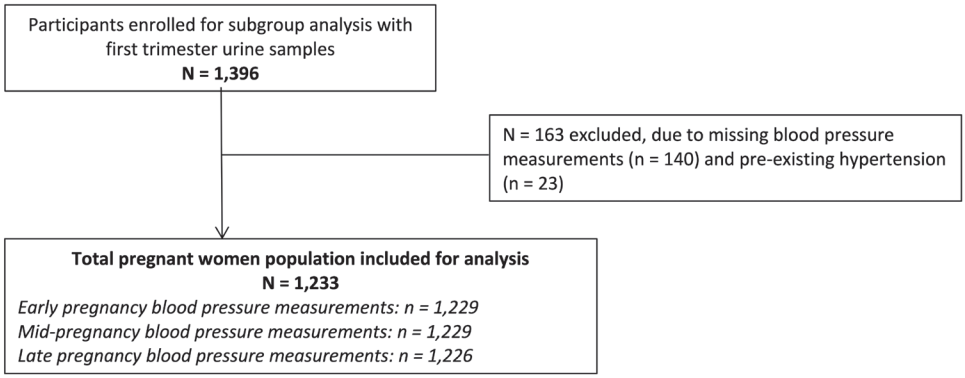
*P-value<0.05.

mmHg: millimeters of mercury.

Supplementary Table S8 can be found online

Supplementary Table S9 can be found online

Supplementary Figure S1. Flowchart for participants in the study of bisphenol and phthalate metabolites, maternal hemodynamics, and gestational hypertensive disorders



Supplementary Figure S2. Longitudinal associations of bisphenol A concentrations and placental hemodynamic function marker Z-scores

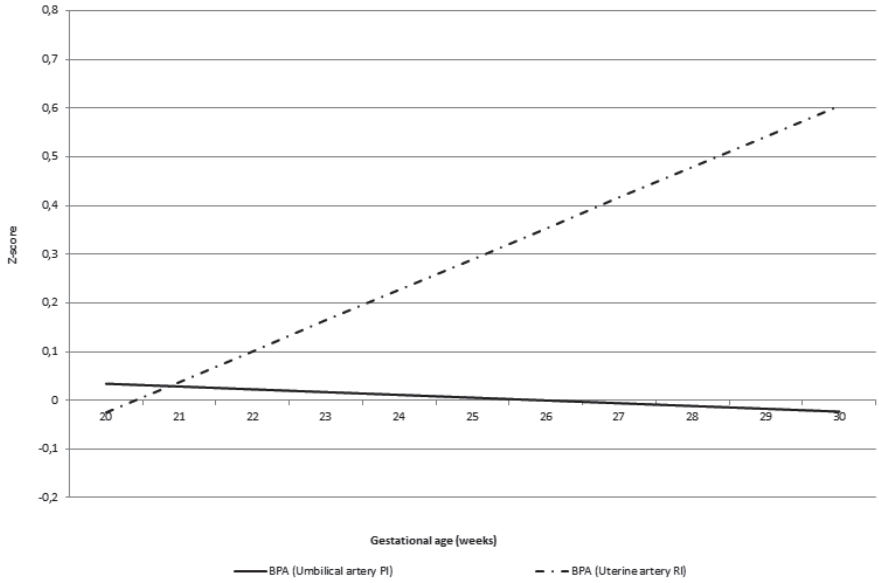


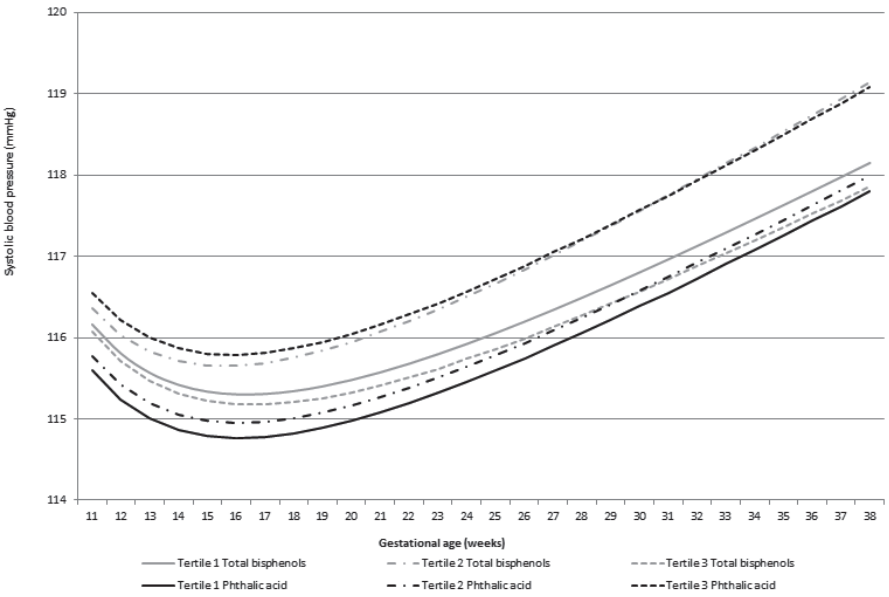
Figure represents the change in umbilical artery pulsatility index Z-score and uterine artery resistance index Z-score per each natural log unit increase in bisphenol A (BPA). Based on repeated measurement analysis (hemodynamic function marker Z-score = β_0 + β_1 * log unit compound + β_2 * gestational age + β_3 * log unit compound * gestational age (+ β_x * additional covariates)).

BPA was associated with both umbilical artery pulsatility index Z-score pattern as uterine artery resistance index Z-score pattern, but contradictory effects were observed.

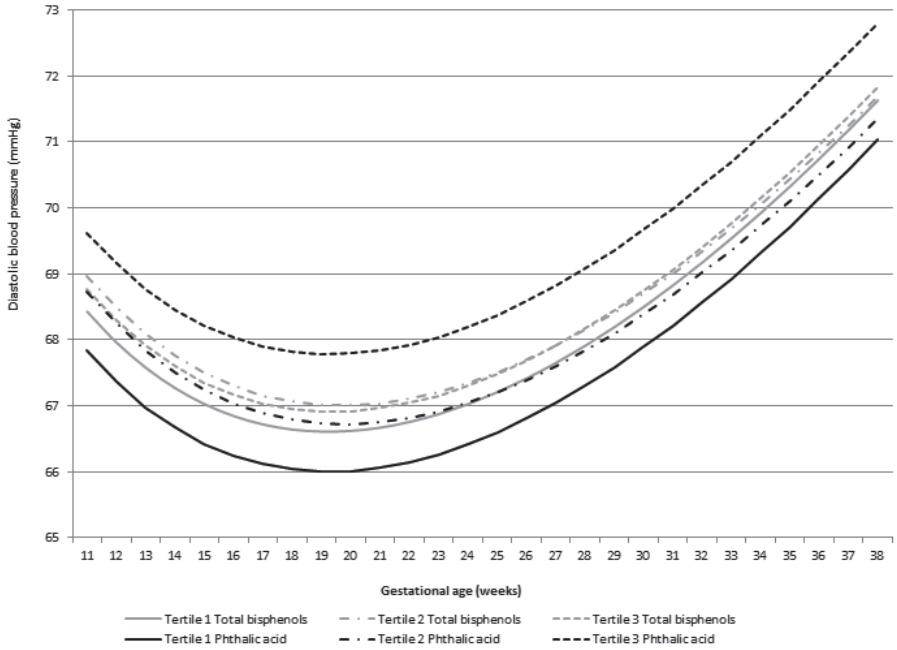
The exact regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction tertile and gestational age) are given for all tested bisphenols and phthalate concentrations in Supplementary Table SVI.

Supplementary Figure S3. Blood pressure patterns for tertiles of total bisphenol and phthalic acid concentrations per gram creatinine

A



B



No significant differences were found for both the intercept and slope of tertiles of total bisphenols and phthalic acid for both systolic and diastolic blood pressure development during pregnancy.

A. Systolic blood pressure during pregnancy. Change in systolic blood pressure in mmHg for women in the three tertiles of total bisphenols and phthalic acid metabolites based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$ * tertile + β_2 * gestational age + β_3 * gestational age^{-2} + β_4 * tertile * gestational age). Concentrations of both total bisphenols and phthalic acid metabolites were not associated with systolic blood pressure patterns during pregnancy. The exact regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction tertile and gestational age) are given for all tested bisphenols and phthalate concentrations in Supplementary Table SVIII.

B. Diastolic blood pressure during pregnancy. Change in diastolic blood pressure in mmHg for women in the three tertiles of total bisphenols and phthalic acid metabolites based on repeated measurement analysis (diastolic blood pressure = $\beta_0 + \beta_1$ * tertile + β_2 * gestational age + β_3 * gestational age$^{0.5}$ + β_4 * tertile * gestational age). Concentrations of both total bisphenol and phthalic acid metabolites were not associated with diastolic blood pressure patterns during pregnancy. The exact regression coefficients for gestational age-independent (intercept) and gestational age-dependent differences (interaction tertile and gestational age) are given for all tested bisphenols and phthalate concentrations in Supplementary Table SVIII.

Supplementary Figure S4 can be found online

2.4

CHAPTER

Bisphenol and phthalate urine concentrations during pregnancy and gestational weight gain

Environ Int. 2019 Dec 18;135:105342.

Abstract

Background: Insufficient or excessive gestational weight gain are associated with increased risks of adverse birth and childhood outcomes. Increasing evidence suggests that exposure to bisphenols and phthalates may disrupt hormonal pathways and thereby influence gestational weight gain.

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

Methods: In a population-based prospective cohort study among 1,213 pregnant women, we measured early and mid-pregnancy bisphenol and phthalate urine concentrations. Maternal anthropometrics before pregnancy were obtained by questionnaire and repeatedly measured at our research center during pregnancy. We used linear and logistic regressions to evaluate the associations of bisphenols and phthalates with total and period-specific gestational weight gain.

Results: Higher maternal total bisphenols and bisphenol S were associated with a lower total gestational weight gain at nominal level. Stratification by body mass index group showed that higher total bisphenols and bisphenol S were associated with lower total gestational weight gain specifically in normal weight women (respectively-509 g [95% CI-819,-198] and-398 g [95% CI-627,-169]). Each log unit increase in early pregnancy total bisphenol and bisphenol A urine concentrations were associated with lower mid- to late pregnancy gestational weight gain in the whole group (effect estimates-218 g/log unit increase [95% CI-334,-102] and-132 g/log unit increase [95% CI-231,-34], respectively). These associations were independent of mid-pregnancy compounds. Mid-pregnancy bisphenols and phthalates concentrations were not associated with gestational weight gain.

Discussion: Higher maternal bisphenol urine concentrations in early pregnancy may lead to reduced gestational weight in second half of pregnancy. Further research is needed to assess the effects of maternal bisphenols and phthalates urine concentrations on placental and fetal growth and development.

Background

Insufficient or excessive gestational weight gain are associated with increased risks of adverse birth and childhood outcomes. The US Institute of Medicine and others have established criteria for excessive as well as insufficient gestational weight gain, recognizing a substantial literature documenting increases in adverse pregnancy, birth and offspring outcomes among women with excessive and insufficient gestational weight gain.[1-5]

Gestational weight gain is a multifactorial phenotype. Risk factors for excessive gestational weight gain include nulliparity, higher total energy intake and smoking during pregnancy.[3,6] Studies reporting associations of increased maternal progesterone and leptin levels with greater gestational weight gain suggest that hormonal responses may be important mechanisms contributing to insufficient or excessive gestational weight gain.[7,8] A substantial literature has suggested that synthetic chemicals, such as bisphenols and phthalates, can disrupt hormones and thereby influence gestational weight gain.[9-14] For example, mono-ethyl phthalate (MEP) has been associated with lower maternal progesterone levels in the second trimester of pregnancy.[15] Higher maternal progesterone levels have been associated with increased gestational weight gain.[7] A study in mice reported increased leptin concentrations in pregnant mice exposed to bisphenol A (BPA).[16] Exposure to bisphenols and phthalates can be modified through behavioral modifications as well as regulatory action.[17-20] To our knowledge, the associations of bisphenol and phthalate concentrations with maternal gestational weight gain have not been studied yet.

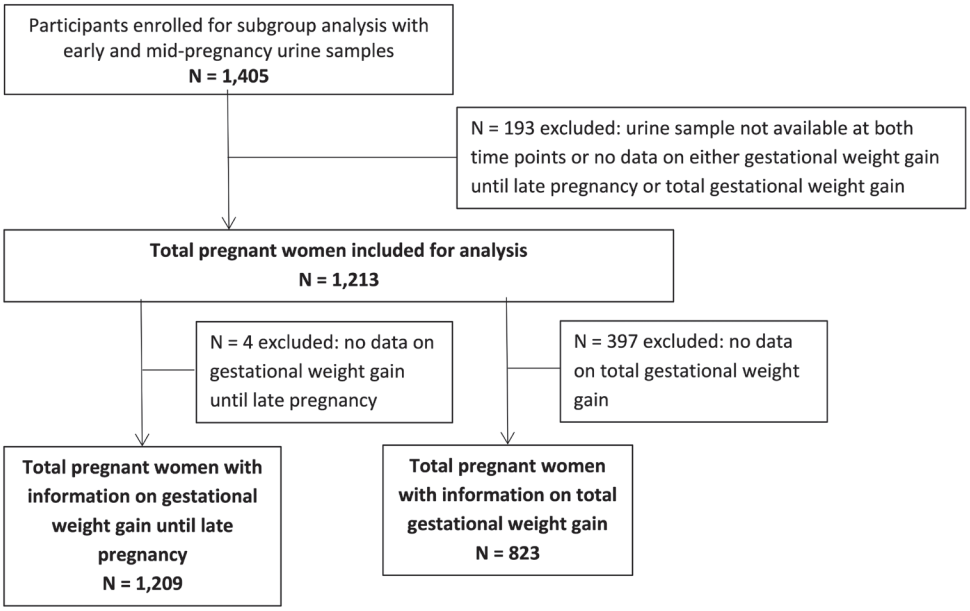
We examined among 1,213 women participating in a population-based prospective cohort study the associations of early and mid-pregnancy bisphenol and phthalate urine concentrations with total and period-specific gestational weight gain and the risks of insufficient or excessive gestational weight gain.

Methods

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 onwards.[21] In total, 8,879 women were enrolled in pregnancy, of which 76% before a gestational age of 18 weeks. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam. Written consent was obtained from all participating women.[22] Bisphenol and phthalate urine concentrations were measured in a subgroup study among 1,405 mothers with an available early or mid-pregnancy urine sample and whose children participated in postnatal studies. This subgroup included singleton pregnancies only. We excluded women without an available urine sample at both time points, without information on gestational weight gain until late pregnancy or total gestational weight gain (n=192), which led to 1,213 women included in the analysis. For analysis on total gestational weight gain and clinical gestational weight gain categories, we excluded women without information on total gestational weight gain (n=397), leading to 823 women included in those analyses (Figure 1).

Figure 1. Flowchart



Bisphenol and phthalate urine concentrations

As previously described, bisphenol and phthalate concentrations were measured in a spot urine sample obtained from each subject during the early and mid-pregnancy measurement (median gestational age 13.1 weeks [inter-quartile range (IQR) 12.1-14.5 weeks] and 20.4 weeks [IQR 19.9-20.9], respectively). All urine samples were collected between February 2004 and October 2005. Details on collection, transportation and analysis methodology are provided elsewhere.[23]

We grouped urinary biomarkers for exposure to phthalates according to their use in product categories. These product categories were first personal care products, and second plasticizers to impart flexibility to plastics. Based on these categories, phthalates we grouped in low and high molecular weight phthalates. We calculated the weighted molar sums for low molecular weight (LMW) phthalate, high molecular weight (HMW) phthalate, di-2-ethylhexylphthalate (DEHP) metabolites, and di-n-octylphthalate metabolites. Phthalic acid (PA) was used separately as a proxy of total phthalate exposure. Among HMW phthalates, DEHP is of particular interest because of its widespread use in food packaging.[24] DNOP is also of concern because, although banned from use in the European Union since 2005, its primary metabolite, mono(3-carboxypropyl)phthalate (mCPP), is still detectable in biosamples.[23,25] Individual compounds were included in if they were detected in ≥20% of the samples. Also, bisphenols that were detected in ≥50% of the samples were analyzed separately. For bisphenol and phthalate concentrations below the level of detection we substituted the level of detection divided by the square root of 2, as routinely performed in bisphenols and phthalates.[26] Table 1 shows the metabolites that were included in all separate groups, their values and detection rates.

Maternal anthropometrics

Maternal height (cm) and weight (kg) were measured at enrollment without shoes and heavy clothing and body mass index (kg/m²) was calculated. Weight was measured repeatedly during subsequent visits at the research center (early pregnancy median gestational age 13.1 weeks [IQR 12.1, 14.5], mid pregnancy median 20.4 weeks [IQR 19.9, 20.9], and late pregnancy median 30.2 weeks [IQR 29.9, 30.8]). Information on maternal weight just before pregnancy was obtained by questionnaire. In our population for analysis, 68.2% of all women were enrolled before a gestational of 14 weeks. Information on total weight during pregnancy was assessed by questionnaire 2 months after delivery (median gestational age at delivery 40.3 [IQR 39.3, 41.0]). Total gestational weight gain was calculated as the difference between the highest weight before birth and pre-pregnancy weight and was available in a subgroup of 823 mothers. For sensitivity analysis, gestational weight gain until the late pregnancy visit was calculated as the difference between late pregnancy weight and pre-pregnancy weight and was available for 1,209 mothers. Correlation of late pregnancy weight and total weight was 0.96 (P-value <0.001).

According to the IOM guidelines, we classified total gestational weight gain as insufficient, sufficient and excessive in relation to maternal pre-pregnancy BMI.[27] Weight gain was further analyzed in specific periods of pregnancy (weight gain between the measured weight at the early and mid-pregnancy visit; weight gain between the measured weight at the mid- and late pregnancy visit; and weight gain between the measured weight at the late pregnancy visit and reported total pregnancy weight).

Table 1. Bisphenol and phthalate urinary concentrations (n=1,213)

	Early pregnancy (<18 weeks)		Mid-pregnancy (18-25 weeks)	
	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[1]	9.31 (3.61, 20.85)		6.31 (3.04, 13.87)	
Bisphenol A (BPA)	1.67 (0.71, 3.61)	21.2	1.46 (0.74, 3.19)	6.7
Bisphenol S (BPS)	0.35 (0.17, 1.09)	31.9	0.24 (0.12, 0.49)	70.9
Bisphenol F (BPF)	0.58 (0.30, 1.31)	59.6	NA	88.5
Phthalic acid (PA) metabolites	57.38 (31.03, 123.45)	0.3	149.68 (61.74, 280.94)	0.1
Low molecular weight (LMW) metabolites[1]	1080.01 (425.05, 2940.32)		586.77 (238.87, 1444.95)	
Monomethylphthalate (mMP)	5.59 (2.75, 9.85)	0.2	3.47 (1.84, 6.21)	0.2
Monoethylphthalate (mEP)	136.55 (41.15, 488.49)	0.1	72.64 (25.05, 222.41)	-
Mono-isobutylphthalate (mIBP)	20.93 (9.52, 45.65)	0.2	8.88 (4.59, 17.80)	-
Mono-n-butylphthalate (mBP)	16.08 (7.01, 30.94)	0.7	9.68 (5.51, 18.91)	-
High molecular weight (HMW) metabolites[1]	219.09 (112.60, 403.22)		131.83 (73.85, 242.94)	
Di-2-ethylhexylphthalate (DEHP) metabolites[1]	171.59 (89.23, 323.30)		96.82 (53.12, 183.72)	
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)	16.09 (8.25, 31.29)	0.2	10.45 (5.77, 19.98)	0.1
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)	11.84 (5.76, 22.80)	0.2	5.57 (2.96, 10.68)	0.1
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)	7.75 (3.54, 15.34)	0.1	7.44 (3.68, 16.30)	-
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)	14.06 (7.60, 26.36)	0.1	4.02 (2.27, 7.38)	0.2
Di-n-octylphthalate (DNOP)	5.78 (3.17, 10.81)		3.53 (2.06, 6.77)	
Mono(3-carboxypropyl)phthalate (mCPP)	1.45 (0.80, 2.71)	0.2	0.89 (0.52, 1.70)	0.1
Other high molecular weight metabolites				
Monobenzylphthalate (mBzP)	6.40 (3.06, 12.55)	8.0	5.27 (2.29, 11.19)	1.5
Mono-hexylphthalate (mHxP)	0.33 (0.16, 0.62)	23.9	NA	98.7
Mono-2-heptylphthalate (mHpP)	1.09 (0.58, 2.33)	35.4	NA	96.8

[1]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.
NA: not applicable; bisphenol or phthalate is not included in the group due to >80% below the limit of detection.

Covariates

Covariates were selected based on previous analyses of potential determinants of first trimester bisphenol and phthalate concentrations.[23] Information on maternal age at enrollment, educational level, ethnicity, parity, pre-pregnancy weight, and folic acid supplementation use was obtained from the first questionnaire at enrollment. Information on smoking and alcohol consumption was assessed by questionnaires in each trimester.[28] 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.*[29] The FFQ covered the average dietary intake over the previous three months, covering the dietary intake in the first trimester of pregnancy.[30] We used caloric intake derived from the FFQ as a covariate in statistical analyses.

Statistical analysis

Differences in subject characteristics between groups of gestational weight gain were assessed using one-way ANOVA tests for continuous variables and chi-square tests for proportions. Non-response analysis was performed to assess distributions of maternal characteristics and investigated outcomes. For the main analyses, all bisphenol and phthalate urinary metabolite concentrations were log-transformed to account for right skewness in the distribution.

We performed multivariable linear and multinomial logistic regressions to evaluate associations of early and mid-pregnancy urinary concentrations with total gestational weight gain continuously, gestational weight gain per pregnancy period and clinical categories of gestational weight gain. To investigate total gestational weight gain continuously and in clinical categories, early and mid-pregnancy and bisphenol and phthalate groupings were used simultaneously to examine the relative influence of early versus mid-pregnancy urinary concentrations. When testing associations of gestational weight gain in specific pregnancy periods, metabolite concentrations of all earlier time points were added simultaneously to the model to adjust for measures at other visits. Therefore, models for early-to-mid-pregnancy gestational weight gain included metabolite concentrations in early pregnancy only. Because detection rates of bisphenol S (BPS) dropped below 50% in mid-pregnancy, early pregnancy BPS concentrations were adjusted for total bisphenol concentrations in mid-pregnancy.

For all significant models, subanalyses of individual bisphenol compounds or phthalate metabolites were performed to determine which metabolites were driving the association. Subanalysis of significant models with early and mid-pregnancy concentrations of bisphenols and phthalates used simultaneously were performed with the separate compounds of the significant group together with the total group of the other pregnancy period, to keep models comparable. As a sensitivity analysis, we used multivariable linear regression models to examine the associations between the logs of molar concentrations of the metabolite groups with gestational weight gain until late pregnancy.

In all models, urinary concentrations of each bisphenol or phthalate compound or grouping were converted to µg/g or µmol/g creatinine to adjust for dilution.[31] All models were adjusted for maternal age, educational level, ethnicity, parity, daily dietary caloric intake, folic acid supplement use, smoking, alcohol consumption. Higher pre-pregnancy BMI has been associated with a lower gestational weight

gain.[32] Our previous studies showed that higher pre-pregnancy BMI was associated with higher bisphenol and phthalate concentrations in early pregnancy.[23] Therefore, models with gestational weight gain as outcome were additionally adjusted for pre-pregnancy BMI. To investigate potential effect modification by pre-pregnancy BMI of the associations of bisphenol and phthalate concentrations with gestational weight gain, we have tested interaction terms with categories of pre-pregnancy BMI. Additionally stratified analyses have been performed for significant interactions. Non-linear effects of early and mid-pregnancy metabolite concentrations on total gestational weight gain were assessed using quartiles.

Missing data of the covariates were imputed using multiple imputation. Five imputed data sets were created. Effect estimates were pooled to obtain the overall result, taking into account the within and between imputation variance according to Rubin's Rules.[33] The percentage of missing values within the population for analysis were lower than or equal to 10%, except for maternal folic acid supplementation use (17.0%) and daily dietary caloric intake (23.8%). 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 0.011).[34] All analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Mid-pregnancy urine concentrations of bisphenols and phthalates were generally lower than in early pregnancy. Also, detection rates of BPS, bisphenol F (BPF), mono-hexylphthalate (mHxP) and mono-2-heptylphthalate (mHpP) urine concentrations were considerably lower in mid-pregnancy (Table 1). Characteristics of the included mothers are given in Table 2. Of all women, 19.1%, 30.0%, and 50.9% had insufficient, sufficient, and excessive gestational weight gain, respectively. Women with excessive gestational weight gain had a higher pre-pregnancy BMI and were more often younger, smokers, and nulliparous. As shown in Supplementary Table S1, nonresponse analysis showed similar distributions of sociodemographic factors and other risk factors for gestational weight gain in the subgroup study population as in the entire study cohort. However, the subgroup of mothers with information on total gestational weight gain tended to be slightly higher educated and healthier.

Table 2. Subject characteristics by gestational weight gain classification[1]

	Total	Insufficient gestational weight gain	Sufficient gestational weight gain	Excessive gestational weight gain	p-value[2]
	n = 1,213	n = 157	n = 247	n = 419	
Maternal age (years)	30.6 (4.8)	31.2 (4.5)	31.8 (4.1)	30.6 (4.6)	0.005
Pre-pregnancy BMI (kg/m²)*	22.7 (20.8, 25.3)	22.1 (20.7, 23.9)	21.9 (20.2, 23.7)	23.2 (21.0, 25.7)	0.000
Educational level					0.020
Low	583 (48.1)	70 (45.2)	85 (34.7)	187 (45.3)	
High	596 (49.1)	85 (54.8)	160 (65.3)	226 (54.7)	
Missings	35 (2.8)	-	-	-	
Ethnicity					0.751
Dutch/European	756 (62.3)	107 (69.0)	176 (71.3)	302 (72.2)	
Non-European	452 (37.3)	48 (31.0)	71 (28.7)	116 (27.8)	
Missings	5 (0.4)	-	-	-	
Parity					0.001
Nulliparous	742 (61.2)	85 (54.1)	152 (61.5)	293 (69.9)	
Multiparous	471 (38.8)	72 (45.9)	95 (38.5)	126 (30.1)	
Missings	-	-	-	-	
Daily dietary caloric intake (kcal)	2080 (508)	2035 (548)	2145 (490)	2113 (485)	0.163
Creatinine early pregnancy (<18 weeks) (µg/mL)*	1030 (491, 1661)	958 (522, 1582)	1026 (450, 1705)	1032 (472, 1642)	0.647
Creatinine mid-pregnancy (18-25 weeks) (µg/mL)*	1164 (740, 1818)	1291 (814, 2025)	1222 (676, 1861)	1106 (709, 1654)	0.375
Smoking					0.000
Never	867 (71.5)	136 (86.6)	192 (77.7)	286 (68.3)	
Until pregnancy was known	108 (8.9)	7 (4.5)	19 (7.7)	50 (11.9)	

	Total	Insufficient gestational weight gain	Sufficient gestational weight gain	Excessive gestational weight gain	p-value[2]
	n = 1,213	n = 157	n = 247	n = 419	
Continued	160 (13.2)	7 (4.5)	21 (8.5)	57 (13.6)	
Missings	78 (6.4)	7 (4.5)	15 (6.1)	26 (6.2)	
Alcohol consumption					0.566
Never	491 (40.5)	64 (40.8)	84 (34.0)	157 (37.5)	
Until pregnancy was known	193 (15.9)	24 (15.3)	47 (19.0)	62 (14.8)	
Continued	451 (37.2)	63 (40.1)	101 (40.9)	172 (41.1)	
Missings	78 (6.4)	6 (3.8)	15 (6.1)	28 (6.7)	
Folic acid supplementation					0.893
No	191 (15.7)	18 (11.5)	28 (11.3)	51 (12.2)	
Start first 10 weeks	330 (27.2)	40 (25.5)	62 (25.1)	122 (29.1)	
Start periconceptional	484 (39.9)	72 (45.9)	116 (47.0)	189 (45.1)	
Missings	208 (17.1)	27 (17.2)	41 (16.6)	57 (13.6)	
Early to-mid pregnancy weight gain (kg)*	3.0 (2.0, 5.0)	-	-	-	
Mid- to late pregnancy weight gain (kg)*	5.0 (3.5, 7.0)	-	-	-	
Late to total pregnancy weight gain (kg)*	4.5 (3.0, 7.0)	-	-	-	
Total gestational weight gain(kg)*	15.0 (12.0, 18.0)	-	-	-	
Gestational weight gain until late pregnancy (kg)*	10.0 (8.0, 13.0)	-	-	-	

[1] Values are means (standard deviation) or numbers of subjects (percentage). Only women with available information on total gestational weight gain were classified in a total gestational weight gain category (n = 823).
[2]Differences between groups of insufficient, sufficient and excessive gestational weight gain were assessed using one-way ANOVA tests for continuous variables and chi-square tests for proportions. *Median (IQR range)

Bisphenol and phthalate urine concentrations and gestational weight gain

Early and mid-pregnancy phthalates were not associated with total gestational weight gain (Table 3). For total bisphenols and BPS, associations with a decreased total gestational weight gain were observed at nominal level.

We observed effect modification by pre-pregnancy BMI of the associations of bisphenol and phthalate concentrations with total gestational weight gain (statistical interaction *p-value* <0.1) for early pregnancy total bisphenols, BPA, BPS, PA, LMW phthalate metabolites and DEHP metabolites (*data not shown*). Further stratification yielded significant results for total bisphenols and BPS in the normal weight group with a decreased total gestational weight gain (respectively -509 g (95% CI -819, -198) and -398 g (95% CI -627, -169), both p-value=0.001). To illustrate, an interquartile range increase in total bisphenols was associated with -864 g (95% CI -1391, -336) decrease in total gestational weight gain among normal weight women. Because the numbers per stratum were low for underweight and obese women these analyses were not presented as main analyses. Assessment of potential non-linear association of early and mid-pregnancy bisphenol and phthalate concentrations using quartiles did not reveal any indications of non-linearity (*data not shown*).

Each log unit increase in early pregnancy total bisphenol urine concentrations was associated with -218 g (95% CI -334, -102) gestational weight gain in mid- to late pregnancy. Analysis of individual bisphenol compounds in early pregnancy showed that maternal BPA concentrations were driving this association with a -132 g (95% CI -231, -34) lower mid- to late pregnancy weight gain/log unit increase. The associations of early pregnancy BPS and BPF urine concentrations with gestational weight gain in mid-to-late pregnancy tended toward nominal significance (Table 3 and Supplementary Table S2). Early pregnancy DNOP metabolite concentrations were associated with mid- to late pregnancy weight gain at nominal level. Bisphenol and phthalate concentrations in early and mid-pregnancy were not associated with early-to-mid-pregnancy weight gain or late pregnancy-to-total gestational weight gain. We did not observe effect modification by pre-pregnancy BMI for the analyses on gestational weight gain during specific periods of pregnancy (*data not shown*).

Table 3. Associations of early and mid-pregnancy bisphenol and phthalate urine concentrations with gestational weight gain (n = 1,213)

	Gestational weight gain (grams)			
	Early to mid-pregnancy, (95% Confidence Interval) (n=1,205)	**Mid- to late pregnancy, (95% Confidence Interval) (n=1,207)[1]**	**Late pregnancy to total, (95% Confidence Interval) (n=819)[1]**	**Total (95% Confidence Interval) (n=823)[1]**
Early pregnancy (<18 weeks)				
Total bisphenols	0 (-98, 98)	-218 (-334,-102)*†	-82 (-261, 98)	-354 (-641,-68)*
Bisphenol A	17 (-66, 100)	-132 (-231,-34)*†	-54 (-205, 98)	-125 (-367, 117)
Bisphenol S[2]	-26 (-97, 44)	-76 (-160, 7)	-41 (-169, 87)	-261 (-466,-56)*
Phthalic acid	32 (-84, 147)	-139 (-277, 0)	-131 (-334, 71)	-50 (-375, 274)
LMW phthalate metabolites	63 (-33, 159)	-110 (-230, 9)	-196 (-375,-17)*	-191 (-478, 96)
HMW phthalate metabolites	13 (-113, 140)	-133 (-285, 18)	-175 (-411, 61)	-268 (-646, 111)
DEHP metabolites	24 (-100, 147)	-122 (-270, 27)	-183 (-413, 47)	-259 (-627, 109)
DNOP metabolites	40 (-83, 162)	-176 (-324,-29)*	-218 (-436,-1)*	-319 (-666, 29)
Mid-pregnancy (18-25 weeks)				
Total bisphenols	-	-119 (-251, 14)	161 (-35, 356)	143 (-168, 453)
Bisphenol A	-	-112 (-238, 14)	151 (-36, 338)	147 (-150, 444)
Bisphenol S	-	-	-	-
Phthalic acid	-	-125 (-271, 21)	217 (-6, 440)	33 (-323, 389)
LMW phthalate metabolites	-	-86 (-221, 49)	145 (-56, 346)	60 (-262, 381)
HMW phthalate metabolites	-	-149 (-304, 5)	112 (-129, 353)	-30 (-415, 354)
DEHP metabolites	-	-140 (-292, 11)	156 (-81, 393)	64 (-315, 444)
DNOP metabolites	-	-68 (-235, 99)	96 (-149, 342)	-112 (-505, 280)

Estimates are based on multivariate regression analyses. Increases are per log unit increase in early and mid-pregnancy urinary Total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gram creatinine. All models are adjusted for maternal age, maternal pre-pregnancy BMI, daily dietary caloric intake, parity, ethnicity, education, maternal smoking, maternal alcohol, and folic acid supplementation. In total, 1,213 women are included in the analyses in this table. Due to random nonresponse, not all women had available information about all the weights. [1]Early and mid-pregnancy compounds have been used in the model simultaneously, yielding estimates adjusted for compounds at the other time point. [2]For models of early pregnancy BPS, the total group of mid-pregnancy bisphenols has been used in the model simultaneously, if applicable. Estimates for mid-pregnancy total bisphenols in these models are not presented. *p-value<0.05 †significant after multiple testing correction

Bisphenol and phthalate levels and clinical categories of gestational weight gain

Table 4 shows that bisphenol and phthalate urine concentrations in early and mid-pregnancy were not associated with insufficient or excessive gestational weight gain. Early pregnancy LMW phthalate metabolites were associated with higher odds of insufficient gestational weight gain, However, this associations attenuated into non-significance correction for multiple testing.

Table 4. Associations of early and mid-pregnancy bisphenol and phthalate urine concentrations with clinical categories of gestational weight gain (n = 823)

	Insufficient weight gain, Odds Ratio (95% Confidence Interval) (n=157)	**Excessive weight gain, Odds Ratio (95% Confidence Interval) (n=419)**
Early pregnancy (<18 weeks)		
Total bisphenols	0.96 (0.82, 1.13)	0.91 (0.80, 1.03)
Bisphenol A	0.97 (0.84, 1.11)	0.96 (0.86, 1.07)
Bisphenol S[1]	1.03 (0.92, 1.15)	0.96 (0.88, 1.06)
Phthalic acid	1.18 (0.98, 1.41)	1.11 (0.96, 1.28)
LMW phthalate metabolites	1.18 (1.01, 1.39)*	1.03 (0.91, 1.17)
HMW phthalate metabolites	1.16 (0.94, 1.43)	1.00 (0.84, 1.18)
DEHP metabolites	1.16 (0.94, 1.42)	1.00 (0.85, 1.18)
DNOP metabolites	1.15 (0.95, 1.40)	0.97 (0.83, 1.13)
Mid pregnancy (18-25 weeks)		
Total bisphenols	0.94 (0.79, 1.12)	1.02 (0.89, 1.17)
Bisphenol A	0.94 (0.79, 1.11)	1.03 (0.90, 1.17)
Bisphenol S	-	-
Phthalic acid	0.97 (0.80, 1.19)	1.00 (0.86, 1.17)
LMW phthalate metabolites	0.97 (0.81, 1.16)	0.97 (0.84, 1.12)
HMW phthalate metabolites	0.92 (0.75, 1.14)	0.97 (0.82, 1.14)
DEHP metabolites	0.91 (0.74, 1.12)	0.98 (0.83, 1.15)
DNOP metabolites	0.96 (0.78, 1.19)	0.88 (0.74, 1.04)

Estimates are based on multivariate regression analyses. Reference category is sufficient weight gain. Only women with available information on total gestational weight gain were classified in a total gestational weight gain category. Increases are per log unit increase in early and mid-pregnancy urinary total bisphenols/BPA/BPS/Phthalic acid/ LMW/HMW/DEHP/DNOP metabolite concentrations per gram creatinine. Models are adjusted for maternal age, daily dietary caloric intake, parity, ethnicity, education, maternal smoking, maternal alcohol, and folic acid supplementation. Early and mid-pregnancy compounds have been used in the model simultaneously, yielding estimates adjusted for compounds at the other time point. [1]For models of early pregnancy BPS, the total group of mid-pregnancy bisphenols has been used in the model simultaneously, if applicable. Estimates for second trimester total bisphenols in these models are not presented.
*p-value<0.05

Sensitivity analysis

Sensitivity analysis shows that the associations of early pregnancy bisphenols with gestational weight gain until late pregnancy somewhat attenuated but had the same directionality (Supplementary Table S3). Early pregnancy BPS concentrations were associated with gestational weight gain until late pregnancy at nominal level, adjusted for total bisphenol concentrations in mid-pregnancy. However, this associations attenuated into non-significance after correction for multiple testing.

Discussion

Results from this prospective population-based cohort study showed that among normal weight women total gestational weight gain was lower for women with higher total bisphenols or BPS concentrations in early pregnancy, independent of bisphenol concentrations in mid-pregnancy. Early pregnancy total bisphenols and BPA were associated with a lower gestational weight gain in mid- to late pregnancy in the whole group.

