Maternal Lifestyle and Pregnancy Complications

The Generation R Study

Rachel Bakker

Acknowledgements

The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. We gratefully acknowledge the contribution of children and parents, general practitioners, hospitals, midwives and pharmacies in Rotterdam. The general design of Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development (ZonMw), the Netherlands Organisation for Scientific Research (NWO), the Ministry of Health, Welfare and Sport and the Ministry of Youth and Families.

The work presented in this thesis was conducted at the Generation R Study Group, the Department of Epidemiology and the Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, the Netherlands. Publication of this thesis was supported by the Generation R Study Group, the Department of Epidemiology and the Department of Obstetrics and Gynaecology, Erasmus Medical Center, Rotterdam, the Netherlands, the Erasmus University Rotterdam, GE Healthcare and Bayer Healthcare B.V.

ISBN:978-94-6169-064-7

Layout and printing: Optima Grafische Communicatie, Rotterdam, the Netherlands

Maternal Lifestyle and Pregnancy Complications
The Generation R Study

Maternale levensstijl en zwangerschapscomplicaties
Het Generation R Onderzoek

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof.dr. H.G. Schmidt

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op
vrijdag 27 mei 2011 om 11.30 uur

door

Rachel Bakker

geboren te Amersfoort

Promotiecommissie

Promotoren:	Prof.dr. A. Hofman
	Prof.dr. E.A.P. Steegers
Overige leden:	Prof.dr. A.J. van der Heijden
	Prof.dr. J.P. Mackenbach
	Prof. J. Olsen

Paranimfen:	Marinka Bakker
	Claudia J Kruithof

Contents

Manuscripts that form the basis of this thesis

Chapter 2.2
Bakker R, Kruithof CJ, Steegers EAP, Tiemeier H, Mackenbach JP, Hofman A, Jaddoe VWV. Assessment of maternal smoking status during pregnancy and the associations with neonatal outcomes. *Submitted for publication*

Chapter 3.1
Gaillard R, **Bakker R**, Willemsen S, Hofman A, Steegers EAP, Jaddoe VWV. Blood pressure tracking during pregnancy and the risk of gestational hypertensive complications. The Generation R Study. *Submitted for publication*

Chapter 3.2
Gaillard R, **Bakker R**, Steegers EAP, Hofman A, Jaddoe VWV. Maternal age during pregnancy is associated with third trimester blood pressure level. The Generation R Study. *Submitted for publication*

Chapter 3.3
Bakker R, Steegers EAP, Mackenbach JP, Hofman A, Jaddoe VWV. Maternal smoking and blood pressure in different trimesters of pregnancy. The Generation R Study. *J Hypertens.* 2010;28:2210-8.

Chapter 3.4
Bakker R, Steegers EAP, Raat H, Hofman A, Jaddoe VWV. Maternal caffeine intake, blood pressure and the risk of hypertensive complications during pregnancy. The Generation R Study. *Am J Hypertens*. 2010;24:421-8.

Chapter 3.5
Bakker R, Steegers EAP, Hofman A, Jaddoe VWV. Blood pressure in different trimesters, fetal growth and the risks of adverse birth outcomes. The Generation R Study. Am J Epidemiol. *In press*

Chapter 4.1
Bakker R, Steegers EAP, Bihari AA, Mackenbach JP, Hofman A, Jaddoe VWV. Explaining differences in birth outcomes in relation to maternal age. *BJOG*. 2011;118:500-9.

Chapter 4.2
Bakker R, Pluimgraaff LE, Steegers EAP, Raat H, Tiemeier H, Hofman A, Jaddoe VWV. Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy. The Generation R Study. *Int J Epidemiol*. 2010;39:777-89.

Chapter 4.3

Jaddoe VWV, **Bakker R**, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JC. Moderate alcohol consumption during pregnancy and the risk of low birth weight and preterm birth. The Generation R Study. *Ann Epidemiol*. 2007;17:834-40.

Chapter 4.4

Bakker R, Timmermans S, Steegers EAP, Hofman A, Jaddoe VWV. Maternal smoking, folic acid supplement use and the risks of fetal complications. The Generation R Study. *Submitted for publication*

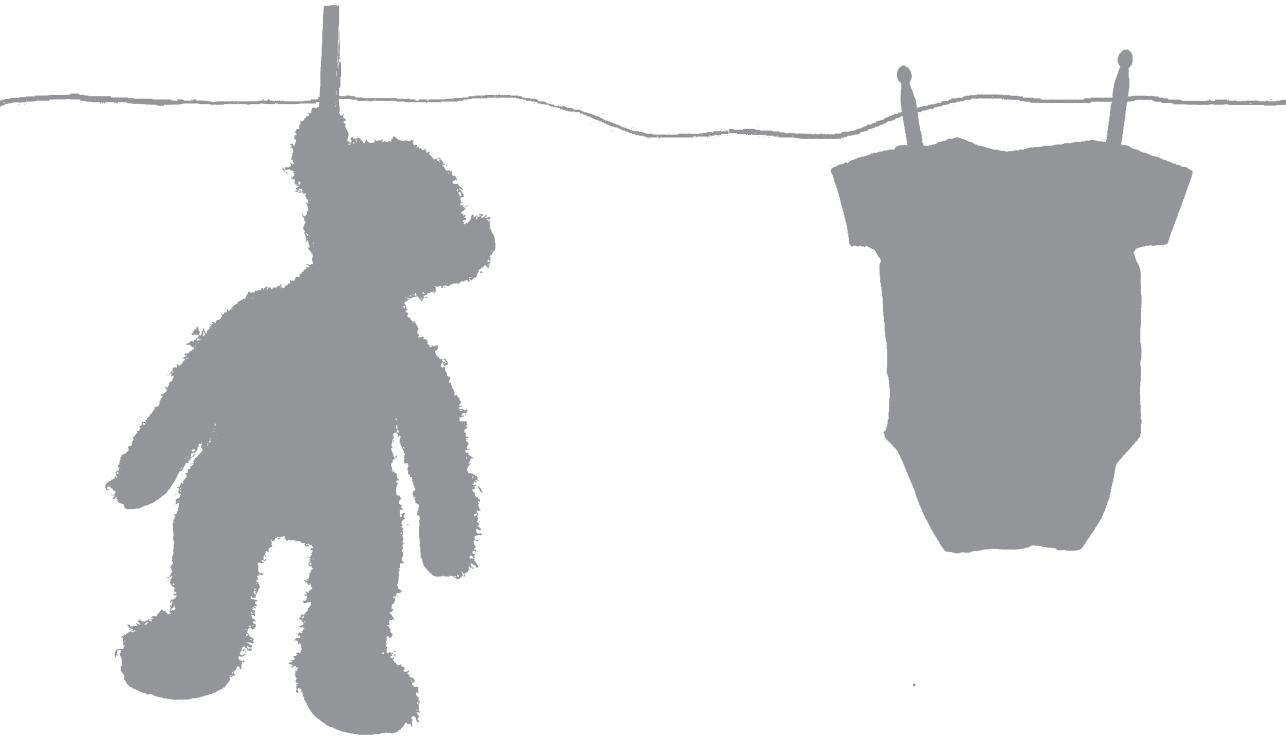
Chapter 4.5

Bakker R, Steegers EAP, Obradov A, Raat H, Hofman A, Jaddoe VWV. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes. The Generation R Study. *Am J Clin Nutr.* 2010;91:1691-98.

List of abbreviations

BMI, body mass index
BL, birth length
BW, birth weight
CI, confidence interval
CRL, crown-rump length
DBP, diastolic blood pressure
EFW, estimated fetal weight
FL, femur length
GSI, global severity index
HC, head circumference
LGA, large-size-for-gestational-age
LWB, low birth weight
OR, odds ratio
PE, preeclampsia
PIH, pregnancy-induced hypertension
PTB, preterm birth
SBP, systolic blood pressure
SD, standard deviation
SGA, small-size-for-gestational-age

Part 1 | **Introduction**



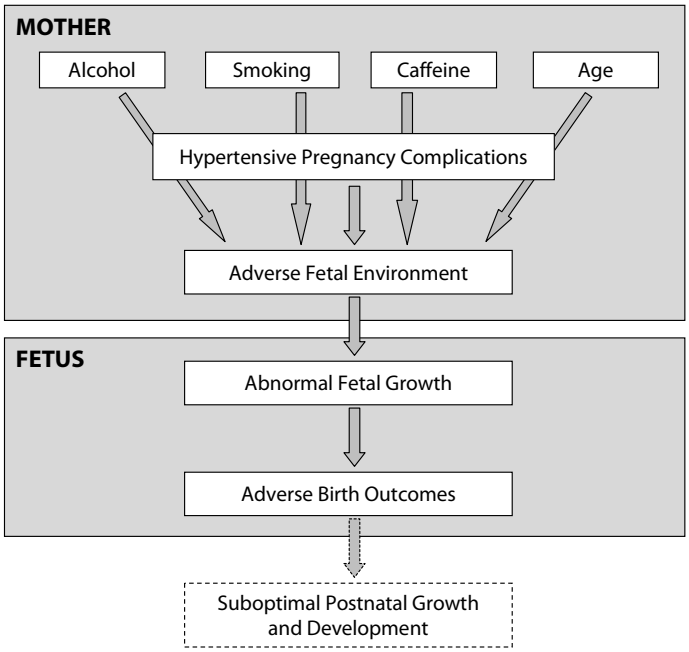
Adverse maternal lifestyle habits during pregnancy are important modifiable risk factors for pregnancy complications in Western countries. Most common adverse maternal lifestyle habits include smoking, alcohol consumption, and caffeine consumption. Although not directly lifestyle related, maternal age is also considered as a modifiable risk factor for adverse pregnancy outcomes.

Maternal cardiovascular adaptations might be influenced by this adverse maternal lifestyle during pregnancy, and increase the risks of maternal gestational hypertensive disorders, including pregnancy-induced hypertension and preeclampsia. Maternal age has also been suggested as risk factor for the development of hypertensive disorders during pregnancy[1-2]. Maternal cardiovascular adaptations might also be involved in pathways leading to an adverse fetal environment and subsequently neonatal complications. As a result of hypertensive complications impaired placental perfusion may occur, and subsequently the oxygen and nutrient supply to the fetus might be limited.

Maternal smoking during pregnancy is a well-established risk factor for various adverse pregnancy outcomes, such as fetal death, preterm birth and fetal growth retardation[3-6]. Women who smoke during pregnancy have offspring with a lower birth weight of 150 to 200 grams[5]. It has been suggested that the effects of maternal smoking during pregnancy on birth outcomes are trimester specific[7-8]. High levels of maternal alcohol consumption during pregnancy are associated with birth and long-term developmental defects, fetal alcohol syndrome and increased risks of low birth weight and preterm delivery[9-15]. Also, previous studies suggested that high caffeine intake during pregnancy is associated with increased risks of miscarriage and fetal death[16-18]. Similarly, previous studies suggested associations of higher levels of maternal caffeine intake during pregnancy with a lower birth weight[19-20]. Previous studies suggested an inverse U-shaped relationship between maternal age and birth weight[21]. Whether maternal lifestyle habits explain these associations is not known.

Thus, previous studies showed robust evidence for the associations of high exposures levels of maternal smoking, alcohol consumption and caffeine intake with the risks of perinatal mortality and morbidity. However, less is known about the effects of low to moderate exposure levels on these outcomes. In addition, most previous studies focussed on birth weight as main outcome measure, but birth weight is just a proxy of fetal growth. Different fetal growth characteristics and body proportions might result in the same birth weight. Exposure to adverse maternal lifestyle habits in different trimesters of pregnancy might also have differential effects on fetal growth characteristics. Therefore, studies on exposure effects in different trimesters might identify specific critical periods. Finally, investigation of factors that may explain the established relationship between maternal age and adverse pregnancy outcomes may help understand the underlying mechanisms. (Figure 1)

FIGURE 1. Overview of proposed pathways of maternal lifestyle, pregnancy complications and adverse birth outcomes



MAIN OBJECTIVES

The main objectives of the studies presented in this thesis are to examine the associations of maternal lifestyle habits with hypertensive complications during pregnancy, and with fetal growth and the risks of neonatal complications.

OUTLINE OF THESIS

These objectives are addressed in several studies presented in this thesis. In **Part 2**, we present the overall design of the study (**Chapter 2.1**), and the potential of misclassification in maternal smoking habits assessment by questionnaires (**Chapter 2.2**).

Part 3 presents studies focused on blood pressure and hypertensive complications during pregnancy. In **Chapter 3.1** we examined whether blood pressure in early pregnancy tracks and whether this tracking is associated with the risk of gestational hypertensive disorders. The influences of maternal age, smoking during pregnancy, and maternal caffeine intake on blood pressure levels and the risks of hypertensive disorders are presented in **Chapters 3.2**, **3.3** and **3.4**, respectively. Finally, in **Chapter 3.5**, the associations of blood pressure levels during pregnancy with fetal growth and neonatal complications are described.

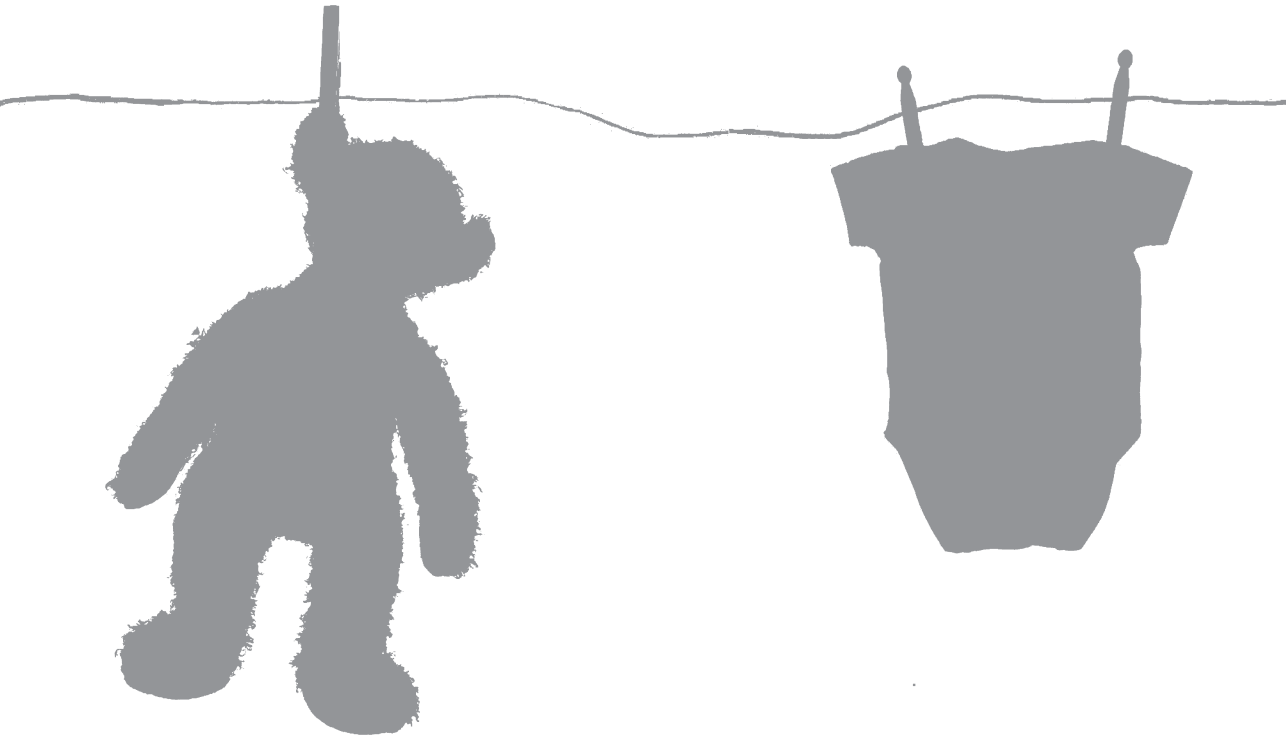
In **Part 4**, we present studies focused on the associations of maternal lifestyle factors with fetal growth patterns and the risks of neonatal complications. In **Chapter 4.1** we examined the differences in birth outcomes in relation to maternal age. Also, we studied the associations of moderate maternal alcohol consumption during pregnancy with fetal growth patterns (**Chapter 4.2**), and the risks of low birth weight and preterm birth (**Chapter 4.3**). In **Chapter 4.4**, we assessed whether the well-known association of maternal smoking with low birth weight can be modified by use of folic acid supplements during pregnancy. The associations of maternal caffeine intake and fetal growth are presented in **Chapter 4.5**.

Finally, in **Part 5** the studies performed on maternal lifestyle factors and pregnancy complications in this thesis are discussed, and suggestions for future research are presented.

REFERENCES

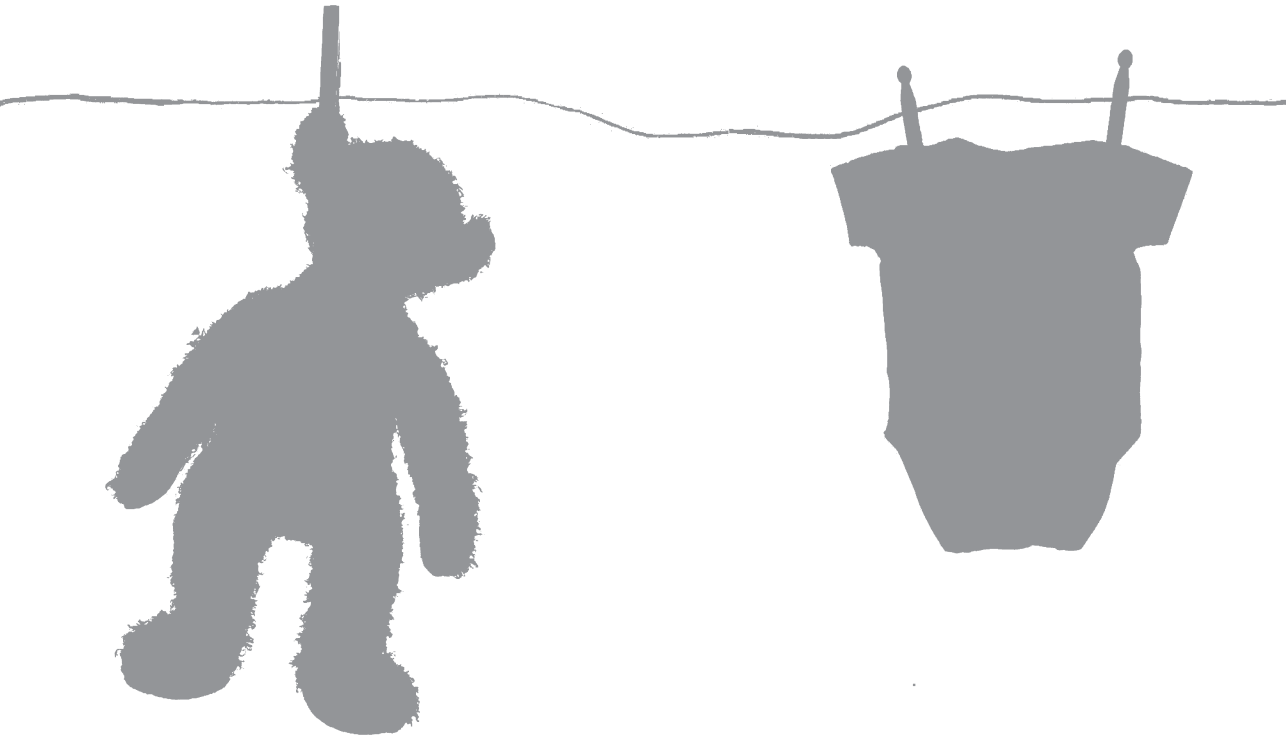
1. Nijdam ME, Janssen KJ, Moons KG, et al. Prediction model for hypertension in pregnancy in nulliparous women using information obtained at the first antenatal visit. J Hypertens. 2010;28:119-26.
2. Steegers EAP, von Dadelszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet. 2010;376:631-44.
3. Kline J, Stein ZA, Susser M, et al. Smoking: a risk factor for spontaneous abortion. N Engl J Med. 1977;297: 793-6.
4. Cnattingius S, Haglund B, Meirik O. Cigarette smoking as risk factor for late fetal and early neonatal death. BMJ. 1988;297:258-61.
5. Butler NR, Goldstein H, Ross EM. Cigarette smoking in pregnancy: its influence on birth weight and perinatal mortality. BMJ. 1972;2:127-30.
6. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the Generation R Study. Am J Epidemiol. 2007;165:1207-15.
7. Jaddoe VW, Troe EJ, Hofman A, 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;22:162-71.
8. McCowan LM, Dekker GA, Chan E, et al; SCOPE consortium. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. BMJ. 2009;338: b1081
9. Barr HM, Bookstein FL, O'Malley KD, et al. Binge drinking during pregnancy as a predictor of psychiatric disorders on the Structured Clinical Interview for DSM-IV in young adult offspring. Am J Psychiatry. 2006;163: 1061–65.
10. Henderson J, Kesmodel U, Gray R. Systematic review of the fetal effects of prenatal binge-drinking. J Epidemiol Community Health. 2007;61:1069–73.
11. Spohr HL, Willms J, Steinhausen HC. Prenatal alcohol exposure and long-term developmental consequences. Lancet. 1993;341:907-10.
12. Kline J, Shrout P, Stein Z, et al. Drinking during pregnancy and spontaneous abortion. Lancet. 1980;2:176-80.
13. Ouellette EM, Rosett HL, Rosman NP, et al. Adverse effects on offspring of maternal alcohol abuse during pregnancy. N Engl J Med. 1977;297:528-30.
14. Sokol RJ, Delaney-Black V, Nordstrom B. Fetal alcohol spectrum disorder. JAMA. 2003;290:2996–99.
15. O'Leary CM, Nassar N, Kurinczuk JJ, et al. The effect of maternal alcohol consumption on fetal growth and preterm birth. BJOG. 2009;116:390–400.
16. Cnattingius S, Signorello LB, Annerén G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med. 2000;343:1839-45.
17. Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gyneacol. 2008;198:279 e1-8.
18. Wisborg K, Kesmodel U, Bech BH, et al. Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study. BMJ. 2003;326:420.
19. CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ 2008;337:a2332.
20. Cook DG, Peacock JL, Feyerabend C, et al. Relation of caffeine intake and blood caffeine concentrations during pregnancy to fetal growth: prospective population based study. BMJ. 1996;313:1358-62.
21. MacLeod S, Kiely JL. The effects of maternal age and parity on birthweight: a population-based study in New York City. Int J Gynaecol Obstet. 1988;26:11-9.

Part 2 | **Design and methods**



Chapter 2.1

Study design: The Generation R Study



INTRODUCTION

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health during fetal life, childhood and adulthood. The study is conducted in Rotterdam, the second largest city in the Netherlands. Rotterdam is situated in the Western part of the Netherlands on almost 80 km south from Amsterdam, the capital of the Netherlands. The total population consists of about 600000 inhabitants of almost 150 different ethnicities. The study area is well defined by postal codes and covers more than half of the cities inhabitants (almost 350000 inhabitants)[1]. The largest ethnic groups in this population are the Dutch (56%), Surinamese (9%), Turkish (7%), Moroccan (6%), Dutch Antillean (3%) and Cape Verdian (3%) groups[2]. The percentages of the non-Dutch groups are higher in younger age groups[2]. The number of children born in this study area is about 4300 per year. Measurements in the prenatal phase of the study were conducted in two well-equipped research centers in the study area, with a close collaboration with midwives and hospitals.