Interpretation of main findings

To the best of our knowledge, this is the first prospective study that examined the associations of maternal bisphenols and phthalates concentrations with gestational weight gain. Our findings suggest that maternal bisphenol concentrations in early pregnancy are associated with a lower gestational weight gain, mainly in second half of pregnancy. Additionally, the findings suggest that women with a normal weight are most vulnerable for effects of early pregnancy bisphenols on gestational weight gain. We did not observe associations of bisphenol and phthalate concentrations with clinical categories of gestational weight gain. An additional analysis suggests that among women with insufficient weight gain each log unit increase in total bisphenols was associated with a stronger reduction in weight gain than in women with sufficient and excessive weight gain (*data not shown*). Since we have only used early and mid-pregnancy bisphenol and phthalate urine concentrations, we cannot rule out that also late pregnancy bisphenol and phthalate concentrations have a certain effect on gestational weight gain. However, this seems unlikely, since the associations of early pregnancy exposures were independent of mid pregnancy exposure concentrations.

Previous cross-sectional studies investigating determinants of bisphenols and phthalates reported associations of higher concentrations of BPA and phthalates in pregnant women with a higher BMI.[11,12,35-37] A recent prospective study of pregnant women reported a negative association between DEHP metabolites in early pregnancy and early gestational weight gain.[38] Persistent organic pollutants (POPs) have also been examined for associations with gestational weight gain. Similar to bisphenols and phthalates, the majority of POPs are lipophilic chemicals, except for perfluoroalkyl substances (PFASs).[39,40] The results from studies investigating effects of POPs on gestational weight gain show different associations with gestational weight gain for the PFASs and other POPs. Higher perfluorooctanesulfonate (PFOS) levels – a perfluoroalkyl substance - before and in early pregnancy have been associated with a higher gestational weight gain in normal and underweight women, while in overweight women this effect was not observed.[41,42] Other POPs, including dichlorodiphenyl

dichloroethene (DDE), polychlorinated bisphenyls (PCBs) in early pregnancy and neonatal DDE, hexachlorocyclohexanes (HCHs), PCBs and polybrominated diphenyl ethers (PBDEs), have been associated with lower or even insufficient gestational weight gain.[43-45] Thus our study results add to previous studies suggesting that various environmental exposures in specifically early pregnancy may influence gestational weight gain.

Gestational weight gain is a complex phenotype.[3,6] Besides increased maternal fat storage, several compartments could be responsible for the observed change in gestational weight gain. Information about maternal fat storage, measurements of body composition during pregnancy would be informative. However, measurements of body composition during pregnancy were not available in the current study. In our previous study, we did not observe associations of early pregnancy bisphenol and phthalate concentrations with placental weight at birth.[46] A previous study within the same cohort suggested lower fetal growth in association with maternal BPA concentrations.[47] Gestational weight gain, in particular in mid- and late pregnancy, is associated with birth weight.[3,48] In a recent rodent study, early pregnancy BPA exposure was associated with impaired remodeling of the uterine spiral arteries and intrauterine growth restriction.[49] Altogether, previous studies and our results suggest that higher maternal bisphenol urine concentrations in early pregnancy may lead to reduced gestational weight in second half of pregnancy. Further research is needed to assess the effects of maternal bisphenol and phthalate urine concentrations on different aspects of gestational weight gain, such as placental and fetal growth and development.

Strengths and limitations

Strengths of this study were the prospective data collection from early pregnancy onwards, large sample size of 1,213 participants with a urine sample in early and mid-pregnancy, and information on gestational weight gain. The subgroup of women with information on total gestational weight gain tended to a slightly higher educated, healthier population, which might have influenced results. However, sensitivity analysis of gestational weight gain until late pregnancy and period-specific gestational weight gain argue against biased estimates. The response rate at baseline was 61%.[21] Although we cannot rule out selection towards a relatively healthy population, selection bias in cohort studies is more likely to arise from loss to follow up rather than from non-response at baseline.[50] Additionally, models have been adjusted for several potential proxies for health, reducing the odds of biased estimates due to selection bias. Less variation in our study population than in the general population may have led to underestimation of effect estimates. Repeated exposures were analyzed using multiple regression analysis, enabling investigation of potential windows of vulnerability.[51] In our analysis, collinearity was not an issue (Supplementary Table S4). 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.[52,53] A single spot urine sample for phthalates could reasonably reflect exposure for up to three months,[54] but bisphenols have a high temporal variability, even over the day.[55] This non-differential misclassification is expected to lead to attenuation bias in dose-response relationships.

A common method to account for dilution of urinary chemical concentrations is via creatinine adjustment.[56] Endogenous creatinine clearance, measured by 24-hr urine collection, remains the most

precise estimation of the glomerular filtration rate in pregnant women.[57] A recent study suggested that specific gravity adjustment is a better correction method in pregnant women.[58] Unfortunately, specific gravity measurements were not available. Additional analysis of models without creatinine adjustment yielded similar results (*data not shown*).

Maternal weight was measured during the visits at our research center. Information on maternal pre-pregnancy weight and total weight during pregnancy was self-reported. Self-reported weight tends to be underestimated, leading to misclassification. Consequently, this might have led to biased estimates. In the period-specific analysis, early-to-mid and mid-to-late pregnancy analyses were based on measured weights only and provide therefore the most reliable estimates. Detailed information on a large number of potential confounding factors was available. Nonetheless, due to the observational design of the study, residual confounding due to unmeasured environmental exposures, socio-demographic or lifestyle factors still might still be an issue.

Conclusion

Higher maternal bisphenol urine concentrations in early pregnancy may lead to reduced gestational weight in second half of pregnancy. Further research is needed to assess the effects of maternal bisphenols urine concentrations on placental and fetal growth and development.

References

1. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. Obes Rev. 2015;16(8):621-38.
2. Hrolfsdottir L, Rytter D, Olsen SF, Bech BH, Maslova E, Henriksen TB, et al. Gestational weight gain in normal weight women and offspring cardio-metabolic risk factors at 20 years of age. Int J Obes (Lond). 2015;39(4):671-6.
3. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring). 2013;21(5):1046-55.
4. Kaimura M, Oda M, Mitsubuchi H, Ohba T, Katoh T. Participant Characteristics in the Kumamoto University Regional Center of Japan Environment and Children's Study (JECS): Association of Pregnancy Outcomes with Pregestational Maternal Body Mass Index and Maternal Weight Gain during Pregnancy. Nihon Eiseigaku Zasshi. 2017;72(2):128-34.
5. Santos S, Voerman E, Amiano P, Barros H, Beilin LJ, Bergstrom A, et al. Impact of maternal body mass index and gestational weight gain on pregnancy complications: an individual participant data meta-analysis of European, North American and Australian cohorts. BJOG. 2019;126(8):984-95.
6. Nohr EA, Vaeth M, Baker JL, Sorensen T, Olsen J, Rasmussen KM. Combined associations of prepregnancy body mass index and gestational weight gain with the outcome of pregnancy. Am J Clin Nutr. 2008;87(6):1750-9.
7. Lof M, Hilakivi-Clarke L, Sandin SS, de Assis S, Yu W, Weiderpass E. Dietary fat intake and gestational weight gain in relation to estradiol and progesterone plasma levels during pregnancy: a longitudinal study in Swedish women. BMC Womens Health. 2009;9:10.
8. Lacroix M, Battista MC, Doyon M, Moreau J, Patenaude J, Guillemette L, et al. Higher maternal leptin levels at second trimester are associated with subsequent greater gestational weight gain in late pregnancy. BMC Pregnancy Childbirth. 2016;16:62.
9. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA. 2012;308(11):1113-21.
10. Harley KG, Aguilar Schall R, Chevrier J, Tyler K, Aguirre H, Bradman A, et al. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. Environ Health Perspect. 2013;121(4):514-20.
11. Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, et al. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environ Int. 2014;62:1-11.
12. Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, et al. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int J Hyg Environ Health. 2015;218(2):220-31.
13. Buser MC, Murray HE, Scinicariello F. Age and sex differences in childhood and adulthood obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health. 2014;217(6):687-94.
14. Philips EM, Jaddoe VW, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2017;68:105-18.
15. Johns LE, Ferguson KK, Soldin OP, Cantonwine DE, Rivera-Gonzalez LO, Del Toro LV, et al. Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis. Reprod Biol Endocrinol. 2015;13:4.
16. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, et al. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect. 2010;118(9):1243-50.

17. Carwile JL, Luu HT, Bassett LS, Driscoll DA, Yuan C, Chang JY, et al. Polycarbonate Bottle Use and Urinary Bisphenol A Concentrations. Env Health Persp. 2009;9(117):1368-72.
18. Carwile JL, Ye X, Zhou X, Calafat AM, Michels KB. Canned Soup Consumption and Urinary Bisphenol A: A Randomized Crossover Trial. JAMA. 2011;306(20):2218-20.
19. Harley KG, Kogut K, Madrigal DS, Cardenas M, Vera IA, Meza-Alfaro G, et al. Reducing Phthalate, Paraben, and Phenol Exposure from Personal Care Products in Adolescent Girls: Findings from the HERMOSA Intervention Study. Environ Health Perspect. 2016.
20. Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, et al. Food Packaging and Bisphenol A and Bis(2-Ethyhexyl) Phthalate Exposure: Findings from a Dietary Intervention. Environ Health Perspect. 2011;119(7).
21. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.
22. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
23. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ Res. 2018;161:562-72.
24. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ Health. 2014;13(1):43.
25. Casas L, Fernandez MF, Llop S, Guxens M, Ballester F, Olea N, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011;37(5):858-66.
26. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.
27. Institute of Medicine and National Research Council Committee to Reexamine IOM Pregnancy Weight Guidelines. Weight gain during pregnancy: reexamining the guidelines. 2009.
28. Jaddoe VW, van Duijn CM, van der Heijden AJ, Mackenbach JP, Moll HA, Steegers EA, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25(11):823-41.
29. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, Geleijnse JM, Hofman A, Grobbee DE, et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr. 1998;52(8):588-96.
30. Tielemans MJ, Steegers EA, Voortman T, Jaddoe VW, Rivadeneira F, Franco OH, et al. Protein intake during pregnancy and offspring body composition at 6 years: the Generation R Study. Eur J Nutr. 2016.
31. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005;113(2):192-200.
32. Santos S, Eekhout I, Voerman E, Gaillard R, Barros H, Charles MA, et al. Gestational weight gain charts for different body mass index groups for women in Europe, North America, and Oceania. BMC Med. 2018;16(1):201.
33. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York: John Wiley and Sons, Inc.; 1987.
34. Li MX, Yeung JM, Cherny SS, Sham PC. Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. Hum Genet. 2012;131(5):747-56.
35. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, et al. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int. 2014;68:55-65.

36. Lewin A, Arbuckle TE, Fisher M, Liang CL, Marro L, Davis K, et al. Univariate predictors of maternal concentrations of environmental chemicals: The MIREC study. Int J Hyg Environ Health. 2017;220(2 Pt A):77-85.
37. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol analogue exposures are widely prevalent in pregnant women in a population-based cohort in the Netherlands, 2004-5. Environmental International. 2017;submitted.
38. Bellavia A, Hauser R, Seely EW, Meeker JD, Ferguson KK, McElrath TF, et al. Urinary phthalate metabolite concentrations and maternal weight during early pregnancy. Int J Hyg Environ Health. 2017;220(8):1347-55.
39. Stockholm Convention. Protecting human health and the environment from persistent organic pollutants: United Nations Environment Programme; [Available from: http://chm.pops.int/.
40. DeWitt JC. Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances: Humana Press, Springer; 2015.
41. Jaacks LM, Boyd Barr D, Sundaram R, Grewal J, Zhang C, Buck Louis GM. Pre-Pregnancy Maternal Exposure to Persistent Organic Pollutants and Gestational Weight Gain: A Prospective Cohort Study. Int J Environ Res Public Health. 2016;13(9).
42. Ashley-Martin J, Dodds L, Arbuckle TE, Morisset AS, Fisher M, Bouchard MF, et al. Maternal and Neonatal Levels of Perfluoroalkyl Substances in Relation to Gestational Weight Gain. Int J Environ Res Public Health. 2016;13(1).
43. Vizcaino E, Grimalt JO, Glomstad B, Fernandez-Somoano A, Tardon A. Gestational weight gain and exposure of newborns to persistent organic pollutants. Environ Health Perspect. 2014;122(8):873-9.
44. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al. Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in an urban population. Environ Health Perspect. 2007;115(12):1794-800.
45. Vafeiadi M, Vrijheid M, Fthenou E, Chalkiadaki G, Rantakokko P, Kiviranta H, et al. Persistent organic pollutants exposure during pregnancy, maternal gestational weight gain, and birth outcomes in the mother-child cohort in Crete, Greece (RHEA study). Environ Int. 2014;64:116-23.
46. Philips EM, Trasande L, Kahn LG, Gaillard R, Steegers EAP, Jaddoe VWV. Early pregnancy bisphenol and phthalate metabolite levels, maternal hemodynamics and gestational hypertensive disorders. Hum Reprod. 2019;34(2):365-73.
47. Snijder CA, Heederik D, Pierik FH, Hofman A, Jaddoe VW, Koch HM, et al. Fetal growth and prenatal exposure to bisphenol A: the generation R study. Environ Health Perspect. 2013;121(3):393-8.
48. Ay L, Kruithof CJ, Bakker R, Steegers EA, Witteman JC, Moll HA, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG. 2009;116(7):953-63.
49. Muller JE, Meyer N, Santamaria CG, Schumacher A, Luque EH, Zenclussen ML, et al. Bisphenol A exposure during early pregnancy impairs uterine spiral artery remodeling and provokes intrauterine growth restriction in mice. Sci Rep. 2018;8(1):9196.
50. Nohr EA, Frydenberg M, Henriksen TB, Olsen J. Does low participation in cohort studies induce bias? Epidemiology. 2006;17(4):413-8.
51. Chen YH, Ferguson KK, Meeker JD, McElrath TF, Mukherjee B. Statistical methods for modeling repeated measures of maternal environmental exposure biomarkers during pregnancy in association with preterm birth. Environ Health. 2015;14:9.
52. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.

53. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.

54. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.

55. Vernet C, Philippat C, Agier L, Calafat AM, Ye X, Lyon-Caen S, et al. An Empirical Validation of the Within-subject Biospecimens Pooling Approach to Minimize Exposure Misclassification in Biomarker-based Studies. Epidemiology. 2019;30(5):756-67.

56. O'Brien KM, Upson K, Cook NR, Weinberg CR. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. Environ Health Perspect. 2016;124(2):220-7.

57. Ahmed SB, Bentley-Lewis R, Hollenberg NK, Graves SW, Seely EW. A comparison of prediction equations for estimating glomerular filtration rate in pregnancy. Hypertens Pregnancy. 2009;28(3):243-55.

58. MacPherson S, Arbuckle TE, Fisher M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J Expo Sci Environ Epidemiol. 2018.

Supplementary Table S1. Nonresponse analysis[1]

	Sample n= 823 Information on maximum gestational weight gain	**Sample n = 1,213 Information on maximum gestational weight gain or gestational weight gain until late pregnancy**	**Sample n = 3,927 Sample from original cohort not included in subgroup study on maternal bisphenol and phthalate urine concentrations**
Maternal pre-pregnancy BMI (kg/m^2)[2]	22.4 (20.7, 24.8)	22.7 (20.8, 25.3)	22.6 (20.8, 25.3)
Maximum GWG (kg)	15.0 (5.6)	15.0 (5.6)	15.0 (5.8)
GWG until late pregnancy (kg)	10.5 (4.3)	10.4 (4.7)	10.5 (4.8)
IOM classification			
Insufficient weight gain	19.1	19.1	18.4
Sufficient weight gain	30.0	30.0	30.4
Excessive weight gain	50.9	50.9	51.3
Maternal age (y)	31.0 (4.5)	30.6 (4.8)	30.4 (4.9)
Daily dietary caloric intake	2109 (499)	2080 (508)	2054 (544)
Parity (% nulliparous)	64.4	61.2	59.9
Ethnicity (% Dutch/European)	71.3	62.6	64.7
Education (% high)	58.0	50.6	48.8
Maternal smoking (% nonsmoking)	79.2	76.4	73.8
Maternal alcohol use (% no alcohol)	39.4	43.3	42.4
Maternal folic acid supplement use (% periconceptional)	54.0	48.2	45.6

[1]Values represent means (standard deviation) or valid percentages
[2]Median (IQR)

Supplementary Table S2. Subanalysis of associations of early pregnancy bisphenol F with mid- to late gestational weight gain

	Gestational weight gain mid- to late pregnancy (grams) (95% Confidence Interval) (n=1,207)
Bisphenol F	-116 (-234, 1)

Estimates are based on multivariate regression analyses. Increases are per log unit increase in early pregnancy urinary BPF concentrations per gram creatinine, adjusted for mid-pregnancy total bisphenols concentration. Models are adjusted for maternal age, maternal pre-pregnancy BMI, daily dietary caloric intake, parity, ethnicity, education, maternal smoking, maternal alcohol, and folic acid supplementation.
*p-value<0.05

Supplementary Table S3. Associations of early and mid-pregnancy bisphenol and phthalate urine concentrations with gestational weight gain until late pregnancy (n=1,209)

	Gestational weight gain until late pregnancy (grams) (95% Confidence Interval)
Early pregnancy (<18 weeks)	
Total bisphenols	-159 (-354, 36)
Bisphenol A	-47 (-212, 119)
Bisphenol S[1]	-142 (-282,-2)*
Phthalic acid	32 (-200, 265)
LMW phthalate metabolites	26 (-174, 226)
HMW phthalate metabolites	-52 (-306, 203)
DEHP metabolites	-13 (-261, 235)
DNOP metabolites	-101 (-348, 147)
Mid-pregnancy (18-25 weeks)	
Total bisphenols	-79 (-302, 144)
Bisphenol A	-58 (-270, 155)
Bisphenol S	-
Phthalic acid	-217 (-462, 28)
LMW phthalate metabolites	-59 (-286, 168)
HMW phthalate metabolites	-127 (-386, 131)
DEHP metabolites	-52 (-306, 202)
DNOP metabolites	-261 (-541, 19)

Estimates are based on multivariate regression analyses. Increases are per log unit increase in early and mid-pregnancy urinary total bisphenols/BPA/BPS/Phthalic acid/LMW/HMW/DEHP/DNOP metabolite concentrations per gram creatinine. Models are adjusted for maternal age, maternal pre-pregnancy BMI, daily dietary caloric intake, parity, ethnicity, education, maternal smoking, maternal alcohol, and folic acid supplementation. Early and mid-pregnancy compounds have been used in the model simultaneously, yielding estimates adjusted for compounds at the other time point. [1]For models of early pregnancy BPS, the total group of mid-pregnancy bisphenols has been used in the model simultaneously, if applicable. Estimates for second trimester total bisphenols in these models are not presented. *p-value<0.05

Supplementary Table S4. Within and between correlations for early and mid-pregnancy compounds (n=1,213)

	Mid-pregnancy							
Early pregnancy	**Total bisphenols**	**Bisphenol A**	**Bisphenol S**	**Phthalic Acid**	**LMW phthalate metabolites**	**HMW phthalate metabolites**	**DEHP phthalate metabolites**	**DNOP phthalate metabolites**
Total bisphenols	**0.027**	0.988*†	-	0.254*†	0.085*	0.250*†	0.265*†	0.232*†
Bisphenol A	0.829*†	**0.064***	-	0.240*†	0.078*	0.242*†	0.255*†	0.231*†
Bisphenol S	0.489*†	0.192*†	-	-	-	-	-	-
Phthalic Acid	0.340*†	0.314*†	0.228*†	**0.128*†**	0.583*†	0.534*†	0.527*†	0.516*†
LMW phthalate metabolites	0.202*†	0.185*†	0.147*†	0.701*†	**0.325*†**	0.315*†	0.281*†	0.286*†
HMW phthalate metabolites	0.340*†	0.307*†	0.258*†	0.546*†	0.404*†	**0.119*†**	0.942*†	0.669*†
DEHP phthalate metabolites	0.335*†	0.303*†	0.254*†	0.532*†	0.376*†	0.979*†	**0.104*†**	0.647*†
DNOP phthalate metabolites	0.299*†	0.254*†	0.277*†	0.512*†	0.366*†	0.735*†	0.710*†	**0.180*†**

Spearman's correlation coefficients for early and mid-pregnancy compounds and compound groups, used as µg/g or µmol/g creatinine. Correlations of compounds or compound groups within early pregnancy are displayed in darker grey. Correlations of compounds or compound groups within mid-pregnancy are displayed in light grey. Correlations of compounds between the pregnancy periods are bold.
Bisphenol S for mid-pregnancy is excluded for this table, as is for the total paper, because of a low detection rate.

2.5

CHAPTER

Exposures to phthalates and bisphenols in pregnancy and postpartum weight gain

Environ Int. 2020 Jul 31;144:106002.

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 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.

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.[1] 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.[2] Although lifestyle and behavioral factors, notably diet and physical activity, are strong predictors of retention of pregnancy-related weight gain,[3] exposures to other environmental factors, such as endocrine disrupting chemicals, may have a causal role.[4] 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.[5] 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.[6] 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.[7] Peroxisome proliferator-activated receptor γ (PPARγ), which is highly expressed in adipose tissue, has a key role in adipogenesis, lipid metabolism and insulin sensitivity.[8] 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.[9]

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.[10,11] 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.[11-15] 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.[16-20] 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.[21] In the Women's Health Initiative, researchers observed associations of several phthalates with short term weight gain in postmenopausal women.[22] Obesogenic effects of bisphenols other than BPA in women, specifically, have not been investigated, though it is thought they have similar endocrine disrupting capabilities.[23,24]

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.[25] 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.[26] 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.[27] In women, monoethylphthalate (mEP) was associated with impaired glucose tolerance and excessive gestational weight gain,[28] which is considered the strongest risk factor of postpartum weight retention.[29] 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.[30] 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.

Methods

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.[31] 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.[32]

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 1,405 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.

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.[33] 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.[34]

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.[35] For bisphenol and phthalate concentrations below the LOD we substituted values with a LOD value divided by the square root of 2 (LOD/V2), as performed earlier [36]. Bisphenol and phthalate compounds included in the weighted molar sums for early and mid-pregnancy groupings are shown in Supplementary Table S1.

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/m²) before pregnancy was calculated.

Covariates

Potential covariates, effect modifiers, and variables for sensitivity analyses were selected based on previous research, literature review and causal diagram (Supplementary Figure S1).[34] 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.*[37] The FFQ covered the average dietary intake over the previous three months, covering the dietary intake in the first trimester of pregnancy.[38] 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.

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 [39]).

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.[40,41] 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 1,405 participants and both covariates and outcomes as predictors.[42] Five imputed datasets were created and pooled for analyses, taking into account the within and between imputation variance according to Rubin's Rules.[43] 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.

Results

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.

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

	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)**
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
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 areincluded only if less than 80% of values was below the LOD. GA : gestational age.

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).

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.

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*).

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.

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.

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.

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.[16-20] 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.[25] 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.[21] 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.

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.[44,45] A single spot urine sample for phthalates could reasonably reflect exposure for up to three months,[46] but bisphenols have a high temporal variability, even over the day.[47] 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.[48] The present study relies on a single time point, in contrast to the biannual evaluations performed in the Nurses' Health Study.[21] 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 g (PPARg) activation.[49,50] PPARg 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 PPARg activators, while butylbenzylphthalate (BBP) and its main metabolite mBzP showed strong activation of PPARg. 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,[51] though it should be noted these are thought to have mainly anti-androgenic effects.[52] 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.[53,54] Our previous study did not show strong associations of nutrition related factors in the previous three months with bisphenol and phthalate urine concentrations.[34] 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.[55] 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.[39] Endogenous creatinine clearance, measured by 24-hr urine collection, remains the most precise estimation of the glomerular filtration rate in pregnant women.[56] 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.[57] 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.[39] 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.[58,59] 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 [60,61] as well as regulatory action. We note substantial reductions in DEHP metabolites in the US between 2001-2010 [62] due to additional regulatory attention that perhaps explain the greater attributable obesity and costs in the EU compared to US.[58,59] 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.[63,64] Additional studies will be needed with newer populations to assess whether these replacements have the same obesogenic effects.

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.

References

1. Flegal KM, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. JAMA. 2016;315(21):2284-91.
2. Rasmussen KM, Abrams B, Bodnar LM, Butte NF, Catalano PM, Maria Siega-Riz A. Recommendations for weight gain during pregnancy in the context of the obesity epidemic. Obstet Gynecol. 2010;116(5):1191-5.
3. Amorim Adegboye AR, Linne YM. Diet or exercise, or both, for weight reduction in women after childbirth. Cochrane Database Syst Rev. 2013(7):CD005627.
4. Heindel JJ, Newbold R, Schug TT. Endocrine disruptors and obesity. Nat Rev Endocrinol. 2015;11(11):653-61.
5. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36(6):E1-E150.
6. Hirschberg AL. Sex hormones, appetite and eating behaviour in women. Maturitas. 2012;71(3):248-56.
7. Herrera E, Ortega-Senovilla H. Maternal lipid metabolism in normal pregnancy and its implications for fetal development. Clinical Lipidology. 2010;5(6):899-911.
8. Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). Public Health Nutr. 2007;10(10A):1132-7.
9. Clewell RA, Kremer JJ, Williams CC, Campbell JL, Jr., Andersen ME, Borghoff SJ. Tissue exposures to free and glucuronidated monobutylyphthalate in the pregnant and fetal rat following exposure to di-n-butylphthalate: evaluation with a PBPK model. Toxicol Sci. 2008;103(2):241-59.
10. Sathyanarayana S. Phthalates and children's health. Curr Probl Pediatr Adolesc Health Care. 2008;38(2):34-49.
11. Philips EM, Jaddoe VW, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2017;68:105-18.
12. Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, et al. Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol. 2017;68:3-33.
13. Desvergne B, Feige JN, Casals-Casas C. PPAR-mediated activity of phthalates: A link to the obesity epidemic? Molecular and Cellular Endocrinology. 2009;304(1-2):43-8.
14. Nunez AA, Kannan K, Giesy JP, Fang J, Clemens LG. Effects of Bisphenol A on energy balance and accumulation in brown adipose tissue in rats. Chemosphere. 2001;42(8):917-22.
15. Wei J, Lin Y, Li Y, Ying C, Chen J, Song L, et al. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology. 2011;152(8):3049-61.
16. Carwile JL, Michels KB. Urinary bisphenol A and obesity: NHANES 2003–2006. Environmental Research. 2011;111(6):825-30.
17. Hatch E, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environmental health : a global access science source. 2008;7:27.
18. Yaghjyan L, Sites S, Ruan Y, Chang SH. Associations of urinary phthalates with body mass index, waist circumference and serum lipids among females: National Health and Nutrition Examination Survey 1999-2004. Int J Obes (Lond). 2015;39(6):994-1000.
19. Buser MC, Murray HE, Scinicariello F. Age and sex differences in childhood and adulthood obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health. 2014;217(6):687-94.
20. Liu B, Lehmler H-J, Yangbo S, Xu G, Liu, Yuewei, Zong G, Sun, Qi, Hu FB, et al. Bisphenol A substitutes and obesity in US adults: analysis of a population-based, cross-sectional study. 2017;1(3):e114–e22.

21. Song Y, Hauser R, Hu FB, Franke AA, Liu S, Sun Q. Urinary concentrations of bisphenol A and phthalate metabolites and weight change: a prospective investigation in US women. Int J Obes (Lond). 2014.

22. Diaz Santana MV, Hankinson SE, Bigelow C, Sturgeon SR, Zoeller RT, Tinker L, et al. Urinary concentrations of phthalate biomarkers and weight change among postmenopausal women: a prospective cohort study. Environmental health : a global access science source. 2019;18(1):20.

23. Usman A, Ahmad M. From BPA to its analogues: Is it a safe journey? Chemosphere. 2016;158:131-42.

24. Trasande L. Exploring regrettable substitution: replacements for bisphenol A. 2017;1(3):e88–e9.

25. Rodriguez-Carmona Y, Cantoral A, Trejo-Valdivia B, Tellez-Rojo MM, Svensson K, Peterson KE, et al. Phthalate exposure during pregnancy and long-term weight gain in women. Environ Res. 2019;169:26-32.

26. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, et al. Bisphenol A Exposure during Pregnancy Disrupts Glucose Homeostasis â€¨in Mothers and Adult Male Offspring. Environ Health Perspect. 2010;118(9).

27. Schmidt JS, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di(2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N mice. Environ Health Perspect. 2012;120(8):1123-9.

28. James-Todd TM, Meeker JD, Huang T, Hauser R, Ferguson KK, Rich-Edwards JW, et al. Pregnancy urinary phthalate metabolite concentrations and gestational diabetes risk factors. Environ Int. 2016;96:118-26.

29. Rong K, Yu K, Han X, Szeto IM, Qin X, Wang J, et al. Pre-pregnancy BMI, gestational weight gain and postpartum weight retention: a meta-analysis of observational studies. Public Health Nutr. 2015;18(12):2172-82.

30. Philips EM, Santos S, Steegers EAP, Asimakopoulos AG, Kannan K, Trasande L, et al. Maternal bisphenol and phthalate urine concentrations and weight gain during pregnancy. Environ Int. 2019;135:105342.

31. Kooijman MN, Kruithof CJ, van Duijn CM, Duijts L, Franco OH, van IMH, et al. The Generation R Study: design and cohort update 2017. Eur J Epidemiol. 2016;31(12):1243-64.

32. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.

33. Asimakopoulos AG, Xue J, De Carvalho BP, Iyer A, Abualnaja KO, Yaghmoor SS, et al. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. Environ Res. 2016;150:573-81.

34. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ Res. 2018;161:562-72.

35. Bang du Y, Lee IK, Lee BM. Toxicological characterization of phthalic Acid. Toxicol Res. 2011;27(4):191-203.

36. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.

37. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, Geleijnse JM, Hofman A, Grobbee DE, et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. Eur J Clin Nutr. 1998;52(8):588-96.

38. Tielemans MJ, Steegers EA, Voortman T, Jaddoe VW, Rivadeneira F, Franco OH, et al. Protein intake during pregnancy and offspring body composition at 6 years: the Generation R Study. Eur J Nutr. 2016.

39. O'Brien KM, Upson K, Cook NR, Weinberg CR. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. Environ Health Perspect. 2016;124(2):220-7.

40. Hayes AF. *Introduction to mediation, moderation, and conditional process analysis.* A regression-based approach. New York, NY: The Guilford Press; 2013.

41. Hayes AF, Rockwood NJ. Regression-based statistical mediation and moderation analysis in clinical research: Observations, recommendations, and implementation. Behav Res Ther. 2017;98:39-57.

42. Moons KG, Donders RA, Stijnen T, Harrell FE, Jr. Using the outcome for imputation of missing predictor values was preferred. J Clin Epidemiol. 2006;59(10):1092-101.
43. Rubin DB. Multiple Imputation for Nonresponse in Surveys. New York: John Wiley and Sons, Inc.; 1987.
44. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.
45. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.
46. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.
47. Vernet C, Philippat C, Agier L, Calafat AM, Ye X, Lyon-Caen S, et al. An Empirical Validation of the Within-subject Biospecimens Pooling Approach to Minimize Exposure Misclassification in Biomarker-based Studies. Epidemiology. 2019;30(5):756-67.
48. Jaddoe V, Mackenbach J, Moll H, Steegers E, Tiemeier H, Verhulst F, et al. The Generation R Study: Design and cohort profile. European journal of epidemiology. 2006;21(6):475-84.
49. Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, et al. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One. 2013;8(10):e77481.
50. Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. Toxicol Sci. 2003;74(2):297-308.
51. Grün F, Blumberg B. Minireview: The Case for Obesogens. Molecular Endocrinology. 2009;23(8):1127-34.
52. Takeuchi S, Iida M, Kobayashi S, Jin K, Matsuda T, Kojima H. Differential effects of phthalate esters on transcriptional activities via human estrogen receptors α and β, and androgen receptor. Toxicology. 2005;210(2–3):223-33.
53. Schecter A, Lorber M, Guo Y, Wu Q, Yun SH, Kannan K, et al. Phthalate concentrations and dietary exposure from food purchased in New York State. Environ Health Perspect. 2013;121(4):473-94.
54. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9; discussion 81-5.
55. Pacyga DC, Sathyanarayana S, Strakovsky RS. Dietary Predictors of Phthalate and Bisphenol Exposures in Pregnant Women. Adv Nutr. 2019;10(5):803-15.
56. Ahmed SB, Bentley-Lewis R, Hollenberg NK, Graves SW, Seely EW. A comparison of prediction equations for estimating glomerular filtration rate in pregnancy. Hypertens Pregnancy. 2009;28(3):243-55.
57. MacPherson S, Arbuckle TE, Fisher M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J Expo Sci Environ Epidemiol. 2018.
58. Attina TM, Hauser R, Sathyanarayana S, Hunt PA, Bourguignon JP, Myers JP, et al. Exposure to endocrine-disrupting chemicals in the USA: a population-based disease burden and cost analysis. The lancet Diabetes & endocrinology. 2016;4(12):996-1003.
59. Legler J, Fletcher T, Govarts E, Porta M, Blumberg B, Heindel JJ, et al. Obesity, diabetes, and associated costs of exposure to endocrine-disrupting chemicals in the European union. The Journal of clinical endocrinology and metabolism. 2015;100(4):1278-88.
60. Rudel RA, Gray JM, Engel CL, Rawsthorne TW, Dodson RE, Ackerman JM, et al. Food Packaging and Bisphenol A and Bis(2-Ethyhexyl) Phthalate Exposure: Findings from a Dietary Intervention. Environ Health Perspect. 2011;119(7).
61. Harley KG, Kogut K, Madrigal DS, Cardenas M, Vera IA, Meza-Alfaro G, et al. Reducing Phthalate, Paraben, and Phenol Exposure from Personal Care Products in Adolescent Girls: Findings from the HERMOSA Intervention Study. Environ Health Perspect. 2016.

62. Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001-2010. Environ Health Perspect. 2014;122(3):235-41.

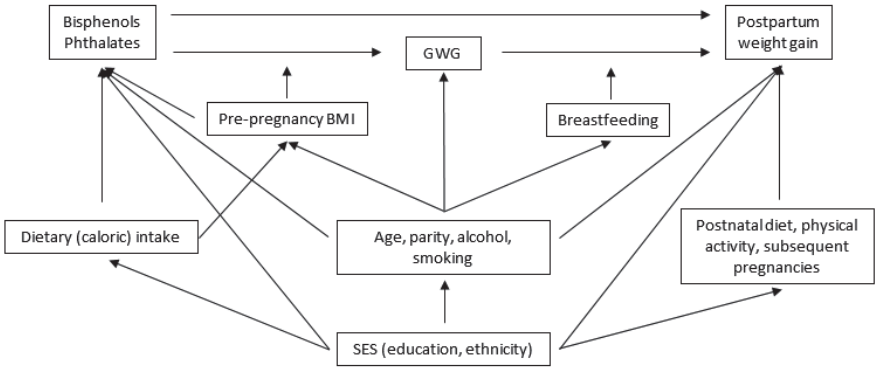
63. Trasande L, Attina TM. Association of Exposure to Di-2-Ethylhexylphthalate Replacements with Increased Insulin Resistance in Adolescents from NHANES 2009-2012. The Journal of clinical endocrinology and metabolism. 2015:jc20151686.

64. Attina TM, Trasande L. Association of Exposure to Di-2-Ethylhexylphthalate Replacements With Increased Insulin Resistance in Adolescents From NHANES 2009-2012. The Journal of clinical endocrinology and metabolism. 2015;100(7):2640-50.

Supplementary Table S1. Bisphenol and phthalate groupings as used in the main analyses (n=1,192)

EARLY PREGNANCY	MID-PREGNANCY
Total bisphenols	**Total bisphenols**
Bisphenol A (BPA)	Bisphenol A (BPA)
Bisphenol S (BPS)	Bisphenol S (BPS)
Bisphenol F (BPF)	
Phthalic acid (PA) metabolites	**Phthalic acid (PA) metabolites**
Low molecular weight (LMW) metabolites	**Low molecular weight (LMW) metabolites**
Monomethylphthalate (mMP)	Monomethylphthalate (mMP)
Monoethylphthalate (mEP)	Monoethylphthalate (mEP)
Mono-isobutylphthalate (mIBP)	Mono-isobutylphthalate (mIBP)
Mono-n-butylphthalate (mBP)	Mono-n-butylphthalate (mBP)
High molecular weight (HMW) metabolites	**High molecular weight (HMW) metabolites**
Di-2-ethylhexylphthalate (DEHP) metabolites	***Di-2-ethylhexylphthalate (DEHP) metabolites***
Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)	Mono-(2-ethyl-5-carboxypentyl)phthalate (mECPP)
Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)	Mono-(2-ethyl-5-hydroxyhexyl)phthalate (mEHHP)
Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)	Mono-(2-ethyl-5-oxohexyl)phthalate (mEOHP)
Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)	Mono-[(2-carboxymethyl)hexyl]phthalate (mCMHP)
Di-n-octylphthalate (DNOP)	***Di-n-octylphthalate (DNOP)***
Mono(3-carboxypropyl)phthalate (mCPP)	Mono(3-carboxypropyl)phthalate (mCPP)
Other high molecular weight metabolites	***Other high molecular weight metabolites***
Monobenzylphthalate (mBzP)	Monobenzylphthalate (mBzP)
Mono-hexylphthalate (mHxP)	
Mono-2-heptylphthalate (mHpP)	

Supplementary Figure S1. Causal diagram describing the hypothesized relations among bisphenols and phthalate metabolites, postpartum weight gain and potential covariates



Supplementary Table S2. Nonresponse analysis[a]

	Sample n = 1,192 (information on maternal weight gain between pre-pregnancy and 6 y postpartum)	**Sample = 373 (information on maternal weight gain between pre-pregnancy and 6 y postpartum and no subsequent pregnancies)**	**Generation R n = 3,909 (singleton live born children + inclusion <18w + visit F@5 + information on maternal weight gain between pre-pregnancy and 6 y postpartum)**
Maternal age (years)	36.8 (4.7)	38.7 (4.6)	37.0 (4.8)
Missing	NA	NA	NA
Gestational weight gain (kg)	10.3 (4.7)	9.9 (5.0)	10.5 (4.8)
Missing	0.4	0.1	3.0
Pre-pregnancy BMI[b]	22.7 (20.8, 25.3)	22.7 (20.8, 25.8)	22.6 (20.8, 25.3)
Missing	NA	NA	0.2
Daily dietary caloric intake during pregnancy	2077 (508)	2044 (656)	2038 (546)
Missing	23.7	25.2	17.7
Parity (% nulliparous)	61.2	35.9	59.6
Missing	NA	NA	0.1
Ethnicity (% Dutch/European)	62.2	60.6	64.6
Missing	0.4	0.3	0.4
Maternal education (% high)	49.2	44.2	47.7
Missing	2.9	3.5	2.2
Smoking during pregnancy (% yes)	22.2	25.5	25.0
Missing	6.5	8.3	4.4
Alcohol use during pregnancy (% yes)	53.3	54.7	54.8
Missing	6.5	8.6	4.6
Breastfeeding (months) [b]	3.5 (1.5, 6.5)	3.5 (1.5, 8.5)	3.5 (1.5, 6.5)
Missing	19.5	19.3	33.5
Maternal weight change (6 y postpartum – pre-prenancy weight)		4.1 (7.1)	5.1 (7.5)
Missing	NA	NA	NA

[a]Values represent means (standard deviation) or percentages
[b]Median (IQR range)

Supplementary Table S3. Univariate associations of potential covariates with weight change

	Maternal weight change, g (95% CI)	**p-value**
Maternal age 6 years postpartum (years)	-162 (-247,-76)	<0.001
Daily dietary caloric intake (kcal)	0 (-1, 1)	0.637
Pre-pregnancy BMI (kg/m^2)	-126 (-219,-32)	0.018
Gestational weight gain (kg)	456 (373, 538)	<0.001
Parity		0.010
Nulliparous	Reference	
Multiparous	-1081 (-1904,-258)	
Ethnicity		<0.001
Dutch/European	Reference	
non-Dutch/non-European	2294 (1472, 3116)	
Education		<0.001
Low	2357 (1559, 3155)	
High	Reference	
Alcohol use during pregnancy		<0.001
No	1734 (914, 2553)	
Yes	Reference	
Smoking during pregnancy		<0.001
No	Reference	
Yes	1934 (996, 2873)	
Breastfeeding (months)	-19 (-176, 137)	0.751
Early pregnancy creatinine concentration (ng/mL)	0 (0, 1)	0.325
Mid-pregnancy creatinine concentration (ng/mL)	0 (0, 1)	0.066

Univariate analysis of potential confounders with maternal weight change between pre-pregnancy weight and maternal weight 6 years postpartum. Analysis performed with multiply imputed data.

Supplementary Table S4. Unadjusted associations of averaged early and mid-pregnancy bisphenol and phthalate urine concentrations with maternal weight change (n=1,192)

	Maternal weight change, g (95% CI)
Total bisphenols	418 (16, 820)
Bisphenol A	414 (53, 775)
Phthalic acid	947 (483, 1411)
LMW phthalate metabolites	864 (512, 1216)
HMW phthalate metabolites	973 (479, 1466)
DEHP metabolites	794 (314, 1273)
DNOP metabolites	885 (380, 1390)

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 (in ng/mL).

Supplementary Table S5. 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 stratified for pre-pregnancy BMI (n=1,192)

	Maternal weight change, g (95% CI)			
	Underweight (n=30)	**Normal weight (n=834)**	**Overweight (n=228)**	**Obese (n=100)**
Total bisphenols	1268 (-405, 2942)	71 (-298, 439)	516 (-472, 1504)	2179 (-454, 4813)
DNOP metabolites	1403 (-1928, 4735)	671 (226, 1116)	854 (-413, 2122)	3893 (2, 7784)

Interaction terms of BPA, PA, LMW, HMW and DEHP metabolites with pre-pregnancy BMI were p-value>0.1. Increases are per natural log unit increase in averaged early and mid-pregnancy urinary total bisphenols/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.

Supplementary Table S6. Multivariable associations of averaged early and mid-pregnancy individual phthalate metabolite urine concentrations with maternal weight change from pre-pregnancy up to until 6 years postpartum (n=1,192)

	Maternal weight change, g (95% CI)
mMP	856 (433, 1279)
mEP	437 (153, 722)
mIBP	531 (115, 947)
mBP	720 (252, 1187)
mECPP	650 (189, 1112)
mEHHP	389 (-70, 848)
mEOHP	501 (62, 939)
mCMHP	453 (-25, 930)
mCPP	840 (347, 1332)
mBzP	583 (205, 961)

Increases are per natural log unit increase in averaged early and mid-pregnancy urinary phthalate 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, and maternal alcohol use and smoking during pregnancy.

Supplementary Table S7. Multivariable associations of averaged early and mid-pregnancy individual phthalate metabolite 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)
mMP	1378 (608, 2147)
mEP	473 (-27, 973)
mIBP	1197 (475, 1918)
mBP	1202 (447, 1958)
mECPP	723 (-120, 1566)
mEHHP	463 (-357, 1282)
mEOHP	639 (-157, 1436)
mCMHP	329 (-545, 1203)
mCPP	1007 (211, 1803)
mBzP	530 (-187, 1247)

Increases are per natural log unit increase in averaged early and mid-pregnancy urinary phthalate 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, and maternal alcohol use and smoking during pregnancy

Supplementary Table S8. 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 stratified for pre-pregnancy BMI (n=373)

	Maternal weight change, g (95% CI)		
	Normal weight (n=249)	**Overweight (n=77)**	**Obese (n=39)**
Total bisphenols	-175 (-848, 498)	796 (-944, 2536)	2661 (-1304, 6626)
Phthalic acid	281 (-506, 1068)	3168 (802, 5535)	5502 (-1775, 12779)
LMW phthalate metabolites	389 (-144, 922)	1723 (183, 3262)	5939 (1326, 10553)
DNOP metabolites	374 (-327, 1076)	1270 (-864, 3404)	8184 (1916, 14453)

Interaction terms of BPA, HMW and DEHP metabolites with pre-pregnancy BMI were p-value>0.1. Increases are per natural log unit increase in averaged early and mid-pregnancy urinary total bisphenols/ Phthalic acid/LMW/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. Estimates for underweight (n=8) could not be provided due to insufficient number of samples.

Supplementary Table S9. Within and between correlations for early and mid-pregnancy compounds (n=1,192)

	Mid-pregnancy							
Early pregnancy	**Total bisphenols**	**Bisphenol A**	**Bisphenol S**	**Phthalic Acid**	**LMW phthalate metabolites**	**HMW phthalate metabolites**	**DEHP phthalate metabolites**	**DNOP phthalate metabolites**
Total bisphenols	**0.025**	0.987**	-	0.255**	0.086*	0.253**	0.267**	0.230**
Bisphenol A	0.832**	**0.058***	-	0.249**	0.079*	0.244**	0.256**	0.229**
Bisphenol S	0.483**	0.188**	-	-	-	-	-	-
Phthalic Acid	0.334**	0.312**	0.224**	**0.135****	0.580**	0.537**	0.530**	0.515**
LMW phthalate metabolites	0.196**	0.186**	0.144**	0.702**	**0.330****	0.318**	0.282**	0.285**
HMW phthalate metabolites	0.337**	0.311**	0.258**	0.546**	0.402**	**0.124****	0.941**	0.667**
DEHP phthalate metabolites	0.332**	0.307**	0.254**	0.532**	0.375**	0.979**	**0.110****	0.644**
DNOP phthalate metabolites	0.295**	0.256**	0.277**	0.509**	0.361**	0.735**	0.709**	**0.188****

Spearman's correlation coefficients for early and mid-pregnancy compounds and compound groups, used as µg/g or µmol/g creatinine. Correlations of compounds or compound groups within early pregnancy are displayed in darker grey. Correlations of compounds or compound groups within mid-pregnancy are displayed in light grey. Correlations of compounds between the pregnancy periods are bold.
Bisphenol S for mid-pregnancy is excluded for this table, as is for the total paper, because of a low detection rate.
*P-value <0.05. **P-value <0.001.

CHAPTER 3

Parental smoking

3.1

CHAPTER

Changes in parental smoking during pregnancy and risks of adverse birth outcomes and childhood overweight

PLOS Medicine. 2020 Aug 18;17(8):e1003182.

Abstract

Background: Fetal smoke exposure is a common and key avoidable risk factor for birth complications and seems to influence later risk of overweight. It is unclear whether this increased risk is also present if mothers smoke during the first trimester only or reduce the number of cigarettes during pregnancy, or when only fathers smoke. We aimed to assess the associations of parental smoking during pregnancy, specifically of quitting or reducing smoking and maternal and paternal smoking combined, with preterm birth, small size for gestational age, and childhood overweight.

Methods and findings: We performed an individual participant data meta-analysis among 229,158 families from 28 pregnancy/birth cohorts from Europe and North America. All 28 cohorts had information on maternal smoking, and 16 also had information on paternal smoking. In total, 22 cohorts were population-based, with birth years ranging from 1991 to 2015. The mothers' median age was 30.0 years, and most mothers were medium or highly educated. We used multilevel binary logistic regression models adjusted for maternal and paternal sociodemographic and lifestyle-related characteristics. Compared with nonsmoking mothers, maternal first trimester smoking only was not associated with adverse birth outcomes but was associated with a higher risk of childhood overweight (odds ratio [OR] 1.17 [95% CI 1.02–1.35], *P* value = 0.030). Children from mothers who continued smoking during pregnancy had higher risks of preterm birth (OR 1.08 [95% CI 1.02–1.15], *P* value = 0.012), small size for gestational age (OR 2.15 [95% CI 2.07–2.23], *P* value < 0.001), and childhood overweight (OR 1.42 [95% CI 1.35–1.48], *P* value < 0.001). Mothers who reduced the number of cigarettes between the first and third trimester, without quitting, still had a higher risk of small size for gestational age. However, the corresponding risk estimates were smaller than for women who continued the same amount of cigarettes throughout pregnancy (OR 1.89 [95% CI 1.52–2.34] instead of OR 2.20 [95% CI 2.02–2.42] when reducing from 5–9 to ≤4 cigarettes/day; OR 2.79 [95% CI 2.39–3.25] and OR 1.93 [95% CI 1.46–2.57] instead of OR 2.95 [95% CI 2.75–3.15] when reducing from ≥10 to 5–9 and ≤4 cigarettes/day, respectively [*P* values < 0.001]). Reducing the number of cigarettes during pregnancy did not affect the risks of preterm birth and childhood overweight. Among nonsmoking mothers, paternal smoking was associated with childhood overweight (OR 1.21 [95% CI 1.16–1.27], *P* value < 0.001) but not with adverse birth outcomes. Limitations of this study include the self-report of parental smoking information and the possibility of residual confounding. As this study only included participants from Europe and North America, results need to be carefully interpreted regarding other populations.

Conclusions: We observed that as compared to nonsmoking during pregnancy, quitting smoking in the first trimester is associated with the same risk of preterm birth and small size for gestational age, but with a higher risk of childhood overweight. Reducing the number of cigarettes, without quitting, has limited beneficial effects. Paternal smoking seems to be associated, independently of maternal smoking, with the risk of childhood overweight. Population strategies should focus on parental smoking prevention before or at the start, rather than during, pregnancy.

Introduction

One in five women of reproductive age are expected to be tobacco users by 2025.[1] Although strategies to prevent smoking are globally implemented, up to 25% of women in Western countries smoke during pregnancy.[2] This is a major public health concern, particularly since smoking during pregnancy not only affects women's own health but is also associated with adverse birth and offspring outcomes, such as preterm birth, low birth weight, and childhood overweight.[3-13] Preterm birth and low birth weight are major causes of perinatal morbidity and mortality, and childhood overweight is related to a higher risk of cardiovascular disease, premature death, and disability in adulthood.[14-16]

A vast number of studies observed consistent associations of continued maternal smoking during pregnancy with increased risks of preterm birth, low birth weight, and childhood overweight.[7,10,11] However, evidence on critical windows of vulnerability to maternal smoking and changes in smoking behavior during pregnancy remain inconclusive, potentially reflecting between-study heterogeneity of outcome measures and small study sample sizes. Previous studies focused on maternal smoking in first trimester of pregnancy only, consistently showing no associations with preterm birth, but showed conflicting results for the risks of low birth weight and childhood overweight.[8,9,17-21] Also, the associations of paternal smoking during pregnancy with preterm birth, low birth weight, and childhood overweight have been scarcely studied and remain unclear.[20,22,23] Paternal smoking might affect offspring outcomes through direct gamete or passive smoking intrauterine effects. However, comparisons of maternal and paternal smoking associations can also be used to disentangle direct uterine programming effects and confounding by shared or family-based lifestyle or socioeconomic variables. To our knowledge, no large sample size studies assessed the associations of maternal smoking during first trimester only, of reducing the number of cigarettes during pregnancy, or of paternal smoking only with birth and childhood outcomes.

We conducted an individual participant data meta-analysis among 229,158 singleton births from 28 pregnancy and birth cohort studies in Europe and North America to assess the associations of parental smoking during pregnancy with preterm birth, small size for gestational age (SGA), and childhood overweight. We were specifically interested in the associations of quitting or reducing smoking during pregnancy and of combined maternal and paternal smoking patterns with birth and offspring outcomes.

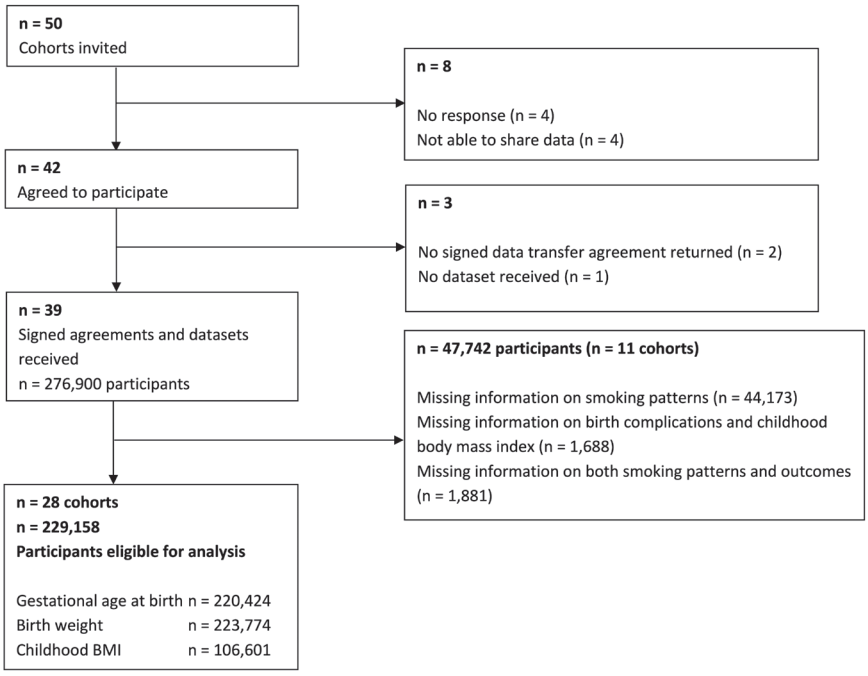
Methods

Inclusion criteria and participating cohorts

This study was part of an international LifeCycle Project (https://lifecycle-project.eu) collaboration on maternal obesity and childhood outcomes.[24-28] Pregnancy and birth cohort studies were eligible for inclusion if they included mothers with singleton live-born children who were born from 1989 onwards, had information available on maternal prepregnancy/early-pregnancy body mass index (BMI), and had at least one offspring measurement (birth weight or childhood BMI). We identified eligible cohorts from existing collaborations on childhood health (EarlyNutrition Project, CHICOS Project, www.birthcohorts.net assessed until July 2014). Fifty cohorts from Europe, North America, and Oceania were identified

and invited, of which 39 cohorts agreed to participate. The cohorts were approved by their local institutional review boards, and written informed consent from all participants or parents was obtained. Eleven cohorts were excluded from the current analysis because there was no information on maternal smoking patterns or only nonsmoking mothers in their cohort. In total, 28 cohorts comprising data on 229,158 singleton births were included (Figure 1). Twenty-two of the 28 cohorts defined themselves as regionally or nationally based studies, four as hospital-based (Co.N.ER, EDEN, GASPII, LUKAS), one as internet users–based (NINFEA), and one as studying selected populations (FCOU). The plan for analyses given to the cohorts when inviting them to participate in this paper from the LifeCycle Project collaboration is provided in S1 Text. Based on data availability and additional research questions, it was decided among the collaborators to refine the existing questions and to extend the project with additional questions to be addressed. Analyses that were not in the original plan are marked in S1 Text. Associations of smoking with early- and late-childhood BMI were excluded because of low numbers. All cohorts provided written informed consent for using their data. Anonymized datasets were stored on a single central secured data server with access for the main analysts (EP, SS) only. This study is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (S1 PRISMA Checklist).

Figure 1. Flowchart of the cohorts and participants



BMI, body mass index.

Parental tobacco smoking

Parental smoking information was obtained by questionnaires (cohort-specific information in S1 Table). We used trimester-specific maternal smoking information to categorize smoking during pregnancy in three groups (nonsmoking; first-trimester-only smoking; continued smoking [as being any second or third trimester smoking]). Trimester-specific maternal smoking information was categorized into nonsmoking, ≤4 cigarettes/day, 5–9 cigarettes/day, and ≥10 cigarettes/day. We combined the information about maternal smoking in first and third trimester to examine the change in smoking behavior. Information on paternal nonsmoking/smoking was used. To explore the combined effects of maternal and paternal smoking, we combined the maternal and paternal smoking information into six categories: maternal and paternal nonsmoking (used as reference category); maternal nonsmoking and paternal smoking; maternal first-trimester-only smoking and paternal nonsmoking; maternal first-trimester-only smoking and paternal smoking; maternal continued smoking and paternal nonsmoking; and maternal continued smoking and paternal smoking.

Birth complications and childhood overweight

Information on gestational age at birth, birth weight, and childhood weight and height was measured, derived from clinical records, or reported (cohort-specific information in S1 Table). Preterm birth was defined as <37 weeks of gestation, and full-term birth (≥37 weeks) was used as the reference group in the analyses.[29] We created sex- and gestational age–adjusted birth weight standard deviation scores (SDSs) based on a North European reference chart.[30] SGA at birth was defined per cohort as sex- and gestational age–adjusted birth weight below the 10th percentile. The reference group used in the analyses comprises children born at appropriate and large size for gestational age (i.e., cohort-specific sex- and gestational age–adjusted birth weight above the 10th percentile). BMI measurements in mid-childhood (≥5 to <10 years) were used. If there were multiple measurements of a child available within the age interval, we used the measurement at the highest age. We created sex- and age-adjusted SDSs of childhood BMI using World Health Organization (WHO) reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[31,32] Childhood normal weight, overweight, and obesity were defined using WHO cutoffs.[31,32] For the analyses, we combined the overweight and obesity group, hereafter referred to as the overweight group. Normal weight was used as the reference group in childhood overweight analyses.

Covariates

Information on covariates was mostly assessed using questionnaires. Most covariates were provided by cohorts as categorical variables: child's sex, maternal educational level (low, medium, high), parity (nulliparous, multiparous), and alcohol consumption during pregnancy (yes, no). To allow handling of missing data, continuous covariates were categorized: maternal age (defined on the basis of data availability: <25.0 years, 25.0–29.9 years, 30.0–34.9 years, and ≥35.0 years) and prepregnancy or early-pregnancy maternal and paternal BMI (underweight [<18.5 kg/m^2], normal weight [18.5–24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obesity [≥30.0 kg/m^2]). Maternal ethnicity was not included, since most cohorts were largely of European descent and there was a high percentage of missing data. Covariates per cohort are described in S2 Table.

Statistical analysis

We conducted 1-stage meta-analyses, in which we analyzed individual participant data from all cohorts simultaneously in binary logistic multilevel mixed-effects models, accounting for clustering of participants within cohorts.[33] First, we examined the associations of maternal smoking (across different trimesters; dose-response) with the risks of preterm birth, SGA, and childhood overweight. When examining the dose-response effects of first trimester maternal smoking, mothers who continued smoking were excluded from the analysis. Second, we used similar models to investigate the associations of change in maternal smoking behavior from first to third trimester with the risks of preterm birth, SGA, and childhood overweight. Finally, we used similar models to investigate the combined associations of both maternal and paternal smoking with the risks of these outcomes. We assessed whether the risk estimates between categories statistically differed using the formula $Z = \frac{\beta 1 - \beta 2}{\sqrt{(SE\beta 1)^2 + (SE\beta 2)^2}}$[34].

We adjusted all analyses focused on maternal smoking for maternal age, educational level, parity, prepregnancy or early-pregnancy BMI, alcohol consumption during pregnancy, and paternal smoking. We adjusted all analyses focused on combined maternal and paternal smoking for the same covariates and paternal BMI. As sensitivity analyses, we repeated all models for gestational age at birth, sex- and gestational age–adjusted birth weight SDSs, and childhood sex- and age-adjusted BMI SDSs. Also, we conducted two-stage random-effects meta-analyses for the core associations and tested for heterogeneity between the cohorts estimates with the I^2 test.[33,35] To express the uncertainty associated with I^2 estimates, we calculated the corresponding 95% confidence intervals (CIs).[36] All covariates were categorized and missing values were added as an additional group to prevent exclusion of noncomplete cases. If information on a covariate was available for less than 50% of the cohort sample used for each analysis, available information was not used and the corresponding data for that full cohort sample were assigned to the missing category. We conducted a sensitivity analysis with complete cases only. Also, to explore the influence on our results of using maternal age and BMI as categorical covariates, we repeated the complete cases' analysis using these covariates continuously. The statistical analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (SPSS, Chicago, IL, United States of America) and Review Manager (RevMan) version 5.3 of the Cochrane Collaboration (The Nordic Cochrane Centre, Copenhagen, Denmark).

Results

Participants' characteristics

Information about the main characteristics per cohort is given in Table 1. Overall, 14.4% (range 5.5–26.8) of mothers and 27.5% (range 16.9–83.8) of fathers smoked during pregnancy. Children were born at a median gestational age of 40.0 weeks (95% range 35.7–42.3) and a median birth weight of 3,530 grams (95% range 2,390–4,580). In total, 4.7% of children were born preterm, 10.0% were SGA at birth, and 20% were in the overweight group. Additional information about maternal smoking is given in S3 Table.

Table 1. Characteristics of the participating pregnancy and birth cohorts (n = 229,158)

	Maternal smoking			Paternal smoking		Birth outcomes				Childhood BMI		
Cohort name, number of participants, birth years (country)	**No**	**First trimester only**	**Continued**	**No**	**Yes**	**Gestational age at birth (weeks)**	**Preterm birth**	**Birth weight (g)**	**Small size for gestational age at birth**	**Age (months)**	**BMI (SDS)**	**Overweight**
ABCD, *n* = 7,324, 2003–2004 (the Netherlands)	6,571 (89.7)	NA	753 (10.3)	NA	NA	40.0 (35.0–42.0)	385 (5.3)	3,460 (2,270–4,500)	732 (10.1)	68.1 (61.6–82.1)	0.09 (−1.69 to 2.29)	706 (16.6)
ALSPAC, *n* = 12,148, 1991–1992 (United Kingdom)	9,581 (78.9)	NA	2,567 (21.1)	7,397 (63.2)	4,301 (36.8)	40.0 (35.0–42.0)	650 (5.4)	3,440 (2,240–4,420)	1,190 (10.0)	115.0 (88.0–119.0)	0.24 (−1.61 to 2.66)	1,960 (26.3)
BAMSE, *n* = 4,057, 1994–1996 (Sweden)	3,533 (87.1)	72 (1.8)	452 (11.1)	2,756 (83.1)	560 (16.9)	40.0 (35.0–42.0)	212 (5.3)	3,545 (2,334–4,550)	396 (9.9)	101.0 (89.0–109.0)	0.52 (−1.20 to 2.63)	814 (31.2)
BIB, *n* = 1,641, 2007–2010 (UK)	1,398 (85.2)	NA	243 (14.8)	NA	NA	39.7 (35.3–41.9)	83 (5.1)	3,200 (2,180–4,280)	163 (10.0)	NA	NA	NA
Co.N.ER, *n* = 641, 2004–2005 (Italy)	549 (85.6)	30 (4.7)	62 (9.7)	441 (68.9)	199 (31.1)	39.0 (36.0–41.0)	29 (4.5)	3,340 (2,420–4,230)	63 (9.9)	95.0 (86.6–111.1)	0.69 (−1.29 to 2.92)	102 (35.5)
DNBC, *n* = 71,710, 1996–2002 (Denmark)	59,030 (82.3)	NA	12,680 (17.7)	49,534 (70.5)	20,756 (29.5)	40.1 (35.9–42.4)	3,168 (4.4)	3,600 (2,420–4,640)	7,124 (10.0)	85.0 (75.1–89.5)	0.01 (−1.95 to 2.07)	5,644 (15.5)
EDEN, *n* = 1,880, 2003–2005 (France)	1,376 (73.2)	148 (7.9)	356 (18.9)	999 (59.5)	679 (40.5)	39.0 (35.0–41.0)	106 (5.6)	3,300 (2,158–4,200)	187 (10.0)	67.6 (65.0–72.4)	−0.01 (−1.52 to 2.02)	145 (12.9)
FCOU, *n* = 4,003, 1993–1996 (Ukraine)	3,647 (91.1)	NA	356 (8.9)	461 (16.2)	2,382 (83.8)	NA	NA	3,400 (2,100–4,300)	393 (10.2)	84.0 (75.0–93.0)	−0.02 (−2.02 to 2.06)	119 (12.7)

	Maternal smoking			Paternal smoking		Birth outcomes				Childhood BMI		
Cohort name, number of participants, birth years (country)	**No**	**First trimester only**	**Continued**	**No**	**Yes**	**Gestational age at birth (weeks)**	**Preterm birth**	**Birth weight (g)**	**Small size for gestational age at birth**	**Age (months)**	**BMI (SDS)**	**Overweight**
GASPII, *n* = 680, 2003–2004 (Italy)	599 (88.1)	23 (3.4)	58 (8.5)	510 (75.2)	168 (24.8)	40.0 (36.0–42.0)	28 (4.1)	3,350 (2,401–4,320)	67 (9.9)	104.0 (98.0–113.0)	0.70 (−1.37 to 2.66)	172 (37.1)
GENERATION R, *n* = 7,934, 2002–2006 (The Netherlands)	6,190 (78.0)	461 (5.8)	1,283 (16.2)	2,833 (56.5)	2,183 (43.5)	40.1 (35.4–42.3)	474 (6.0)	3,420 (2,190–4,480)	788 (10.0)	115.3 (69.4–119.4)	0.35 (−1.52 to 2.67)	1,578 (27.1)
GENERATION XXI, *n* = 7,541, 2005–2006 (Portugal)	5,766 (76.5)	540 (7.2)	1,235 (16.4)	NA	NA	39.0 (35.0–41.0)	557 (7.4)	3,200 (2,130–4,095)	747 (10.0)	85.0 (70.2–95.0)	0.63 (−1.38 to 3.23)	1,991 (37.9)
GENESIS, *n* = 2,261, 2003–2004 (Greece)	1,842 (81.5)	30 (1.3)	389 (17.2)	NA	NA	40.0 (34.0–40.0)	224 (10.0)	3,250 (2,100–4,200)	213 (10.0)	61.9 (60.1–71.9)	0.93 (−1.43 to 4.11)	39 (43.3)
GINIplus, *n* = 2,086, 1995–1998 (Germany)	1,903 (91.2)	NA	193 (8.8)	NA	NA	NA	NA	NA	NA	62.9 (60.2–74.4)	0.01 (−1.77 to 1.93)	215 (10.3)
HUMIS, *n* = 986, 2002–2009 (Norway)	932 (94.5)	NA	54 (5.5)	NA	NA	40.1 (33.2–42.9)	86 (8.7)	3,580 (1,822–4,703)	98 (10.0)	84.0 (60.0–92.0)	0.02 (−2.03 to 2.14)	58 (17.5)
INMA, *n* = 2,406, 1997–2008 (Spain)	1,988 (82.6)	NA	418 (17.4)	1,395 (58.0)	1,009 (42.0)	39.9 (36.0–42.0)	98 (4.1)	3,250 (2,300–4,200)	238 (10.0)	83.6 (75.1–94.5)	0.55 (−1.37 to 3.31)	489 (37.7)
KOALA, *n* = 2,800, 2000–2002 (the Netherlands)	2,594 (92.6)	NA	206 (7.4)	NA	NA	40.0 (36.0–42.0)	89 (3.2)	3,500 (2,478–4,510)	277 (10.0)	106.2 (61.5–119.3)	−0.17 (−2.16 to 1.77)	199 (11.4)

Cohort name, number of participants, birth years (country)	Maternal smoking			Paternal smoking		Birth outcomes				Childhood BMI		
	No	First trimester only	Continued	No	Yes	Gestational age at birth (weeks)	Preterm birth	Birth weight (g)	Small size for gestational age at birth	Age (months)	BMI (SDS)	Overweight
LISAplus, *n* = 1,965, 1997–1999 (Germany)	1,697 (86.4)	87 (4.4)	181 (9.2)	1,557 (82.0)	342 (18.0)	NA	NA	NA	NA	62.7 (60.2–74.0)	−0.09 (−1.92 to 1.88)	201 (10.2)
LUKAS, *n* = 441, 2002–2005 (Finland)	371 (84.1)	35 (7.9)	35 (7.9)	NA	NA	NA	NA	3,630 (2,790–4,689)	44 (10.0)	73.2 (68.6–76.0)	0.52 (−1.08 to 3.33)	114 (31.4)
MoBa, *n* = 80,116, 1999–2009 (Norway)	72,466 (90.5)	NA	7,650 (9.5)	63,071 (79.2)	16,523 (20.8)	40.1 (36.1–42.4)	3,312 (4.1)	3,620 (2,521–4,640)	7,967 (10.0)	85.9 (61.0–100.9)	0.15 (−2.05 to 2.30)	6,002 (19.5)
NINFEA, *n* = 2,259, 2005–2010 (Italy)[a]	2,085 (92.3)	29 (1.3)	145 (6.4)	NA	NA	39.7 (35.9–41.9)	91 (4.0)	3,240 (2,271–4,189)	220 (10.0)	86.1 (84.8–93.1)	−0.02 (−2.16 to 2.43)	95 (21.5)
PÉLAGIE, *n* = 1,353, 2002–2005 (France)	1,022 (75.2)	172 (12.7)	159 (11.8)	597 (61.8)	369 (38.2)	40.0 (36.0–41.0)	44 (3.3)	3,400 (2,460–4,315)	135 (10.0)	NA	NA	NA
Piccolipiù, *n* = 3,292, 2011–2015 (Italy)	2,572 (78.1)	374 (11.4)	346 (10.5)	1,496 (71.4)	598 (28.6)	39.0 (36.0–41.0)	93 (2.9)	3,340 (2,470–4,229)	323 (10.0)	NA	NA	NA
PRIDE Study, *n* = 1,616, 2011–2015 (the Netherlands)	1,519 (94.0)	39 (2.4)	58 (3.6)	NA	NA	39.0 (35.6–41.0)	77 (4.9)	3,484 (2,280–4,500)	154 (9.9)	NA	NA	NA
Project Viva, *n* = 2,001, 1999–2002 (USA)	1,784 (89.2)	124 (6.2)	93 (4.6)	NA	NA	39.7 (34.7–41.9)	142 (7.1)	3,487 (2,155–4,536)	199 (10.0)	92.2 (82.5–116.5)	0.42 (−1.38 to 3.04)	315 (30.6)

Cohort name, number of participants, birth years (country)	Maternal smoking			Paternal smoking		Birth outcomes				Childhood BMI		
	No	First trimester only	Continued	No	Yes	Gestational age at birth (weeks)	Preterm birth	Birth weight (g)	Small size for gestational age at birth	Age (months)	BMI (SDS)	Overweight
REPRO_PL, *n* = 1,434, 2007–2011 (Poland)	1,215 (84.7)	83 (5.8)	136 (9.5)	866 (63.0)	509 (37.0)	39.0 (36.0–41.0)	64 (4.5)	3,350 (2,376–4,290)	142 (10.0)	88.0 (84.3–94.0)	0.64 (−1.55 to 3.64)	19 (38.8)
RHEA, *n* = 651, 2007–2008 (Greece)	544 (83.6)	NA	107 (16.4)	287 (48.6)	303 (51.4)	38.0 (35.0–40.0)	73 (11.3)	3,190 (2,312–4,059)	63 (9.9)	NA	NA	NA
SCOPE BASELINE, *n* = 1,216, 2009–2011 (Ireland)	1,078 (88.7)	NA	138 (11.3)	739 (78.5)	203 (21.5)	40.3 (35.2–41.7)	60 (4.9)	3,460 (2,353–4,485)	121 (10.0)	NA	NA	NA
SWS, *n* = 2,716, 1998–2007 (UK)	2,316 (85.3)	NA	400 (14.7)	NA	NA	40.1 (35.1–42.1)	154 (5.7)	3,450 (2,330–4,475)	268 (10.0)	80.3 (74.7–87.2)	0.21 (−1.51 to 2.47)	368 (22.0)
Total group	196,168 (85.6)	2,247 (1.0)	30,743 (13.4)	134,939 (72.5)	51,084 (27.5)	40.0 (35.7–42.3)	10,299 (4.7)	3,530 (2,390–4,580)	22,312 (10.0)	85.2 (61.0–117.7)	0.13 (−1.86 to 2.43)	21,345 (20.0)

Values are expressed as number of participants (valid %) or medians (95% range). First trimester refers to mothers who smoked during first trimester only. Childhood overweight also includes obesity and includes information at child age ≥5 to <10 years. Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex- and gestational age–adjusted birth weight SDS per cohort.

[a] Subset of participants with follow-up completed at 4 years of child's age by the time of data transfer (March 2015).

Abbreviations: BMI, body mass index; NA, not available (not collected or not provided) or not applicable (gestational age at birth [FCOU, GINIplus, LISAplus, LUKAS] and birth weight [GINIplus, LISAplus] due to study samples restricted to specific ranges of gestational age and weight at birth); SDS, standard deviation score

Changes in maternal smoking habits during pregnancy and the risks of preterm birth, SGA, and childhood overweight

Table 2 shows that maternal first trimester smoking only was not associated with adverse birth outcomes but was associated with higher risks of childhood overweight (odds ratio [OR] 1.17 (95% CI 1.02–1.35), *P* value = 0.030). Compared with children from mothers who did not smoke during pregnancy, those from mothers who continued smoking had higher risks of preterm birth (OR 1.08 [1.02–1.15], *P* value = 0.012), SGA (OR 2.15 [2.07–2.33], *P* value < 0.001), and childhood overweight (OR 1.42 [1.35–1.48], *P* value < 0.001). We observed dose-response relationships for third trimester smoking starting at ≤4 cigarettes/day. We observed similar results when we used the continuous outcomes, except for the association of first-trimester-only smoking with childhood BMI SDS, which was in the same direction but no longer significant (S4 Table). We observed similar results when using two-stage random-effects models (Figure 2, 3, and 4). We observed low to moderate heterogeneity between the cohorts' estimates (I^2 estimates range from 0% to 47%; corresponding CIs are presented in the footnotes of Figures 2, 3, and 4). Only the cohort-specific results for the associations of maternal continued smoking with SGA showed high heterogeneity between estimates (I^2 75% [95% CI 56%–86%]). Almost all cohorts were included in the analyses for continued smoking, whereas only roughly half had information on first-trimester-only smoking. When restricting the two-stage continued smoking models to the cohorts also with information on first-trimester-only smoking, we observed a lower heterogeneity between estimates (I^2 23% [95% CI 0%–65%]), but the pooled risk estimate remained similar (S1 Figure).