STUDY DESIGN

Overview

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. Mothers with a delivery date between April 2002 and January 2006 were eligible. Extensive assessments have been carried out in mothers and partners and are currently performed in their children (Table 1). Assessments in pregnancy were planned in early pregnancy (gestational age <18.0 weeks), mid-pregnancy (gestational age 18.0–24.9 weeks) and late pregnancy (gestational age ≥25 weeks). These measurements are considered as first, second and third trimester measurements. The partners were assessed once in pregnancy. The children form a prenatally recruited birth-cohort that will be followed until young adulthood.

Eligibility and enrolment

Eligible mothers were those who were resident in the study area at their delivery date and had a delivery date from April 2002 until January 2006. We aimed to enrol mothers in early pregnancy (gestational age <18.0 weeks) but enrolment was allowed until birth of their child. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care, handed out the information package and asked these mothers to make an appointment for the first ultrasound examination. The study staff contacted these mothers by phone for additional information about the study and in person at the ultrasound examination to obtain informed consent. Mothers, who were not approached in pregnancy, were approached and enrolled in the first months after birth of their child when newborns visit the routine child health centers[2]. The

TABLE 1. Assessments in mothers, partners and their children in the prenatal phase

	Early pregnancy[1]	Mid-pregnancy[1]	Late pregnancy[1]	Birth
Mother				
Physical examination	+	+	+	
Questionnaire	+	+	+	
Interview			S	
Fetal ultrasound examination	+	+	+	
Detailed fetal ultrasound			S	
Blood sample	+	+		
Urine sample	+	+	+	
Partner				
Physical examination	+			
Questionnaire		+		
Interview				
Blood sample	+			
Child				
Physical examination				+
Cord blood				+

+ = Assessment in whole cohort.
S = assessment only in subgroup.
[1]Early pregnancy: gestational age <18.0 weeks; mid-pregnancy: gestational age 18.0–24.9 weeks; late pregnancy: gestational age ≥25 weeks.

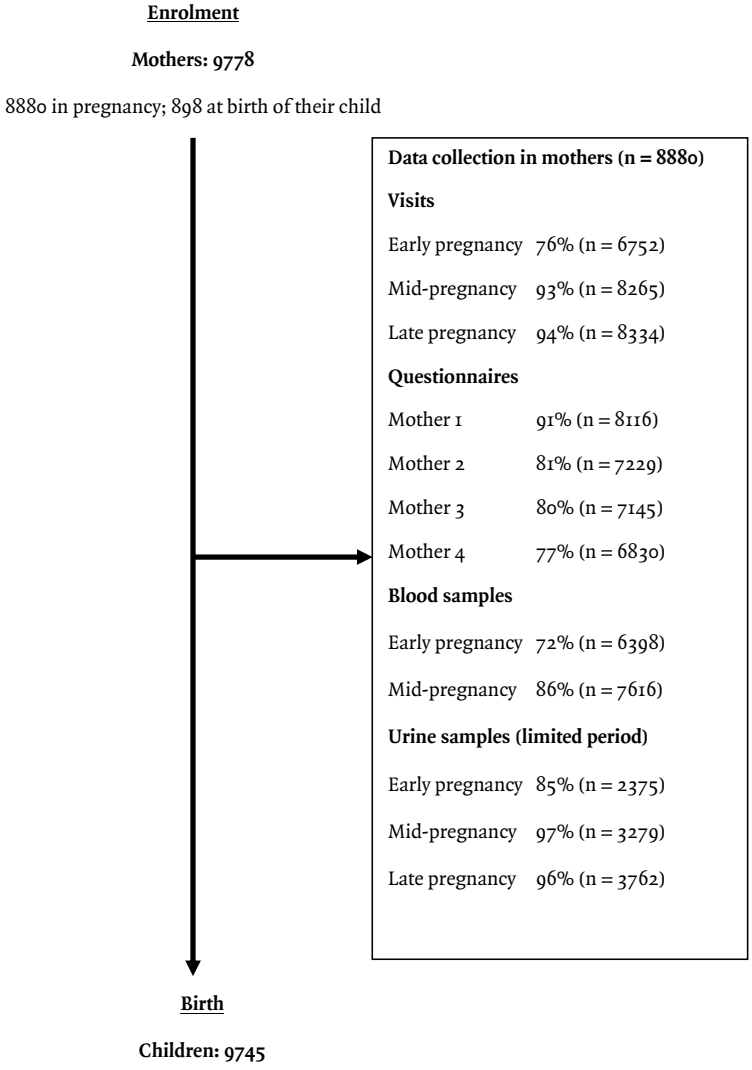
partners were not approached directly by the study staff but the mothers were informed about the importance of involvement of the partners in the study.

STUDY COHORT

Parents

In total, 9778 mothers were enrolled in the study (Figure 1). Of these mothers, 91% (n = 8880) was enrolled in pregnancy. Only partners from mothers enrolled in pregnancy were invited to participate. In total, 71% (n = 6347) of all partners was enrolled. The general characteristics of the mothers and partners are presented in Table 2. Of all participating mothers, enrolment was in early pregnancy in 69% (n = 6691), in mid-pregnancy in 19% (n = 1918), in late pregnancy in 3% (n = 271) and at birth of their child in 9% (n = 898). Of all pregnant women enrolled, 94% (n = 8356), 6% (n = 516) and 0.1% (n = 8) were first, second and third pregnancies in the study, respectively. The largest ethnic groups were the Dutch, Surinamese, Turkish and Moroccan groups, according to the classification of Statistics Netherlands[3,4]. The ethnic distribution differed only moderately from that of the population in the study area[3]. Mean household income in Rotterdam is about

FIGURE 1. Enrolment and measurements during pregnancy



€1600 per month and the percentage subjects with a secondary or higher education level in Rotterdam is 56%[3]. The educational level of participating mothers and their partners was classified in groups, according to the classification of Statistics Netherlands[5]. Ethnic background, educational level and occupational status are of major interest and are studied as determinants of health and behavioural outcomes[6-23]. Both household income and highest followed educational level in mothers and partners in the study cohort suggest a selection towards a higher socioeconomic status than in the whole study. This pattern is similar as in other large-scale cohort studies[24]. However, differences between the population and cohort characteristics may also be due to selective missing values of ethnicity and socio-economic status in the questionnaires. Socio-economic

TABLE 2. Characteristics of mothers and their partners[1]

	Mothers	Partners
	n=9778	n=6347
Gestational age at enrolment, %		
Early pregnancy	69	-
Mid-pregnancy	19	-
Late pregnancy	3	-
Birth	9	-
Pregnancy number in study, %		
1st pregnancy	94	-
2nd pregnancy	6	-
3rd pregnancy	0.1	-
Age at enrolment (years)[2]	29.9 (5.4)	32.7 (5.7)
Parity, %		
0	55	-
1	30	-
≥2	15	-
Ethnicity, %		
Dutch, other-European	59	68
Surinamese	9	7
Moroccan	6	4
Turkish	9	7
Dutch Antilles	3	3
Cape verdian	4	2
Others	10	9
Highest completed education, %		
Primary school	11	8
Secundary school	46	41
Higher education	43	51

[1]Values are percentages.
[2]Mean (standard deviation).

status is related to various perinatal and postnatal health outcomes and of major interest in the study[25-41].

Children at birth and overall response

Among the live births, 51% were male and 49% female. These percentages are similar to the population figures in the Netherlands and in Rotterdam[3]. The percentages of children born preterm or with low birth weight are smaller than expected based on the population figures. This seems to reflect a selection toward a relative more healthy study population. Estimation of the precise number of eligible pregnant women in the study area is difficult since there is no satisfactory

registry of pregnancies. Therefore, it was not attempted to identify overall response rates of pregnant women. Since the children form a prenatally recruited birth-cohort, the overall response of the study has been established at birth and is 61%.

DATA COLLECTION DURING PREGNANCY

Physical examinations

Physical examinations were planned at each visit in early pregnancy, mid-pregnancy and late pregnancy and included height, weight and blood pressure measurements of both parents. Overall response rates for these specific measurements in mothers and partners are similar as the visit percentages presented in Figure 1. Since there was a wide range of gestational age at each visit, these measurements are used in the analyses as gestational-age-adjusted measurements[42].

Questionnaires

Mothers received four postal questionnaires and father received one postal questionnaire in the prenatal phase (Table 1). All questionnaires are available in three languages (Dutch, English and Turkish). If needed, further support for verbal translation of questionnaires is available in Arabic, Portuguese and French. Each questionnaire comprises about 25 pages and takes about 30 to 45 minutes to be completed[2]. Topics in these questionnaires were:

– Mother 1: lifestyle habits, including smoking, alcohol consumption, tea and coffee consumption
– Mother 2: folic acid supplement use
– Mother 3: lifestyle habits, including smoking, alcohol consumption, tea and coffee consumption
– Mother 4: lifestyle habits, including smoking, alcohol consumption, tea and coffee consumption

Overall response rates for these questionnaires varied from 77 to 91% (Figure 1). However, the response rates of specific questions may be lower due to missing values within questionnaires.

Fetal ultrasound examinations

Fetal ultrasound examinations were performed at each prenatal visit. Overall response rates for these ultrasound examinations were in general similar to the visit percentages given in Figure 1. These ultrasound examinations were used for both establishing gestational age and assessing fetal growth patterns. These methods have previously been described in detail[43,44]. Establishing gestational age by using the first day of the last menstrual period is not reliable for a variety of reasons including the large number of women who do not know their exact date, have irregular menstrual cycles or amenorrhea, use oral contraceptive pills or bleed in early pregnancy[45]. Using fetal ultrasound data such as crown-rump length or biparietal diameter for pregnancy dating seems to overcome these problems but does not allow growth studies of these measurements since no growth variability between subjects is assumed. Pregnancy dating-curves have been derived in

a subsample of the cohort including subjects with complete data on both the first day of the last menstrual period and crown-rump length or biparietal diameter and used to date the gestational age[43]. Subsequently, longitudinal curves of all fetal growth measurements (head circumference, biparietal diameter, abdominal circumference and femur length) were created resulting in standard deviation scores for all of these specific growth measurements. Various socio-demographic and lifestyle related determinant seems to affect these fetal growth and birth outcomes[46-49]. Also, specific fetal growth patterns seem to be associated with outcomes in childhood[50-53]. We have demonstrated, in a subgroup study among mothers with a known and reliable last menstrual period, that various lifestyle related factors affect first trimester growth[54]. Placental haemodynamics including resistance indices of the uterine and umbilical arteries have been assessed in second and third trimester[55]. Detailed measurements of fetal brain, cardiac and kidney development have been performed in the subcohort[56-59].

Pregnancy complications and outcomes

The obstetric records of mothers have been looked up in the hospitals and mid-wife practices. Specialists in the relevant field code items in these records, and used for validation studies for maternal reported data[60,61]. The major pregnancy outcomes, including live births, induced abortion fetal or perinatal loss, pregnancy-induced hypertension, preeclampsia, and gestational diabetes are known in 99% of all enrolled mothers. These outcomes are related to various exposures of interest[62-77]. In all children known to be born alive, information about sex, birth weight and gestational age is available.