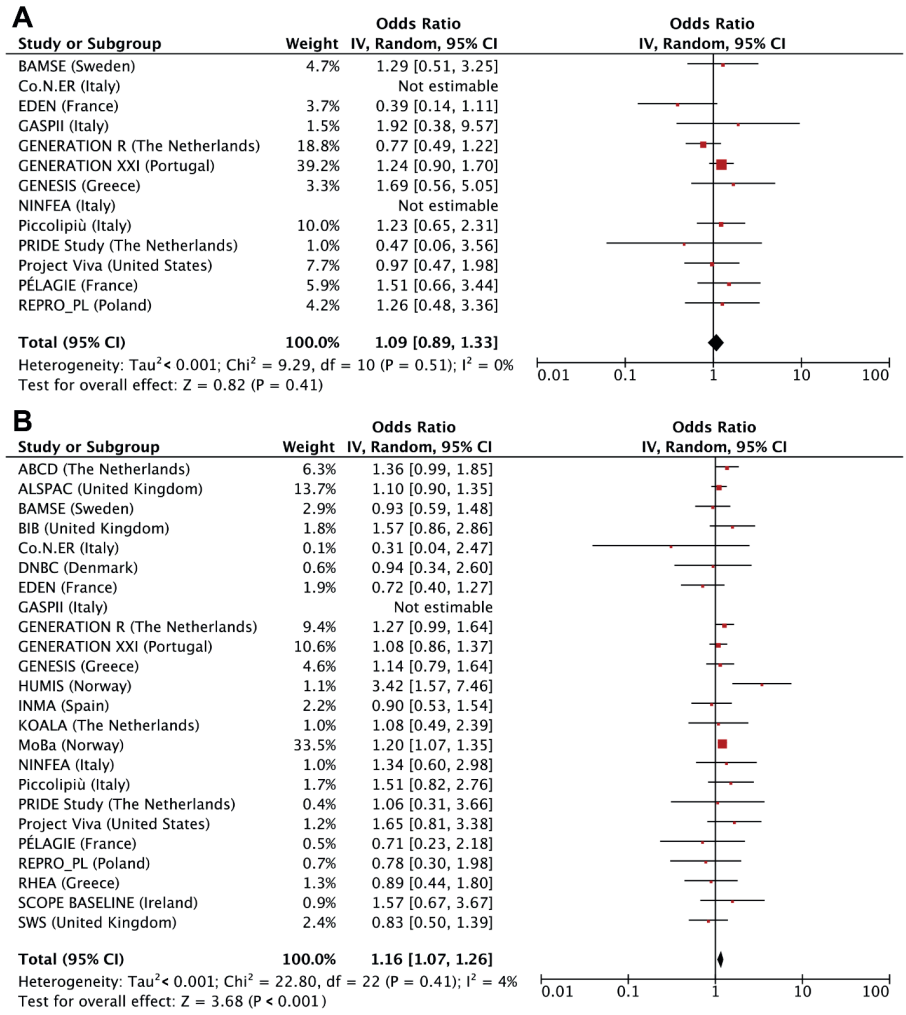
Table 3 shows that, compared with mothers who did not smoke during pregnancy, mothers who quit smoking from first to third trimester had similar risks of delivering SGA infants. Reducing the number of cigarettes, without quitting, from first to third trimester lowered the risks of delivering SGA infants, but risks were still higher compared with those of nonsmoking mothers (OR 1.89 [1.52–2.34] when reducing from 5–9 to ≤4 cigarettes/day; 2.79 [2.39–3.25] and 1.93 [1.46–2.57] when reducing from ≥10 to 5–9 and ≤ 4 cigarettes/day, respectively [all *P* values < 0.001]). Mothers who increased the number of cigarettes from first to third trimester increased their risks of delivering SGA infants (OR 2.43 [2.05–2.89] and 2.47 [1.71–3.58] when increasing from ≤4 to 5–9 and ≥10 cigarettes/day, respectively; and 2.70 [2.35–3.10] when increasing from 5–9 to ≥10 cigarettes/day [all *P* value < 0.001]). Changes in maternal smoking from first to third trimester did not influence the risks of preterm birth and childhood overweight. Similar results were observed when assessing the associations of the changes in maternal smoking during pregnancy with the continuous outcomes (S5 Table).

Table 2. Maternal smoking with risks of birth complications and childhood overweight

Maternal smoking	Preterm birth	Small size for gestational age at birth	Childhood overweight
	Odds ratio (95% confidence interval)	**Odds ratio (95% confidence interval)**	**Odds ratio (95% confidence interval)**
No maternal smoking	Reference $n_{cases/total}$ = 8,586/188,357	Reference $n_{cases/total}$ = 16,879/190,873	Reference $n_{cases/total}$ = 17,530/92,434
Only first trimester smoking	1.03 (0.85–1.25) $n_{cases/total}$ = 120/2,116	0.99 (0.85–1.15) $n_{cases/total}$ = 200/2,144	1.17 (1.02–1.35)* $n_{cases/total}$ = 329/1,084
First trimester dosage			
≤4 cigarettes/day	0.99 (0.70–1.39) $n_{cases/total}$ = 36/828	0.96 (0.75–1.22) $n_{cases/total}$ = 77/826	1.02 (0.78–1.33) $n_{cases/total}$ = 78/340
5–9 cigarettes/day	1.00 (0.58–1.72) $n_{cases/total}$ = 14/288	0.90 (0.59–1.36) $n_{cases/total}$ = 25/288	1.37 (0.92–2.06) $n_{cases/total}$ = 35/136
≥10 cigarettes/day	0.81 (0.45–1.46) $n_{cases/total}$ = 12/273	0.88 (0.57–1.35) $n_{cases/total}$ = 23/271	1.31 (0.89–1.93) $n_{cases/total}$ = 40/152
Continued smoking	1.08 (1.02–1.15)* $n_{cases/total}$ = 1,593/29,951	2.15 (2.07–2.23)** $n_{cases/total}$ = 5,233/30,125	1.42 (1.35–1.48)** $n_{cases/total}$ = 3,486/13,083
Continued smoking dosage			
≤4 cigarettes/day	1.01 (0.89–1.14) $n_{cases/total}$ = 288/5,866	1.57 (1.45–1.70)** $n_{cases/total}$ = 836/6,034	1.30 (1.18–1.42)** $n_{cases/total}$ = 688/2,792
5–9 cigarettes/day	1.07 (0.95–1.19) $n_{cases/total}$ = 367/7,115	2.40 (2.25–2.56)** $n_{cases/total}$ = 1,341/7,162	1.42 (1.30–1.55)** $n_{cases/total}$ = 813/3,284
≥10 cigarettes/day	1.11 (1.01–1.22)* $n_{cases/total}$ = 524/9,771	2.93 (2.76–3.10)** $n_{cases/total}$ = 2,001/9,743	1.55 (1.43–1.67)** $n_{cases/total}$ = 1,137/4,139

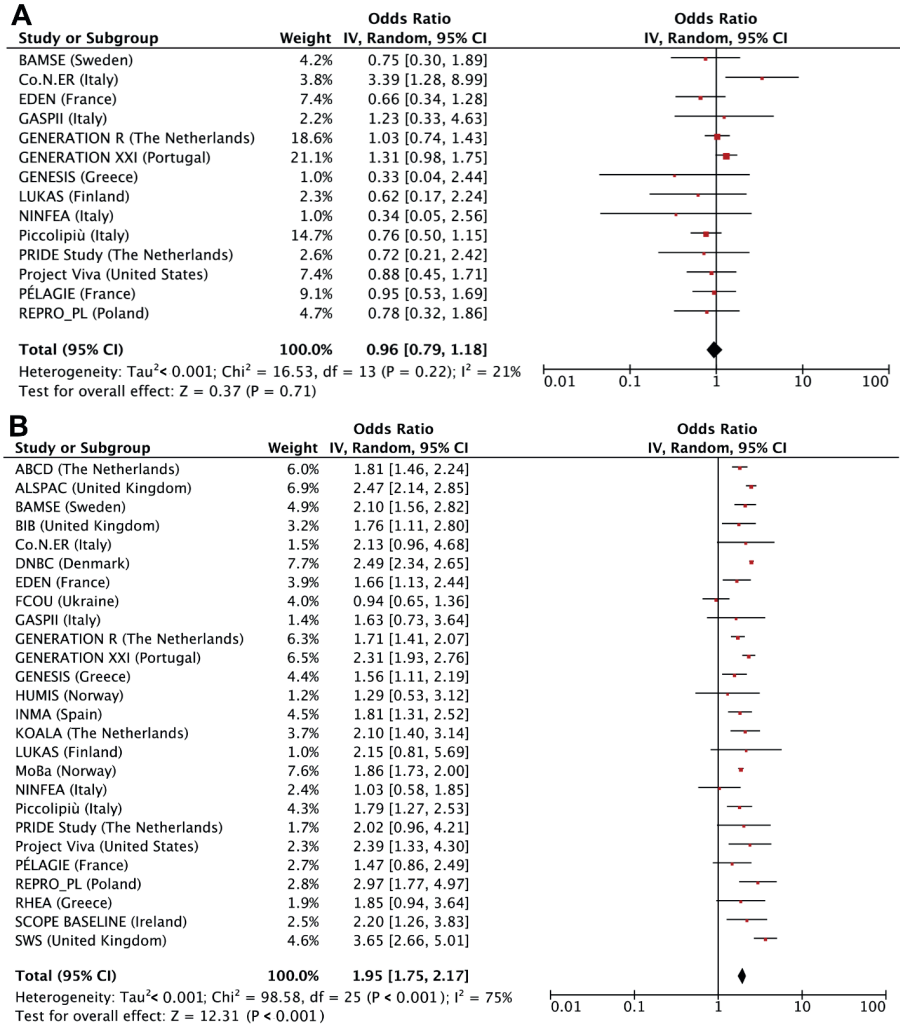
Values are odds ratios (95% confidence intervals) from multilevel binary logistic mixed-effects models that reflect the risk of preterm birth, small size for gestational age, and childhood overweight per smoking group compared with the reference group (no maternal smoking).
Number of cigarettes used as continued smoking dosage was based on third trimester information. Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex- and gestational age–adjusted birth weight standard deviation score per cohort. Childhood overweight is overweight and obesity together according to the World Health Organization criteria. Models are adjusted for maternal age, educational level, parity, prepregnancy or early-pregnancy body mass index, alcohol consumption during pregnancy, and paternal smoking.
**P* value < 0.05.
***P* value < 0.001.

Figure 2. Maternal smoking with risks of preterm birth assessed by 2-stage random-effects models



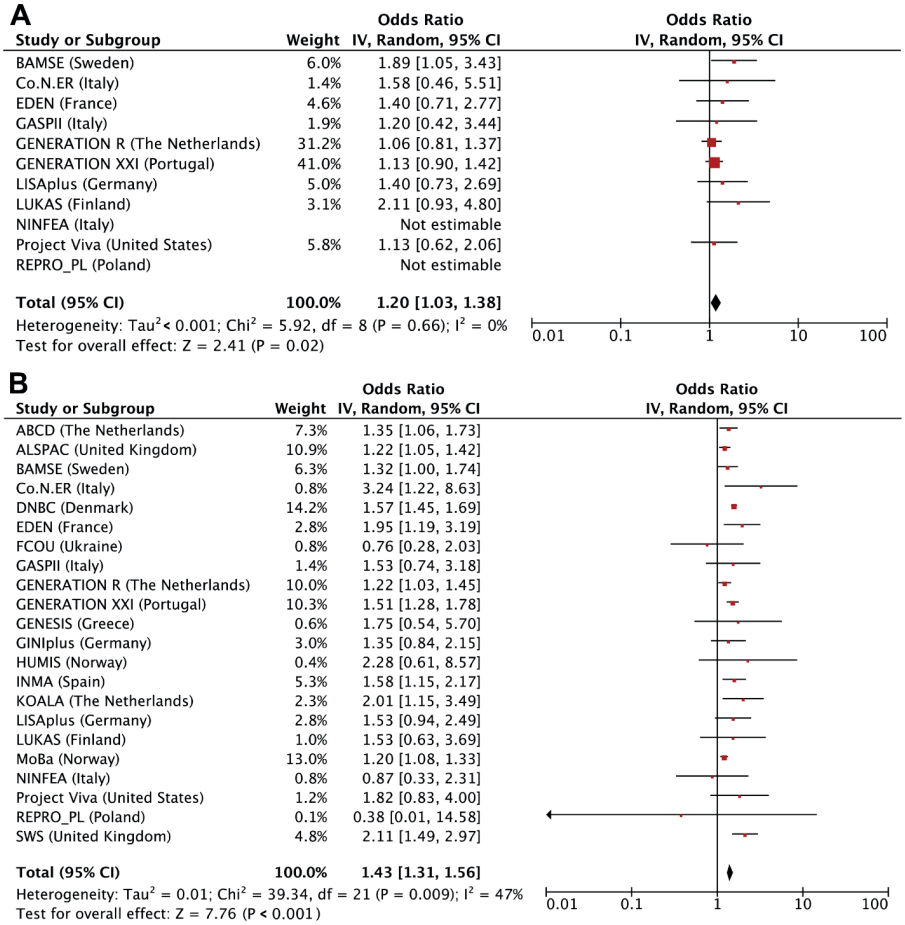
(A) First trimester smoking versus nonsmoking, (B) continued smoking versus nonsmoking. Values are odds ratios (95% CIs) per cohort and pooled from binary logistic regression models that reflect the risk of preterm birth per smoking pattern (first-trimester-only smoking or continued smoking) compared to that of nonsmoking. Models are adjusted for maternal age, educational level, parity, prepregnancy or early-pregnancy body mass index, alcohol consumption during pregnancy, and paternal smoking. The cohorts for which no estimate was provided had no data available for that particular analysis. The heterogeneity between the estimates of each cohort was 0% (95% CI 0%–57%) and 4% (95% CI 0%–47%) for first-trimester-only smoking and continued smoking, respectively. CI, confidence interval, IV, instrumental variable

Figure 3. Maternal smoking with risks of small size for gestational age assessed by two-stage random-effects models



(A) First trimester smoking versus nonsmoking, (B) continued smoking versus nonsmoking. Values are odds ratios (95% CIs) per cohort and pooled from binary logistic regression models that reflect the risk of small size for gestational age per smoking pattern (first-trimester-only smoking or continued smoking) compared to that of nonsmoking. Models are adjusted for maternal age, educational level, parity, prepregnancy or early-pregnancy body mass index, alcohol consumption during pregnancy, and paternal smoking. The cohorts for which no estimate was provided had no data available for that particular analysis. The heterogeneity between the estimates of each cohort was 21% (95% CI 0%–65%) and 75% (95% CI 56%–86%) for first-trimester-only smoking and continued smoking, respectively. CI, confidence interval, IV, instrumental variable

Figure 4. Maternal smoking with risks of childhood overweight assessed by two-stage random-effects models



(A) First trimester smoking versus nonsmoking, (B) continued smoking versus nonsmoking. Values are odds ratios (95% CIs) per cohort and pooled from binary logistic regression models that reflect the risk of childhood overweight per smoking pattern (first-trimester-only smoking or continued smoking) compared to that of nonsmoking. Models are adjusted for maternal age, educational level, parity, prepregnancy or early-pregnancy body mass index, alcohol consumption during pregnancy, and paternal smoking. The cohorts for which no estimate was provided had no data available for that particular analysis. The heterogeneity between the estimates of each cohort was 0% (95% CI 0%–60%) and 47% (95% CI 1%–72%) for first-trimester-only smoking and continued smoking, respectively. CI, confidence interval, IV, instrumental variable

Table 3. Change in maternal smoking habits during pregnancy and risks of birth complications and childhood overweight

Maternal smoking	Preterm birth Odds ratio (95% confidence interval)	Small size for gestational age at birth Odds ratio (95% confidence interval)	Childhood overweight Odds ratio (95% confidence interval)
No maternal smoking in first trimester			
Third trimester no smoking	Reference $n_{cases/total}$ = 4,527/100,634	Reference $n_{cases/total}$ = 8,698/103,740	Reference $n_{cases/total}$ = 11,177/59,070
Third trimester ≤4 cigarettes/day	0.73 (0.40–1.34) $n_{cases/total}$ = 11/278	1.20 (0.81–1.78) $n_{cases/total}$ = 28/274	1.31 (0.90–1.92) $n_{cases/total}$ = 41/147
Third trimester 5–9 cigarettes/day	1.07 (0.48–2.48) $n_{cases/total}$ = 6/104	2.02 (1.18–3.46)* $n_{cases/total}$ = 16/103	1.27 (0.64–2.37) $n_{cases/total}$ = 13/51
Third trimester ≥10 cigarettes/day	1.51 (0.65–3.49) $n_{cases/total}$ = 6/80	1.74 (0.91–3.32) $n_{cases/total}$ = 11/79	1.60 (0.72–3.55) $n_{cases/total}$ = 10/31
Maternal smoking in first trimester ≤4 cigarettes/day			
Third trimester quit	0.96 (0.69–1.35) $n_{cases/total}$ = 38/862	1.04 (0.82–1.31) $n_{cases/total}$ = 84/859	1.20 (0.94–1.53) $n_{cases/total}$ = 98/388
Third trimester ≤4 cigarettes/day	1.05 (0.86–1.27) $n_{cases/total}$ = 114/2,261	1.54 (1.37–1.74)** $n_{cases/total}$ = 328/2,457	1.32 (1.14–1.52)** $n_{cases/total}$ = 289/1,169
Third trimester 5–9 cigarettes/day	1.15 (0.85–1.55) $n_{cases/total}$ = 47/885	2.43 (2.05–2.89)** $n_{cases/total}$ = 170/880	1.81 (1.45–2.25)** $n_{cases/total}$ = 121/440
Third trimester ≥10 cigarettes/day	1.37 (0.76–2.47) $n_{cases/total}$ = 12/186	2.47 (1.71–3.58)** $n_{cases/total}$ = 36/185	1.31 (0.79–2.19) $n_{cases/total}$ = 21/86
Maternal smoking in first trimester 5–9 cigarettes/day			
Third trimester quit	1.04 (0.62–1.73) $n_{cases/total}$ = 16/304	0.95 (0.64–1.42) $n_{cases/total}$ = 27/304	1.32 (0.91–1.92) $n_{cases/total}$ = 41/165
Third trimester ≤4 cigarettes/day	0.86 (0.58–1.28) $n_{cases/total}$ = 27/657	1.89 (1.52–2.34)** $n_{cases/total}$ = 102/654	1.53 (1.17–2.00)* $n_{cases/total}$ = 80/307
Third trimester 5–9 cigarettes/day	1.00 (0.85–1.18) $n_{cases/total}$ = 163/3,551	2.21 (2.02–2.42)** $n_{cases/total}$ = 630/3,617	1.43 (1.26–1.61)** $n_{cases/total}$ = 403/1,704

Maternal smoking	Preterm birth Odds ratio (95% confidence interval)	Small size for gestational age at birth Odds ratio (95% confidence interval)	Childhood overweight Odds ratio (95% confidence interval)
Third trimester ≥10 cigarettes/day	0.99 (0.76–1.30) $n_{cases/total}$ = 59/1,330	2.70 (2.35–3.10)** $n_{cases/total}$ = 265/1,319	1.40 (1.15–1.69)* $n_{cases/total}$ = 149/632
Maternal smoking in first trimester ≥10 cigarettes/day			
Third trimester quit	0.82 (0.46–1.43) $n_{cases/total}$ = 13/285	1.06 (0.71–1.57) $n_{cases/total}$ = 28/283	1.34 (0.96–1.88) $n_{cases/total}$ = 52/194
Third trimester ≤4 cigarettes/day	1.26 (0.82–1.95) $n_{cases/total}$ = 22/358	1.93 (1.46–2.57)** $n_{cases/total}$ = 59/354	1.14 (0.81–1.61) $n_{cases/total}$ = 48/192
Third trimester 5–9 cigarettes/day	1.26 (0.97–1.63) $n_{cases/total}$ = 62/1,078	2.79 (2.39–3.25)** $n_{cases/total}$ = 224/1,072	1.46 (1.18–1.80)** $n_{cases/total}$ = 128/503
Third trimester ≥10 cigarettes/day	1.16 (1.04–1.31)* $n_{cases/total}$ = 364/6,949	2.95 (2.75–3.15)** $n_{cases/total}$ = 1,434/6,940	1.67 (1.53–1.83)** $n_{cases/total}$ = 849/2,976

Values are odds ratios (95% confidence intervals) from multilevel binary logistic mixed-effects models that reflect the risk of preterm birth, small size for gestational age, and childhood overweight per change in smoking group compared with that of the reference group (nonsmoking in first and third trimester). Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex- and gestational age–adjusted birth weight standard deviation score per cohort. Childhood overweight is overweight and obesity together according to the World Health Organization criteria. Models are adjusted for maternal age, educational level, parity, prepregnancy body mass index, alcohol consumption during pregnancy, and paternal smoking.

*P value < 0.05.

**P value < 0.001.

Parental smoking during pregnancy and the risks of preterm birth, SGA, and childhood overweight

Among mothers who did not smoke during pregnancy, paternal smoking tended to be associated with higher risks of preterm birth (OR 1.06 [1.00–1.12], *P* value = 0.05), SGA (OR 1.04 [1.00–1.09], *P* value = 0.05), and childhood overweight (OR 1.21 [1.16–1.27], *P* value < 0.001) (Table 4). Among mothers who smoked during first trimester only, paternal smoking was not associated with preterm birth or SGA but was associated with a higher risk of childhood overweight (OR 1.36 [1.02–1.80], *P* value = 0.036). Among mothers who continued smoking during pregnancy, paternal smoking further increased the risks of SGA and childhood overweight (both Z-score *P* value for differences in effect sizes between categories <0.0001) but not the risk of preterm birth. Children whose mothers continued smoking during pregnancy and whose fathers also smoked had the highest risks of being born preterm (OR 1.10 [1.02–1.19], *P* value = 0.016) and SGA (OR 2.37 [2.26–2.49], *P* value < 0.001) and of childhood overweight (OR 1.76 [1.65–1.87], *P* value < 0.001). Similar results were observed for the combined maternal and paternal smoking with the continuous outcomes (S6 Table).

Discussion

In this study, maternal continued smoking during pregnancy was associated, in a dose-response manner, to higher risks of preterm birth, being SGA at birth, and childhood overweight. Maternal smoking during the first trimester of pregnancy only was not associated with risks of preterm birth and SGA but was associated with a higher risk of childhood overweight. Reducing the number of cigarettes during pregnancy without quitting may be beneficial for the risk of SGA but seems not to influence the risks of preterm birth and childhood overweight. Paternal smoking seems to be associated, independently of maternal smoking, with the risks of childhood overweight.

Maternal smoking is a major public health concern.[1] The associations of maternal continued smoking during pregnancy and increased risks of preterm birth and SGA are well established.[7,10,18] Also, several studies have suggested associations of fetal smoke exposure with childhood overweight and obesity.[11,22] In line with these previous studies, we observed that children whose mothers continued smoking during pregnancy have higher risks of preterm birth, being SGA at birth, and overweight in childhood. The risks of preterm birth were somewhat weaker than reported previously,[7,9,18] potentially because no information was available about induced or spontaneous preterm birth.

Results from previous studies focused on the associations of maternal early smoking cessation and of reducing the number of cigarettes during pregnancy with child health outcomes are inconsistent.[8,17,19,21,22] Results from prospective studies in the Netherlands and Australia previously suggested that quitting smoking after the first trimester was not associated with risks of adverse birth outcomes.[18,19] A large US study with more than 21,000 first trimester smokers reported that smoking of any duration during pregnancy was associated with an increased risk of fetal growth restriction with decreasing risk the earlier that cessation occurred.[17] Similarly, a recent study from the UK Millennium Cohort Study suggested that two-thirds of the total adverse smoking impact on birth weight occurs in the second trimester and that cutting smoking intensity by the third month in pregnancy leads to infants of the

Table 4. Associations of maternal and paternal smoking with risks of birth complications and childhood overweight

Maternal and paternal smoking	Preterm birth	Small size for gestational age at birth	Childhood overweight
	Odds ratio (95% confidence interval)	Odds ratio (95% confidence interval)	Odds ratio (95% confidence interval)
Maternal nonsmoking			
Paternal nonsmoking	Reference $n_{cases/total}$ = 5,232/123,666	Reference $n_{cases/total}$ = 10,746/123,328	Reference $n_{cases/total}$ = 10,298/59,395
Paternal smoking	1.06 (1.00–1.12) $n_{cases/total}$ = 1,505/31,890	1.04 (1.00–1.09) $n_{cases/total}$ = 3,030/33,691	1.21 (1.16–1.27)** $n_{cases/total}$ = 3,199/15,474
Maternal first trimester smoking			
Paternal nonsmoking	0.64 (0.36–1.15) $n_{cases/total}$ = 12/412	0.78 (0.53–1.13) $n_{cases/total}$ = 30/412	1.36 (0.98–1.87) $n_{cases/total}$ = 54/233
Paternal smoking	1.03 (0.70–1.51) $n_{cases/total}$ = 29/626	1.05 (0.80–1.39) $n_{cases/total}$ = 59/625	1.36 (1.02–1.80)* $n_{cases/total}$ = 70/305
Maternal continued smoking			
Paternal nonsmoking	1.04 (0.93–1.15) $n_{cases/total}$ = 405/8,768	2.06 (1.94–2.20)** $n_{cases/total}$ = 1,366/8,723	1.33 (1.23–1.44)** $n_{cases/total}$ = 877/3,872
Paternal smoking	1.10 (1.02–1.19)* $n_{cases/total}$ = 810/15,806	2.37 (2.26–2.49)** $n_{cases/total}$ = 2,896/15,967	1.76 (1.65–1.87)** $n_{cases/total}$ = 1,785/6,661

Values are odds ratios (95% confidence intervals) from multilevel binary logistic mixed-effects models that reflect the risk of preterm birth, small size for gestational age, and childhood overweight per smoking group compared with the reference group (no parental smoking).
Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex- and gestational age–adjusted birth weight standard deviation score per cohort. Childhood overweight is overweight and obesity together according to the World Health Organization criteria. Models are adjusted for maternal age, maternal body mass index, paternal body mass index, maternal education, parity, and maternal alcohol consumption during pregnancy.
*P value < 0.05.
**P value < 0.001.

same weight as those infants born to persistent light smokers.[37] A recent study investigating associations of parental smoking with fetal growth using additional methods of mendelian randomization and parental negative control showed consistent linear dose-dependent associations of maternal smoking with fetal growth from early second trimester onward.[38] These studies suggest that smoking cessation programs should focus on the benefit of quitting as early in pregnancy as possible. A previous analysis using data from the Nurses' Health Study showed that first-trimester-only maternal smoking was not, or was only to a limited extent, associated with obesity in later life.[20] However, in the same cohort, first-trimester-only maternal smoking was associated with type 2 diabetes in the offspring.[39] In the current study, maternal first-trimester-only smoking was not associated with the risks of preterm birth or SGA but was associated with an increased risk of childhood overweight. A biological explanation might be that maternal first-trimester-only smoking already leads to specific adaptations, which might have lifelong consequences for body composition and metabolic health in later life, but the fetal smoke exposure is not long enough to affect birth outcomes. Reducing the number of cigarettes from first to

third trimester lowered the risks of SGA, but risks were still elevated compared with those in infants born to nonsmoking mothers. This association was not observed for preterm birth and childhood overweight. Thus, our findings suggest that quitting smoking in the first trimester of pregnancy might optimize birth outcomes but might not reduce the risk of adverse metabolic effects in the offspring to the level of nonsmoking. Also, reducing the number of cigarettes from first trimester onward may reduce risks of fetal growth restriction.

The role of paternal smoking during pregnancy on child health outcomes remains unclear.[23,40,41] Paternal smoking has been associated with reduced semen quality and fertility and higher risks of spontaneous abortion, birth defects, and, in the long-term, attention-deficit/hyperactivity disorder and several cancers.[42-45] A recent meta-analysis showed that paternal smoking was associated with increased risks of preterm birth and SGA.[44] In a previous Dutch study, paternal smoking during pregnancy among nonsmoking mothers was associated with higher childhood BMI.[12] A small study from the US using self-reported smoking and serum cotinine measurements found a higher BMI at 2 and 3 years of age in children whose mothers were exposed to passive smoking during pregnancy.[40] In the current study, paternal smoking among nonsmoking mothers was associated with a higher risk of childhood overweight and tended to be associated with higher risks of preterm birth and SGA. This suggests that paternal smoking may be, independently of maternal smoking, associated with childhood overweight. However, we cannot exclude the possibility of residual confounding by factors not or insufficiently measured in the studies. Previous studies used comparisons of maternal and paternal smoking associations to explore potential mechanisms.[12,46] In the current study, if only one parent smoked, the risks of SGA were much higher among maternal smokers than among paternal smokers, whereas the risks of preterm birth for maternal and paternal smoking were similar. The similar associations of maternal and paternal smoking and preterm birth may suggest that the underlying mechanisms include shared family-based characteristics, such as environmental exposures and lifestyle. The stronger associations of maternal smoking, compared with paternal smoking, with SGA may suggest that these associations are mainly explained by intrauterine mechanisms. Since paternal smoking among nonsmoking mothers was not associated with SGA, the risk increase when both parents smoked may represent an additional mechanistic pathway through shared family-based characteristics. The risk of overweight was slightly higher among children whose mothers smoked than whose fathers smoked. However, the risks increased significantly if both parents smoked. These findings suggest that, although intrauterine programming mechanisms might play a role, shared family-based lifestyle and genetic characteristics are potential underlying mechanisms. Whether these findings also reflect transgenerational epigenetic inheritance through the gametes needs to be further studied.

Various components of tobacco smoke might be involved in the mechanistic pathway toward adverse birth outcomes and childhood overweight. Both nicotine and carbon monoxide are reported to reduce placental blood flow.[47] Nicotine stimulates acetylcholine receptors, which release a multitude of vasoactive catecholamines and peptides, which in turn reduce blood flow through vasoconstriction.[47] Carbon monoxide competes with oxygen for binding sites on the transport protein hemoglobin, causing hypoxia.[48] Chronic hypoxia interferes with the maternal circulatory adjustments to pregnancy which can be another cause of reduced placental blood flow.[49] Uterine blood flow is essential for uterine, placental, and fetal growth. Several mechanisms for nicotine-induced alterations in overweight risks

have been proposed, including stimulation of the fetal hypothalamic-pituitary axis.[50] It has been suggested that cadmium, present in tobacco smoke, modulates oxytocin receptor function, proposing a role in the pathophysiology of preterm birth.[48] Recent studies have found an association between maternal smoking during pregnancy and birth weight with a mediating role of DNA methylation.[51-53] Further research is needed to assess such possible mechanisms. During the last few years, e-cigarettes have been widely used as substitutes for smoking. Evidence from recently started cohorts is needed to clarify whether e-cigarettes are any safer during pregnancy.

We performed an individual participant data meta-analysis of prospective cohort studies to investigate the associations of parental smoking during pregnancy with preterm birth, SGA, and childhood overweight. We included data from cohort studies in Europe and North America, so our findings are mainly applicable to populations in developed countries. Inclusion of data from other regions could have led to differences in prevalence of maternal and paternal smoking, birth complications, childhood overweight, and ethnic and sociodemographic characteristics, complicating or limiting the possibility of doing a meta-analysis. Among study limitations, our outcomes might not be generalizable to populations from low-income and middle-income countries, which need to be further studied. The large sample size enabled us to investigate the effects of changing smoking habits and paternal smoking. However, our study might have been underpowered to detect associations in the analyses looking at maternal-only first trimester smoking and the change in smoking habits from first to third trimester, due to small sample sizes. Since we used original, individual participant data, we did not formally assess the quality of the individual studies included. We are aware that our study cannot overcome potential limitations of individual studies in terms of their design and conduct, differences in the definitions of exposure and outcome data, and variation in missing data. Parental smoking information during pregnancy was self-reported. For active smoking, correlations between cotinine measurements and self-reported smoking habits are high.[54] We have no information on the specific question asked or the timing in which it was asked, which might have differed across cohorts and influenced our results. It has been suggested that using maternal nonsmokers as a reference group without considering the impact of passive smoke exposure may contribute to an underestimation of the estimated effects.[40] To limit this misclassification, all analyses on maternal smoking were adjusted for paternal smoking. Although smoking in the preconception period has been reported not to be associated with fetal growth restriction, studies considering its effect on childhood overweight are lacking.[17] In the current study, information on smoking in the preconception period was missing. Further research is needed to assess the associations of smoking in the preconception period with offspring outcomes. It has been suggested that exposure to smoking during childhood amplifies the association between prenatal smoke exposure and childhood BMI outcomes.[55] Many women resume smoking shortly after birth. Six weeks after birth, approximately 25% of women resumed smoking, and 1 year after birth these numbers are up to 80%.[56] In our study, information on exposure to smoking during childhood was not available for most cohorts. Further research is needed to assess whether childhood BMI outcomes are additionally influenced by exposure to smoking during childhood. Overall, we observed low to moderate heterogeneity in the 2-stage random-effects models, which might be due to the inclusion of mostly high-income and Caucasian cohorts. However, we observed high heterogeneity between the cohorts for the associations of maternal continued smoking with SGA. This might be in part explained by differences in pattern and dosage of maternal and paternal smoking between cohorts. When we restricted the 2-stage continued

smoking models to the cohorts that also had information on first-trimester-only smoking, we observed a substantially lower heterogeneity between estimates. Missing values of covariates were used as an additional group. This approach has been commonly used in large meta-analyses of individual participant data because of the constraints in applying more advanced imputation strategies. Although we cannot disregard the possibility of bias, we consider it unlikely considering the relatively small percentage of missing data.[57] We observed similar results when we conducted a complete case analysis (S7 Table). Also, similar associations were observed when adjusting for maternal age and BMI as categorical or continuous covariates (S7 and S8 Tables). Although we adjusted for multiple lifestyle-related factors, we cannot exclude residual confounding by other environmental lifestyle-related factors. From the current observational data, no conclusions can be drawn on the causality of the observed associations.

Our results suggest that as compared to mothers who continued smoking throughout pregnancy, mothers who quit smoking during the first trimester have a reduced risk of birth complications. Reducing the number of cigarettes without quitting during pregnancy is still associated with an increased risk of birth complications. The observed risk estimates were small to moderate but are important from a public health perspective, since smoking is a common adverse exposure and preterm birth and SGA are among the most frequent birth complications. Also, preterm birth, SGA, and childhood obesity are related with adverse health consequences later in life. Our findings suggest that it is of great importance to invest in prevention of smoking in women of reproductive age before or at the start of pregnancy. Pregnant women should still be motivated to reduce smoking, even later in pregnancy. The current guidelines focus only on quitting smoking and not reducing, which can be discouraging for women who find it difficult to quit smoking. These women should be provided with sufficient information about the risks of continued smoking but also about the benefits of reducing their number of cigarettes. Future research should investigate whether quitting smoking in the first trimester or reducing the number of cigarettes during pregnancy is also beneficial for other adverse birth and offspring outcomes. Although we cannot exclude a role of residual confounding and shared family-based characteristics in the associations of paternal smoking with childhood overweight, we recommend that fathers are more closely involved in preconception and pregnancy consultations focused on smoking reduction.

Our results suggest that maternal smoking during the first trimester only is not associated with the risks of SGA and preterm birth but is associated with a higher risk of childhood overweight. Reducing the number of cigarettes during pregnancy without quitting may be beneficial for the risk of SGA but does not influence the risks of preterm birth and childhood overweight. Paternal smoking seems to be associated, independently of maternal smoking, with the risks of childhood overweight. Population strategies should focus on parental smoking prevention before or at the start of, rather than during, pregnancy.

References

1. Samet JM, Yoon SY. Women and the tabacco epidemic: challenges for the 21st century: WHO, Institute for Global Tabacco Control, Johns Hopkins School of Public Health; 2011.
2. Lange S, Probst C, Rehm J, Popova S. National, regional, and global prevalence of smoking during pregnancy in the general population: a systematic review and meta-analysis. Lancet Glob Health. 2018;6(7):e769-e76.
3. Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. Nicotine Tob Res. 2004;6 Suppl 2:S125-40.
4. Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update. 2011;17(5):589-604.
5. Marufu TC, Ahankari A, Coleman T, Lewis S. Maternal smoking and the risk of still birth: systematic review and meta-analysis. BMC Public Health. 2015;15:239.
6. Zhang K, Wang X. Maternal smoking and increased risk of sudden infant death syndrome: a meta-analysis. Leg Med (Tokyo). 2013;15(3):115-21.
7. Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol. 2000;182(2):465-72.
8. Raisanen S, Sankilampi U, Gissler M, Kramer MR, Hakulinen-Viitanen T, Saari J, et al. Smoking cessation in the first trimester reduces most obstetric risks, but not the risks of major congenital anomalies and admission to neonatal care: a population-based cohort study of 1,164,953 singleton pregnancies in Finland. J Epidemiol Community Health. 2014;68(2):159-64.
9. Moore E, Blatt K, Chen A, Van Hook J, DeFranco EA. Relationship of trimester-specific smoking patterns and risk of preterm birth. Am J Obstet Gynecol. 2016.
10. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65(5):663-737.
11. Rayfield S, Plugge E. Systematic review and meta-analysis of the association between maternal smoking in pregnancy and childhood overweight and obesity. J Epidemiol Community Health. 2016.
12. Durmus B, Heppe DH, Taal HR, Manniesing R, Raat H, Hofman A, et al. Parental smoking during pregnancy and total and abdominal fat distribution in school-age children: the Generation R Study. Int J Obes (Lond). 2014;38(7):966-72.
13. Albers L, Sobotzki C, Kuss O, Ajslev T, Batista RF, Bettiol H, et al. Maternal smoking during pregnancy and offspring overweight: is there a dose-response relationship? An individual patient data meta-analysis. Int J Obes (Lond). 2018;42(7):1249-64.
14. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. Lancet. 2008;371(9608):261-9.
15. Daniels SR, Jacobson MS, McCrindle BW, Eckel RH, Sanner BM. American Heart Association Childhood Obesity Research Summit Report. Circulation. 2009;119(15):e489-517.
16. Ludvigsson JF, Lu D, Hammarstrom L, Cnattingius S, Fang F. Small for gestational age and risk of childhood mortality: A Swedish population study. PLoS Med. 2018;15(12):e1002717.
17. Blatt K, Moore E, Chen A, Van Hook J, DeFranco EA. Association of reported trimester-specific smoking cessation with fetal growth restriction. Obstet Gynecol. 2015;125(6):1452-9.
18. Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, et al. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. Paediatr Perinat Epidemiol. 2008;22(2):162-71.
19. McCowan LM, Dekker GA, Chan E, Stewart A, Chappell LC, Hunter M, et al. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. BMJ. 2009;338:b1081.