REFERENCES

1. Wiesbauer F, Blessberger H, Goliasch G, et al. Elevated risk of myocardial infarction in very young immigrants from former Yugoslavia. Eur J Epidemiol. 2009;24:691-6.
2. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update until the age of 4 years. Eur J Epidemiol. 2008;23:801-11.
3. Centre for Research and Statistics, Rotterdam (COS); http:// www.cos.rotterdam.nl. 2005.
4. Statistics Netherlands. Allochtonen in Nederland 2004. Voorburg/Heerlen: Statistics Netherlands; 2004.
5. Troe EJ, Raat H, Jaddoe VW, et al. Explaining differences in birthweight between ethnic populations. The Generation R Study. BJOG. 2007;114:1557-65.
6. Statistics Netherlands. Standaard Onderwijsindeling 2003. Voorburg/Heerlen: Statistics Netherlands; 2004.
7. Troe EJ, Raat H, Jaddoe VW, et al. Smoking during pregnancy in ethnic populations: the Generation R study. Nicotine Tob Res. 2008;10:1373-84.
8. Chote AA, Koopmans GT, Redekop WK, et al. Explaining Ethnic Differences in Late Antenatal Care Entry by Predisposing, Enabling and Need Factors in the Netherlands. The Generation R Study. Matern Child Health J. 2010. doi: 10.1007/s10995-010-0619-2
9. Chote AA, de Groot CJ, Bruijnzeels MA, et al. Ethnic differences in antenatal care use in a large multi-ethnic urban population in the Netherlands. Midwifery. 2011;27:36-41.
10. El Marroun H, Tiemeier H, Jaddoe VW, et al. Demographic, emotional and social determinants of cannabis use in early pregnancy: the Generation R study. Drug Alcohol Depend. 2008;98:218-26.
11. Timmermans S, Jaddoe VW, Mackenbach JP, et al. Determinants of folic acid use in early pregnancy in a multi-ethnic urban population in The Netherlands: the Generation R study. Prev Med. 2008;47:427-32.
12. van Rossem L, Vogel I, Steegers E, et al. Breastfeeding patterns among ethic minorities: The generation R study. J Epidemiol Community Health. 2010;64:1080-5.
13. Silva LM, Steegers EA, Burdorf A, et al. No midpregnancy fall in diastolic blood pressure in women with a low educational level: the Generation R Study. Hypertension. 2008;52:645-51.
14. Silva LM, Coolman M, Steegers EA, et al. Low socioeconomic status is a risk factor for preeclampsia: the Generation R Study. J Hypertens. 2008;26:1200-8.
15. Silva L, Coolman M, Steegers E, et al. Maternal educational level and risk of gestational hypertension: the Generation R Study. J Hum Hypertens. 2008;22:483-92.
16. Silva LM, Jansen PW, Steegers EA, et al. Mother's educational level and fetal growth: the genesis of health inequalities. Int J Epidemiol. 2010;39:1250-61.
17. Jansen PW, Tiemeier H, Jaddoe VW, et al. Explaining educational inequalities in preterm birth: the generation r study. Arch Dis Child Fetal Neonatal Ed. 2009;94:F28-34.
18. Jansen PW, Tiemeier H, Looman CW, et al. Explaining educational inequalities in birthweight: the Generation R Study. Paediatr Perinat Epidemiol. 2009;23:216-28.
19. Jansen PW, Tiemeier H, Verhulst FC, et al. Employment status and the risk of pregnancy complications: the Generation R Study. Occup Environ Med. 2010;67:387-94.
20. Jansen PW, Raat H, Mackenbach JP, et al. Socioeconomic inequalities in infant temperament: the generation R study. Soc Psychiatry Psychiatr Epidemiol. 2009;44:87-95.
21. Jansen PW, Raat H, Mackenbach JP, et al. National Origin and Behavioural Problems of Toddlers: The Role of Family Risk Factors and Maternal Immigration Characteristics. J Abnorm Child Psychol. 2010;38:1151-64
22. van Rossem L, Oenema A, Steegers EA, et al. Are starting and continuing breastfeeding related to educational background? The generation R study. Pediatrics. 2009;123:e1017-27.
23. van Rossem L, Silva LM, Hokken-Koelega A, et al. Socioeconomic Status is not Inversely Associated with Overweight in Preschool Children. J Pediatr. 2010;157:929-935.e1.
24. Jacobsen TN, Nohr EA, Frydenberg M. Selection by socioeconomic factors into the Danish National Birth Cohort. Eur J Epidemiol. 2010;25:349-55.
25. Rodrigues T, Barros H. Maternal unemployment: an indicator of spontaneous preterm delivery risk. Eur J Epidemiol. 2008;23:689-93.

26. Quansah R, Gissler M, Jaakkola JJ. Work as a physician and adverse pregnancy outcomes: a Finnish nationwide population-based registry study. Eur J Epidemiol. 2009;24:531-6.
27. McFadden E, Luben R, Wareham N, et al. Occupational social class, risk factors and cardiovascular disease incidence in men and women: a prospective study in the European Prospective Investigation of Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort. Eur J Epidemiol. 2008;23:449-58.
28. McFadden E, Luben R, Khaw KT. Different measures of social class in women and mortality. Eur J Epidemiol 2009;24:231-6.
29. McFadden E, Luben R, Wareham N, et al. How far can we explain the social class differential in respiratory function? A cross-sectional population study of 21,991 men and women from EPIC-Norfolk. Eur J Epidemiol 2009;24:193-201.
30. McFadden E, Luben R, Wareham N, et al. Occupational social class, educational level, smoking and body mass index, and cause-specific mortality in men and women: a prospective study in the European Prospective Investigation of Cancer and Nutrition in Norfolk (EPIC-Norfolk) cohort. Eur J Epidemiol. 2008;23:511-22.
31. Abatih E, Van Oyen H, Bossuyt N, et al. Variance estimation methods for health expectancy by relative socio-economic status. Eur J Epidemiol. 2008;23:243-9.
32. Heraclides A, Witte D, Brunner EJ. The association between father's social class and adult obesity is not explained by educational attainment and an unhealthy lifestyle in adulthood. Eur J Epidemiol. 2008;23:573-9.
33. Lallukka T, Sarlio-Lahteenkorva S, Kaila-Kangas L, et al. Working conditions and weight gain: a 28-year follow-up study of industrial employees. Eur J Epidemiol. 2008;23:303-10.
34. Laszlo KD, Janszky I, Ahnve S. Income and recurrent events after a coronary event in women. Eur J Epidemiol. 2008;23:669-80.
35. Schlaefer K, Schlehofer B, Schuz J. Validity of self-reported occupational noise exposure. Eur J Epidemiol. 2009;24:469-75.
36. Pfefferle PI, Kramer A. Helicobacter pylori-infection status and childhood living conditions are associated with signs of allergic diseases in an occupational population. Eur J Epidemiol. 2008;23:635-40.
37. Baste V, Riise T, Moen BE. Radiofrequency electromagnetic fields; male infertility and sex ratio of offspring. Eur J Epidemiol. 2008;23:369-77.
38. Muller-Riemenschneider F, Reinhold T, Berghofer A, et al. Health-economic burden of obesity in Europe. Eur J Epidemiol. 2008;23:499-509.
39. Hublin C, Partinen M, Koskenvuo K, et al. Shift-work and cardiovascular disease: a population-based 22-year follow-up study. Eur J Epidemiol. 2010;25:315-23.
40. Leclerc A. Shift-work and cardiovascular disease. Eur J Epidemiol. 2010;25:285-6
41. Thomas C, Power C. Shift work and risk factors for cardiovascular disease: a study at age 45 years in the 1958 British birth cohort. Eur J Epidemiol. 2010;25:305-14.
42. Ay L, Kruithof CJ, Bakker R, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG. 2009;116:953-63.
43. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31: 388-96.
44. Verburg BO, Mulder PG, Hofman A, et al. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenat Diagn. 2008;28:323-31.
45. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol. 1996;8:178-85.
46. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R study. Am J Epidemiol. 2007;165:1207-15.
47. El Marroun H, Tiemeier H, Steegers EA, et al. Intrauterine Cannabis Exposure Affects Fetal Growth Trajectories: The Generation R Study. J Am Acad Child Adolesc Psychiatry. 2009;48:1173-1181.
48. Henrichs J, Schenk JJ, Roza SJ, et al. Maternal psychological distress and fetal growth trajectories: the Generation R Study. Psychol Med. 2010;40:633-43.

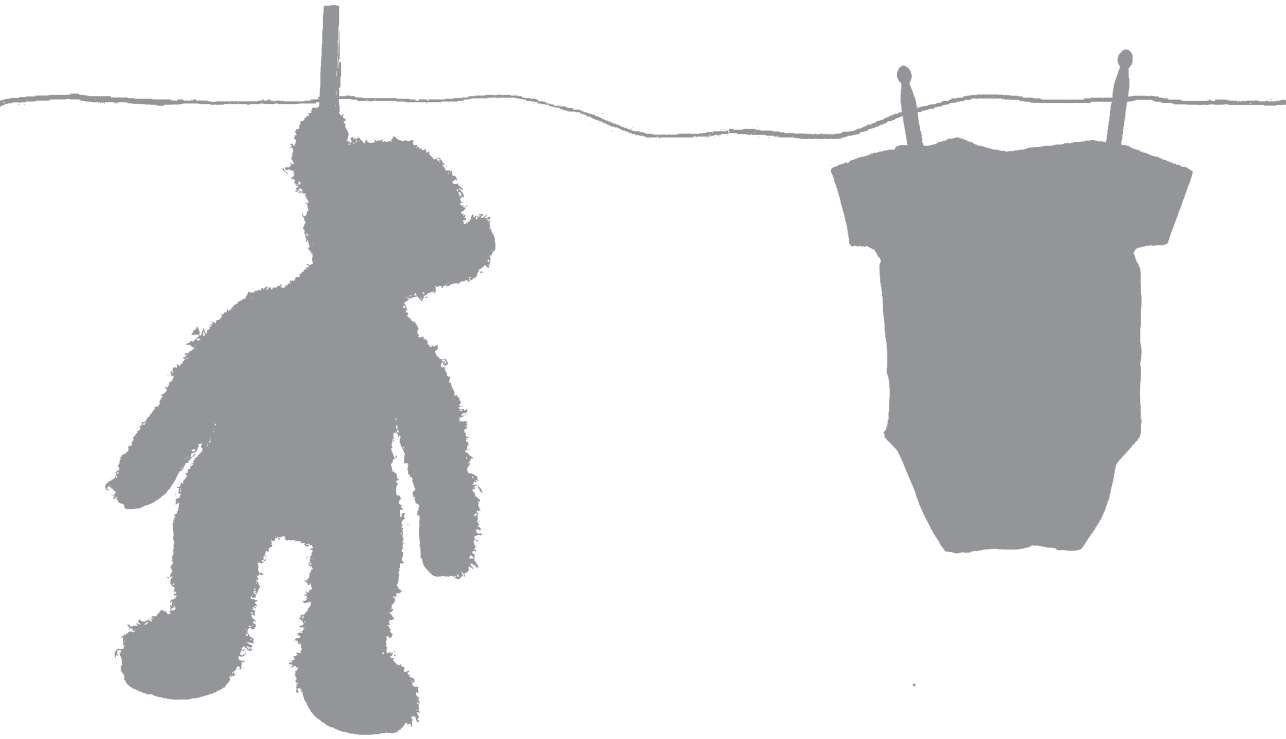
49. Roza SJ, Verburg BO, Jaddoe VW, et al. Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study. Eur J Neurosci. 2007;25:611-7.
50. Roza SJ, van Lier PA, Jaddoe VW, et al. Intrauterine growth and infant temperamental difficulties: the Generation R Study. J Am Acad Child Adolesc Psychiatry. 2008;47:264-72.
51. Henrichs J, Schenk JJ, Schmidt HG, et al. Fetal size in mid- and late pregnancy is related to infant alertness: the generation R study. Dev Psychobiol. 2009;51:119-30.
52. Henrichs J, Schenk JJ, Barendregt CS, et al. Fetal growth from mid- to late pregnancy is associated with infant development: the Generation R Study. Dev Med Child Neurol. 2010;52:644-51.
53. Timmermans S, Jaddoe VW, Hofman A, et al. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102:777-85.
54. Mook-Kanamori DO, Steegers EA, Eilers PH, et al. Risk factors and outcomes associated with first-trimester fetal growth restriction. JAMA. 2010;303:527-34.
55. Timmermans S, Jaddoe VW, Silva LM, et al. Folic acid is positively associated with uteroplacental vascular resistance: The Generation R Study. Nutr Metab Cardiovasc Dis. 2011;21:54-61.
56. Verburg BO, Jaddoe VW, Wladimiroff JW, et al. Fetal hemodynamic adaptive changes related to intrauterine growth: the Generation R Study. Circulation. 2008;117:649-59.
57. Verburg BO, Geelhoed JJ, Steegers EA, et al. Fetal kidney volume and its association with growth and blood flow in fetal life: The Generation R Study. Kidney Int. 2007;72:754-61.
58. Roza SJ, Steegers EA, Verburg BO, et al. What is spared by fetal brain-sparing? Fetal circulatory redistribution and behavioral problems in the general population. Am J Epidemiol. 2008;168:1145-52.
59. El Marroun H, Tiemeier H, Steegers EA, et al. A prospective study on intrauterine cannabis exposure and fetal blood flow. Early Hum Dev. 2010;86:231-236.
60. Coolman M, de Groot CJ, Jaddoe VW, et al. Medical record validation of maternally reported history of pre-eclampsia. J Clin Epidemiol. 2010;63:932-7.
61. Troude P, L'Helias LF, Raison-Boulley AM, et al. Perinatal factors reported by mothers: do they agree with medical records? Eur J Epidemiol. 2008;23:557-64.
62. Jaddoe VW, Troe EJ, Hofman A, 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;22:162-71.
63. van den Hooven EH, Jaddoe VW, de Kluizenaar Y, et al. Residential traffic exposure and pregnancy-related outcomes: a prospective birth cohort study. Environ Health. 2009;8:59.
64. Both MI, Overvest MA, Wildhagen MF, et al. The association of daily physical activity and birth outcome: a population-based cohort study. Eur J Epidemiol. 2010;25:421-9.
65. Khashan AS, Kenny LC.The effects of maternal body mass index on pregnancy outcome. Eur J Epidemiol. 2009; 24:697-705.
66. Abdullah NA, Pearce MS, Parker L, et al. Evidence of an environmental contribution to the aetiology of cryptorchidism and hypospadias? Eur J Epidemiol. 2007;22:615-20.
67. Astolfi P, De Pasquale A, Zonta LA. Gestational age shortening in single births at term. Italy 1990-1998. Eur J Epidemiol. 2007;22:263-5.
68. Bartels DB, Wenzlaff P, Poets CF. Obstetrical volume and early neonatal mortality in preterm infants. Eur J Epidemiol. 2007;22:791-8.
69. Nabet C, Lelong N, Ancel PY, et al. Smoking during pregnancy according to obstetric complications and parity: results of the EUROPOP study. Eur J Epidemiol. 2007;22:715-21.
70. Tu YK, Manda SO, Ellison GT, et al. Revisiting the interaction between birth weight and current body size in the foetal origins of adult disease. Eur J Epidemiol. 2007;22:565-75.
71. Banhidy F, Acs N, Puho EH, et al. Maternal acute respiratory infectious diseases during pregnancy and birth outcomes. Eur J Epidemiol. 2008;23:29-35.
72. Hernandez-Diaz S, Wilcox AJ, Schisterman EF, et al. From causal diagrams to birth weight-specific curves of infant mortality. Eur J Epidemiol. 2008;23:163-6.
73. See LC, Shen YM, Lin SR. Lower early neonatal mortality among singletons in transnational marriage families: Taiwan Birth Registry. Eur J Epidemiol. 2008;23:483-9.

74. Strandberg-Larsen K, Rod Nielsen N, Nybo Andersen AM, et al. Characteristics of women who binge drink before and after they become aware of their pregnancy. Eur J Epidemiol. 2008;23:565-72.
75. Zhu JL, Vestergaard M, Madsen KM, et al. Paternal age and mortality in children. Eur J Epidemiol. 2008;23: 443-7.
76. Julihn A, Ekbom A, Modeer T. Maternal overweight and smoking: prenatal risk factors for caries development in offspring during the teenage period. Eur J Epidemiol. 2009;24:753-62.
77. Greenwood DC, Alwan N, Boylan S, et al. Caffeine intake during pregnancy, late miscarriage and stillbirth. Eur J Epidemiol. 2010;25:275-80.

Chapter 2.2

Assessment of smoking status and neonatal outcomes

Rachel Bakker
Claudia J Kruithof
Eric AP Steegers
Henning Tiemeier
Johan P Mackenbach
Albert Hofman
Vincent WV Jaddoe
Submitted for publication



ABSTRACT

Single assessment of smoking during pregnancy may lead to misclassification, due to underreporting or failure of smoking cessation. We examined the percentage of mothers who were misclassified in smoking status based on single assessment, as compared to repeated assessment, and whether this misclassification leads to altered effect estimates for the associations between maternal smoking and neonatal complications. This study was performed in 5389 mothers participating in a prospective population-based cohort study in the Netherlands. Smoking status was assessed three times during pregnancy using questionnaires. Information on birth weight and neonatal complications was obtained from hospital records. For categorizing mothers per smoking status, Cohen's Kappa coefficient was 0.86 (P<0.001) between single and repeated assessment. Of all mothers who reported non-smoking or first trimester only smoking in early pregnancy, 1.7% (70 of 4141), and 33.7% (217 of 643), respectively, were reclassified to continued smoking based on repeated assessment. Younger, shorter, lower educated mothers who had non-European ethnicity, experienced more stress, consumed more alcohol and did not use folic acid supplements had higher risk of underreporting their smoking status or failure of smoking cessation. Marginal differences were found on the associations of maternal smoking with neonatal complications between single or repeated assessment. Our results suggest that single assessment of smoking during pregnancy, leads to underestimation of the continued smoking prevalence, especially among mothers who reported quitting smoking in first trimester. However, this underestimation does not materially change the effect estimates for the associations between maternal smoking and neonatal outcomes.

INTRODUCTION

Maternal smoking is associated with impaired fetal growth from early pregnancy onwards and increased risks of neonatal complications. Birth weight is 150-250 grams lower among children of mothers who continued smoking during pregnancy[1-5].

In large epidemiological studies, assessment of maternal smoking during pregnancy is mainly performed by questionnaires. Although assessing smoking during pregnancy by questionnaires seems to be valid method, misclassification may occur[6]. Underreporting of maternal smoking across the various smoking categories may be present. To overcome these limitations, previous studies have used biomarkers of tobacco exposure, including cotinine, in maternal urine samples[7-8]. However, it has been demonstrated that use of cotinine levels is not superior to the use of self-reporting questionnaires in studying the effect of maternal smoking in pregnancy on birth weight[9]. For this and practical and financial reasons, most large-scale population based cohort studies use self-reported questionnaire data for assessing maternal smoking status. Assessing continued maternal smoking by one questionnaire during early pregnancy may lead to underestimation due to changing smoking status. Mothers who report non-smoking or first trimester only smoking may start smoking again later in pregnancy. This type of misclassification is due to failure of smoking cessation, instead of underreporting smoking status. In general, failure of smoking cessation leading to misclassification of smoking status during pregnancy may affect association studies of maternal smoking and neonatal complications. Previous studies studied failure of smoking cessation and misclassification rates for smoking status[10,11]; however, less studies are available with a large population-based sample size, and examined the effect of failure of smoking cessation and misclassification on the associations between smoking and neonatal complications.

Therefore, in 5389 mothers participating in a population-based prospective cohort study performed in Rotterdam, the Netherlands, we examined the percentage of mothers who were misclassified based on single assessment only, as compared to repeated, in each trimester, assessment, and whether any misclassification leads to changes in the effect estimates for the associations between maternal smoking and neonatal complications.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[12]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating mothers. All mothers were enrolled during pregnancy between 2001 and 2005. Three assessments during pregnancy

were planned in first, second and third trimester. In total, 8880 mothers were enrolled during pregnancy. For the present study, we excluded mothers without complete smoking information, missing in either first, second or third trimester, (n=3178), leading to 5702 mothers. Also, we excluded pregnancies not leading to singleton live births (n = 242), missing birth weights (n = 70), and missing gestational age at birth (n = 1). Thus, the cohort for analysis comprised 5389 mothers (Supplementary Figure S1).

Non response analysis showed that women excluded from the analysis were younger, and shorter, had higher body mass index, were more often multiparous, lower educated, and of non-European ethnicity, experienced more stress, consumed less alcohol and caffeine, used less often preconceptional folic acid supplements, were enrolled in the study later, and gave earlier birth to smaller children (data not shown).

Maternal smoking

Information on smoking was obtained by self-reported questionnaires sent in the first, second, and third trimester. Response rates for these questionnaires were 91%, 80%, and 77%, respectively[12]. Maternal smoking at enrolment was assessed in the first questionnaire by asking each mother whether she smoked during pregnancy thus far (no; first trimester only; continued). This questionnaire was sent to all included mothers, regardless of the gestational age at enrolment. To assess smoking status in second and third trimester, mothers were asked whether they smoked in the past 2 months (no; yes) in the second and third questionnaire.

Neonatal outcomes

Information about fetal sex, gestational age, and birth weight was obtained from medical records and hospital registries. Preterm birth was defined as a gestational age of less than 37 weeks at delivery. Low birth weight was defined as birth weight below 2500 grams. Small-size-for-gestational-age at birth was defined as a gestational-age-adjusted birth weight below 5th percentile in this study cohort (less than -1.73 SD).

Covariates

Information on maternal age (continuous), educational level (primary school; secondary school; higher education), ethnicity (European; non-European), parity (nulliparous; multiparous) and folic acid supplementation use (preconceptional use; first trimester only; no use) was obtained at enrolment. Information about alcohol consumption (no; first trimester only; continued) and daily caffeine intake (0 to <2 units; 2 to 5.9 units; ≥6 units) was assessed by questionnaires in each trimester, and, subsequently, combined to an overall alcohol consumption and caffeine intake during pregnancy. At enrolment height (cm) and weight (kg) were measured without shoes and heavy clothing. Body mass index (kg/m^2) was calculated with these measurements. Maternal distress (continuous) was measured by questionnaire at 20 weeks of gestation using the Brief

Symptom Inventory, which gives a Global Severity Index (GSI)[13]. Higher GSI reflects more stress mothers experience.

Statistical analyses

First, we explored the agreement (Cohen's Kappa coefficient) between smoking categories based on single and repeated assessment of smoking during pregnancy. Second, we assessed the percentage of mothers misclassified and identified risk factors for misclassification. Third, we assessed the associations of maternal smoking status with birth weight using linear regression models for both single and repeated assessment of smoking. The associations of maternal smoking status with the risks of preterm birth, low birth weight, and small-size-for-gestational-age were assessed using multiple logistic regression models for both single and multiple assessment of smoking. All models were adjusted for maternal age, body mass index, height, educational level, ethnicity, parity, alcohol consumption, daily caffeine intake, folic acid supplement use, maternal stress, gestational age at birth and fetal sex. The percentages of missing values within the population for analysis were lower than 6%, except for folic acid supplement use (13%). This higher percentage was due to a large number of mothers who only partially completed the food frequency questionnaire or were enrolled later in pregnancy. We used multiple imputations for missing values in the covariates. Five imputed data sets were created and analyzed together. All analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 shows that, as compared to mothers that did not smoke, mothers who reported continued smoking based on a single assessment were younger, had a higher body mass index, were lower educated, more often of non-European ethnicity, experienced more stress, consumed more caffeine per day, and used less folic acid supplements. Children of these mothers had lower birth weight, were born at less weeks of gestation, and were more often boys. Mothers who smoked first trimester only were, as compared to continued smoking mothers, older, had lower body mass index, more often nulliparous, higher educated, experienced less stress, consumed more alcohol and less caffeine, used more often preconceptional folic acid supplements, enrolled earlier in pregnancy, and had children with higher birth weight. Similar characteristics of continued smoking mothers were found after classifying smoking status based on multiple assessments during pregnancy (data not shown).