20. Harris HR, Willett WC, Michels KB. Parental smoking during pregnancy and risk of overweight and obesity in the daughter. Int J Obes (Lond). 2013;37(10):1356-63.
21. Grzeskowiak LE, Hodyl NA, Stark MJ, Morrison JL, Clifton VL. Association of early and late maternal smoking during pregnancy with offspring body mass index at 4 to 5 years of age. J Dev Orig Health Dis. 2015;6(6):485-92.
22. Durmus B, Kruithof CJ, Gillman MH, Willemsen SP, Hofman A, Raat H, et al. Parental smoking during pregnancy, early growth, and risk of obesity in preschool children: the Generation R Study. Am J Clin Nutr. 2011;94(1):164-71.
23. Inoue S, Naruse H, Yorifuji T, Kato T, Murakoshi T, Doi H, et al. Impact of maternal and paternal smoking on birth outcomes. J Public Health (Oxf). 2016.
24. LifeCycle Project-Maternal O, Childhood Outcomes Study G, Voerman E, Santos S, Inskip H, Amiano P, et al. Association of Gestational Weight Gain With Adverse Maternal and Infant Outcomes. JAMA. 2019;321(17):1702-15.
25. Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. PLoS Med. 2019;16(2):e1002744.
26. Santos S, Voerman E, Amiano P, Barros H, Beilin LJ, Bergstrom A, et al. Impact of maternal body mass index and gestational weight gain on pregnancy complications: an individual participant data meta-analysis of European, North American and Australian cohorts. BJOG. 2019;126(8):984-95.
27. Santos S, Eekhout I, Voerman E, Gaillard R, Barros H, Charles MA, et al. Gestational weight gain charts for different body mass index groups for women in Europe, North America, and Oceania. BMC Med. 2018;16(1):201.
28. Patro Golab B, Santos S, Voerman E, Lawlor DA, Jaddoe VWV, Gaillard R, et al. Influence of maternal obesity on the association between common pregnancy complications and risk of childhood obesity: an individual participant data meta-analysis. Lancet Child Adolesc Health. 2018;2(11):812-21.
29. Tucker J, McGuire W. Epidemiology of preterm birth. BMJ. 2004;329(7467):675-8.
30. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand. 1991;90(8-9):756-62.
31. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl. 2006;450:76-85.
32. de Onis M, Onyango AW, Borghi E, Siyam A, Nishida C, Siekmann J. Development of a WHO growth reference for school-aged children and adolescents. Bull World Health Organ. 2007;85(9):660-7.
33. Debray TP, Moons KG, Abo-Zaid GM, Koffijberg H, Riley RD. Individual participant data meta-analysis for a binary outcome: one-stage or two-stage? PLoS One. 2013;8(4):e60650.
34. Paternoster R, Brame R, Mazerolle P, Piquero A. Using the correct statistical test for equality of regression coefficients Criminology. 1998;36(4):859-66.
35. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.
36. Thorlund K, Imberger G, Johnston BC, Walsh M, Awad T, Thabane L, et al. Evolution of heterogeneity (I2) estimates and their 95% confidence intervals in large meta-analyses. PLoS One. 2012;7(7):e39471.
37. Yan J, Groothuis PA. Timing of prenatal smoking cessation or reduction and infant birth weight: evidence from the United Kingdom Millennium Cohort Study. Matern Child Health J. 2015;19(3):447-58.
38. Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Voerman E, et al. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. PLoS Med. 2019;16(11):e1002972.

39. Jaddoe VW, de Jonge LL, van Dam RM, Willett WC, Harris H, Stampfer MJ, et al. Fetal exposure to parental smoking and the risk of type 2 diabetes in adult women. Diabetes Care. 2014;37(11):2966-73.
40. Braun JM, Daniels JL, Poole C, Olshan AF, Hornung R, Bernert JT, et al. Prenatal environmental tobacco smoke exposure and early childhood body mass index. Paediatr Perinat Epidemiol. 2010;24(6):524-34.
41. Qiu J, He X, Cui H, Zhang C, Zhang H, Dang Y, et al. Passive smoking and preterm birth in urban China. Am J Epidemiol. 2014;180(1):94-102.
42. Borges E, Jr., Braga D, Provenza RR, Figueira RCS, Iaconelli A, Jr., Setti AS. Paternal lifestyle factors in relation to semen quality and in vitro reproductive outcomes. Andrologia. 2018:e13090.
43. Wang L, Yang Y, Liu F, Yang A, Xu Q, Wang Q, et al. Paternal smoking and spontaneous abortion: a population-based retrospective cohort study among non-smoking women aged 20-49 years in rural China. J Epidemiol Community Health. 2018;72(9):783-9.
44. Oldereid NB, Wennerholm UB, Pinborg A, Loft A, Laivuori H, Petzold M, et al. The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. Hum Reprod Update. 2018;24(3):320-89.
45. Zhu JL, Olsen J, Liew Z, Li J, Niclasen J, Obel C. Parental smoking during pregnancy and ADHD in children: the Danish national birth cohort. Pediatrics. 2014;134(2):e382-8.
46. Brion MJ, Leary SD, Smith GD, Ness AR. Similar associations of parental prenatal smoking suggest child blood pressure is not influenced by intrauterine effects. Hypertension. 2007;49(6):1422-8.
47. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. Semin Perinatol. 1996;20(2):115-26.
48. Ion R, Bernal AL. Smoking and Preterm Birth. Reprod Sci. 2015;22(8):918-26.
49. Moore LG. Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. High Alt Med Biol. 2003;4(2):141-56.
50. Koshy G, Delpisheh A, Brabin BJ. Dose response association of pregnancy cigarette smoke exposure, childhood stature, overweight and obesity. Eur J Public Health. 2011;21(3):286-91.
51. Richmond RC, Simpkin AJ, Woodward G, Gaunt TR, Lyttleton O, McArdle WL, et al. Prenatal exposure to maternal smoking and offspring DNA methylation across the lifecourse: findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). Hum Mol Genet. 2015;24(8):2201-17.
52. Kupers LK, Xu X, Jankipersadsing SA, Vaez A, la Bastide-van Gemert S, Scholtens S, et al. DNA methylation mediates the effect of maternal smoking during pregnancy on birthweight of the offspring. Int J Epidemiol. 2015;44(4):1224-37.
53. Morales E, Vilahur N, Salas LA, Motta V, Fernandez MF, Murcia M, et al. Genome-wide DNA methylation study in human placenta identifies novel loci associated with maternal smoking during pregnancy. Int J Epidemiol. 2016;45(5):1644-55.
54. Mattsson K, Kallen K, Rignell-Hydbom A, Lindh CH, Jonsson BA, Gustafsson P, et al. Cotinine Validation of Self-Reported Smoking During Pregnancy in the Swedish Medical Birth Register. Nicotine Tob Res. 2016;18(1):79-83.
55. Moller SE, Ajslev TA, Andersen CS, Dalgard C, Sorensen TI. Risk of childhood overweight after exposure to tobacco smoking in prenatal and early postnatal life. PLoS One. 2014;9(10):e109184.
56. Feeney A, Britton G. Counseling Women on Smoking Relapse Prevention During Postpartum. MCN Am J Matern Child Nurs. 2016;41(5):287-92.
57. Groenwold RH, White IR, Donders AR, Carpenter JR, Altman DG, Moons KG. Missing covariate data in clinical research: when and when not to use the missing-indicator method for analysis. CMAJ. 2012;184(11):1265-9.

S1 Text can be found online

S2 Text can be found online

S1 PRISMA Checklist can be found online

S1 Table. Cohort-specific methods of data collection for parental smoking, birth outcomes, and childhood BMI

Cohort name, number of participants, birth years (country)	Maternal smoking	Paternal smoking	Gestational age at birth	Birth weight	Childhood weight and height
ABCD, n=7,324, 2003-2004 (The Netherlands)	Self-reported	NA	Clinical records	Clinical records	Measured
ALSPAC, n=12,148,1991-1992 (United Kingdom)	Self-reported	Self-reported	Clinical records	Measured	Measured
BAMSE, n=4,057, 1994-1996 (Sweden)	Self-reported	Self-reported	Medical Birth Registry	Medical Birth Registry	Measured
BIB, n=1,641, 2007-2010 (United Kingdom)	Self-reported	NA	Clinical records	Clinical records	NA
Co.N.ER, n=641, 2004-2005 (Italy)	Self-reported	Self-reported	Self-reported	Clinical records	Self-reported
DNBC, n=71,710, 1996-2002 (Denmark)	Self-reported	Self-reported	Self-reported or National Medical Birth Registry	National Medical Birth Registry	Self-reported or measured
EDEN, n=1,880, 2003-2005 (France)	Self-reported	Self-reported	Clinical records	Clinical records	Measured or clinical records
FCOU, n=4,003, 1993-1996 (Ukraine)	Self-reported	Self-reported	NA	Clinical records	Clinical records
GASPII, n=680 (Italy)	Self-reported	Self-reported	Clinical records	Clinical records	Measured
GENERATION R, 7,934, 2002-2006 (The Netherlands)	Self-reported	Self-reported	Clinical records	Clinical records	Measured

Cohort name, number of participants, birth years (country)	Maternal smoking	Paternal smoking	Gestational age at birth	Birth weight	Childhood weight and height
GENERATION XXI, n=7,541, 2005-2006 (Portugal)	Self-reported	NA	Clinical records	Clinical records	Measured
GENESIS, n=2,261 , 2003-2004 (Greece)	Self-reported	NA	Clinical records	Clinical records	Measured
GINIplus, n=2,086 , 1995-1998 (Germany)	Self-reported	NA	NA	NA	Clinical records at 4y, measured and self-reported at 10 and 15y
HUMIS, n=986 , 2002-2009 (Norway)	Self-reported	NA	Clinical records	Clinical records	Self-reported
INMA, n=2,406, 1997-2008 (Spain)	Self-reported	Self-reported	Clinical records	Clinical records	Measured
KOALA, n=2,800, 2000-2002 (The Netherlands)	Self-reported	NA	Clinical records	Clinical records	Reported
LISAplus, n=1,965, 1997-1999 (Germany)	Self-reported	Self-reported	NA	NA	Clinical records at 4y, measured and self-reported at 10 and 15y
LUKAS, n=441, 2002-2005 (Finland)	Self-reported	NA	NA	Clinical records	Self-reported
MoBa, n=80,116, 1999-2009 (Norway)	Self-reported	Self-reported	Clinical records	Clinical records	Self-reported
NINFEA, n=2,259, 2005-2010 (Italy)	Self-reported	NA	Self-reported	Self-reported	Self-reported
PÉLAGIE, n=1,494, 2002-2005 (France)	Self-reported	Self-reported	Clinical records	Clinical records	NA
Piccolipiù, n=3,292, 2011-2015 (Italy)	Self-reported	Self-reported	Clinical records	Clinical records	NA

Cohort name, number of participants, birth years (country)	Maternal smoking	Paternal smoking	Gestational age at birth	Birth weight	Childhood weight and height
PRIDE Study, n=1,616, 2011-2015 (The Netherlands)	Self-reported	NA	Self-reported or clinical records	Self-reported or clinical records	NA
Project Viva, n=2,001, 1999-2002 (United States)	Self-reported	NA	Self-reported or clinical records	Clinical records	Measured
REPRO_PL, n=1,434, 2007-2011 (Poland)	Self-reported	Self-reported	Clinical records	Clinical records	Measured
RHEA, n=651, 2007-2008 (Greece)	Self-reported	Self-reported	Self-reported or clinical records	Clinical records	NA
SCOPE BASELINE, n=1,216, 2009-2011 (Ireland)	Self-reported	Self-reported	Measured	Measured	NA
SWS, n=2,716, 1998-2007 (United Kingdom)	Self-reported	NA	Measured	Clinical records	Measured

NA: Not available or not applicable. BMI, body mass index.

S2 Table. Cohort-specific description of available covariates

Cohort name, number of participants	Maternal age (years)	Maternal educational level				Maternal parity		Maternal pre- or early pregnancy BMI (kg/m²)	Paternal BMI (kg/m²)	Maternal alcohol consumption	
		Low	Medium	High	Missing	Nulliparous	Missing			Yes	Missing
ABCD, n=7,324	31.0 (20.0, 40,0)	1547 (21.1)	2799 (38.2)	2916 (39.8)	62 (0.8)	4101 (56.0)	NA	22.2 (17.9, 33.8)	NA	1562 (21.3)	4 (0.1)
ALSPAC, n=12,148	28.0 (19.0, 38.0)	7132 (58.7)	2547 (21.0)	1506 (12.4)	963 (7.9)	5269 (43.4)	217 (1.8)	22.3 (17.9, 33.9)	24.9 (19.8, 32.7)	NA	12148 (100.0)
BAMSE, n=4,057	30.0 (22.0, 40.0)	1377 (33.9)	999 (24.6)	1655 (40.8)	26 (0.6)	2208 (54.4)	48 (1.2)	22.4 (18.2, 31.6)	NA	NA	4057 (100.0)
BIB, n=1,641	27.0 (17.0, 39.0)	412 (25.1)	609 (37.1)	614 (37.4)	6 (0.4)	624 (38.0)	24 (1.5)	24.7 (17.8, 39.8)	NA	308 (18.8)	7 (0.4)
Co.N.ER, n=641	33.7 (24.5, 41.9)	116 (18.1)	284 (44.3)	240 (37.4)	1 (0.2)	283 (44.1)	2 (0.3)	21.1 (17.6, 30.5)	24.8 (20.0, 31.1)	287 (44.8)	3 (0.5)
DNBC, n=71,710	30.3 (22.5, 39.2)	6625 (9.2)	27085 (37.8)	37742 (52.6)	258 (0.4)	34959 (48.8)	47 (0.1)	22.7 (18.0, 34.5)	24.8 (20.0, 32.4)	31754 (44.3)	79 (0.1)
EDEN, n=1,880	29.4 (20.3, 39.6)	531 (28.2)	334 (17.8)	1005 (53.5)	10 (0.5)	1039 (55.3)	3 (0.2)	22.1 (17.4, 34.6)	24.6 (19.3, 33.6)	944 (50.2)	101 (5.4)
FCOU, n=4,003	23.0 (17.0, 36.0)	242 (6.0)	2554 (63.8)	945 (23.6)	262 (6.5)	2594 (64.8)	307 (7.7)	21.7 (17.3, 31.6)	23.9 (19.6, 31.0)	837 (20.9)	193 (4.8)
GASPII, n=680	33.0 (22.0, 41.0)	96 (14.1)	341 (50.1)	243 (35.7)	NA	399 (58.7)	NA	21.3 (17.6, 31.2)	25.0 (20.7, 32.9)	240 (35.3)	3 (0.4)
GENERATION R, n=7,934	30.4 (19.3, 39.3)	821 (10.3)	3446 (43.4)	3280 (41.3)	387 (4.9)	4440 (56.0)	42 (0.5)	22.8 (18.0, 35.1)	24.9 (19.5, 33.0)	3642 (45.9)	700 (8.8)

Cohort name, number of participants	Maternal age (years)	Maternal educational level				Maternal parity		Maternal pre- or early pregnancy BMI (kg/m²)	Paternal BMI (kg/m²)	Maternal alcohol consumption	
		Low	Medium	High	Missing	Nulliparous	Missing			Yes	Missing
GENERATION XXI, n=7,541	29.0 (18.0, 40.0)	2473 (32.8)	3282 (43.5)	1753 (23.2)	33 (0.4)	4213 (55.9)	95 (1.3)	22.9 (18.1, 34.7)	NA	847 (11.2)	364 (4.8)
GENESIS, n=2,261	30.4 (21.2, 39.2)	98 (4.3)	1135 (50.2)	947 (41.9)	81 (3.6)	1122 (49.6)	NA	21.9 (17.6, 31.1)	NA	108 (4.8)	NA
GINIplus, n=2,086	31.0 (24.0, 40.0)	243 (11.6)	874 (41.9)	964 (46.2)	5 (0.2)	NA	2086 (100.0)	22.1 (18.0, 31.6)	NA	NA	2086 (100.0)
HUMIS, n=986	30.0 (22.0, 39.0)	99 (10.0)	166 (16.8)	601 (61.0)	120 (12.2)	425 (43.1)	NA	23.4 (18.4, 35.1)	25.7 (21.0, 33.3)	72 (7.3)	400 (40.6)
INMA, n=2,406	30.0 (21.0, 39.0)	750 (31.2)	913 (37.9)	713 (29.6)	30 (1.2)	1305 (54.2)	2 (0.1)	22.5 (18.0, 34.9)	25.3 (20.3, 33.2)	219 (9.1)	29 (1.2)
KOALA, n=2,800	32.0 (25.0, 40.0)	286 (10.2)	1047 (37.4)	1328 (47.4)	139 (5.0)	1206 (43.1)	81 (2.9)	22.7 (18.4, 33.9)	NA	457 (16.3)	NA
LISAplus, n=1,965	32.0 (23.0, 40.0)	142 (7.2)	721 (36.7)	1089 (55.4)	13 (0.7)	855 (43.5)	7 (0.4)	21.7 (17.9, 32.8)	NA	1143 (58.2)	39 (2.0)
LUKAS, n=441	30.8 (21.2, 42.1)	21 (4.8)	334 (75.7)	86 (19.5)	NA	151 (34.2)	NA	24.1 (18.5, 36.4)	26.1 (20.1, 34.7)	NA	441 (100.0)
MoBa, n=80,116	30.0 (21.0, 39.0)	25127 (31.4)	32959 (41.1)	20428 (25.5)	1602 (2.0)	36477 (45.5)	NA	23.1 (18.4, 34.9)	25.4 (20.5, 33.4)	10259 (12.8)	607 (0.8)
NINFEA, n=2,259[a]	33.0 (25.0, 41.0)	92 (4.1)	774 (34.3)	1388 (61.4)	5 (0.2)	1519 (67.2)	2 (0.1)	21.5 (17.4, 32.0)	24.5 (19.7, 31.7)	895 (39.6)	5 (0.2)

Cohort name, number of participants	Maternal age (years)	Maternal educational level				Maternal parity		Maternal pre- or early pregnancy BMI (kg/m²)	Paternal BMI (kg/m²)	Maternal alcohol consumption	
		Low	Medium	High	Missing	Nulliparous	Missing			Yes	Missing
PÉLAGIE, n=1,353	30.2 (22.8, 39.6)	190 (14.0)	238 (17.6)	992 (68.1)	3 (0.2)	591 (43.7)	5 (0.4)	21.6 (17.7, 32.2)	NA	202 (14.9)	13 (1.0)
Piccolipiù, n=3,292	34.0 (23.0, 42.0)	393 (11.9)	1415 (43.0)	1473 (44.7)	11 (0.3)	1916 (58.2)	8 (0.2)	21.7 (17.6, 33.0)	24.8 (20.2, 32.7)	1452 (44.1)	37 (1.1)
PRIDE Study, n=1,616	30.0 (24.0, 38.0)	31 (1.9)	329 (20.4)	1219 (75.4)	37 (2.3)	973 (60.2)	5 (0.3)	22.6 (18.4, 33.5)	24.1 (19.4, 31.2)	300 (18.6)	219 (13.6)
Project Viva, n=2,001	32.3 (19.5, 40.9)	662 (33.1)	724 (36.2)	603 (30.1)	12 (0.6)	959 (47.9)	NA	23.4 (18.1, 39.2)	25.9 (20.0, 35.2)	1245 (62.2)	137 (6.8)
REPRO_PL, n=1,434	28.0 (20.0, 37.0)	171 (11.9)	429 (29.9)	820 (57.2)	14 (1.0)	804 (56.1)	13 (0.9)	21.5 (17.3, 31.2)	NA	116 (8.1)	108 (7.5)
RHEA, n=651	30.0 (20.0, 40.0)	109 (16.7)	322 (49.5)	213 (32.7)	7 (1.1)	NA	651 (100.0)	23.4 (18.1, 36.1)	26.8 (21.2, 36.3)	126 (19.4)	201 (30.9)
SCOPE BASELINE, n=1,216	31.0 (20.0, 39.0)	NA	162 (13.3)	1045 (85.9)	9 (0.7)	1216 (100.0)	NA	23.9 (19.1, 34.9)	26.5 (20.9, 33.9)	431 (35.4)	36 (3.0)
SWS, n=2,716	30.2 (22.8, 36.3)	334 (12.3)	1594 (58.7)	782 (28.8)	6 (0.2)	1339 (49.3)	2 (0.1)	24.2 (18.9, 37.5)	25.5 (19.8, 34.1)	2093 (77.1)	254 (9.4)
Total group	30.0 (20.3, 39.2)	50120 (21.9)	88416 (38.6)	86520 (37.8)	4102 (1.8)	110987 (48.4)	3647 (1.6)	22.7 (18.1, 34.6)	25.1 (20.1, 33.1)	59539 (26.0)	22271 (9.7)

Values are expressed as number of participants (%) or medians (95% range). BMI, body mass index.
[a]Subset of participants with follow-up completed at 4 years of child's age by the time of data transfer (March 2015).

S3 Table. Cohort-specific description of maternal smoking variables

Cohort name, number of participants	Maternal first trimester smoking		Categories (cigarettes/day)			Maternal third trimester smoking		Categories (cigarettes/day)		
	No	Yes	<1-4	5-9	≥10	No	Yes	<1-4	5-9	≥10
ABCD, n=7,324[a]	5451 (90.8)	550 (9.2)	NA	NA	NA	4439 (90.1)	490 (9.9)	253 (3.6)	118 (1.7)	94 (1.3)
ALSPAC, n=12,148	9687 (79.7)	2461 (20.3)	404 (3.4)	559 (4.7)	1473 (12.3)	9581 (78.9)	2567 (21.1)	498 (4.1)	622 (5.1)	1394 (11.5)
BAMSE, n=4,057	3574 (88.1)	483 (11.9)	100 (2.5)	114 (2.8)	267 (6.7)	3671 (90.5)	386 (9.5)	109 (2.7)	87 (2.1)	188 (4.6)
BIB, n=1,641	1418 (86.4)	223 (13.6)	NA	NA	NA	NA	NA	NA	NA	NA
Co.N.ER, n=641	555 (86.6)	86 (13.4)	48 (7.6)	26 (4.1)	12 (1.9)	582 (90.8)	59 (9.2)	29 (4.5)	19 (3.0)	11 (1.7)
DNBC, n=71,710	59316 (82.7)	12391 (17.3)	2351 (3.3)	4103 (5.7)	5896 (8.3)	55659 (81.4)	12680 (18.6)	1631 (2.3)	3885 (5.5)	6066 (8.6)
EDEN, n=1,880	1397 (74.5)	478 (25.5)	109 (5.8)	160 (8.6)	208 (11.2)	1544 (83.2)	312 (16.8)	95 (5.1)	111 (5.9)	98 (5.3)
FCOU, n=4,003	3647 (91.1)	356 (8.9)	223 (5.6)	91 (2.3)	41 (1.0)	3647 (91.1)	356 (8.9)	223 (5.6)	91 (2.3)	41 (1.0)
GASPII, n=680	603 (88.7)	77 (11.3)	45 (6.7)	16 (2.4)	16 (2.4)	627 (92.2)	53 (7.8)	28 (4.1)	14 (2.1)	11 (1.6)
GENERATION R, n=7,934	5662 (79.0)	1503 (21.0)	799 (10.4)	401 (5.2)	303 (3.9)	5596 (84.6)	1020 (15.4)	506 (6.5)	321 (4.1)	193 (2.5)
GENERATION XXI, n=7,541	5796 (76.9)	1739 (23.1)	NA	NA	NA	6405 (85.0)	1129 (15.0)	NA	NA	NA
GENESIS, n=2,261	1894 (83.8)	367 (16.2)	150 (6.8)	116 (5.3)	100 (4.5)	1897 (83.9)	364 (16.1)	158 (7.0)	114 (5.0)	92 (4.1)
GINIplus, n=2,086	1903 (91.8)	171 (8.2)	40 (1.9)	58 (2.8)	72 (3.5)	1903 (92.0)	165 (8.0)	69 (3.3)	59 (2.7)	38 (1.8)
HUMIS, n=986	935 (94.8)	51 (5.2)	6 (0.6)	18 (1.8)	22 (2.2)	759 (93.4)	54 (6.6)	11 (1.1)	17 (1.7)	16 (1.6)
INMA, n=2,406	1992 (82.8)	414 (17.2)	127 (5.3)	116 (4.8)	166 (6.9)	1978 (82.6)	418 (17.4)	169 (7.0)	149 (6.2)	100 (4.2)
KOALA, n=2,800	2616 (93.4)	184 (6.6)	32 (1.2)	82 (3.0)	70 (2.5)	2594 (92.6)	206 (7.4)	42 (1.5)	67 (2.4)	97 (3.5)
LISAplus, n=1,965	1713 (87.2)	252 (12.8)	85 (4.4)	73 (3.8)	90 (4.6)	1743 (91.6)	160 (8.4)	54 (2.8)	53 (2.7)	47 (2.4)
LUKAS, n=441	371 (84.1)	70 (15.8)	NA	NA	NA	407 (92.3)	34 (7.7)	NA	NA	NA
MoBa, n=80,116[a]	72315 (91.1)	7090 (8.9)	NA	NA	NA	51108 (92.0)	4428 (8.0)	1970 (2.5)	1327 (1.7)	1084 (1.4)
NINFEA, n=2,259[b]	2092 (92.6)	167 (7.4)	NA	NA	NA	2129 (94.7)	120 (5.3)	NA	NA	NA

Cohort name, number of participants	Maternal first trimester smoking		Categories (cigarettes/day)			Maternal third trimester smoking		Categories (cigarettes/day)		
	No	Yes	<1-4	5-9	≥10	No	Yes	<1-4	5-9	≥10
PÉLAGIE, n=1,494	1025 (75.8)	328 (24.2)	119 (8.8)	108 (8.0)	97 (7.2)	1160 (89.2)	140 (10.8)	72 (6.2)	47 (4.1)	15 (1.3)
Piccolipiù, n=3,292	2584 (78.5)	707 (21.5)	458 (14.0)	156 (4.8)	93 (2.8)	2983 (90.6)	308 (9.4)	177 (5.4)	81 (2.5)	46 (1.4)
PRIDE Study, n=1,616	1531 (94.9)	82 (5.1)	40 (2.5)	22 (1.4)	19 (1.2)	1402 (97.1)	42 (2.9)	17 (1.1)	11 (0.7)	9 (0.6)
Project Viva, n=2,001	1744 (90.1)	192 (9.9)	NA	NA	NA	1880 (96.4)	70 (3.6)	NA	NA	NA
REPRO_PL, n=1,434	1224 (85.7)	205 (14.3)	52 (3.8)	57 (4.1)	58 (4.2)	1296 (92.0)	113 (8.0)	34 (2.4)	36 (2.5)	36 (2.5)
RHEA, n=651	545 (84.5)	100 (15.5)	29 (4.7)	23 (3.7)	23 (3.7)	NA	NA	NA	NA	NA
SCOPE BASELINE, n=1,216	1079 (88.7)	137 (11.3)	63 (5.2)	40 (3.3)	24 (2.0)	NA	NA	NA	NA	NA
SWS, n=2,716	2350 (86.8)	358 (13.2)	61 (2.3)	102 (3.8)	195 (7.3)	2155 (84.3)	400 (15.7)	75 (2.8)	96 (3.5)	228 (8.4)
Total group	117253 (83.3)	23582 (16.7)	5341 (2.5)	6441 (3.0)	9245 (4.3)	167145 (86.5)	26074 (13.5)	6220 (2.8)	7322 (3.3)	9904 (4.4)

Values are expressed as number of participants (valid %). NA, not available or not applicable. Maternal first trimester smoking refers to all maternal smoking in first trimester no matter if mothers stop or continue smoking after first trimester.
[a]Numbers of smoking (no/yes) in first trimester are second trimester smoking numbers. To prevent exclusion due to no information on first trimester smoking, nonsmokers from second trimester were used as nonsmoking during pregnancy (ABCD and MoBa).
[b]Subset of participants with follow-up completed at 4 years of child's age by the time of data transfer (March 2015). Data on number of cigarettes/day smoked in pregnancy were not used for this manuscript.

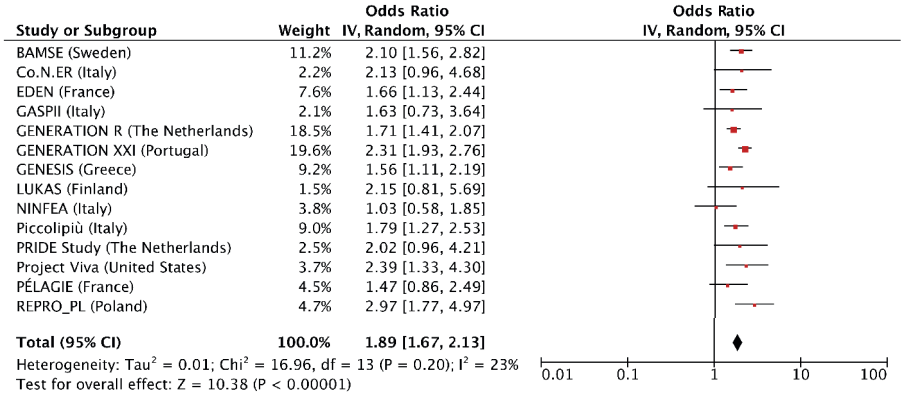
S4 Table. Associations of maternal smoking with gestational age at birth, birth weight, and childhood BMI

	Gestational age at birth in weeks (95% Confidence Interval)	**Gestational age-adjusted birth weight SDS (95% Confidence Interval)**	**Childhood BMI SDS (95% Confidence Interval)**
No maternal smoking	*Reference* n=188,357	*Reference* n=190,873	*Reference* n=92,434
Only first trimester smoking	0.06 (-0.01, 0.14) n=2,116	0.02 (-0.02, 0.06) n=2,144	0.04 (-0.02, 0.10) n=1,084
First trimester dosage			
≤4 cigarettes/day	0.09 (-0.03, 0.21) n=828	0.04 (-0.03, 0.11) n=826	-0.05 (-0.16, 0.06) n=340
5-9 cigarettes/day	0.04 (-0.16, 0.24) n=288	0.02 (-0.09, 0.13) n=288	0.05 (-0.13, 0.22) n=136
≥10 cigarettes/day	-0.02 (-0.22, 0.19) n=273	0.09 (-0.02, 0.21) n=271	0.06 (-0.11, 0.22) n=152
Continued smoking	-0.05 (-0.07,-0.03)** n=29,951	-0.37 (-0.38,-0.36)** n=30,125	0.19 (0.17, 0.21)** n=13,083
Continued smoking dosage			
≤4 cigarettes/day	-0.08 (-0.12,-0.03)* n=5,866	-0.22 (-0.25,-0.20)** n=6,034	0.16 (0.12, 0.20)** n=2,792
5-9 cigarettes/day	-0.11 (-0.15,-0.07)** n=7,115	-0.43 (-0.46,-0.41)** n=7,162	0.18 (0.14, 0.21)** n=3,284
≥10 cigarettes/day	-0.15 (-0.19,-0.12)** n=9,771	-0.55 (-0.57,-0.53)** n=9,743	0.23 (0.19, 0.26)** n=4,139

Values are beta's (95% confidence intervals) from multilevel linear mixed effects models that reflect the differences in gestational age at birth in weeks, gestational age-adjusted birth weight in standard deviation scores and childhood body mass index in standard deviation scores per smoking group compared with the reference group (no maternal smoking). Number of cigarettes used as continued smoking dosage were based on third trimester information. Models are adjusted for maternal age, educational level, parity, pre- or early pregnancy body mass index, alcohol consumption during pregnancy and paternal smoking.
*P-value<0.05; **P-value<0.001.
BMI, body mass index; SDS, standard deviation score.

S1 Figure. Maternal continued smoking with risks of small size for gestational age assessed by two-stage random-effects models

Continued smoking vs non-smoking



Values are odds ratios (95% confidence intervals) per cohort and pooled from binary logistic regression models that reflect the risk of small size for gestational age for continued smoking compared to non-smoking. Models are adjusted for maternal age, educational level, parity, pre- or early pregnancy body mass index, alcohol consumption during pregnancy and paternal smoking. Analysis was restricted to cohorts with information on first trimester only smoking. The heterogeneity between the estimates of each cohort was 23% (95% CI 0%-65%).

S5 Table. Change in maternal smoking habits during pregnancy, gestational age at birth, birth weight, and childhood BMI

	Gestational age at birth in weeks (95% Confidence Interval)	Gestational age-adjusted birth weight SDS (95% Confidence Interval)	Childhood BMI SDS (95% Confidence Interval)
No maternal smoking in first trimester			
Third trimester no smoking	*Reference* n=100,634	*Reference* n=103,740	*Reference* n=59,070
Third trimester ≤4 cigarettes/day	-0.04 (-0.28, 0.16) n=278	-0.10 (-0.22, 0.01) n=274	0.25 (0.09, 0.42)* n=147
Third trimester 5-9 cigarettes/day	0.07 (-0.25, 0.39) n=104	-0.28 (-0.46,-0.09)* n=103	0.18 (-0.09, 0.46) n=51
Third trimester ≥10 cigarettes/day	0.03 (-0.34, 0.40) n=80	-0.35 (-0.56,-0.14)* n=79	0.19 (-0.16, 0.54) n=31
Maternal smoking in first trimester ≤4 cigarettes/day			
Third trimester quitted	0.09 (-0.03, 0.20) n=862	-0.01 (-0.08, 0.06) n=859	0.01 (-0.09, 0.11) n=388
Third trimester ≤4 cigarettes/day	0.02 (-0.05, 0.09) n=2,261	-0.23 (-0.27,-0.20)** n=2,457	0.17 (0.11, 0.23)** n=1,169
Third trimester 5-9 cigarettes/day	-0.10 (-0.21, 0.02) n=885	-0.40 (-0.46,-0.33)** n=880	0.26 (0.16, 0.35)** n=440
Third trimester ≥10 cigarettes/day	-0.21 (-0.46, 0.03) n=186	-0.46 (-0.60,-0.32)** n=185	0.20 (-0.01, 0.41) n=86
Maternal smoking in first trimester 5-9 cigarettes/day			
Third trimester quitted	0.04 (-0.16, 0.23) n=304	-0.06 (-0.17, 0.05) n=304	0.06 (-0.09, 0.22) n=165
Third trimester ≤4 cigarettes/day	0.03 (-0.10, 0.16) n=657	-0.28 (-0.36,-0.21)** n=654	0.21 (0.10, 0.33)** n=307
Third trimester 5-9 cigarettes/day	-0.05 (-0.11, 0.01) n=3,551	-0.42 (-0.45,-0.39)** n=3,617	0.17 (0.12, 0.22)** n=1,704

	Gestational age at birth in weeks (95% Confidence Interval)	Gestational age-adjusted birth weight SDS (95% Confidence Interval)	Childhood BMI SDS (95% Confidence Interval)
Third trimester ≥10 cigarettes/day	-0.10 (-0.20,-0.01)*	-0.51 (-0.56,-0.46)**	0.20 (0.13, 0.28)**
	n=1,330	n=1,319	n=632
Maternal smoking in first trimester ≥10 cigarettes/day			
Third trimester quitted	0.02 (-0.18, 0.22)	0.03 (-0.08, 0.14)	0.13 (-0.02, 0.27)
	n=285	n=283	n=194
Third trimester ≤4 cigarettes/day	-0.05 (-0.22, 0.13)	-0.24 (-0.34,-0.14)*	0.16 (0.01, 0.30)*
	n=358	n=354	n=192
Third trimester 5-9 cigarettes/day	-0.12 (-0.22,-0.02)*	-0.51 (-0.57,-0.45)**	0.18 (0.09, 0.27)**
	n=1,078	n=1,072	n=503
Third trimester ≥10 cigarettes/day	-0.15 (-0.20,-0.11)**	-0.55 (-0.58, 0.53)**	0.25 (0.22, 0.07)**

Values are beta's (95% confidence intervals) from multilevel linear mixed effects models that reflect the differences in gestational age at birth in weeks, gestational age-adjusted birth weight in standard deviation scores and childhood BMI in standard deviation scores per smoking group compared with the reference group (non-smoking in first and third trimester). Models are adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, alcohol consumption during pregnancy and paternal smoking. *P-value<0.05; **P-value<0.001. BMI, body mass index; SDS, standard deviation score.

S6 Table. Associations of maternal and paternal smoking with gestational age at birth, birth weight, and childhood BMI

	Gestational age at birth in weeks (95% Confidence Interval)	Gestational age-adjusted birth weight SDS (95% Confidence Interval)	Childhood BMI SDS (95% Confidence Interval)
Maternal non-smoking			
Paternal non-smoking	*Reference* n=123,666	*Reference* n=123,328	*Reference* n=59,395
Paternal smoking	-0.03 (-0.05,-0.01)* n=31,890	-0.01 (-0.02, 0.00) n=33,691	0.06 (0.04, 0.08)** n=15,474
Maternal first trimester smoking			
Paternal non-smoking	0.09 (-0.08, 0.26) n=412	0.04 (-0.06, 0.13) n=412	0.01 (-0.12, 0.14) n=233
Paternal smoking	0.03 (-0.10, 0.17) n=626	0.04 (-0.04, 0.11) n=625	0.04 (-0.08, 0.15) n=305
Maternal continued smoking			
Paternal non-smoking	-0.03 (-0.07, 0.00) n=8,768	-0.34 (-0.36,-0.32)** n=8,723	0.14 (0.10, 0.17)** n=3,872
Paternal smoking	-0.08 (-0.11, -0.06)** n=15,806	-0.42 (-0.44,-0.40)** n=15,967	0.26 (0.23, 0.28)** n=6,661

Values are beta's (95% confidence intervals) from multilevel linear mixed effects models that reflect the differences in gestational age at birth in weeks, gestational age-adjusted birth weight in standard deviation scores and childhood BMI in standard deviation scores per smoking group compared with the reference group (no parental smoking). Models are adjusted for maternal age, maternal BMI, paternal BMI, maternal education, parity and maternal alcohol consumption during pregnancy.
*P-value<0.05; **P-value<0.001. BMI, body mass index; SDS, standard deviation score.