Table 2 shows that, based on single and repeated smoking assessment, 11.2% (n = 605) and 16.6% (n = 892) of all mothers, respectively, were classified as continued smoking during pregnancy. Of all mothers who reported non-smoking in the first questionnaire (n = 4141), 1.7% (n = 70), reported smoking in the second or third questionnaire. Furthermore, of all mothers who

TABLE 1. Maternal and fetal characteristics according to smoking status based single assessment (N=5389)[1,2]

	Non-smoking n=4141	First trimester only smoking n=643	Continued smoking n=605
Age, yrs	30.5 (4.9)	29.5 (5.3)**	28.8 (5.8)**
Height, cm	168.1 (7.4)	168.6 (7.1)	167.6 (6.9)
Weight, kg	69.2 (12.9)	69.7 (13.3)	70.6 (13.4)*
Body mass index, kg/m^2	24.5 (4.3)	24.5 (4.4)	25.1 (4.6)**
Parity ≥1, %	42.0	30.5**	56.1
Education, %			
Primary school	6.8	8.6**	15.7**
Secondary school	39.6	47.6**	65.1**
Higher education	52.2	43.1**	18.2**
Missing	1.4	0.7**	1.0**
Ethnicity, %			
European	64.2	67.2**	64.1**
Non- European	35.6	32.5**	35.6**
Missing	0.1	0.3**	0.3**
Maternal stress, index[3]	0.2 (0.0-1.2)	0.3 (0.0-1.7)**	0.4 (0.0-1.7)**
Alcohol consumption, %			
None	46.3	24.9**	43.8
First trimester only	12.3	21.5**	11.9
Continued	41.1	53.5**	43.6
Missing	0.3	0.1**	0.7
Daily caffeine intake, %			
0 to <2 units	62.3	54.9**	41.8**
2 to 5.9 units	35.3	44.0**	52.1**
≥6 units	0.8	0.6**	5.8**
Missing	1.5	0.5**	0.3**
Folic acid supplement use, %			
Preconceptional use	42.6	30.8**	20.2**
First trimester only	25.1	36.7**	35.4**
No use	19.1	18.8**	29.4**
Missing	13.2	13.7**	15.0**
Enrolment in early pregnancy, %	78.7	86.8**	78.8
Birth weight, g	3473 (547)	3418 (555)*	3274 (500)**
Gestational age, wks[3]	40.1 (36.0-42.4)	40.0 (35.7-42.3)	40.0 (35.5-42.3)*
Fetal sex, %			
Male	49.6	50.2	54.9*
Female	50.4	49.8	45.1*

[1]Values are means (standard deviation) or percentage. [2]Differences in characteristics between the smoking categories (first trimester only; continued) compared to the non-smoking category were estimated by an independent samples t-test for continuous variables, and chi-square tests for categorical variables. [3]Median (95% range). *P-value<0.05, **P-value<0.01

TABLE 2. Misclassification of smoking status during pregnancy (N=5389)[1]

Smoking status based on single assessment	Smoking status based on repeated assessment			
	Non-smoking	First trimester only smoking	Continued smoking	Total
Non-smoking	98.3 (4071)	n.a.	1.7 (70)[2]	4141
First trimester only smoking	n.a.	66.3 (426)	33.7 (217)[2]	643
Continued smoking	n.a.	n.a.	100 (605)	605
Total	4071	426	892	5389
Cohen's Kappa coefficient = 0.86 (P<0.001)				

[1]Values are percentages of mothers (absolute number) who answered the question on smoking habits in one questionnaire early pregnancy (rows) and in repeated questionnaires (columns).
[2]Percentage of mothers (number) with misclassified smoking habits based on repeated assessments during pregnancy.

reported in the first questionnaire that they smoked in first trimester only and quitted thereafter (n = 643), 33.7% (n = 217) reported smoking in a second or third questionnaire and were subsequently reclassified as continued smoking during pregnancy. In total, 287 mothers (5.3% of 5389) were reclassified in different smoking groups as a consequence of the repeated smoking assessment. For categorizing mothers in non-, first trimester only, and continued smoking Cohen's Kappa coefficient was 0.86 (P<0.001) between single first trimester and repeated each trimester assessment. Mothers who reported non- or first trimester only smoking based on single assessment, but reported smoking in the second or third trimester questionnaire were younger, shorter, lower educated, more often of non-European ethnicity, experienced more stress, consumed more alcohol and did not use folic acid supplements (data not shown).

Table 3 shows lower birth weight in the offspring of mothers who continued smoking during pregnancy (-157 grams (95% confidence interval (CI): -194, -120) and -143 grams (95% CI: -175, -111)) based on single and repeated smoking assessment, respectively, as compared to non-smoking mothers. An increased risk of small-size-for-gestational-age at birth in mothers who smoked in first trimester only was found (Odds ratio (OR), 1.55 (95% CI: 1.08, 2.22)) for single assessment of smoking. No associations were found for the risks of preterm birth and low birth weight. Continued smoking mothers had higher risks on children with low birth weight (OR, 1.67 (95% CI: 1.01, 2.76) and OR, 1.49 (95% CI: 0.96, 2.33)) for single and repeated smoking assessment, respectively. Similar results were found for the risk of delivering small-size-for-gestational-age children (OR, 2.10 (95% CI: 1.47, 2.99) and OR, 2.11 (95% CI: 1.55, 2.88)) for single and repeated smoking assessment, respectively.

DISCUSSION

In Western countries, maternal smoking is one of the most important adverse exposures for pregnant women. Maternal smoking during pregnancy is associated with impaired fetal growth from early pregnancy onwards and increased risks of neonatal complications[1-2,4-5]. Findings from most epidemiological studies are based on self-reported smoking status once during pregnancy. Assessing maternal smoking status by questionnaire during first trimester may lead to misclassification due to underreporting and changing smoking status, caused by failure of smoking cessation. We found that mothers who reported non- or first trimester only smoking in the first questionnaire, but were reclassified as continued smoking based on second or third trimester questionnaire were younger, taller, lower educated, used more alcohol and less preconceptional folic acid supplements during pregnancy. These results are comparable to a study of Pickett *et al.*, who also observed that women who would have been misclassified as non-smokers differed significantly from rightly classified non-smokers in maternal, family, and neighborhood characteristics[14]. We also found that reclassified mothers in our study had children with lower birth weight on average, compared to mothers who were non-reclassified. These results were not found in the study of Pickett *et al.*[14]

In total, 33.7% of all mothers who reported smoking first trimester only in the first questionnaire were subsequently classified to continued smokers due to reported smoking in the second or third questionnaire. So, a large percentage of mothers who apparently reported to have quitted smoking during early pregnancy start smoking again. A study of England *et al.* reported similar findings[15]. They validated self-reported quit status of mothers later in pregnancy with cotinine levels from urine samples. In total, 21.6% had evidence of active smoking. Also, a study of Wells *et al.* reported differences in misclassification percentages of smoking status stratified by sex and US minority majority status[10]. According to Verkerk *et al.* underestimation more than overestimation is likely to occur in studies in which self-reports are used to obtain data on activities which participants voluntarily engage in and which are socially not acceptable or known to be potentially dangerous[16]. We found differences among ethnic groups, which might have been caused by cultural differences. Language differences were resolved within the Generation R Study by providing translated questionnaires (English, French, Turkish and Portuguese) to the participants or translation assistance by one of our employees. Mothers who reported smoking cessation after first trimester of pregnancy had different characteristics as compared to mothers who did not smoke or continued smoking during pregnancy. These associations might reflect sociodemographic and cultural differences, but do not reflect causal associations.

Only marginal differences were found between single and multiple assessment of maternal smoking during pregnancy. Overall, continued smoking is associated with lower birth weight and increased risks of low birth weight and small-size-for-gestational-age at birth[17]. England *et al.* reported, after reclassification of misclassified non-smoking mothers to smokers, lower effect estimates on the associations of smoking and the risk of small-size-for-gestational-age and low

TABLE 3. Association of maternal smoking during pregnancy and neonatal outcomes (N=5389)[1]

	Difference in birth weight (grams)[2]	
Smoking status	Single assessment	Repeated assessment
Non-smoking	Reference n=4141	Reference n=4071
First trimester only smoking	-14 (-49, 20) n=643	38 (-3, 79) n=426
Continued smoking	-157 (-194, -120)** n=605	-143 (-175, -111)** n=892
	Preterm birth (Odds ratio) (n_{cases}=239)[3]	
Smoking status	Single assessment	Repeated assessment
Non-smoking	Reference n_{cases}=179	Reference n_{cases}=177
First trimester only smoking	0.88 (0.57, 1.36) n_{cases}=26	0.66 (0.37, 1.17) n_{cases}=13
Continued smoking	1.33 (0.88, 1.99) n_{cases}=34	1.25 (0.88, 1.78) n_{cases}=49
	Low birth weight (Odds ratio) (n_{cases}=222)[3]	
Smoking status	Single assessment	Repeated assessment
Non-smoking	Reference n_{cases}=157	Reference n_{cases}=155
First trimester only smoking	0.91 (0.51, 1.61) n_{cases}=26	0.65 (0.30 1.41) n_{cases}=12
Continued smoking	1.67 (1.01, 2.76)* n_{cases}=39	1.49 (0.96, 2.33) n_{cases}=55
	Small-size-for-gestational-age (Odds ratio) (n_{cases}=269)[3]	
Smoking status	Single assessment	Repeated assessment
Non-smoking	Reference n_{cases}=174	Reference n_{cases}=170
First trimester only smoking	1.55 (1.08, 2.22)* n_{cases}=42	1.17 (0.73, 1.88) n_{cases}=21
Continued smoking	2.10 (1.47, 2.99)** n_{cases}=53	2.11 (1.55, 2.88)** n_{cases}=78

[1]Models are adjusted for maternal age, body mass index, height, educational level, ethnicity, parity, alcohol consumption, daily caffeine intake, folic acid supplement use, maternal stress, gestational age at birth (only in birth weight and low birth weight model) and fetal sex.
[2]Values are regression coefficients (95% confidence interval) that reflect the difference in birth weight in grams per smoking habit category compared to the reference group of non-smoking mothers.
[3]Values are odds ratios (95% confidence interval) that reflect the difference in risk of adverse birth outcomes per smoking habit category compared to the reference group of non-smoking mothers.
*P-value<0.05
**P-value<0.01

birth weight[15]. According to Verkerk *et al.* it is generally believed that non-differential misclassification will lead to a bias toward the null-value. However, they show in their study that non-differential misclassification may also lead to a bias away from the null-value instead of toward the null[16,18]. Based on our results, we would suggest using repeated assessment of smoking status in population-based studies, which would lead to less underestimation of continued smoking prevalence. We did not observe differences between single versus repeated measurement of smoking for the associations of smoking status during pregnancy with the risks of neonatal outcomes. However, there may be long term consequences on maternal health and postnatal child growth and development[19-21].

Some methodological issues need to be addressed. This study is based on a prospective data collection, which started in early pregnancy. We had a large sample size of 5389 participants and a wide range of potential confounding factors available. Smoking assessment was performed three times during pregnancy, which gave us the opportunity to study misclassification of smoking status during pregnancy. Among mothers with information about smoking status we had a limited loss-to-follow-up. Therefore, we do not expect biased results in our analyses on neonatal outcomes[22]. A limitation is that all the comparisons were based on self-reported smoking status. Our results should therefore only be used for interpretation of self-reported data. Furthermore, we used the following method of classifying based on repeated measurements; if reports of smoking status were consistent throughout the assessment, we classified these mothers accordingly in that specific smoking category. However, reporting consistently false smoking status may have occurred. We did not take this possibility into account. To overcome possible underreporting of smoking, previous studies have used biomarkers of tobacco exposure, including cotinine, in maternal urine samples[7, 8]. Cotinine levels to validate the self-reported smoking status were not available in this study. Haddow et al. have demonstrated that use of cotinine levels is not superior to the use of self-reporting questionnaires in studying the effect of maternal smoking in pregnancy on birth weight[9].

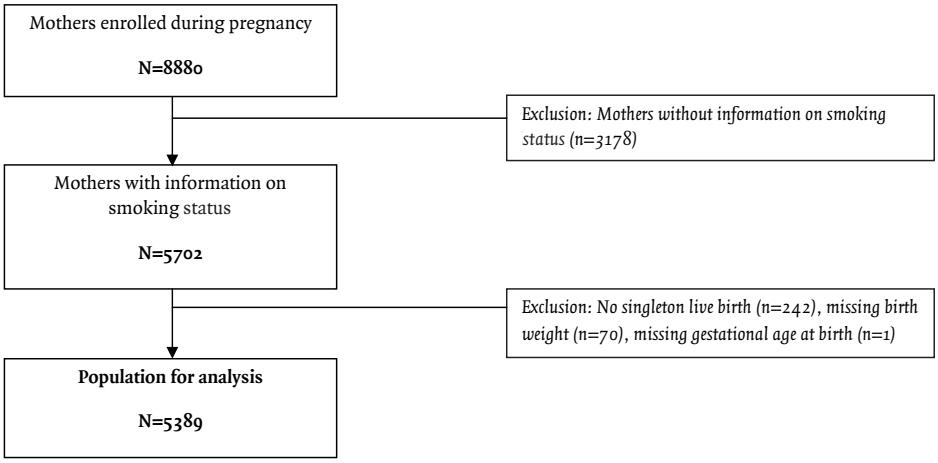
Conclusion

In conclusion, our results suggest that, as compared to repeated smoking assessment, single smoking assessment during pregnancy leads to underestimation of smoking prevalence, due to failure of smoking cessation or underreporting. This may lead to misclassification is epidemiological studies focused on the influences of early or late maternal smoking in pregnancy on health outcomes in mothers and their children. Also, in clinical practice, repeated assessment of smoking status may help to identify women with failure of smoking cessation during pregnancy.

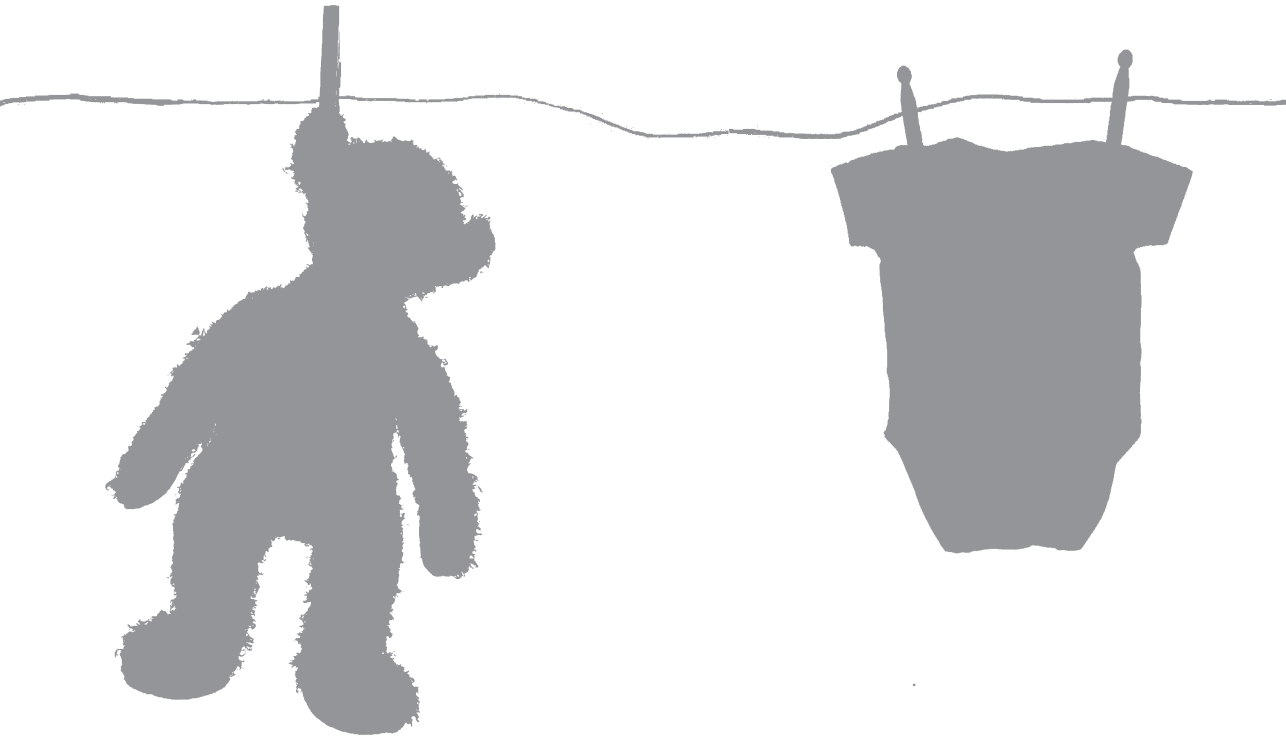
REFERENCES

1. Horta BL, Victora CG, Menezes AM, et al. Low birthweight, preterm births and intrauterine growth retardation in relation to maternal smoking. Paediat Perinat Epidemiol. 1997;11:140-51.
2. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R study. Am J Epidemiol. 2007;165:1207-15.
3. Jaddoe VW, Troe EJ, Hofman A, et al. Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. Paediat Perinat Epidemiol. 2008;22:162-71.
4. McCowan LME, Dekker GA, Chan E, et al; SCOPE consortium. Spontaneous preterm birth and small for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. BMJ. 2009; 338:b1081
5. Mook-Kanamori DO, Steegers EA, Eilers PH, et al. Risk factors and outcomes associated with first-trimester fetal growth restriction. JAMA. 2010;303:527-34.
6. Klebanoff MA, Levine RJ, Morris CD, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediat Perinat Epidemiol. 2001;15:140-3.
7. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol. 1988;41:483-9.
8. Wang X, Tager IB, Van Vunakis H, et al. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol. 1997;26:978-88.
9. Haddow JE, Knight GJ, Palomaki GE, et al. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gynaecol. 1987;94:678-81.
10. Wells AJ, English PB, Posner SF, et al. Misclassification rates for current smokers misclassified as nonsmokers. Am J Public Health. 1998;88:1503-9.
11. Shipton D, Tappin DM, Vadiveloo T, et al. Reliability of self reported smoking status by pregnant women for estimating smoking prevalence: a retrospective, cross sectional study. BMJ 2009;339:b4347
12. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
13. Derogatis LR. Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition. Minneapolis, MN. 1993.
14. Pickett KE, Kasza K, Biesecker G, et al. Women who remember, women who do not: a methodological study of maternal recall of smoking in pregnancy. Nicotine Tob Res. 2009;11:1166-74.
15. England LJ, Grauman A, Qian C, et al. Misclassification of maternal smoking status and its effects on an epidemiologic study of pregnancy outcomes. Nicotine Tob Res. 2007;9:1005-13.
16. Verkerk PH, Buitendijk SE. Non-differential underestimation may cause a threshold effect of exposure to appear as a dose-response relationship. J Clin Epidemiol. 1992;45:543-5.
17. Cnattingius S. The epidemiology of smoking during pregnancy: smoking prevalence, maternal characteristics, and pregnancy outcomes. Nicotine Tob Res. 2004;6 Suppl 2:S125-40.
18. Blair A, Stewart P, Lubin JH, et al. Methodological issues regarding confounding and exposure misclassification in epidemiological studies of occupational exposures. Am J Industr Med. 2007;50:199-207.
19. Bakker R, Steegers EA, Mackenbach JP, et al. Maternal smoking and blood pressure in different trimesters of pregnancy: the Generation R study. J *Hypertens*. 2010;28:2210-8.
20. Geelhoed J, El Marroun H, Verburg B, et al. Maternal smoking during pregnancy, fetal arterial resistance adaptations and cardiovascular function in childhood. *BJOG*. 2011. doi: 10.1111/j.1471-0528.2011.02900.x.
21. Shea AK, Steiner M. Cigarette smoking during pregnancy. Nicotine Tob Res. 2008;10:267-78.
22. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.

SUPPLEMENTARY FIGURE S1. Flow chart of participants included for analysis



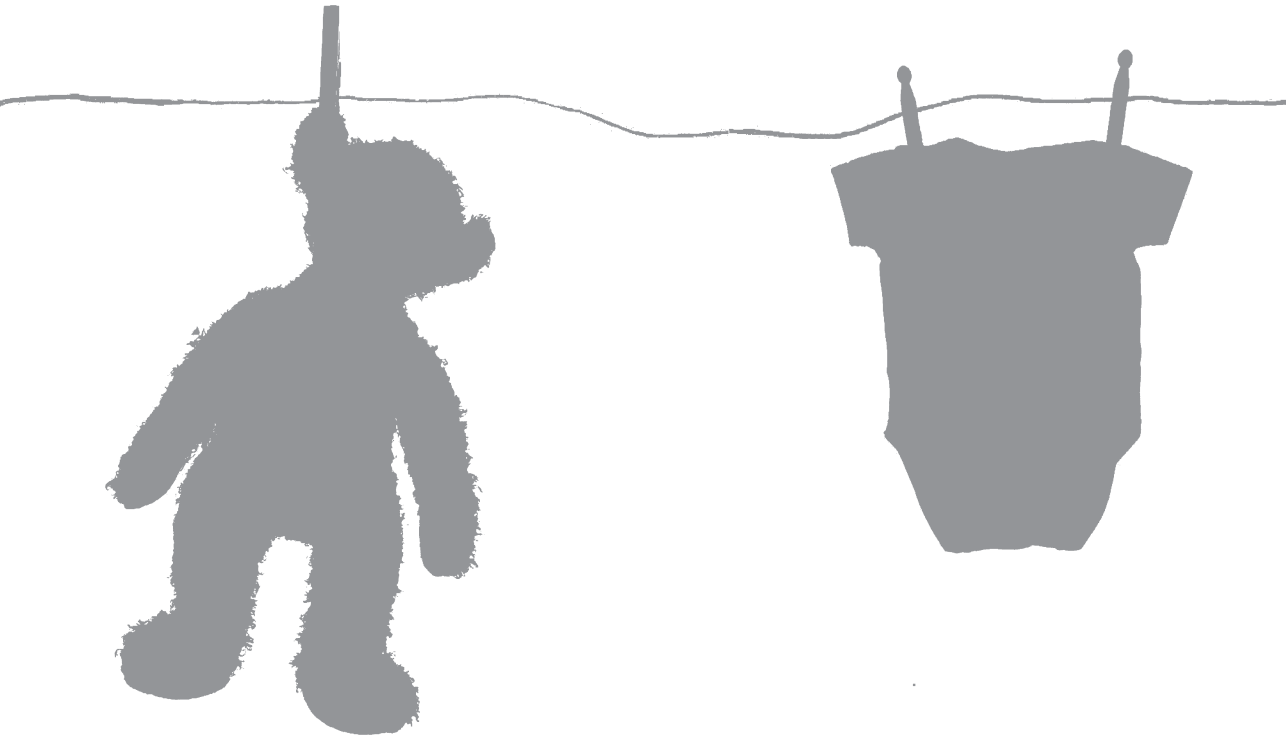
Part 3 | **Hypertensive complications during pregnancy**



Chapter 3.1

Blood pressure tracking and the risks of hypertensive complications

Romy Gaillard
Rachel Bakker
Sten Willemsen
Albert Hofman
Eric AP Steegers
Vincent VW Jaddoe
Submitted for publication



ABSTRACT

Blood pressure tracking can be used to examine the predictability of future values by early measurements. In a population-based prospective cohort study, among 8482 pregnant women, we examined whether blood pressure in early pregnancy tracks to third trimester and whether this tracking is influenced by maternal characteristics and is associated with the risk of gestational hypertensive disorders. Blood pressure was measured in each trimester of pregnancy. Information about doctor diagnosed pregnancy-induced hypertension and preeclampsia was obtained from medical records. Correlation coefficients between first and third trimester for systolic and diastolic blood pressure were 0.47 and 0.46, respectively. The odds ratio (OR) for staying in the highest tertile from first to third trimester for systolic blood pressure was 3.09 (95% Confidence Interval (CI): 2.73, 3.50) and for diastolic blood pressure 3.28 (95% CI: 2.90, 3.69). Blood pressure tracking coefficients were lower in younger, shorter and non-European women and in women with higher gestational weight gain. Systolic and diastolic blood pressure changes from second to third trimester, but not from first to second trimester, were positively associated with the risks of pregnancy-induced hypertension and preeclampsia. Blood pressure tracks moderately during pregnancy and is influenced by maternal characteristics. Second to third trimester increases in systolic and diastolic blood pressure are associated with an increased risk of gestational hypertensive disorders.