S7 Table. Complete cases analysis of maternal smoking with risks of birth complications and childhood overweight (with maternal age and BMI in categories)

	Preterm birth Odds Ratio (95% Confidence Interval)	**Small size for gestational age at birth Odds Ratio (95% Confidence Interval)**	**Childhood overweight Odds Ratio (95% Confidence Interval)**
No maternal smoking	*Reference* $n_{cases/total}$ =5,859/138,839	*Reference* $n_{cases/total}$ =12,340/140,086	*Reference* $n_{cases/total}$ = 10,932/63,318
Only first trimester smoking	0.76 (0.53, 1.09) $n_{cases/total}$ =33/941	0.92 (0.73, 1.17) $n_{cases/total}$ =82/941	1.13 (0.89, 1.43) $n_{cases/total}$ =102/473
Continued smoking	1.02 (0.94, 1.10) $n_{cases/total}$ =980/20,943	2.16 (2.06, 2.26)** $n_{cases/total}$ =3,621/21,068	1.41 (1.33, 1.50)** $n_{cases/total}$ =2,110/8,667

Values are odds ratios (95% confidence intervals) from multilevel binary logistic mixed effects models that reflect the risk of preterm birth, small size for gestational age and childhood overweight per smoking group compared with the reference group (no maternal smoking).
Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex and gestational age adjusted birth weight standard deviation score per cohort. Childhood overweight is overweight and obesity together according to the World Health Organization criteria. Models are adjusted for maternal age, educational level, parity, pre- or early pregnancy BMI, alcohol consumption during pregnancy and paternal smoking, all categorized. *P-value<0.05; **P-value<0.001. BMI, body mass index.

S8 Table. Complete cases analysis of maternal smoking with risks of birth complications and childhood overweight (with maternal age and BMI continuously)

	Preterm birth Odds Ratio (95% Confidence Interval)	**Small size for gestational age at birth Odds Ratio (95% Confidence Interval)**	**Childhood overweight Odds Ratio (95% Confidence Interval)**
No maternal smoking	*Reference* $n_{cases/total}$ =5,859/138,839	*Reference* $n_{cases/total}$ =12,340/140,086	*Reference* $n_{cases/total}$ = 10,932/63,318
Only first trimester smoking	0.76 (0.53, 1.10) $n_{cases/total}$ =33/941	0.92 (0.72, 1.16) $n_{cases/total}$ =82/941	1.13 (0.89, 1.43) $n_{cases/total}$ =102/473
Continued smoking	1.03 (0.96, 1.12) $n_{cases/total}$ =980/20,943	2.18 (2.08, 2.28)** $n_{cases/total}$ =3,621/21,068	1.42 (1.34, 1.51)** $n_{cases/total}$ =2,110/8,667

Values are odds ratios (95% confidence intervals) from multilevel binary logistic mixed effects models that reflect the risk of preterm birth, small size for gestational age and childhood overweight per smoking group compared with the reference group (no maternal smoking).
Preterm birth is defined as birth before the gestational age of 37 weeks. Small size for gestational age is defined as the lowest 10% of sex and gestational age adjusted birth weight standard deviation score per cohort. Childhood overweight is overweight and obesity together according to the World Health Organization criteria. Models are adjusted for maternal age, educational level, parity, pre- or early pregnancy BMI, alcohol consumption during pregnancy and paternal smoking. Except for maternal age and pre- or early pregnancy BMI, all covariates were categorized. *P-value<0.05; **P-value<0.001. BMI, body mass index.

4

CHAPTER

General discussion

Introduction

Environmental exposures, including environmental pollutants and adverse lifestyle behavior, have a significant contribution to the etiology of chronic non-communicable diseases leading to morbidity and mortality worldwide.[1] Among those, endocrine disrupting chemicals (EDCs) such as bisphenols and phthalates are of increasing interest. In the last decade, an increasing body of evidence suggests that exposure to bisphenols and phthalates may contribute to the development of cardiometabolic diseases.[2,3] Growing concern over human exposure has led to several governmental embargoes concerning bisphenol A and several phthalates, concurrently stimulating the industries to switch to synthetic bisphenol analogues and phthalate replacements.[4] Information on adverse effects from synthetic bisphenol analogues and phthalate replacements is still limited. Following the developmental origins of health and disease (DOHaD) hypothesis, the bulk of studies investigated effects of prenatal exposure on fetal programming. However, exposure effects of bisphenols and phthalates in adults have been scarcely examined. A growing body of evidence indicates that for women pregnancy is a period of increased susceptibility to potential short and long-term physiological changes due to exposure to bisphenols and phthalates, some with persistent effects.[5] Therefore, studies in this thesis were focused on associations of bisphenols and phthalate exposure during pregnancy with maternal pregnancy and postpartum outcomes.

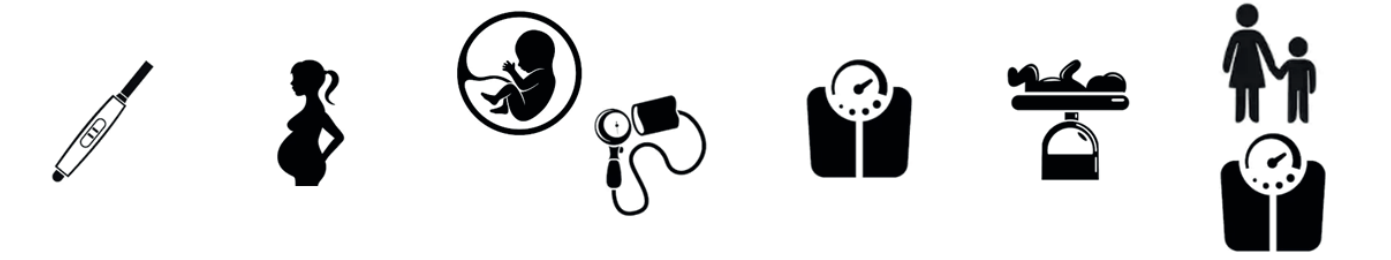
Although smoking prevalence is generally declining, globally smoking is still in the top five of attributable disease-adjusted life-years (DALYs).[6] In the last few decades, smoking prevalence is increasing among women. One in five women of reproductive age is expected to be smoking by 2025.[7] Effects of maternal smoking during pregnancy on birth outcomes have been examined in a large number of studies, showing strong associations tending to causal pathways.[8-12] Also, maternal smoking has been associated with childhood overweight.[13-15] However, the specific windows of vulnerability during pregnancy and the role of paternal tobacco usage are not yet clear. Further insight into these windows of vulnerability to parental smoking during pregnancy are needed to develop adequate preventive strategies.

The general aim of this thesis was to investigate the associations of well-known adverse exposures, namely endocrine disruptors and parental smoking during pregnancy with maternal, fetal and childhood outcomes. The main findings have been presented and discussed in the previous chapters of this thesis. This chapter will provide a general discussion of main findings of the studies in this thesis, consider the general methodological issues, and give suggestions for future research and implications for clinical practice and policy.

Interpretation of main findings

The paragraphs below give an interpretation of the main findings. Figure 1 presents the most important findings presented in this thesis.

Figure 1. Presentation of the most important findings presented in this thesis



Exposure to bisphenols and phthalates during pregnancy

Effects of early exposure on cardiometabolic outcomes in pregnancy and childhood, a narrative review

Exposure to bisphenols and phthalates is believed to induce pathways towards several adverse health effects. In this narrative review (**Chapter 1.2**), we outlined the available evidence on bisphenol and phthalate routes of exposure, metabolism and mechanisms of action. Further, we summarized associations of exposure to bisphenols and phthalates during pregnancy with effects on maternal, fetal and childhood cardiometabolic outcomes. Little is known about maternal health outcomes during pregnancy. Exposure during fetal life may have a substantial influence on perinatal and postnatal cardiometabolic programming, although evidence concerning the majority of investigated outcomes shows contradictory results. However, the majority of the existing human evidence is limited by methodological difficulties that complicate interpretation. Most studies determined exposure in only one single sample, not giving a reliable estimate of tissue exposure due to short biological half-lives and within person-variability leading to attenuation bias in dose-response relationships. Variations in the timing of exposure measurement further complicate conclusions regarding causal inference.

Conclusions on effects of early exposure to bisphenols and phthalates and cardiometabolic outcomes

- Exposure to bisphenols and phthalates during pregnancy may affect maternal health and offspring perinatal and postnatal cardiometabolic programming
- The majority of existing human evidence is limited by methodological difficulties that complicate interpretation

Determinants of bisphenol and phthalate concentrations

Bisphenols and phthalate metabolites have been observed in various Western studies, including a significant number of studies among pregnant women.[16-20] During the last decade, several changes have been observed in exposure levels, mainly due to several governmental embargoes and subsequent use of replacements.[21-23] The study as presented in this thesis (**Chapter 2.1**) is the only human biomonitoring study before 2010 reporting on bisphenol S and bisphenol F.

Diet is considered the most important source of phthalate exposure and, to a lesser extent, of bisphenols, predominantly through food packaging.[24-28] Previous observational studies reported several associations, including of higher bisphenol A concentrations among women with higher canned food consumption and of higher DEHP metabolite concentrations among women with frequent consumption of meat, poultry and fish.[16,29-31] A recent review reported that lower concentrations of bisphenol A and phthalates were associated with healthy food choices.[32] In the study presented in this thesis, we did not observe any association of nutrition related factors, including daily dietary caloric intake and food groups, with bisphenol and phthalate metabolite urine concentrations. However, we did detect some trends towards lower compound concentrations among healthy food consumption patterns. In our

study, we have used a food frequency questionnaire capturing the average intake in the past three months. Dietary sources of phthalate exposure are known to be more variable than other phthalate sources.[33] Given the short biological half-lives of bisphenols and phthalates, this might have resulted in undetectable exposure-response associations.

Overall, previous studies identified younger maternal age, lower socio-economic status and unhealthy behavior such as overweight and smoking as determinants of higher bisphenol and phthalate concentrations during pregnancy.[16,34-37] Also among postmenopausal women such a pattern was observed.[38] Some studies, however, observed differences for LMW and HMW phthalate metabolites, with unhealthy behavior patterns as determinants for higher levels of LMW phthalate metabolites and healthy behavior patterns as determinants for higher levels of HMW phthalate metabolites.[30,39] We observed that women with adverse lifestyle related factors, such as obesity and lack of folic acid supplement use, had higher first trimester bisphenol and phthalate metabolite urine concentrations. In line with previous studies, a trend was observed for multiparity, low educational level, non-European descent, and smoking with higher bisphenol and phthalate metabolite concentrations. Again, previous studies did not apply multiple testing corrections and may consequently have reported associations that we interpreted as trends. Similar to our results, a few studies also observed higher concentrations of bisphenol A and phthalate metabolites among non-European women.[35,40] Several association studies have identified ethnicity-specific associations of bisphenols and phthalates with childhood BMI and diabetes risks.[41-43] Although these associations might indicate variations in bisphenol and phthalate metabolism, we cannot rule out that this is a result from residual confounding by unmeasured lifestyle related factors.

The observed associations of bisphenols and phthalates with smoking might reflect lifestyle related factors, but may also be a direct effect from smoking as bisphenol A and phthalates are used in some cigarette filters.[44] While previous studies showed inconsistent associations between higher maternal BMI and levels of HMW phthalate metabolite concentrations, we observed strong associations between pre-pregnancy BMI in the obesity range and higher HMW phthalate metabolite concentrations.[30,34,37,39,45] Obesogenic effects of phthalate exposure might have induced a higher pre-pregnancy BMI. Phthalates, and bisphenols to some extent, are lipophilic chemicals and might therefore accumulate in adipose tissue.[46] However, we cannot rule out that obese women are more exposed to HMW phthalates or that this is a result from residual confounding by unmeasured lifestyle or nutrition related factors. Further investigation of the toxicokinetics of these chemicals is needed to unravel these associations.

To date, the study presented in this thesis is the only study investigating maternal folic acid supplement use as a determinant of bisphenol and phthalate metabolite concentrations. Several studies, including one of our own (**Chapter 2.2**) identified insufficient folic acid supplement use as an effect modifier in several associations of bisphenols and phthalates.[47,48] Although epigenetic effects of bisphenols and phthalates are not yet clear, methyl donors have been proposed as a potential point of engagement to prevent potential epigenetic effects.[49] It is most likely that the observed association between a lack of folic acid supplement use and higher DNOP metabolite concentrations reflects unmeasured lifestyle related factors., but a lack of folic acid supplement use might increase the vulnerability of these women and their children to health related outcomes in the long term.

A recent study comparing the contribution of dietary and non-dietary exposure found diet to be responsible for >99.9% of bisphenol A and 63% of DEHP exposure.[28] For phthalates, several studies found associations with the use of personal care products, household products and materials used for flooring and walls.[34,50] Therefore, we cannot rule out that the associations of bisphenols and phthalates with lifestyle related factors in this study are rather a proxy for unhealthy lifestyle habits in general, comprising unmeasured food consumption patterns and harmful product exposure than that these factors actually increase exposure or urinary excretion.

Conclusions on determinants of bisphenol and phthalate concentrations

- Bisphenol S and F exposure was highly prevalent among pregnant women as early as 2004-5.
- Nutritional determinants were not associated with first trimester bisphenol or phthalate metabolite urine concentrations.
- Adverse lifestyle related factors, such as obesity and lack of folic acid supplement use, seem to be associated with higher phthalate metabolite urine concentrations in pregnant women.

Time to pregnancy

Previous studies did not observe associations of bisphenols with time to pregnancy.[51-53] For phthalates, results have been inconsistent, both among prospective cohorts and pregnancy studies using recalled time to pregnancy.[51-55] A recent study among women undergoing infertility treatment reported that high urinary bisphenol A concentrations were associated with lower probabilities of implantation, clinical pregnancy, and live birth, among women who consumed <400 µg/day of dietary folate.[56] In line with this study, we observed (**Chapter 2.2**) reduced fecundability ratios for those without preconception folic acid supplementation supporting our hypothesis that bisphenols and phthalates may influence fecundability by inducing changes in DNA methylation. We did not observe associations of first trimester urinary bisphenol and phthalate metabolite concentrations with fecundability in unstratified models. Null findings in previous studies might be explained by dismissed diet interactions. DNA methylation has been identified as a potential mechanism in the association of persistent organic pollutants and reduced fecundability by global DNA methylation in sperm and methylation of the implantation-associated gene Homeobox A10 (HOXA10).[57,58] However, the role of preconception folic acid supplementation has not been examined in this association.

Cross-sectional studies are at risk for reversed causation. Women who experience difficulties conceiving are more likely to obey preconceptional recommendations, including the use of folic acid supplementation. Therefore, the observed interaction with folic acid supplementation use negates potential reversed causation induced by preconceptional behavioral changes in women taking longer to conceive. We cannot rule out that the reduced fecundability ratios for women without preconceptional folic acid supplementation use reflect unmeasured lifestyle related factors. Maternal pre-pregnancy BMI was not selected as a confounder in this association for the fitted models, even though a higher maternal pre-pregnancy BMI is generally associated with a longer time to pregnancy and women in the obesity range tend to have higher chemical concentrations (**Chapter 2.1**). However, obesity has been

reported to affect short-term folate pharmacokinetics leading to higher folate requirements.[59] The use of reported folic acid supplementation use instead of maternal folate concentrations might therefore have led to an underestimation of the observed effects. Thus, results from this thesis showed that preconceptional folic acid supplementation may counteract effects from bisphenols and phthalates on fecundability. This finding is hypothesis generating for methods of action of bisphenols and phthalates and should be tested in other potential associations.

Conclusions on time to pregnancy

- Higher concentrations of bisphenols and phthalates were associated with a longer time to pregnancy among mothers who did not use preconception folic acid supplementation.

Maternal hemodynamics and gestational hypertensive disorders

Gestational hypertension and preeclampsia are hypothesized to originate in early pregnancy.[60] Bisphenols and phthalates may induce early placental maladaptation, subsequently leading to increased risks of gestational hypertensive disorders and higher blood pressure in pregnancy. Previous studies, however, did not show consistent associations of phthalates with gestational hypertensive disorders, although the larger part of studies investigating bisphenol A reported positive associations between bisphenol A and gestational hypertensive disorders.[61-65] Several studies showed associations of bisphenol A and phthalate metabolite concentrations with higher blood pressure in both adults and children,[66-69] although results among pregnant women have been inconsistent.[61,70,71] Pro- and anti-angiogenic factors are important for placental growth and angiogenesis. An imbalance in these factors has been associated with impaired placentation, subsequent placental dysfunction and increased risks of gestational hypertensive disorders.[72] Only one previous study investigated associations of bisphenols and phthalates with placental function markers, observing associations of urinary bisphenol A and DEHP metabolite concentrations with altered placental angiogenic markers.[73] Indices of placental vascular resistance of the uterine and umbilical artery have been used to identify placental dysfunction, leading to adverse pregnancy outcomes such as preeclampsia and fetal growth restriction.[74] Thus far, the role of bisphenols and phthalates in placental hemodynamics has not been investigated in humans. In rodents, early pregnancy bisphenol A exposure was not associated with increased placental resistance indices.[75] To our knowledge, only one other study examined associations of bisphenols and phthalates with placental weight, reporting an association of monocarboxyisononylphthalate and lower placental weight.[76] However, both small and large placentas have been associated with preeclampsia.[77] In our study (**Chapter 2.3**), we observed that bisphenols and phthalate metabolites in early pregnancy were not associated with longitudinal changes in blood pressure or increased risks of gestational hypertensive disorders. Early pregnancy phthalate exposure was associated with altered placental angiogenic markers, while bisphenol A was associated with altered placental hemodynamic function measures, both at a subclinical level. Dissimilar results might be explained by differences between our and other study populations in disease prevalence and other characteristics.

Several potential mechanisms have been proposed to support the observed associations. Bisphenols and phthalates are reported to induce oxidative stress, which plays a role in the onset of preeclampsia, potentially through the release of anti-angiogenic factors.[60,78] However, discrepant associations

between oxidative stress and placental angiogenic factors have been reported.[79,80] Bisphenol A has been accounted for antiproliferative and pro-apoptotic effects on human trophoblastic cells, potentially through estrogen-related receptor γ, proinflammatory cytokines (tumor necrosis factor α, interleukin-1β and interleukin-6) and anti-inflammatory mediators.[81-83] Phthalates have been found to inhibit extravillous trophoblast invasion through the peroxisome proliferator-activated receptor γ and to inhibit cell proliferation through aberrant progesterone secretion.[84,85] Early pregnancy bisphenol A and phthalate exposure have been reported to induce altered methylation of placental genes changing the placental transcriptome, potentially leading to impaired implantation.[86,87]

Conclusions on maternal hemodynamics and gestational hypertensive disorders

- Bisphenols and phthalate metabolites were not associated with longitudinal changes in blood pressure in pregnancy.
- Phthalates were associated with the sFlt-1/PlGF ratio and bisphenol A with placental hemodynamics.

Gestational weight gain

To our knowledge, there are no previous studies investigating associations of maternal bisphenols and phthalate metabolite concentrations with gestational weight gain over the course of pregnancy. A recent study investigating phthalate metabolites and early gestational weight gain reported a negative association between DEHP metabolites in early pregnancy and early gestational weight gain.[88] Another novel study among Mexican pregnant women also observed an inverse association between phthalate metabolite concentrations during pregnancy and maternal weight at delivery.[89] Previous cross-sectional studies, including our own (**Chapter 2.1**), reported associations of higher concentrations of BPA and phthalates among pregnant women with a higher BMI.[30,34,36,37,39,90] A recent study on determinants of phenols and phthalates observed higher BPA concentrations among women with a greater gestational weight gain in univariate associations.[91] Among other environmental exposures, persistent organic pollutants (POPs) have been examined for associations with gestational weight gain. Although the direction of effect differs by the specific POP, the majority of studies consistently identified exposure during early pregnancy as a window of vulnerability for associations with gestational weight gain.[92-96] In line with these findings, we observed (**Chapter 2.4**) associations of early pregnancy higher total bisphenols and bisphenol S with a lower gestational weight gain, especially among normal weight women. Investigation of weight gain per pregnancy period showed the strongest reduction in weight gain for mid-to-late pregnancy, driven by bisphenol A. Several body compartments could be responsible for the observed change in gestational weight gain, including maternal fat, intra- and extravascular fluid volumes, breast mass and intrauterine components being the fetus, placenta and amniotic fluid. As mentioned previously, we did not observe an association between first trimester bisphenols or phthalate metabolites and placental weight (**Chapter 2.3**). Gestational weight gain, particularly in mid- and late pregnancy, is associated with birth weight.[97,98] However, a recent meta-analysis of eight studies did not observe associations between fetal bisphenol A exposure and birth weight.[99] Contradicting our results, a very recent subsequent study among the same cohort as presented in this thesis observed associations

of higher maternal bisphenol S concentrations, especially in the first trimester of pregnancy, with higher fetal weight.[100] We therefore conclude that the findings from this thesis suggest that early pregnancy maternal bisphenol exposure might affect gestational weight gain through changes in maternal body composition.

Several mechanisms might be responsible for the associations of bisphenols and reduced gestational weight gain. Bisphenols are weak xenoestrogens and have been reported to increase the level of 17β-estradiol and are also reported to promote progesterone levels. Although the evidence is limited, estradiol levels have not been associated with gestational weight gain and a small positive effect has been observed for progesterone, contradicting our results.[101] Bisphenols tend to have anti-androgen capacities and to decrease cortisol levels. Both maternal testosterone levels and cortisol levels have been associated with increased gestational weight gain, however both associations were with late pregnancy hormone levels and associations have not been replicated so far.[102,103] Also other potential mechanisms, such as through oxidative stress, adipocytokines and peroxisome proliferator-activated receptor activation are not likely to lead to reduced gestational weight gain. Potential mechanisms should be further investigated in future studies.

Conclusions on maternal weight gain

- Higher maternal total bisphenols and bisphenol S concentrations in early pregnancy were associated with a lower total gestational weight gain, especially among normal weight women.

Postpartum weight gain

Phthalates have been associated with increased body mass in mainly cross-sectional studies.[104-108] A longitudinal study nested within the Nurses' Health Study I and II identified a 170-210 g/year increased weight gain among those most highly exposed for BPA, PA, mBzP and mBP metabolites. For mEP and DEHP metabolites non-monotonic associations were observed.[109] A recent study among Mexican pregnant women reported a slower rate of weight loss in the first year postpartum for women that were higher exposed to bisphenol A and several phthalates.[89] The only previous study among pregnant women with a longer follow up duration observed an association of higher mCPP with 300 g/year maternal weight gain during 10 years postpartum.[110] We observed (**Chapter 2.5**) associations of averaged early and mid-pregnancy phthalate exposure with increased weight gain 6 years postpartum. PA, LMW phthalate and DNOP metabolites were associated with increased weight gain among women without subsequent pregnancies, with similar annual increases as observed in the Nurses' Health Study. Yet, our associations were all linear. Stratification revealed these associations to be driven by overweight and obese women. 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 make less healthy food choices leading to higher bisphenol and phthalate exposure and weight increase independent of exposure levels.

Results from this thesis suggest that exposure to phthalates may have long-term effects on weight gain. The similarity in annual increases with the Nurses' Health Study might suggest that although pregnancy

might be a period with increased susceptibility to these compounds, the observed associations may be independent of pregnancy status. Obesogenic effects of bisphenols and phthalates have been linked to peroxisome proliferator-activated receptor γ activation.[111,112] However, the phthalate metabolites that are reported as activators of this pathway were not associated with increased weight gain among women without subsequent pregnancies. Other proposed mechanisms may be by sex-steroid dysregulation, epigenetics or metabolomics. Further studies are needed to evaluate these potential mechanisms.

Conclusions on postpartum weight gain

- Averaged early and mid-pregnancy phthalate exposure was associated with increased maternal weight gain 6 years postpartum, especially among overweight and obese women.

Parental smoking

Adverse birth outcomes and childhood overweight

Maternal smoking during pregnancy has been associated with increased risks of adverse birth outcomes, such as congenital abnormalities, stillbirth, sudden infant death syndrome, preterm birth and low birth weight, and overweight in childhood.[11-13,113] Evidence on critical windows of vulnerability and effects of changes in maternal smoking behavior during pregnancy remain inconclusive.[113-119] Results from the individual participant meta-analysis presented in this thesis (**Chapter 3.1**) confirm associations of maternal continued smoking during pregnancy with increased risks of preterm birth, being small size for gestational age at birth and overweight in childhood. The risks of preterm birth were somewhat weaker than reported previously, possibly due to no information available about induced or spontaneous preterm birth.[11,114,116] A recent study using additional methods of Mendelian randomization and parental negative control reported consistent linear dose-dependent associations of maternal smoking with fetal growth from early second trimester onwards.[120] In line with these findings, we observed that maternal smoking in first trimester only was not associated with small size for gestational age and preterm birth. Reducing the number of cigarettes from first-to-third trimester lowered the risks of small size for gestational age but not preterm birth. However, risks of small size for gestational age among mothers with reduced smoking intake were still elevated compared to infants born to non-smoking mothers. These findings confirm previous reports that the majority of maternal smoking impact on birth weight occurs after the first trimester of pregnancy.[120,121] Maternal smoking in first trimester only was associated with an increased risk of overweight in childhood and reducing maternal smoking intake did not seem beneficial. In the Nurses' Health Study, adiposity risks at the age of 18 years old of daughters whose mothers smoked during the first trimester only were not increased, but the risk of type 2 diabetes was.[122,123] Altogether, these findings suggest that maternal first trimester smoking might already lead to metabolic adaptations with lifelong consequences for body composition and metabolic health. Thus, findings from this thesis suggest that quitting smoking in the first trimester of pregnancy might optimize birth outcomes, but might not reduce the risk of adverse metabolic effects in the offspring to the level of non-smoking. For risks of fetal growth restriction it might still be beneficial to reduce smoking consumption from first trimester onwards.

In literature, passive smoking and paternal smoking are being used interchangeably, whilst being two different exposures. Paternal smoking is of particular interest because besides a potential direct intrauterine effect through maternal secondhand smoking, paternal smoking might additionally induce mutagenic effects. The role of paternal smoking during pregnancy on preterm birth, low birth weight and childhood overweight has been scarcely examined and remains unclear.[119,122,124] A recent meta-analysis reported that paternal smoking was associated with increased risks of preterm birth and small size for gestational age.[125] In a previous Dutch study, paternal smoking during pregnancy among non-smoking mothers was associated with a higher childhood BMI.[14] We observed that paternal smoking among non-smoking mothers was associated with a higher risk of childhood overweight and tended to be associated with higher risks of preterm birth and small size for gestational age. Among mothers who continued smoking during pregnancy, paternal smoking further increased the risks of small size for gestational age and childhood overweight, but not the risk of preterm birth. These results suggest that paternal smoking might be associated with childhood overweight, independent of maternal smoking. We cannot exclude that paternal smoking also represents passive smoking of the mothers, which might explain the observed associations.

Various components of tobacco smoke might be involved in the mechanistic pathways towards adverse birth outcomes and childhood overweight. With regards to fetal growth restriction, both nicotine and carbon monoxide are reported to reduce placental blood flow, respectively through vasoconstriction and hypoxia.[126-128] In the last few years, several studies found a mediating role of DNA methylation in the association between maternal smoking and birth weight.[129,130] Several mechanisms for nicotine-induced alterations in overweight risks have been proposed, including stimulation of the fetal hypothalamic-pituitary axis.[131] Previous studies used comparisons of maternal and paternal smoking associations to explore potential mechanisms.[14,132] The study as presented in this thesis observed higher risks of small size for gestational age among maternal smokers than among paternal smokers, while risks of preterm birth for maternal and paternal smoking were similar. The similarity of associations of maternal and paternal smoking with preterm birth may indicate that underlying mechanisms must be seen in the light of shared family-based characteristics, such as environmental exposures and lifestyle, rather than the actual smoking exposure. However, we cannot exclude that smoking does affect risks of preterm birth. Stronger maternal than paternal associations for small size for gestational age may suggest an intrauterine effect. However, the risk increase when both parents smoked may represent an additional mechanistic pathway through shared family-based characteristics, since paternal smoking among non-smoking mothers was not associated with small size for gestational age. Comparison of maternal and paternal smoking effect estimates for childhood overweight showed a slightly higher risk for children whose mother smoked. However, risks increased significantly if both parents smoked. These findings suggest that, although intrauterine programming mechanisms play a role, we cannot exclude the role of transgenerational epigenetic inheritance, shared family-based lifestyle and genetic characteristics as potential underlying mechanisms.

Conclusions on parental smoking and adverse birth outcomes and childhood overweight

- Maternal first trimester only smoking was not associated with adverse birth outcomes but was associated with a higher risk of childhood overweight.
- Children from mothers who continued smoking during pregnancy had higher risks of preterm birth, small size for gestational age babies and childhood overweight.
- Women who reduced the number of cigarettes after first trimester still had an increased but lower risk of small size for gestational age babies.
- Among non-smoking mothers, paternal smoking was associated with childhood overweight, but not with adverse birth outcomes.

Methodological considerations

Strengths and limitations for each study individually are described in **Chapters 2 and 3** of this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias, confounding and causality are discussed.

Selection bias

Selection bias may arise from the procedure used to select study participants or factors that influence the study participation, leading to a difference in the relation between exposure and outcome for those who participate and those that were eligible but were not included. Selection bias within the Generation R study may have occurred either from selective non-response at baseline or from selective loss to follow up. Of all children eligible at birth, 61% participated at baseline. This non-response is not likely to be random. Participating women were less often from ethnic minorities and had a higher socio-economic status than would have been expected from population numbers in the study population. Furthermore, participating women had less medical complications during pregnancy and unfavorable pregnancy outcomes, such as gestational hypertensive disorders or low birth weight, suggesting a more healthy study population.[133] Selection bias may also arise from selective loss to follow up. Participation rate at the child age of 6 years old was 85% of the original cohort, of whom 80% visited the research center.[134] Bisphenol and phthalate metabolite concentrations were measured in a subgroup of women comprising 16% of the total study sample who were included in early pregnancy and whose children participated in postnatal studies. This subgroup included singleton pregnancies only. Maternal characteristics were similarly distributed between participants and non-participants that would have been eligible for inclusion. However, mothers that did not participate in the follow up measurements at the child age of 6 years old had a lower educational level and more unhealthy lifestyle habits than the women who did participate in follow up studies. This selection in the Generation R Study and more profoundly in our subgroup towards a more affluent and healthy study population could lead to biased estimates if determinants of participation are related to both exposure and outcome measures. Several studies showed that in cohort studies the influence of non-response at baseline on effect estimates

was limited and that additional adjustment for covariates associated with loss to follow up decreases selection bias.[135] Therefore, we do not assume that this non-response has influenced our results. However, this selection towards a healthier population will probably lower the prevalence rates of the outcomes of interest and thereby reduce statistical power and the generalizability of our results.

Information bias

Information bias may arise from measurement error of a determinant or outcome and is also referred to as misclassification. Two types of information bias can occur: differential and non-differential misclassification. Differential misclassification may occur when the probability of the determinant being misclassified is non-random and dependent on the outcome, and vice versa. As a result, this can lead to underestimation or overestimation of the results. Non-differential misclassification is a random error and unrelated to other study variables, leading to a bias towards the null value.

Information on the determinants and outcomes in the studies described in this thesis were obtained prospectively by physical and ultrasound examinations, blood and urine analyses and parental questionnaires. In environmental exposure research, an important concern is correct assessment of the exposure. For this thesis, we have used urinary bisphenols and phthalate metabolites to estimate tissue exposure. Urinary bisphenols and phthalate metabolites were analyzed independent of outcome measures. Therefore, differential misclassification of bisphenol and phthalate exposure seems unlikely. However, several issues concerning potential non-differential misclassification should be addressed.

Urinary samples have been stored at-20°C for approximately 10 years. It has been suggested that-80°C would be a more optimal storage temperature,[136] however there are no studies investigating differences between these specific temperatures and effects of long-term storage. Therefore, we cannot exclude that there has been some biological activity during the storage period leading to potential non-differential misclassification and underestimation of the effects. The analytical techniques allowed for detection of both unconjugated (free) compounds as well as glucuronidated and sulfated bisphenol and phthalate conjugates. As diet is the primary source of bisphenol and phthalate exposure, ingestion will be followed by a first-pass metabolism.[28] For phthalates, and for bisphenols to a lesser extent, a substantial fraction of exposure is through non-dietary sources such as transdermal exposure to personal care products and thermal paper or inhalation of indoor dust. Following exposure through other routes than ingestion, the first-pass effect is initially bypassed, provoking higher bioavailability and tissue exposure. Measurement of urinary metabolites might therefore underestimate tissue exposure to phthalates, leading to non-differential misclassification and subsequent bias towards the null.[137]

Quantitative detection of environmental compounds, such as bisphenols and phthalates, is always complicated by left censoring due to non-detectable concentrations. Several methods have been proposed to handle left-censored data, including deletion, simple replacement, extrapolation or maximum likelihood estimation. In the studies comprising this thesis we applied a simple replacement technique by substituting the values below the limit of detection (LOD) for LOD/√2, as standard practice.[138,139] Such replacement techniques are convenient but may affect the variability of the distribution and may thereby distort regression coefficients and reduce power. However, when only a small percentage of values have been censored, simple replacement techniques are adequate.[140] In

the studies comprising this thesis, individual bisphenols or phthalate metabolites were included in the groups when >20% of values were detected and bisphenols were analyzed individually when >50% of the values were detected. Almost all included phthalate metabolites had very high detection rates, allowing for simple replacement techniques. Detection rates for bisphenols were lower, which might have complicated the detection of associations.

Urinary concentrations of bisphenols and phthalate metabolites were measured in a single spot urine sample in the majority of studies in this thesis. A few studies used two spot urine samples to measure exposure levels. Both bisphenols and phthalates have short biological half-lives (less than 24h).[141,142] Despite of the reported short biological half-lives, it has been suggested that one single urine sample for phthalate concentrations reasonably reflects exposure for up to three months or even longer.[143,144] Phthalate exposure sources such as personal care products and indoor environments are relatively consistent, while dietary sources of phthalate exposure are known to be more variable.[33] Within-person variability of bisphenol urinary concentrations is reported to be high, constraining the value of one single urine sample for bisphenol measurement.[145] This non-differential misclassification is expected to lead to attenuation bias in dose-response relationships.

It has been suggested that exposure to phthalate esters with long side chains is easily underestimated if only a few metabolites are being measured.[146] For the studies in this thesis, 18 phthalate metabolites were measured including phthalate acid. Phthalate acid is a common endpoint of phthalate metabolism and can be used as a proxy for total phthalate exposure.[147] Therefore, we do not expect underestimation of phthalate exposure due to limited metabolite measurements. Urinary concentrations of bisphenols and phthalate metabolites are subject to dilution. To correct for dilution several methods are applied. In the studies comprising this thesis we applied creatinine adjustments with measured creatinine levels from each spot urine sample. Endogenous creatinine clearance, measured by 24-hr urine collection, remains the most precise estimation of glomerular filtration rate in pregnant women.[148] It has been suggested that specific gravity adjustment is a better correction method in pregnant women.[149] Unfortunately, specific gravity measurements were not available.

Self-reported lifestyle habits, such as parental smoking or lifestyle and nutrition related factors investigated as potential determinants of bisphenols and phthalates, may have been underreported or overreported by the parents since most parents are aware of potential negative effects of these habits. Since parents were unaware of specific research questions, differential misclassification seems unlikely, but non-differential misclassification may have resulted in attenuation of the studied associations.

The majority of outcomes in this thesis were hands-on measurements of weight or growth, medical diagnoses, ultrasound measurements or laboratory analyses. Observers of these outcomes were unaware of exposure status, which makes differential misclassification unlikely. Some of the outcomes were self-reported and recalled, including time to pregnancy, pre-pregnancy weight and highest weight before birth. Self-reported weight tends to be underestimated, leading to non-differential misclassification. Only one study investigated recall error for prospectively measured and recalled time to pregnancy, showing a reasonable validity.[150] Although misclassification might be present, differential misclassification seems unlikely.

Confounding

Confounding is present when all or part of the observed association between the exposure and the outcome is in fact explained by other variables associated with the outcome without being affected by the exposure themselves.[151] If a confounding factor is not taken into account, results might be biased obscuring the true exposure effect. In this thesis, we have used several approaches to explore the role of confounding in the studied associations. To take account for confounding, we adjusted all analyses for multiple potential confounders. We selected covariates based on their associations with the exposures and outcomes of interest in our or previous studies or a change in effect estimate of more than 10%. As in any observational study, residual confounding might still be present due to unknown or unmeasured confounding variables. Some of the unmeasured confounding can relate to other environmental exposures, medical history, genetics, and unmeasured sociodemographic and lifestyle factors, including maternal diet and activity. Also, information about several confounders was self-reported and measurement error might have occurred, contributing to residual confounding and an under- or overestimation of the observed effect estimates. To help understanding whether the associations of maternal smoking with adverse birth outcomes and childhood adiposity are explained by direct intrauterine mechanisms or confounded by environmental, lifestyle or genetic characteristics, we compared associations of maternal and paternal smoking exposure during pregnancy. A similar effect size for the maternal and paternal association would suggest that the association of maternal smoking with adverse birth outcomes and childhood adiposity is explained by shared family-based lifestyle and genetic characteristics, rather than intrauterine programming.

Causality

The causality of the associations observed in this thesis remains to be established. The Bradford Hill criteria can be used to provide evidence of the causal relationship between an exposure and an outcome, including strength, consistency, specificity, temporality, dose-response relationship, biological plausibility, coherence, experiment and analogy.[152] Overall, if an association is observed at all, our associations are relatively small. Notwithstanding that stronger associations are more likely to be causal, weak associations may also be causal. For many of the associations investigated in this thesis, evidence so far is limited, complicating effect interpretation. However, the majority of findings is consistent with previous studies, potential biological mechanisms and animal studies. Environmental exposures are not likely to be specific and give rise to only one single outcome. Except for the studies concerning determinants of bisphenols and phthalates and time to pregnancy, all studies were longitudinal supporting temporality between exposures and outcomes. Although some previous studies suggested non-monotonic effects from bisphenols and phthalates, all our associations were linear showing a dose-response effect. Also for associations of smoking, strong dose-response relationships were observed, consistent with previous studies and potential mechanisms, primarily for fetal growth restriction. As this thesis is based on observational research, the criterion of experiment is not addressed in this thesis and would not be ethically responsible. Also the analogy criterion is not being addressed in this thesis. Altogether, this thesis was not designed to clarify causality of these associations, but some of our observational studies seem to provide some evidence for causal relationships based on the Bradford Hill criteria.

Future research

As stated previously, environmental exposures such as bisphenols, phthalates and smoking have a significant share in chronic non-communicable diseases worldwide and exposure during pregnancy is a window of vulnerability for both mother and child. Findings from this thesis provide some evidence that bisphenol and phthalate exposure during pregnancy may affect maternal health in the short and long term, including time to pregnancy, placental hemodynamics, adequate gestational weight gain and postpartum weight gain. For parental smoking, findings suggest that maternal smoking affects risks for small size for gestational age through a direct intra-uterine effect and may therefore benefit from quitting maternal smoking during pregnancy. Contrariwise, parental smoking associations with childhood adiposity seem rather based on shared family-based and genetic characteristics than a direct intra-uterine effect. However, the following major issues remain to be addressed in future studies.

Causality

Although we found some evidence for causality in our studies based on the Bradford Hill criteria, the causality of the associations of bisphenols and phthalates during pregnancy with maternal health outcomes observed in this thesis remains unclear. Associations as observed between maternal smoking during pregnancy and fetal growth restriction met with multiple Bradford Hill criteria for causality. However, a causal relationship still has not been confirmed. For preterm birth and childhood adiposity the causality of the associations as observed in this thesis remains unclear.

Randomized controlled trials are the golden standard study design to establish causality, however this is not possible for environmental chemicals like bisphenols and phthalates. For the association with fetal growth, previous randomized smoking cessation trials have shown an increased birth weight for the quitters compared to the continued smokers, but do not take into account the time of quitting and do not report on effects in the group that does not quit completely but does reducing their smoking intake.[153,154]

Parental negative control studies and sibling comparison studies allow control for shared family-based characteristics. Sibling comparison studies additionally allow control for maternal genotype that are shared among siblings. This study design is difficult for bisphenol and phthalate exposure. Few studies have used this design to investigate the associations between smoking during pregnancy and fetal growth restriction.[120,155] However, as other characteristics such as lifestyle may differ between parents or siblings, this design may lead to biased estimates.[156]

Mendelian randomization studies use genetic variants that are robustly associated with the exposure of interest, not affected by confounding and therefore are an adequate design to examine causal relationships. Further investigation of genetic variants associated with bisphenol and phthalate metabolism might shed light on the most vulnerable populations and may allow for Mendelian randomization studies. Our study on time to pregnancy revealed an association of bisphenols and phthalates with reduced fecundability among women who did not use preconception folic acid supplements. This finding is hypothesis generating suggesting that the mechanism by which bisphenols

and phthalates impair reproductive potential may involve changes in DNA methylation. Further research is needed to investigate these mechanisms and to obtain more evidence on maternal folate levels that counteract these effects.

Among Europeans, the common variant rs1051730 is robustly associated with smoking quantity and is associated with a reduced ability to quit smoking during pregnancy.[157,158] This genotype was associated with reduced birth weight among current maternal smokers but not among quitters and non-smokers, strengthening the evidence that maternal cigarette smoking during pregnancy is causally related to a lower birth weight.[120,159] However, due to a small number of individuals in the group of quitters, these findings should be taken with caution. Also, the role of smoking in the preconception period has to be further assessed. To further increase evidence for causality, future studies should assess larger groups of quitters, reducers and smoking in the preconception period.

For preterm birth and childhood adiposity, these studies are lacking. For childhood adiposity, further studies are needed to assess the role of exposure to smoking during childhood, as many women who quitted during pregnancy resume smoking shortly after birth.[160] While potential mechanisms between smoking and fetal growth restriction are rather established, mechanisms for preterm birth and childhood adiposity are not yet clear. Further research is needed to assess such possible mechanisms.

Assessment of exposures and outcomes

As mentioned previously, potential misclassification of bisphenol and phthalate exposure might be present in the studies comprising this thesis. To reduce this risk of misclassification and bias due to high exposure temporal variability, future studies should include a large number of urine samples, preferably also during the preconception period and in very early pregnancy. To reduce the additional costs of laboratory analysis of a large number of samples, samples could be pooled as reported previously.[33] However, the adequate number of samples for bisphenols and phthalates with variable exposure sources such as diet should be further investigated. As urinary samples are often stored before laboratory analysis, further studies should investigate optimal storage conditions and effects of long-term storage. The studies in this thesis use creatinine correction to correct for urinary dilution. It has been suggested that specific gravity is a better correction method in pregnant women.[149] Future studies of maternal urinary bisphenol and phthalate metabolite concentrations should preferably use specific gravity measurements to correct for urinary dilution. Parental smoking information was self-reported. Measured cotinine levels might be used to confirm smoking status and to quantify passive smoking in order to reduce the risk for misclassification and bias.

During pregnancy, women are not only exposed to bisphenols or phthalates but to a whole spectrum of environmental chemicals. Not much is known about these chemical mixtures, e.g. the value of individual chemical agents, potential interactions and potential cumulative exposure to multiple agents.[161] Only few studies have quantified the impact of aggregate exposure to multiple chemicals, but none have for the outcomes investigated in this thesis. Several methods to investigate chemical mixtures have been proposed,[162] however it still remains difficult to address exposure variability issues at the same time. Moreover, not only exposures during pregnancy may affect maternal health during and after pregnancy, but the whole exposome might. The exposome can be defined as the totality of exposures an individual

experiences in a lifetime and how those exposures relate to health.[163] This exposome does not only include environmental chemicals, but also the ecosystem and socio-economic and lifestyle factors. Investigation of the exposome is challenging and complex, but might reveal further insight in disease etiology and give opportunities for prevention.

Concerning the outcomes, many were self-reported and some were even recalled, such as time to pregnancy, pre-pregnancy weight and highest weight during pregnancy before birth. Prospectively measured outcomes might reduce the risk for misclassification and bias. For outcomes like weight gain and adiposity, measurements of body composition such as magnetic resonance imaging measurements might provide further insight into the underlying mechanism. To optimize, repeated measurements of weight, growth and body composition are needed to allow for investigation of specific weight gain and growth patterns. Additional measures of cardiometabolic function postpartum and during childhood, such as blood pressure, insulin/glucose metabolism and endothelial function might be of interest to gain more knowledge about long-term cardiometabolic changes.

Implications for clinical practice and policy

Maternal exposure to bisphenols and phthalates during pregnancy might affect maternal health on the short and long term. The observed effect estimates were, if an association was observed, small. However, these small effects might contribute to the total burden of disease due to environmental exposures during the human life course. Even subclinical differences in maternal health during pregnancy might influence fetal outcomes and potentially induce transgenerational effects. As pregnancy is a window of vulnerability to metabolic changes in the mother, small effects during or shortly after pregnancy might track into later life contributing to the development of cardiovascular and metabolic disease. Further studies are needed to investigate potential causal effects. Although further governmental embargoes may seem beneficial, it may also stimulate the use of even more harmful substitutes. As exposure to bisphenols and phthalates seems associated with adverse lifestyle habits, preventive strategies or interventions should focus on motivating women to make lifestyle changes during the preconception period and pregnancy. Although lifestyle medicine is an emerging field of interest and governmental policies follow slowly, the focus is rather on secondary prevention than on primary prevention among fertile man and women. Since preconception consultations are not standard in the Netherlands, health professionals should address these issues during regular appointments, even when the woman is already pregnant. Governmental campaigns including promotion of preconception consultations might increase awareness among man and women who are planning to start a family.

The study in this thesis provides solid evidence that maternal smoking during pregnancy affects fetal growth through a direct intra-uterine effect with beneficial effects from quitting and even reducing smoking intake during pregnancy. For childhood adiposity, parental smoking effects seem rather based on shared family-based lifestyle and genetic characteristics than a direct intra-uterine effect. The associations as observed for preterm birth were small. However, our results might be important from an etiological and preventive perspective since preterm birth, fetal growth restriction and childhood adiposity are related with adverse health consequences in later life. These findings imply that it is of the greatest importance to invest in prevention of smoking in women of reproductive age, but they

also vote for an ongoing support of smoking pregnant women to motivate them to reduce smoking, even in advanced pregnancy. Current guidelines focus only on quitting and not reducing smoking, while reducing could be beneficial for women who find it difficult to quit completely. Our results concerning childhood adiposity advocate for preventive strategies or interventions focused on lifestyle changes in the preconception period for both parents. Fathers should therefore be more closely involved in preconception and also pregnancy consultations.

Conclusion

Findings from this thesis suggest that environmental exposures during pregnancy, such as bisphenols, phthalates and smoking may affect maternal and child health outcomes in the short and long term. The observed associations are relatively small, but may contribute to the total burden of morbidity and mortality due to non-communicable diseases. Further research with more precise exposure assessment including assessment of chemical mixtures and exposure during the periconception, pregnancy and postpartum periods is needed to provide more solid information about potential causal effects as a basis for preventive measures.

References

1. Pruss-Ustun A, van Deventer E, Mudu P, Campbell-Lendrum D, Vickers C, Ivanov I, et al. Environmental risks and non-communicable diseases. BMJ. 2019;364:l265.
2. Ranciere F, Lyons JG, Loh VH, Botton J, Galloway T, Wang T, et al. Bisphenol A and the risk of cardiometabolic disorders: a systematic review with meta-analysis of the epidemiological evidence. Environmental health : a global access science source. 2015;14:46.
3. James-Todd TM, Huang T, Seely EW, Saxena AR. The association between phthalates and metabolic syndrome: the National Health and Nutrition Examination Survey 2001-2010. Environmental health : a global access science source. 2016;15:52.
4. Russo G, Barbato F, Mita DG, Grumetto L. Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Food Chem Toxicol. 2019;131:110575.
5. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. Endocr Rev. 2015;36(6):E1-E150.
6. GBD.Risk.Factors.Collaborators;. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016;388(10053):1659-724.
7. Samet JM, Yoon SY. Women and the tabacco epidemic: challenges for the 21st century: WHO, Institute for Global Tabacco Control, Johns Hopkins School of Public Health; 2011.
8. Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update. 2011;17(5):589-604.
9. Marufu TC, Ahankari A, Coleman T, Lewis S. Maternal smoking and the risk of still birth: systematic review and meta-analysis. BMC Public Health. 2015;15:239.
10. Zhang K, Wang X. Maternal smoking and increased risk of sudden infant death syndrome: a meta-analysis. Leg Med (Tokyo). 2013;15(3):115-21.
11. Shah NR, Bracken MB. A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery. Am J Obstet Gynecol. 2000;182(2):465-72.
12. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65(5):663-737.
13. Rayfield S, Plugge E. Systematic review and meta-analysis of the association between maternal smoking in pregnancy and childhood overweight and obesity. J Epidemiol Community Health. 2016.
14. Durmus B, Heppe DH, Taal HR, Manniesing R, Raat H, Hofman A, et al. Parental smoking during pregnancy and total and abdominal fat distribution in school-age children: the Generation R Study. Int J Obes (Lond). 2014;38(7):966-72.
15. Albers L, Sobotzki C, Kuss O, Ajslev T, Batista RF, Bettiol H, et al. Maternal smoking during pregnancy and offspring overweight: is there a dose-response relationship? An individual patient data meta-analysis. Int J Obes (Lond). 2018;42(7):1249-64.
16. Casas M, Valvi D, Luque N, Ballesteros-Gomez A, Carsin AE, Fernandez MF, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int. 2013;56:10-8.
17. Philippat C, Botton J, Calafat AM, Ye X, Charles MA, Slama R, et al. Prenatal exposure to phenols and growth in boys. Epidemiology. 2014;25(5):625-35.
18. Valvi D, Casas M, Mendez MA, Ballesteros-Gomez A, Luque N, Rubio S, et al. Prenatal bisphenol a urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology. 2013;24(6):791-9.
19. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ Health Perspect. 2011;119(6):878-85.

20. Harley KG, Berger K, Rauch S, Kogut K, Claus Henn B, Calafat AM, et al. Association of prenatal urinary phthalate metabolite concentrations and childhood BMI and obesity. Pediatr Res. 2017;82(3):405-15.
21. Wu H, Kupsco AJ, Deierlein AL, Just AC, Calafat AM, Oken E, et al. Trends and Patterns of Phthalates and Phthalate Alternatives Exposure in Pregnant Women from Mexico City during 2007-2010. Environ Sci Technol. 2020;54(3):1740-9.
22. Rodriguez-Carmona Y, Ashrap P, Calafat AM, Ye X, Rosario Z, Bedrosian LD, et al. Determinants and characterization of exposure to phthalates, DEHTP and DINCH among pregnant women in the PROTECT birth cohort in Puerto Rico. J Expo Sci Environ Epidemiol. 2020;30(1):56-69.
23. Ashrap P, Watkins DJ, Calafat AM, Ye X, Rosario Z, Brown P, et al. Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends. Environ Int. 2018;121(Pt 1):990-1002.
24. Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006;29(1):134-9; discussion 81-5.
25. Schecter A, Lorber M, Guo Y, Wu Q, Yun SH, Kannan K, et al. Phthalate concentrations and dietary exposure from food purchased in New York State. Environ Health Perspect. 2013;121(4):473-94.
26. Liao C, Kannan K. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J Agric Food Chem. 2013;61(19):4655-62.
27. Serrano SE, Braun J, Trasande L, Dills R, Sathyanarayana S. Phthalates and diet: a review of the food monitoring and epidemiology data. Environmental health : a global access science source. 2014;13(1):43.
28. Martinez MA, Rovira J, Prasad Sharma R, Nadal M, Schuhmacher M, Kumar V. Comparing dietary and non-dietary source contribution of BPA and DEHP to prenatal exposure: A Catalonia (Spain) case study. Environ Res. 2018;166:25-34.
29. Braun JM, Kalkbrenner AE, Calafat AM, Bernert JT, Ye X, Silva MJ, et al. Variability and predictors of urinary bisphenol A concentrations during pregnancy. Environ Health Perspect. 2011;119(1):131-7.
30. Cantonwine DE, Cordero JF, Rivera-Gonzalez LO, Anzalota Del Toro LV, Ferguson KK, Mukherjee B, et al. Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors. Environ Int. 2014;62:1-11.
31. Trasande L, Sathyanarayana S, Jo Messito M, R SG, Attina TM, Mendelsohn AL. Phthalates and the diets of U.S. children and adolescents. Environ Res. 2013;126:84-90.
32. Pacyga DC, Sathyanarayana S, Strakovsky RS. Dietary Predictors of Phthalate and Bisphenol Exposures in Pregnant Women. Adv Nutr. 2019;10(5):803-15.
33. Shin HM, Bennett DH, Barkoski J, Ye X, Calafat AM, Tancredi D, et al. Variability of urinary concentrations of phthalate metabolites during pregnancy in first morning voids and pooled samples. Environ Int. 2019;122:222-30.
34. Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, et al. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. Int J Hyg Environ Health. 2015;218(2):220-31.
35. Wenzel AG, Brock JW, Cruze L, Newman RB, Unal ER, Wolf BJ, et al. Prevalence and predictors of phthalate exposure in pregnant women in Charleston, SC. Chemosphere. 2018;193:394-402.
36. Lewin A, Arbuckle TE, Fisher M, Liang CL, Marro L, Davis K, et al. Univariate predictors of maternal concentrations of environmental chemicals: The MIREC study. Int J Hyg Environ Health. 2017;220(2 Pt A):77-85.
37. Polinski KJ, Dabelea D, Hamman RF, Adgate JL, Calafat AM, Ye X, et al. Distribution and predictors of urinary concentrations of phthalate metabolites and phenols among pregnant women in the Healthy Start Study. Environ Res. 2018;162:308-17.
38. Reeves KW, Santana MD, Manson JE, Hankinson SE, Zoeller RT, Bigelow C, et al. Predictors of urinary phthalate biomarker concentrations in postmenopausal women. Environ Res. 2019;169:122-30.

39. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, et al. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int. 2014;68:55-65.
40. James-Todd TM, Meeker JD, Huang T, Hauser R, Seely EW, Ferguson KK, et al. Racial and ethnic variations in phthalate metabolite concentration changes across full-term pregnancies. J Expo Sci Environ Epidemiol. 2017;27(2):160-6.
41. Trasande L, Attina TM, Blustein J. Association between urinary bisphenol A concentration and obesity prevalence in children and adolescents. JAMA. 2012;308(11):1113-21.
42. Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect. 2013;121(4):501-6.
43. Huang T, Saxena AR, Isganaitis E, James-Todd T. Gender and racial/ethnic differences in the associations of urinary phthalate metabolites with markers of diabetes risk: National Health and Nutrition Examination Survey 2001-2008. Environmental health : a global access science source. 2014;13(1):6.
44. Jackson WJ, Darnell WR, inventorsProcess for foaming cellulose acetate rod1985 Mar 26, 1985.
45. Lewin A, Arbuckle TE, Fisher M, Liang CL, Marro L, Davis K, et al. Univariate predictors of maternal concentrations of environmental chemicals: The MIREC study. Int J Hyg Environ Health. 2017.
46. Philips EM, Jaddoe VW, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2016.
47. Oulhote Y, Lanphear B, Braun JM, Webster GM, Arbuckle TE, Etzel T, et al. Gestational Exposures to Phthalates and Folic Acid, and Autistic Traits in Canadian Children. Environ Health Perspect. 2020;128(2):27004.
48. Philips EM, Kahn LG, Jaddoe VWV, Shao Y, Asimakopoulos AG, Kannan K, et al. First Trimester Urinary Bisphenol and Phthalate Concentrations and Time to Pregnancy: A Population-Based Cohort Analysis. J Clin Endocrinol Metab. 2018;103(9):3540-7.
49. Dolinoy DC, Huang D, Jirtle RL. Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development. Proc Natl Acad Sci U S A. 2007;104(32):13056-61.
50. Buckley JP, Palmieri RT, Matuszewski JM, Herring AH, Baird DD, Hartmann KE, et al. Consumer product exposures associated with urinary phthalate levels in pregnant women. J Expo Sci Environ Epidemiol. 2012;22(5):468-75.
51. Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) Study. Fertility and sterility. 2014;101(5):1359-66.
52. Jukic AM, Calafat AM, McConnaughey DR, Longnecker MP, Hoppin JA, Weinberg CR, et al. Urinary Concentrations of Phthalate Metabolites and Bisphenol A and Associations with Follicular-Phase Length, Luteal-Phase Length, Fecundability, and Early Pregnancy Loss. Environ Health Perspect. 2016;124(3):321-8.
53. Velez M, Arbuckle T, Fraser W. Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. 2015;103(4):1011–20.e2.
54. Thomsen AM, Riis AH, Olsen J, Jonsson BA, Lindh CH, Hjollund NH, et al. Female exposure to phthalates and time to pregnancy: a first pregnancy planner study. Human reproduction (Oxford, England). 2017;32(1):232-8.
55. Specht IO, Bonde JP, Toft G, Lindh CH, Jonsson BA, Jorgensen KT. Serum phthalate levels and time to pregnancy in couples from Greenland, Poland and Ukraine. PloS one. 2015;10(3):e0120070.
56. Minguez-Alarcon L, Gaskins AJ, Chiu YH, Souter I, Williams PL, Calafat AM, et al. Dietary folate intake and modification of the association of urinary bisphenol A concentrations with in vitro fertilization outcomes among women from a fertility clinic. Reprod Toxicol. 2016;65:104-12.
57. Consales C, Toft G, Leter G, Bonde JP, Uccelli R, Pacchierotti F, et al. Exposure to persistent organic pollutants and sperm DNA methylation changes in Arctic and European populations. Environ Mol Mutagen. 2016;57(3):200-9.

58. Qu XL, Ming Z, Yuan F, Wang H, Zhang YZ. Effect of 2,3',4,4',5-Pentachlorobiphenyl Exposure on Endometrial Receptivity and the Methylation of HOXA10. Reprod Sci. 2018;25(2):256-68.

59. da Silva VR, Hausman DB, Kauwell GP, Sokolow A, Tackett RL, Rathbun SL, et al. Obesity affects short-term folate pharmacokinetics in women of childbearing age. Int J Obes (Lond). 2013;37(12):1608-10.

60. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631-44.

61. Werner EF, Braun JM, Yolton K, Khoury JC, Lanphear BP. The association between maternal urinary phthalate concentrations and blood pressure in pregnancy: The HOME Study. Environmental health : a global access science source. 2015;14:75.

62. Leclerc F, Dubois MF, Aris A. Maternal, placental and fetal exposure to bisphenol A in women with and without preeclampsia. Hypertens Pregnancy. 2014;33(3):341-8.

63. Cantonwine DE, Meeker JD, Ferguson KK, Mukherjee B, Hauser R, McElrath TF. Urinary Concentrations of Bisphenol A and Phthalate Metabolites Measured during Pregnancy and Risk of Preeclampsia. Environ Health Perspect. 2016.

64. Camara LR, Arbuckle TE, Trottier H, Fraser WD. Associations between Maternal Exposure to Bisphenol A or Triclosan and Gestational Hypertension and Preeclampsia: The MIREC Study. Am J Perinatol. 2019;36(11):1127-35.

65. Ye Y, Zhou Q, Feng L, Wu J, Xiong Y, Li X. Maternal serum bisphenol A levels and risk of pre-eclampsia: a nested case-control study. Eur J Public Health. 2017;27(6):1102-7.

66. Khalil N, Ebert JR, Wang L, Belcher S, Lee M, Czerwinski SA, et al. Bisphenol A and cardiometabolic risk factors in obese children. Sci Total Environ. 2014;470-471:726-32.

67. Trasande L, Sathyanarayana S, Spanier AJ, Trachtman H, Attina TM, Urbina EM. Urinary phthalates are associated with higher blood pressure in childhood. J Pediatr. 2013;163(3):747-53 e1.

68. Shankar A, Teppala S. Urinary bisphenol A and hypertension in a multiethnic sample of US adults. J Environ Public Health. 2012;2012:481641.

69. Shiue I, Hristova K. Higher urinary heavy metal, phthalate and arsenic concentrations accounted for 3-19% of the population attributable risk for high blood pressure: US NHANES, 2009-2012. Hypertens Res. 2014;37(12):1075-81.

70. Han X, Li J, Wang Y, Xu S, Li Y, Liu H, et al. Association between phthalate exposure and blood pressure during pregnancy. Ecotoxicol Environ Saf. 2020;189:109944.

71. Warembourg C, Basagana X, Seminati C, de Bont J, Granum B, Lyon-Caen S, et al. Exposure to phthalate metabolites, phenols and organophosphate pesticide metabolites and blood pressure during pregnancy. Int J Hyg Environ Health. 2019;222(3):446-54.

72. Saito S, Nakashima A. A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling. J Reprod Immunol. 2014;101-102:80-8.

73. Ferguson KK, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. Phthalate metabolites and bisphenol-A in association with circulating angiogenic biomarkers across pregnancy. Placenta. 2015;36(6):699-703.

74. Gaillard R, Arends LR, Steegers EA, Hofman A, Jaddoe VW. Second- and third-trimester placental hemodynamics and the risks of pregnancy complications: the Generation R Study. Am J Epidemiol. 2013;177(8):743-54.

75. Muller JE, Meyer N, Santamaria CG, Schumacher A, Luque EH, Zenclussen ML, et al. Bisphenol A exposure during early pregnancy impairs uterine spiral artery remodeling and provokes intrauterine growth restriction in mice. Sci Rep. 2018;8(1):9196.

76. Philippat C, Heude B, Botton J, Alfaidy N, Calafat AM, Slama R, et al. Prenatal Exposure to Select Phthalates and Phenols and Associations with Fetal and Placental Weight among Male Births in the EDEN Cohort (France). Environ Health Perspect. 2019;127(1):17002.

77. Dahlstrom B, Romundstad P, Oian P, Vatten LJ, Eskild A. Placenta weight in pre-eclampsia. Acta Obstet Gynecol Scand. 2008;87(6):608-11.

78. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta. 2009;30 Suppl A:S43-8.

79. Li H, Gu B, Zhang Y, Lewis DF, Wang Y. Hypoxia-induced increase in soluble Flt-1 production correlates with enhanced oxidative stress in trophoblast cells from the human placenta. Placenta. 2005;26(2-3):210-7.

80. Ouyang YQ, Li SJ, Zhang Q, Xiang WP, Shen HL, Chen HP, et al. Plasma sFlt-1-to-PlGF ratio is correlated with inflammatory but not with oxidative stress in Chinese preeclamptic women. Arch Gynecol Obstet. 2009;280(1):91-7.

81. Morice L, Benaitreau D, Dieudonne MN, Morvan C, Serazin V, de Mazancourt P, et al. Antiproliferative and proapoptotic effects of bisphenol A on human trophoblastic JEG-3 cells. Reprod Toxicol. 2011;32(1):69-76.

82. Benachour N, Aris A. Toxic effects of low doses of Bisphenol-A on human placental cells. Toxicol Appl Pharmacol. 2009;241(3):322-8.

83. Arita Y, Park HJ, Cantillon A, Getahun D, Menon R, Peltier MR. Effect of bisphenol-A (BPA) on placental biomarkers for inflammation, neurodevelopment and oxidative stress. J Perinat Med. 2019;47(7):741-9.

84. Gao F, Hu W, Li Y, Shen H, Hu J. Mono-2-ethylhexyl phthalate inhibits human extravillous trophoblast invasion via the PPARgamma pathway. Toxicol Appl Pharmacol. 2017;327:23-9.

85. Zhang S, Sun C, Zhao S, Wang B, Wang H, Zhang J, et al. Exposure to DEHP or its metabolite MEHP promotes progesterone secretion and inhibits proliferation in mouse placenta or JEG-3 cells. Environ Pollut. 2020;257:113593.

86. Susiarjo M, Sasson I, Mesaros C, Bartolomei MS. Bisphenol a exposure disrupts genomic imprinting in the mouse. PLoS Genet. 2013;9(4):e1003401.

87. Grindler NM, Vanderlinden L, Karthikraj R, Kannan K, Teal S, Polotsky AJ, et al. Exposure to Phthalate, an Endocrine Disrupting Chemical, Alters the First Trimester Placental Methylome and Transcriptome in Women. Sci Rep. 2018;8(1):6086.

88. Bellavia A, Hauser R, Seely EW, Meeker JD, Ferguson KK, McElrath TF, et al. Urinary phthalate metabolite concentrations and maternal weight during early pregnancy. Int J Hyg Environ Health. 2017;220(8):1347-55.

89. Perng W, Kasper NM, Watkins DJ, Sanchez BN, Meeker JD, Cantoral A, et al. Exposure to Endocrine-Disrupting Chemicals During Pregnancy Is Associated with Weight Change Through 1 Year Postpartum Among Women in the Early-Life Exposure in Mexico to Environmental Toxicants Project. J Womens Health (Larchmt). 2020.

90. Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, et al. Bisphenol analogue exposures are widely prevalent in pregnant women in a population-based cohort in the Netherlands, 2004-5. Environmental International. 2017;submitted.

91. Yazdy MM, Coull BA, Gardiner JC, Aguiar A, Calafat AM, Xiaoyun Y, et al. A possible approach to improving the reproducibility of urinary concentrations of phthalate metabolites and phenols during pregnancy. J Expo Sci Environ Epidemiol. 2018;28(5):448-60.

92. Jaacks LM, Boyd Barr D, Sundaram R, Grewal J, Zhang C, Buck Louis GM. Pre-Pregnancy Maternal Exposure to Persistent Organic Pollutants and Gestational Weight Gain: A Prospective Cohort Study. Int J Environ Res Public Health. 2016;13(9).

93. Ashley-Martin J, Dodds L, Arbuckle TE, Morisset AS, Fisher M, Bouchard MF, et al. Maternal and Neonatal Levels of Perfluoroalkyl Substances in Relation to Gestational Weight Gain. Int J Environ Res Public Health. 2016;13(1).

94. Vizcaino E, Grimalt JO, Glomstad B, Fernandez-Somoano A, Tardon A. Gestational weight gain and exposure of newborns to persistent organic pollutants. Environ Health Perspect. 2014;122(8):873-9.

95. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Patterson DG, Halden RU, et al. Determinants of prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in an urban population. Environ Health Perspect. 2007;115(12):1794-800.

96. Vafeiadi M, Vrijheid M, Fthenou E, Chalkiadaki G, Rantakokko P, Kiviranta H, et al. Persistent organic pollutants exposure during pregnancy, maternal gestational weight gain, and birth outcomes in the mother-child cohort in Crete, Greece (RHEA study). Environ Int. 2014;64:116-23.

97. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring). 2013;21(5):1046-55.

98. Ay L, Kruithof CJ, Bakker R, Steegers EA, Witteman JC, Moll HA, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG. 2009;116(7):953-63.

99. Hu CY, Li FL, Hua XG, Jiang W, Mao C, Zhang XJ. The association between prenatal bisphenol A exposure and birth weight: a meta-analysis. Reprod Toxicol. 2018;79:21-31.

100. Sol CM, van Zwol-Janssens C, Philips EM, Asimakopoulos AG, Martinez-Moral MP, Kannan K, et al. Maternal bisphenol urine concentrations, fetal growth and adverse birth outcomes. A population-based prospective study. Submitted.

101. Lof M, Hilakivi-Clarke L, Sandin SS, de Assis S, Yu W, Weiderpass E. Dietary fat intake and gestational weight gain in relation to estradiol and progesterone plasma levels during pregnancy: a longitudinal study in Swedish women. BMC Womens Health. 2009;9:10.

102. Aubuchon-Endsley NL, Bublitz MH, Stroud LR. Pre-pregnancy obesity and maternal circadian cortisol regulation: Moderation by gestational weight gain. Biol Psychol. 2014;102:38-43.

103. Kallak TK, Hellgren C, Skalkidou A, Sandelin-Francke L, Ubhayasekhera K, Bergquist J, et al. Maternal and female fetal testosterone levels are associated with maternal age and gestational weight gain. Eur J Endocrinol. 2017;177(4):379-88.

104. Carwile JL, Michels KB. Urinary bisphenol A and obesity: NHANES 2003–2006. Environmental Research. 2011;111(6):825-30.

105. Hatch E, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999-2002. Environmental health : a global access science source. 2008;7:27.

106. Yaghjyan L, Sites S, Ruan Y, Chang SH. Associations of urinary phthalates with body mass index, waist circumference and serum lipids among females: National Health and Nutrition Examination Survey 1999-2004. Int J Obes (Lond). 2015;39(6):994-1000.

107. Buser MC, Murray HE, Scinicariello F. Age and sex differences in childhood and adulthood obesity association with phthalates: analyses of NHANES 2007-2010. Int J Hyg Environ Health. 2014;217(6):687-94.

108. Liu B, Lehmler H-J, Yangbo S, Xu G, Liu, Yuewei, Zong G, Sun, Qi, Hu FB, et al. Bisphenol A substitutes and obesity in US adults: analysis of a population-based, cross-sectional study. 2017;1(3):e114–e22.

109. Song Y, Hauser R, Hu FB, Franke AA, Liu S, Sun Q. Urinary concentrations of bisphenol A and phthalate metabolites and weight change: a prospective investigation in US women. Int J Obes (Lond). 2014.

110. Rodriguez-Carmona Y, Cantoral A, Trejo-Valdivia B, Tellez-Rojo MM, Svensson K, Peterson KE, et al. Phthalate exposure during pregnancy and long-term weight gain in women. Environ Res. 2019;169:26-32.

111. Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, et al. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PloS one. 2013;8(10):e77481.

112. Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. Toxicol Sci. 2003;74(2):297-308.

113. Raisanen S, Sankilampi U, Gissler M, Kramer MR, Hakulinen-Viitanen T, Saari J, et al. Smoking cessation in the first trimester reduces most obstetric risks, but not the risks of major congenital anomalies and admission to neonatal care: a population-based cohort study of 1,164,953 singleton pregnancies in Finland. J Epidemiol Community Health. 2014;68(2):159-64.

114. Moore E, Blatt K, Chen A, Van Hook J, DeFranco EA. Relationship of trimester-specific smoking patterns and risk of preterm birth. Am J Obstet Gynecol. 2016;215:109.e1-6.

115. Blatt K, Moore E, Chen A, Van Hook J, DeFranco EA. Association of reported trimester-specific smoking cessation with fetal growth restriction. Obstet Gynecol. 2015;125(6):1452-9.

116. Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, et al. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. Paediatr Perinat Epidemiol. 2008;22(2):162-71.

117. McCowan LM, Dekker GA, Chan E, Stewart A, Chappell LC, Hunter M, et al. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. BMJ. 2009;338:b1081.

118. Grzeskowiak LE, Hodyl NA, Stark MJ, Morrison JL, Clifton VL. Association of early and late maternal smoking during pregnancy with offspring body mass index at 4 to 5 years of age. J Dev Orig Health Dis. 2015;6(6):485-92.

119. Durmus B, Kruithof CJ, Gillman MH, Willemsen SP, Hofman A, Raat H, et al. Parental smoking during pregnancy, early growth, and risk of obesity in preschool children: the Generation R Study. Am J Clin Nutr. 2011;94(1):164-71.

120. Brand JS, Gaillard R, West J, McEachan RRC, Wright J, Voerman E, et al. Associations of maternal quitting, reducing, and continuing smoking during pregnancy with longitudinal fetal growth: Findings from Mendelian randomization and parental negative control studies. PLoS Med. 2019;16(11):e1002972.

121. Yan J, Groothuis PA. Timing of prenatal smoking cessation or reduction and infant birth weight: evidence from the United Kingdom Millennium Cohort Study. Matern Child Health J. 2015;19(3):447-58.

122. Harris HR, Willett WC, Michels KB. Parental smoking during pregnancy and risk of overweight and obesity in the daughter. Int J Obes (Lond). 2013;37(10):1356-63.

123. Jaddoe VW, de Jonge LL, van Dam RM, Willett WC, Harris H, Stampfer MJ, et al. Fetal exposure to parental smoking and the risk of type 2 diabetes in adult women. Diabetes Care. 2014;37(11):2966-73.

124. Inoue S, Naruse H, Yorifuji T, Kato T, Murakoshi T, Doi H, et al. Impact of maternal and paternal smoking on birth outcomes. J Public Health (Oxf). 2016;39:1-10.

125. Oldereid NB, Wennerholm UB, Pinborg A, Loft A, Laivuori H, Petzold M, et al. The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. Hum Reprod Update. 2018;24(3):320-89.

126. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. Semin Perinatol. 1996;20(2):115-26.

127. Ion R, Bernal AL. Smoking and Preterm Birth. Reprod Sci. 2015;22(8):918-26.

128. Moore LG. Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. High Alt Med Biol. 2003;4(2):141-56.

129. Witt SH, Frank J, Gilles M, Lang M, Treutlein J, Streit F, et al. Impact on birth weight of maternal smoking throughout pregnancy mediated by DNA methylation. BMC Genomics. 2018;19(1):290.

130. Kupers LK, Xu X, Jankipersadsing SA, Vaez A, la Bastide-van Gemert S, Scholtens S, et al. DNA methylation mediates the effect of maternal smoking during pregnancy on birthweight of the offspring. Int J Epidemiol. 2015;44(4):1224-37.

131. Koshy G, Delpisheh A, Brabin BJ. Dose response association of pregnancy cigarette smoke exposure, childhood stature, overweight and obesity. Eur J Public Health. 2011;21(3):286-91.

132. Brion MJ, Leary SD, Smith GD, Ness AR. Similar associations of parental prenatal smoking suggest child blood pressure is not influenced by intrauterine effects. Hypertension. 2007;49(6):1422-8.

133. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.

134. Jaddoe VW, van Duijn CM, Franco OH, van der Heijden AJ, van Iizendoorn MH, de Jongste JC, et al. The Generation R Study: design and cohort update 2012. Eur J Epidemiol. 2012;27(9):739-56.

135. Nohr EA, Liew Z. How to investigate and adjust for selection bias in cohort studies. Acta Obstet Gynecol Scand. 2018;97(4):407-16.

136. Guo Y, Wang L, Kannan K. Effect of storage time and temperature on levels of phthalate metabolites and bisphenol A in urine. Advances in Environmental Research. 2013;2(1):9-17.

137. Needham LL, Barr DB, Calafat AM. Characterizing children's exposures: beyond NHANES. Neurotoxicology. 2005;26(4):547-53.

138. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. Appl Occup Environ Hyg. 1990;5(1):46-51.

139. Nie L, Chu H, Liu C, Cole SR, Vexler A, Schisterman EF. Linear regression with an independent variable subject to a detection limit. Epidemiology. 2010;21 Suppl 4:S17-24.

140. Croghan CW, Egeghy PP. Methods of Dealing with Values Below the Limit of Detection using SAS. United States Environmental Protection Agency; 2003.

141. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr. 2013;25(2):247-54.

142. Mattison DR, Karyakina N, Goodman M, LaKind JS. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps. Crit Rev Toxicol. 2014;44(8):696-724.

143. Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environ Health Perspect. 2004;112(17):1734-40.

144. Townsend MK, Franke AA, Li X, Hu FB, Eliassen AH. Within-person reproducibility of urinary bisphenol A and phthalate metabolites over a 1 to 3 year period among women in the Nurses' Health Studies: a prospective cohort study. Environmental health : a global access science source. 2013;12(1):80.

145. Pollack AZ, Perkins NJ, Sjaarda L, Mumford SL, Kannan K, Philippat C, et al. Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. Environ Res. 2016;151:513-20.

146. Koch HM, Bolt HM, Preuss R, Angerer J. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol. 2005;79(7):367-76.

147. Bang du Y, Lee IK, Lee BM. Toxicological characterization of phthalic Acid. Toxicol Res. 2011;27(4):191-203.

148. Ahmed SB, Bentley-Lewis R, Hollenberg NK, Graves SW, Seely EW. A comparison of prediction equations for estimating glomerular filtration rate in pregnancy. Hypertens Pregnancy. 2009;28(3):243-55.

149. MacPherson S, Arbuckle TE, Fisher M. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J Expo Sci Environ Epidemiol. 2018.

150. Radin RG, Rothman KJ, Hatch EE, Mikkelsen EM, Sorensen HT, Riis AH, et al. Maternal Recall Error in Retrospectively Reported Time-to-Pregnancy: an Assessment and Bias Analysis. Paediatr Perinat Epidemiol. 2015;29(6):576-88.

151. Porta M, Greenland S, Hernán M, dos Santos Silva I, Last JM. A Dictionary of Epidemiology. 6th ed. New York: Oxford University Press; 2014.

152. Lucas RM, McMichael AJ. Association or causation: evaluating links between "environment and disease". Bull World Health Organ. 2005;83(10):792-5.

153. Sexton M, Hebel JR. A clinical trial of change in maternal smoking and its effect on birth weight. JAMA. 1984;251(7):911-5.

154. McConnachie A, Haig C, Sinclair L, Bauld L, Tappin DM. Birth weight differences between those offered financial voucher incentives for verified smoking cessation and control participants enrolled in the Cessation in Pregnancy Incentives Trial (CPIT), employing an intuitive approach and a Complier Average Causal Effects (CACE) analysis. Trials. 2017;18(1):337.

155. Kuja-Halkola R, D'Onofrio BM, Larsson H, Lichtenstein P. Maternal smoking during pregnancy and adverse outcomes in offspring: genetic and environmental sources of covariance. Behav Genet. 2014;44(5):456-67.

156. Frisell T, Oberg S, Kuja-Halkola R, Sjolander A. Sibling comparison designs: bias from non-shared confounders and measurement error. Epidemiology. 2012;23(5):713-20.

157. Munafo MR, Timofeeva MN, Morris RW, Prieto-Merino D, Sattar N, Brennan P, et al. Association between genetic variants on chromosome 15q25 locus and objective measures of tobacco exposure. J Natl Cancer Inst. 2012;104(10):740-8.

158. Freathy RM, Ring SM, Shields B, Galobardes B, Knight B, Weedon MN, et al. A common genetic variant in the 15q24 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNB4) is associated with a reduced ability of women to quit smoking in pregnancy. Hum Mol Genet. 2009;18(15):2922-7.

159. Tyrrell J, Huikari V, Christie JT, Cavadino A, Bakker R, Brion MJ, et al. Genetic variation in the 15q25 nicotinic acetylcholine receptor gene cluster (CHRNA5-CHRNA3-CHRNB4) interacts with maternal self-reported smoking status during pregnancy to influence birth weight. Hum Mol Genet. 2012;21(24):5344-58.

160. Feeney A, Britton G. Counseling Women on Smoking Relapse Prevention During Postpartum. MCN Am J Matern Child Nurs. 2016;41(5):287-92.

161. Braun JM, Gennings C, Hauser R, Webster TF. What Can Epidemiological Studies Tell Us about the Impact of Chemical Mixtures on Human Health? Environ Health Perspect. 2016;124(1):A6-9.

162. Kalloo G, Wellenius GA, McCandless L, Calafat AM, Sjodin A, Romano ME, et al. Exposures to chemical mixtures during pregnancy and neonatal outcomes: The HOME study. Environ Int. 2020;134:105219.

163. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1847-50.

CHAPTER 5

Summary

Summary

In **Chapter 1**, the background for the studies presented in this thesis is described. **Chapter 1.1** provides the background, hypothesis, aims and design for this thesis. Environmental exposures are omnipresent throughout the human life course and may have a vast influence on human health. An increasing body of evidence suggests that exposure to bisphenols and phthalates may lead to several adverse short and long-term health effects, including the development of cardiometabolic diseases. Thus far, only few studies have assessed the associations of bisphenols and phthalates with the course of pregnancy and maternal health. Also, smoking remains highly prevalent among women of reproductive age, posing a risk for children born to smoking mothers. Maternal smoking has been associated with increased risks of adverse birth outcomes and childhood overweight. It remains unknown to what extent maternal smoking in early pregnancy only, changing maternal smoking behavior and paternal smoking influence these outcomes. The studies presented in this thesis used data from the Generation R Study, a population-based cohort from fetal life onwards in Rotterdam, the Netherlands, and the LifeCycle Project, an international consortium of European cohorts that started during pregnancy or childhood. In **Chapter 1.2**, we summarized available evidence on bisphenols and phthalates, including on their routes of exposure, metabolism and potential mechanisms of effect. We also reviewed existing evidence on the associations of bisphenols and phthalates with maternal, fetal and child growth outcomes. Although the amount of studies focused on maternal outcomes, such as time to pregnancy, gestational diabetes mellitus and gestational hypertensive disorders, is limited, potential adverse effects have already been described. Evidence from observational studies shows a mainly negative effect of phthalates and negative or no effect of bisphenol A on gestational age and body size measures at birth. Studies of effects on childhood growth show contradictory results, but do suggest a potential modifying role for sex, stage of puberty and ethnicity.

Chapter 2 describes studies on the associations of bisphenols and phthalate metabolites with maternal determinants and outcomes. In **Chapter 2.1,** we observed that bisphenol S and F exposure was highly prevalent in pregnant women in the Netherlands as early as 2004-5. Nutritional related factors, including daily dietary caloric intake and food groups, were not associated with bisphenols and phthalate metabolite concentrations when multiple testing corrections were applied. Adverse lifestyle related factors, such as pre-pregnancy obesity and the lack of folic acid supplement use, were associated with higher phthalate metabolite concentrations. In **Chapter 2.2**, we studied the associations of early pregnancy bisphenol and phthalate concentrations with time to pregnancy. We did not observe an association in the whole group, but among women without adequate preconception folic acid supplement use, we observed associations of higher total bisphenols and phthalic acid with reduced fecundability ratios (corresponding to a longer time to pregnancy). In **Chapter 2.3**, we evaluated the associations of early pregnancy bisphenol and phthalate metabolite concentrations with maternal hemodynamics and gestational hypertensive disorders. Bisphenols and phthalate metabolites were not associated with gestational hypertensive disorders or longitudinal changes in blood pressure during pregnancy. We observed subclinical associations of high molecular weight phthalate metabolites with higher early pregnancy sFlt-1/PlGF ratio and of bisphenol A with placental hemodynamics. In **Chapter 2.4**, we focused on the associations of early and mid-pregnancy urinary bisphenol and phthalate metabolite concentrations with gestational weight gain. We showed that higher total bisphenol and

bisphenol S urine concentrations in early pregnancy were associated with reduced gestational weight gain among normal weight women. Among all women, higher total bisphenol and bisphenol A urine concentrations in early pregnancy were associated with a reduced gestational weight gain in the second half of pregnancy. Associations were independent of mid-pregnancy compounds. Bisphenols and phthalate metabolites were not associated with insufficient or excessive gestational weight gain. As a follow-up study, in **Chapter 2.5**, we studied the associations of early and mid-pregnancy urinary bisphenol and phthalate metabolite concentrations with maternal weight gain 6 years postpartum. Among all included women, bisphenol A and all assessed phthalate groupings were associated with increased maternal weight gain. Among women without subsequent pregnancies, phthalic acid, low molecular weight phthalate metabolites and di-n-octylphthalate metabolites remained associated with increased maternal weight gain. Mediation by gestational weight gain was not present and breastfeeding did not modify the effects. In summary, findings from **Chapter 2** suggest that exposure to bisphenols and phthalates during pregnancy may lead to adverse maternal health outcomes in the short and long-term.

In **Chapter 3**, we performed an individual participant data meta-analysis among 229,158 families from 28 pregnancy or birth cohorts from Europe and North America to assess the associations of parental smoking with adverse birth outcomes and childhood overweight. Compared with non-smoking mothers, maternal first trimester smoking only was not associated with adverse birth outcomes but was associated with a higher risk of childhood overweight. Children from mothers who continued smoking during pregnancy had higher risks of preterm birth, being small size for gestational age and childhood overweight. Children from mothers who reduced the number of cigarettes between the first and third trimester, without quitting, still had a higher risk to be born small size for gestational age, but the effect estimate was smaller. Among non-smoking mothers, paternal smoking was associated with childhood overweight, but not with adverse birth outcomes.

Finally, in **Chapter 4**, a general discussion of all studies included in this thesis, suggestions for future research and implications for clinical practice and policy are presented.

In conclusion, findings from this thesis suggest that environmental exposures during pregnancy, such as bisphenols, phthalates and smoking may affect maternal and child health outcomes in the short and long-term. The observed associations are relatively small, but may be important for the burden of morbidity and mortality due to non-communicable diseases on a population level.

Samenvatting

In **Hoofdstuk 1** wordt de achtergrond van de onderzoeken in dit proefschrift besproken. **Hoofdstuk 1.1** beschrijft de achtergrond, hypothese, de doelstellingen en de opzet van dit proefschrift. Mensen worden gedurende het leven blootgesteld aan diverse omgevingsfactoren die van grote invloed op de gezondheid kunnen zijn. Een toenemend aantal studies wijst erop dat blootstelling aan bisfenolen en ftalaten zou kunnen leiden tot verschillende ongunstige gezondheidseffecten op de korte en lange termijn, inclusief het ontwikkelen van cardiometabole aandoeningen. Tot dusver hebben slechts enkele studies onderzoek gedaan naar associaties van bisfenolen en ftalaten met het beloop van de zwangerschap en de maternale gezondheid. Verder blijven veel vrouwen in de vruchtbare leeftijd roken, wat een risico vormt voor de kinderen van deze rokende moeders. Het roken van moeders is geassocieerd met verhoogde risico's op geboorte complicaties en overgewicht op de kinderleeftijd. Het blijft onbekend in welke mate deze uitkomsten beïnvloed worden wanneer moeder enkel tijdens de vroege zwangerschap rookt, haar rookgedrag tijdens de zwangerschap verandert en welk aandeel het roken van vaders hierin heeft. De onderzoeken die in dit proefschrift worden besproken zijn op basis van data van de Generation R Study, een populatie-gebaseerd cohort dat gevolgd wordt vanaf het vroege foetale leven in Rotterdam, Nederland, en het LifeCycle Project, een internationaal consortium van Europese cohorten die gestart zijn tijdens de zwangerschap of op de kinderleeftijd. In **Hoofdstuk 1.2** hebben we de beschikbare studies over bisfenolen en ftalaten samengevat, inclusief de blootstellingsroutes, het metabolisme en het potentiële werkingsmechanisme. Daarnaast hebben we een review gemaakt van de bestaande onderzoeken naar de associaties van bisfenolen en ftalaten met maternale en foetale uitkomsten en groei op de kinderleeftijd. Hoewel er maar een klein aantal onderzoeken gericht was op maternale uitkomsten, zoals hoe lang het duurt om zwanger te worden, zwangerschapsdiabetes en hypertensieve aandoeningen in de zwangerschap, zijn er al potentiële ongunstige effecten beschreven. Observationele studies laten voornamelijk negatieve associaties van ftalaten en negatieve ofwel geen associaties van bisphenol A zien met de zwangerschapsduur of lichaamsafmetingen bij geboorte. Onderzoeken naar effecten op groei op de kinderleeftijd laten tegenstrijdige resultaten zien, maar suggereren wel een potentieel modificerende rol voor geslacht, puberteitsstadium en etniciteit.

Hoofdstuk 2 beschrijft onderzoeken naar de associaties van bisfenolen en ftalaat metabolieten met maternale determinanten en uitkomsten. In **Hoofdstuk 2.1** vonden we dat bisfenol S en F blootstelling onder zwangere vrouwen in Nederland in 2004-5 al wijd verspreid was. Voedingsgerelateerde factoren, inclusief de dagelijkse calorie inname via voeding en groepen van voedingsmiddelen, waren niet geassocieerd met bisfenolen en ftalaat metabolieten wanneer een correctie voor de veelvoud aan testen was toegepast. Ongunstige leefstijlgerelateerde factoren, zoals obesitas voorafgaand aan de zwangerschap en het niet nemen van foliumzuur supplementen, waren geassocieerd met hogere concentraties van ftalaat metabolieten. In **Hoofdstuk 2.2** hebben we de associaties bestudeerd van bisfenol en ftalaat metaboliet concentraties tijdens de vroege zwangerschap met hoe lang het geduurd heeft om zwanger te worden. We vonden geen associaties in de volledige groep, maar onder de vrouwen die geen preconceptionele foliumzuur supplementen hadden genomen vonden we dat een hoger totaal bisfenol en ftaalzuur geassocieerd was met een verminderde vruchtbaarheid (corresponderend met een langere tijd om zwanger te worden). In **Hoofdstuk 2.3** hebben we gekeken of er associaties zijn van bisfenol en ftalaat metaboliet concentraties in de vroege zwangerschap met maternale hemodynamiek en hypertensieve aandoeningen in de zwangerschap. Bisfenolen en ftalaat

metabolieten waren niet geassocieerd met hypertensieve aandoeningen in de zwangerschap of met longitudinale veranderingen in de bloeddruk tijdens de zwangerschap. We vonden subklinische associaties van hoog moleculair gewicht ftalaat metabolieten met een hogere sFlt-1/PlGF ratio in de vroege zwangerschap en van bisfenol A met placenta hemodynamiek. In **Hoofdstuk 2.4** hebben we ons gericht op de associaties van urine concentraties van bisfenolen en ftalaat metabolieten vroeg en halverwege in de zwangerschap met gewichtstoename tijdens de zwangerschap. We vonden dat hogere urine concentraties van totaal bisfenol en bisfenol S in de vroege zwangerschap geassocieerd waren met een verminderde gewichtstoename tijdens de zwangerschap onder vrouwen met een gezond gewicht. Hogere urine concentraties van totaal bisfenol en bisfenol A tijdens de vroege zwangerschap waren geassocieerd met een verminderde gewichtstoename in de tweede helft van de zwangerschap onder alle vrouwen. Deze associaties waren onafhankelijk van het niveau van blootstelling halverwege de zwangerschap. Bisfenolen en ftalaat metabolieten waren niet geassocieerd met inadequate of excessieve gewichtstoename tijdens de zwangerschap. Hierop volgend hebben we in **Hoofdstuk 2.5** gekeken naar associaties van urine concentraties van bisfenolen en ftalaat metabolieten vroeg en halverwege in de zwangerschap met maternale gewichtstoename 6 jaar postpartum. Onder alle geïncludeerde vrouwen waren bisfenol A en alle ftalaat groepen geassocieerd met een toename van het maternale gewicht. Onder vrouwen zonder volgende zwangerschappen bleven ftaalzuur, laag moleculair gewicht ftalaat metabolieten en di-n-octylftalaat metabolieten geassocieerd met een toename van het maternale gewicht. Associaties werden niet gemedieerd door gewichtstoename tijdens de zwangerschap en het geven van borstvoeding modificeerde de effecten niet. Samenvattend suggereren de bevindingen in **Hoofdstuk 2** dat blootstelling aan bisfenolen en ftalaten tijdens de zwangerschap kan leiden tot ongunstige maternale gezondheidsuitkomsten op de korte en lange termijn.

In **Hoofdstuk 3** hebben we een meta-analyse verricht van individuele deelnemersgegevens onder 229.158 gezinnen van 28 zwangerschaps- of geboorte cohorten uit Europa en Noord-Amerika om de associaties te onderzoeken van het roken van ouders met geboortecomplicaties en overgewicht op de kinderleeftijd. Vergeleken met niet rokende moeders was roken enkel tijdens de vroege zwangerschap niet geassocieerd met geboortecomplicaties, maar wel met een hoger risico op overgewicht op de kinderleeftijd. Kinderen van moeders die bleven roken tijdens de zwangerschap hadden een hoger risico op vroeggeboorte, een te laag geboortegewicht voor de zwangerschapsduur en overgewicht op de kinderleeftijd. Kinderen van moeders die bleven roken maar het aantal sigaretten dat zij dagelijks rookten tussen het eerste en derde trimester verminderden hadden nog steeds een verhoogd risico om geboren te worden met een te laag geboortegewicht voor de zwangerschapsduur, maar de effectgrootte was afgenomen. Onder niet rokende moeders was het roken van vaders geassocieerd met overgewicht op de kinderleeftijd, maar niet met geboortecomplicaties.

Tot slot wordt in **Hoofdstuk 4** een algemene discussie over alle studies in dit proefschrift gepresenteerd, samen met suggesties voor verder onderzoek en implicaties voor de klinische praktijk en beleid.

Concluderend suggereren de bevindingen van dit proefschrift dat blootstelling aan omgevingsfactoren tijdens de zwangerschap, zoals bisfenolen, ftalaten en roken, gezondheidsuitkomsten van moeder en kind kunnen beïnvloeden op de korte en lange termijn. De effectgrootte van de geobserveerde associaties is relatief klein, maar op populatieniveau zouden deze associaties van belang kunnen zijn voor het aantal ziekte- en sterftegevallen ten gevolge van niet-overdraagbare ziekten.

CHAPTER 6

Appendices

Abbreviations

AR: androgen receptor
BBP / BBzP: butylbenzyl phthalate
BMI: body mass index
BP: blood pressure
BPA: bisphenol A
BPAF: bisphenol AF
BPAG: bisphenol A monoglucuronide
BPAP: bisphenol AP
BPAS: bisphenol A sulphate
BPB: bisphenol B
BPF: bisphenol F
BPP: bisphenol P
BPS: bisphenol S
BPZ: bisphenol Z
CI: confidence interval
DALYs: disease-adjusted life-years
DBP: di-n-butylphthalate or dibutylphthalate
DDE: dichlorodiphenyl dichloroethene
DEHP: di-2-ethylhexylphthalate
DEP: di-ethylphthalate
DIBP: di-iso-butylphthalate
DIDP: di-isodecylphthalate
DINP: di-isononylphthalate
DMP: di-methylphthalate
DNOP: di-n-octylphthalate
DOHaD: Developmental Origins of Health and Disease
E2: estradiol
ECHA: European Chemicals Agency
EDC: endocrine-disrupting chemical
ER: estrogen receptor
FCS: fully conditional specification
FFA: free fatty acid
FFQ: food-frequency questionnaire
FR: fecundability ratio
GA: gestational age
GMD: gestational diabetes mellitus

GWG: gestational weight gain
GRP30: G-protein-coupled receptor 30
HCHs: hexachlorocyclohexanes
HDL: high-density lipoprotein
HMW: high molecular weight
HOMA-IR: homeostatic model assessment of insulin resistance
HPLC-ESI-MS/MS: high performance liquid chromatography electrospray ionization-tandem mass spectrometry
IGF-1: insulin-like growth factor 1
IOM: Institute of Medicine
IPD: individual participant data
IQR: inter-quartile range
IVF: in vitro fertilization
LLE: liquid-liquid extraction
LOD: limit of detection
LOQ: limit of quantification
LMW: low molecular weight
MAR: missing at random
mBP: mono-n-butylphthalate
mBzP: monobenzyl phthalate
mCHP: monocyclohexylphthalate
mCHpP: mono-(7-carboxy-n-heptyl)phthalate
mCMHP: mono-[(2-carboxymethyl)hexyl]phthalate
mCPP: mono(3-carboxypropyl)phthalate
mECPP: mono(2-ethyl-5-carboxypentyl)phthalate
mEHHP: mono-(2-ethyl-5-hydroxyhexyl)phthalate
mEHP: mono-2-ethylhexylphthalate
mEOHP: mono-(2-ethyl-5-oxohexyl)phthalate
mEP: monoethylphthalate
mHpP: mono-2-heptylphthalate
mHxP: mono-hexylphthalate
mIBP: mono-isobutyl phthalate
mIDP: mono-(8-methyl-1-nonyl)phthalate
mINP: monoisononylphthalate
mMP: monomethylphthalate
mOP: monooctylphthalate
NA: not applicable
NHANES: National Health and Nutrition Examination Survey

OR: odds ratio
PA: phthalic acid
PBDEs: polybrominated diphenyl ethers
PCBs: polychlorinated bisphenyls
PFASs: perfluoroalkyl substances
PFOS: perfluorooctanesulfonate
PI: pulsatility index
PlGF: placental growth factor
POPs: persistant organic pollutions
PPAR: peroxisome proliterator-activated receptor
PVC: polyvinyl chloride plastics
RI: resistance index
RXR: retinoid X receptor
SD: standard deviation
SDS: standard deviation scores
sFlt: soluble fms-like tyrosine kinase
SGA: small size for gestational age
SPE: solid-phase extraction
TTP: time to pregnancy
UGT: UDP-glucuronosyltransferase
WHO: World Health Organization

Publication list

First author

1. **Philips EM**, Jaddoe VWV, Trasande L. Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. Reprod Toxicol. 2017 Mar;68:105-118.

2. **Philips EM**, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, Trasande L.. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ Res. 2018 Feb;161:562-572.

3. **Philips EM**, Kahn LG, Jaddoe VWV, Shao Y, Asimakopoulos AG, Kannan K, Steegers EAP, Trasande L. First trimester urinary bisphenol and phthalate concentrations and time to pregnancy: a population-based cohort analysis. J Clin Endocrinol Metab. 2018 Sept 1;103(9):3540-3547.

4. **Philips EM**, Trasande L, Kahn LG, Gaillard R, Steegers EAP, Jaddoe VWV. Early pregnancy bisphenol and phthalate metabolite levels, maternal hemodynamics and gestational hypertensive disorders. Hum Reprod. 2019 Feb 1;34(2):365-373.

5. **Philips EM**, Santos S, Steegers EAP, Asimakopoulos AG, Kannan K, Trasande L, Jaddoe VWV. Maternal bisphenol and phthalate urine concentrations and gestational weight gain. Environ Int. 2019 Dec 18;135:105342.

6. **Philips EM**, Jaddoe VWV, Deierlein A, Asimakopoulos AG, Kannan K, Steegers EAP, Trasande L. Exposures to Phthalates and Bisphenols in Pregnancy and Postpartum Weight Gain in a Population-Based Longitudinal Birth Cohort. Environ Int. 2020 Jul 31;144:106002.

7. **Philips EM**[*], Santos S[*], Trasande L, Aurrekoetxea JJ, Barros H, von Berg A, Bergström A, Bird PK, Brescianini S, Chaoimh CN, Charles MA, Chatzi L, Chevrier C, Chrousos GP, Costet N, Criswell R, Crozier S, Eggesbø M, Fantini MP, Farchi S, Forastiere F, van Gelder MMHJ, Georgiu V, Godfrey KM, Gori D, Hanke W, Heude B, Hryhorczuk D, Iñiguez C, Inskip H, Karvonen AM, Kenny LC, Kull I, Lawlor DA, Lehmann I, Magnus P, Manios Y, Melén E, Mommers M, Morgen CS, Moschonis G, Murray D, Nohr EA, Nybo Andersen AM, Oken E, Oostvogels AJJM, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Roeleveld N, Rusconi F, Santos AC, Sørensen TIA, Standl M, Stoltenberg C, Sunyer J, Tayler M, Thiering E, Thijs C, Torrent M, Vrijkotte TGM, Wright J, Zvinchuk O, Gaillard R, Jaddoe VWV. Changes in parental smoking during pregnancy and risks of adverse birth outcomes and childhood overweight: an individual participant data meta-analysis of 230,000 families. PLOS Medicine. 2020 Aug 18;17(8):e1003182.

8. **Philips EM**, Peeters B, Teeuw AH, Leenders AG, Boluyt N, Brilleslijper-Kater SN, Benninga MA. Stressful Life Events In Children With Functional Defecation Disorders. J Pediatr Gastroenterol Nutr. 2015 Oct;61(4):384-92.

Co-author

9. Van Zwol-Janssens C, Trasande L, Asimakopoulos AG, Martinez-Moral MP, Kannan K, **Philips EM**, Rivadeneira F, Jaddoe VWV, Santos S. Fetal exposure to bisphenols and phthalates and childhood bone mass: a population-based prospective cohort study. Environ Res. 2020 Jul;186:109602.

10. Van den Dries MA, Guxens M, Spaan S, Ferguson KK, **Philips E**, Santos S, Jaddoe VWV, Ghassabian A, Trasande L, Tiemeier H, Pronk A. Phthalate and bisphenol exposure during pregnancy and offspring nonverbal IQ. Environ Health Perspect. 2020 Jul;128(7):77009.

11. Sol CM, van Zwol-Janssens C, **Philips EM**, Asimakopoulos AG, Martinez-Moral MP, Kannan K, Steegers EAP, Jaddoe VWV, Trasande L, Santos S. Maternal bisphenol urine concentrations, fetal growth and adverse birth outcomes. A population-based prospective cohort study. *Submitted.*

12. Santos S, Sol CM, van Zwol-Janssens C, **Philips EM**, Asimakopoulos AG, Martinez-Moral MP, Kannan K, Steegers EAP, Jaddoe VWV, Trasande L. Maternal bisphenol urine concentrations, fetal growth and adverse birth outcomes. A population-based prospective cohort study. *Submitted.*

13. Sol CM, Santos S, Duijts L, Asimakopoulos AG, Kannan K, **Philips EM**, Trasande L, Jaddoe VWV. Fetal exposure to phthalates and bisphenols and childhood general and organ fat. A population-based prospective cohort study. Int J Obes (Lond). 2020 Sept 12.

14. Derakhshan A, **Philips EM**, Ghassabian A, Santos S, Kortenkamp A, Jaddoe VWV, Trasande L, Peeters RP, Korevaar TIM. Association of urinary bisphenols during pregnancy with maternal, cord blood and childhood thyroid function. Environ Int. 2020 Oct 14; 146: 106160.

15. Kahn L, **Philips EM**, Santos S, van den Dries MA, Gaillard R, Ferguson KK, Jaddoe VWV, Trasande L. Organopesticide exposure during pregnancy, gestational weight gain and long-term postpartum weight retention. *Manuscript preparation.*

16. Peeters B, Noens I, **Philips EM**, Kuppens S, Benninga MA. Autism spectrum disorders in children with functional defecation disorders. J Pediatr. 2013 Sep;163(3):873-8.

About the author

Elise Philips was born on 6 December 1987 in Diemen, The Netherlands. She passed secondary school with an 8 average at the St. Ignatius Gymnasium in Amsterdam in 2006. In the same year, she started studying Greek and Latin language and culture at the University of Amsterdam. After the propaedeutic year, she started medical school at the University of Amsterdam. She completed both her doctoral degree and doctors exam with honors (*cum laude*). After obtaining her medical degree in 2014, she started working as a resident in pediatrics (ANIOS) in the Bronovo hospital, Den Haag (currently Haaglanden Medisch Centrum). After this, she started her PhD project focused on maternal bisphenol, phthalate and smoking exposure during pregnancy under supervision of prof. dr. Vincent Jaddoe, prof. dr. Eric Steegers, prof. dr. Leonardo Trasande and dr. Susana Santos. During her project, she obtained her Master of Science degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences (NIHES) in 2017.

After living in Australia for three months, she started residency to become a general practitioner in March 2019.

Elise lives in Den Haag with her husband Norbert and daughter Emilie.

PhD Portfolio

Summary PhD training

Name PhD student:	Elise Margriet Philips
Erasmus MC Department:	Epidemiology, Generation R
Research School:	Netherlands Institute for Health Sciences
PhD Period:	December 2014 – January 2021
Promotors:	Prof. dr. V.W.V. Jaddoe, Prof. dr. E.A.P. Steegers
Co-promotor:	Dr. S. Santos

PhD training		
	Year	**Workload (ECTS)**
Master of Science Clinical Epidemiology	2015-2017	70.0
Principles of Research in Medicine and Epidemiology		
Methods of Public Health Research		
Clinical Trials		
The Practice of Epidemiologic Analysis		
Study Design		
Biostatistical Methods I: Basic Principles		
Biostatistical Methods II: Classical Regression Models		
Clinical Epidemiology		
Methodologic Topics in Epidemiologic Research		
Principles of Epidemiologic Data-analysis		
Advanced Topics in Decision Making in Medicine		
Joint Models for Longitudinal and Survival Data		
Causal Mediation Analysis		
Logistic Regression		
Causal Inference		
Missing Values in Clinical Research		
Clinical Practice-relevant Therapeutic Trials		
Fundamentals of Medical Decision Making		
Environmental Epidemiology (Cambridge, UK)		
Courses for the Quantitative Researcher		
Human epigenomics		
Development Research Proposal		
Research period PIN Health Sciences		

PhD training		
	Year	**Workload (ECTS)**
General academic skills		
Veiligheidstraining MRI, Erasmus MC, the Netherlands	2014	0.3
Scientific Integrity, Erasmus MC, the Netherlands	2016	0.3
Biomedical English Writing and Communications, Erasmus MC, the Netherlands	2016	4.0
Attended seminars, symposia and conferences		
Generation R Research Meetings	2014-2018	1.0
Maternal and Child Health Meetings	2014-2018	1.0
Pediatric Academic Societies (PAS) Meeting, San Francisco, USA	2017	1.4
DoHAD, Rotterdam, The Netherlands	2017	1.4
(Inter)national conference presentations		
Generation R Research Meeting	2016-2017	1.0
Pediatric Academic Societies (PAS) Meeting, San Francisco, USA – poster presentation	2017	1.4
DoHAD, Rotterdam, The Netherlands – two oral presentations and one poster presentation	2017	4.2
Grants		
Vereniging Trustfonds Erasmus Universiteit Rotterdam, travel grant	2017	
International Society for Developmental Origins of Health and Disease, travel award	2017	
Other		
Incidental Findings MRI Body of Focus @ 9	2015-2017	2.0
Teaching		
Supervising master's thesis 'Associations of first trimester bisphenol and phthalate urinary concentrations with maternal hemodynamics', Elvedin Aganovic	2016-2017	2.0

Dankwoord

Na jaren met veel plezier aan dit proefschrift te hebben gewerkt mag ik nu dan eindelijk dit dankwoord schrijven. Hoewel ik dit dankwoord alleen schrijf, is al het andere in dit boekje tot stand gekomen met de hulp van vele anderen. Graag wil ik een aantal van hen in het bijzonder bedanken.

Allereerst wil ik de ouders en kinderen die deelnemen aan Generation R hartelijk bedanken. Ik heb grote waardering voor de altijd enthousiaste kinderen en ouders die steeds weer tijd investeren in de bezoeken en de vragenlijsten. Zonder jullie deelname was het niet mogelijk geweest dit onderzoek uit te voeren.

Mijn promotoren, Prof. Dr. Jaddoe en Prof. Dr. Steegers. Beste Vincent, dankjewel voor de fijne samenwerking. Ik kan mij nog steeds verbazen over jouw multitask-kwaliteiten. Zo zaten we regelmatig in calls met New York en zat jij ondertussen rustig een manuscript te becommentariëren om vervolgens een treffende opmerking te maken. Dankjewel ook dat je mij de ruimte gaf om aan het MOCO project te beginnen toen de samples maar op zich lieten wachten. Jouw kritische blik en kennis heeft dit proefschrift gevormd tot wat het nu is, dankjewel! Prof. Dr. Steegers, hartelijk dank voor uw bijdrage aan dit promotie traject. Uw commentaar en ideeën hebben een waardevolle bijdrage geleverd aan de inhoud van dit proefschrift.

My unofficial co-promotor, Prof. Leonardo Trasande. Dear Leo, thank you for the pleasant collaboration and your enthusiasm for the project. You always see so many options and you want to explore everything possible. Thank you for sharing your knowledge on these compounds, without you I wouldn't have known where to start this project.

Dr. Susana Santos, dear Susana, you became my official co-promotor a bit later during the project. I was so happy that you became my co-promotor and I could finally ask someone else than Vincent the stupid questions if I needed to. Thank you for the always pleasant collaboration and your critical look. I wish you a great career in research.

Ook veel dank aan de leden van mijn kleine commissie, prof. dr. Reiss, prof. dr. Van Rossum en prof. dr. Vrijheid. Veel dank voor alle tijd die jullie in het lezen en beoordelen van mijn proefschrift hebben gestoken. Prof. Dr. Raat, prof. Trasande en prof. dr. Bierma-Zeinstra, hartelijk dank dat jullie plaats willen nemen in de grote commissie.

Graag wil ik hier ook de gelegenheid nemen om al mijn coauteurs te bedanken voor de prettige samenwerking en alle input in mijn manuscripten. Dear prof. dr. Kannan and dr. Alexandros Asimakopoulos, thank you both for your input and knowledge about these compounds. Dr. Kahn, Dear Linda, it was so nice working with you. Thank you for sharing your amazing writers' skills and for all the great talks we had. Beste dr. Romy Gaillard, dr. Andrea Deierlein, prof. dr. Yongzhao Shao en alle coauteurs van the LifeCycle Project (helaas te veel om hier persoonlijk te noemen), dank jullie wel.

Lieve medewerkers van het Focuscentrum, ik heb met veel plezier met jullie samen gewerkt. Dank jullie wel voor de gezelligheid, jullie betrokkenheid en inzet. Het datamanagement team bestaande uit Marjolein, Claudia en ook Ronald wil ik bedanken voor alle hulp en adviezen rondom datasets en

bellijsten. Claudia, jou in het speciaal wil ik bedanken voor je inzet rondom alle samples die naar de VS moesten en je hulp bij die enorme MOCO dataset. Patricia, veel dank voor jouw hulp bij logistieke vragen.

Lieve collega's van Generation R, dank jullie wel voor alle gezelligheid en jullie bijdrage aan deze onvergetelijke tijd. Hoewel ik natuurlijk eigenlijk alweer even weg ben, mis ik het gezellige samenwerken en alle 'overlegjes' even in de gang of bij de dagelijkse koffie nog steeds. Na de grote kamer, die altijd gezellig druk was, volgde de kleine kamer. Tim, dankjewel voor al je R kennis. Evelien, Suzanne en Marleen, wat hadden wij een gezellige kamer! We konden gezellig kletsen perfect afwisselen met hard werken (en dan even iets lekkers halen). Dank jullie wel voor deze fijne tijd. Annemarijne, Carlijn, Claire, Evelien, Florianne, Hanneke, Laura, Marjolein, Sanne, Simone, Suzanne, Sylvie, Zoe en alle anderen, dank jullie wel voor alle gezellige koffiemomentjes. Ik mis ze nog dagelijks!

Aan alle huisartsgroepsgenootjes en collega's tijdens de huisartsopleiding, dank jullie wel voor alle gezelligheid en jullie interesse in mijn proefschrift.

Vele vrienden en vriendinnen hebben de afgelopen jaren een bijdrage geleverd met alle lunches, borrels, etentjes en gezelligheid die voor ontspanning en afleiding hebben gezorgd. Lieve Tirz, Wies en March, middelbare school vriendinnetjes van het eerste uur! 20 jaar geleden stapten we hetzelfde klaslokaal in. Wat is het heerlijk om jullie, die mij zo goed kennen, na al die jaren nog steeds als mijn vriendinnetjes te hebben. Ignatius meisjes Bente F, Chloé, Elize, Joyce, Laura en Mirjam, dank jullie wel voor jullie vriendschap, interesse, gezelligheid en luisterend oor. We moeten snel weer een weekend weg plannen. Seng & Vivien, Justin & Emma, Domenique & Amanda, Bente B, Anna D, Anna K, Ilse en Ismahaan, dank jullie wel voor jullie vriendschap en alle gezelligheid.

Lieve paranimfen, dank jullie wel dat jullie op deze bijzondere dag naast mij willen staan en mij voorafgaand willen ondersteunen tijdens deze laatste loodjes van mijn promotie. Jonneke, ruim 8 jaar geleden hadden we tegelijkertijd coschap kindergeneeskunde in het toen nog SLAZ, jij vanuit de VU, ik vanuit het AMC. Het klikte meteen en we woonden toevallig ook nog praktisch naast elkaar. Bizar soms hoe wij hetzelfde over dingen denken en ook hetzelfde doen. Wat een eer dat ik naast jou mocht staan. Dankjewel voor je vriendschap, gezelligheid en luisterend oor. Marjolein, hoewel we samen met geneeskunde zijn gestart hebben wij volgens mij nooit samen in een groepje gezeten. Toch kwamen we elkaar steeds weer tegen en groeide daar een waardevolle vriendschap uit. Ik bewonder jouw doorzettingsvermogen om je eigen weg te gaan en uit te zoeken waar jij gelukkig van wordt. Dankjewel voor alle gezellige etentjes en goeie gesprekken.

Rien & Antoinette, mijn lieve schoonouders, dank jullie wel voor de eeuwige gastvrijheid, gezelligheid, vakanties en steun van de afgelopen jaren. Lieve Shier, wat ben jij een fijn schoonzusje, dankjewel! Bart, Simone, Hanke & Hans, Josephine & Juliette, Diederik & Letitia, dank jullie wel voor alle gezelligheid, interesse en steun.

Lieve familie, dank jullie wel voor alle gezelligheid en jullie interesse in mijn proefschrift. Lieve Gemma, wat fijn om aan jou naast een nichtje ook een goeie vriendin te hebben. Zo fijn om alles met jou te kunnen delen.

Lieve papa en mama, zonder jullie zou ik hier nu niet zijn. Jullie hebben de basis gelegd en mij altijd gestimuleerd en gesteund om te dromen en die dromen waar te maken. Dank jullie wel dat jullie er altijd voor mij zijn, jullie eindeloze steun en goede zorgen.

Lieve, lieve Norbert en Emilie, jullie zijn mijn grootste liefdes. Lieve Norbert, bijna 13 jaar geleden vonden we elkaar op de Amsterdamse Achtergracht. Jij bent veruit het beste wat er uit dat jaar Klassieke Talen is voortgekomen. Dankzij jou raakte ik geïnteresseerd in statistiek en epidemiologie. Dankjewel voor je vertrouwen in mij, je steun en je motiverende woorden als dat nodig was. En bovenal, dankjewel voor je liefde. Ik kijk uit naar alle avonturen die nog komen gaan. Ik hou van jou! Lieve Emilie, mijn Blientje, mijn liefde voor jou is met geen pen te beschrijven. Jij maakt alles in dit leven beter en plaatst alles in perspectief. Blijf zoals je bent! Ik hou van jou, voor altijd, het aller- allermeest!