INTRODUCTION

Gestational hypertensive disorders complicate about 7% of all pregnancies and are associated with increased risks of both maternal and perinatal morbidity and mortality[1-2]. Blood pressure measurement is an important screening test used in obstetric care to detect or predict gestational hypertensive disorders[2]. However, the predictive accuracy of blood pressure measurement in early pregnancy still remains controversial[3-4]. A review among 34 studies showed that in first and second trimester systolic and diastolic blood pressure predicted preeclampsia poorly[3]. This review compiled many studies with major methodological differences. The examined populations varied widely in their a priori risk of preeclampsia and blood pressure was measured at very different time-points in pregnancy. Also, many studies used different definitions of gestational hypertensive disorders[5]. Some studies suggested that blood pressure development differs between pregnancies uncomplicated and complicated by gestational hypertensive disorders and that small differences in blood pressure development may already occur in the first half of pregnancy[4,6].

Tracking is used to describe the longitudinal development of a variable and focuses on the maintenance of one's relative position in a distribution of values over time[7-8]. Tracking can also be used to examine the predictability of future values by early measurements[7-8]. Examining tracking during pregnancy might give further insight in the predictive value of blood pressure measurement early in pregnancy. However, to the best of our knowledge, not much is known about blood pressure tracking during pregnancy.

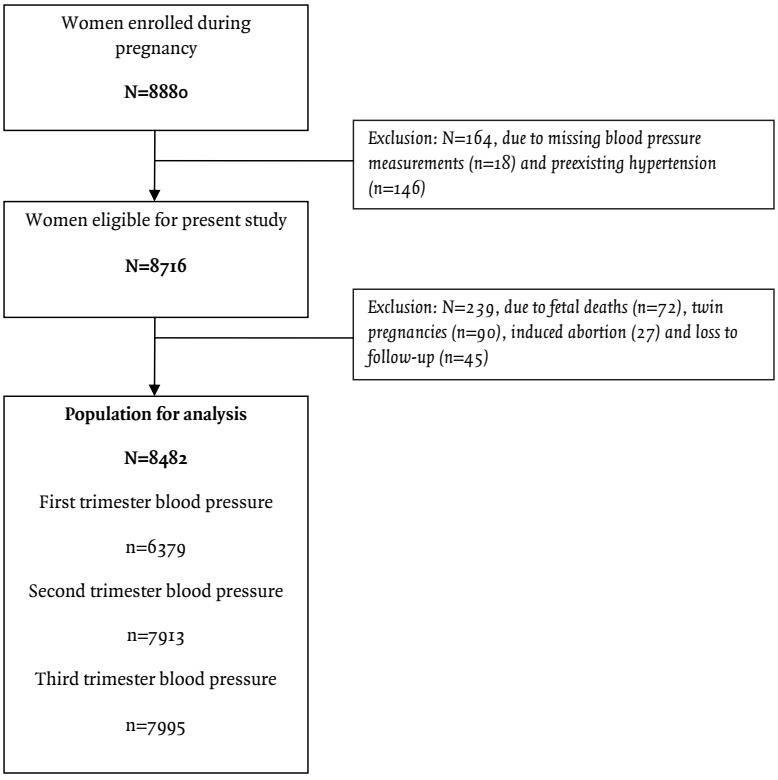
Therefore, we examined in a population-based prospective cohort study among 8482 pregnant women, whether blood pressure in early pregnancy tracks to third trimester, and whether this tracking is influenced by maternal characteristics and is associated with the risk of gestational hypertensive disorders.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards based in Rotterdam, the Netherlands[9-10]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating women. Assessments during pregnancy were planned in first, second and third trimester. The individual timing of these assessments depended on the gestational age at enrolment. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without any blood pressure measurement (n = 18). Also, we excluded women with pre-existent hypertension (n = 146) and pregnancies leading to fetal death (n = 72), induced abortion (n = 27), loss to follow-up (n = 45)

FIGURE 1. Flow chart of the participants included for analysis



and twin pregnancies (n = 90). Thus, the cohort for analysis comprised 8482 pregnant women (Figure 1).

Blood pressure

Blood pressure was measured with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands)[11]. All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 centimeters (cm) a larger cuff (32~42 cm) was used. The mean value of 2 blood pressure readings over a 60-second interval was documented for each participant. In total, blood pressure was measured in 6379 women in first trimester (median, 13.2 weeks of gestation; 95% range, 9.8-17.6), in 7913 women in second trimester (median, 20.4 weeks of gestation; range, 18.5-23.6) and in 7995 women in third trimester (median 30.2, weeks of gestation; 95% range, 28.4-32.9). For the analysis, 22287 blood pressure measurements were available. Three, two and one blood pressure measurements were available for 5857, 2091, 534 women, respectively.

Pregnancy-induced hypertension and preeclampsia

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere[12]. Briefly, the following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia[13]. Information on pregnancy complications was available for 8236 women.

Covariates

Gestational age was established by fetal ultrasound examination during the first ultrasound visit[10]. Maternal age was assessed at enrolment. During visits in first, second and third trimester maternal anthropometrics were measured at one of the research centers. Height (cm) and weight (kg) were measured without shoes and heavy clothing and body mass index (kg/m^2) was calculated for each pregnancy period. We defined gestational weight gain as the difference between weight before pregnancy and weight in third trimester. Information on educational level, ethnicity, and parity was obtained at enrolment. Information about smoking, alcohol consumption and caffeine intake was assessed by questionnaires in each trimester[10].

Statistical analysis

First, we analyzed the longitudinal systolic and diastolic blood pressure patterns in women with uncomplicated pregnancies and women with pregnancies complicated by hypertensive disorders using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data[14]. Using fractional polynomials of gestational age, the best fitting models were constructed. For this analysis, we categorized women in three categories: uncomplicated pregnancy, pregnancy-induced hypertension and preeclampsia. The categories were included in these models as intercept and as an interaction term with gestational age.

To examine whether women maintain their position in the distribution of blood pressure (tracking), we estimated the Pearson's correlation coefficients and categorized systolic blood pressure, diastolic blood pressure and mean arterial pressure in tertiles in first and third trimester. We used logistic regression models to calculate the odds ratio to remain in the same blood pressure tertile from first to third trimester. Next, we examined whether maternal characteristics influence blood pressure tracking. We categorized each maternal characteristic and for each category we estimated Pearson's correlation coefficients and blood pressure tracking coefficients using linear regression models. We further examined the associations of blood pressure change during pregnancy with

the risks of pregnancy-induced hypertension and preeclampsia using multiple logistic regression models.

These models were adjusted for gestational age at intake, gestational age at each pregnancy period, maternal age, educational level, parity, ethnicity, pre-pregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption, and caffeine intake. Missing data of the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than or equal to 15%, except for pre-pregnancy body mass index (19.4%) and gestational weight gain (23.1%). The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Gary NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). P-values are two-tailed. All presented confidence intervals are calculated at the 95% level.

RESULTS

Subject characteristics

Table 1 shows that, of all women, 306 women developed pregnancy-induced hypertension and 168 women developed preeclampsia. Women who developed pregnancy-induced hypertension and preeclampsia were more often nulliparous and had a higher pre-pregnancy body mass index. From first trimester onwards systolic blood pressure, diastolic blood pressure and mean arterial pressure were higher for women who developed pregnancy-induced hypertension and preeclampsia in later pregnancy (Table 2).

Longitudinally measured blood pressure and gestational hypertensive disorders

Figure 2 shows the systolic and diastolic blood pressure development during pregnancy. Systolic blood pressure was higher from first trimester onward in women who developed pregnancy-induced hypertension and preeclampsia. The steepest increase in systolic blood pressure was observed in women who developed preeclampsia. Diastolic blood pressure showed a mid-pregnancy dip, with an increase thereafter in pregnant women without hypertensive disorders. In women with pregnancies complicated by pregnancy-induced hypertension and preeclampsia a minor dip was observed in early pregnancy. Diastolic blood pressure was the highest throughout pregnancy for women who developed pregnancy-induced hypertension, but the steepest increase in diastolic blood pressure was observed for women who developed preeclampsia. The exact regression coefficients for gestational age independent (intercept) and gestational age dependent differences (interaction hypertensive complication and gestational age) are given in the Supplementary Table S1.

TABLE 1. Subject characteristics by pregnancy health (N=8236)[1]

	Non-hypertensive complicated pregnancy	Pregnancy-induced hypertension	Preeclampsia	
	n=7762	n=306	n= 168	P-value[3]
Age, yrs	29.7 (5.3)	30.0 (5.1)	28.8 (5.3)	P=0.086
Height, cm	167.1 (7.4)	168.6 (7.2)	165.7 (7.3)	P<0.001
Weight, kg	65.5 (12.0)	74.9 (18.4)	68.5 (15.0)	P<0.001
Pre-pregnancy body mass index, kg/m^2	23.4 (4.1)	26.3 (6.2)	24.8 (5.3)	P<0.001
Gestational weight gain, kg	10.4 (5.0)	11.5 (6.9)	10.6 (6.5)	P=0.007
Parity, % nulliparous	53.9	74.5	78.0	P<0.001
Gestational age at intake, wks[2]	14.5 (10.4-28.9)	13.7 (9.5-24.0)	14.6 (10.3-24.4)	P=0.011
Highest completed education, %				
Primary school	10.6	7.8	12.5	P=0.016
Secundary school	41.7	48.4	49.4	
Higher education	38.7	39.2	28.0	
Missings	9.1	4.6	10.1	
Ethnicity, %				
European	52.7	70.3	47.6	P<0.001
Non-European	39.7	26.8	44.6	
Missings	7.6	2.9	7.7	
Alcohol consumption, %				
No	42.5	40.5	47.6	P=0.241
Yes	43.4	48.7	41.1	
Missings	14.1	10.8	11.3	
Smoking habits, %				
None	63.8	63.7	63.7	P=0.527
Yes	21.6	25.2	22.6	
Missings	14.5	11.1	13.7	
Caffeine intake, %				
No	4.3	3.6	4.2	P=0.797
Yes	87.4	91.2	85.7	
Missings	8.3	5.2	10.1	

[1]Values are means (standard deviation) or percentages.
[2]Median (95% range).
[3]Differences in subject characteristics between the groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

TABLE 2. Blood pressure levels during pregnancy (N=8236)[1]

	Non-hypertensive complicated pregnancy	Pregnancy-induced hypertension	Preeclampsia	
Pregnancy period	n=7762	n=306	n=168	P-value[2]
First trimester				
Systolic blood pressure	114.7 (11.8)	124.1 (12.3)	119.7 (12.4)	P<0.001
Diastolic blood pressure	67.5 (9.0)	75.7 (10.1)	72.7 (10.2)	P<0.001
Mean arterial pressure	83.2 (8.9)	91.8 (9.8)	88.3 (9.9)	P<0.001
Second trimester				
Systolic blood pressure	115.8 (11.6)	126.2 (12.3)	120.9 (12.9)	P<0.001
Diastolic blood pressure	66.4 (8.9)	75.9 (9.2)	73.4 (9.4)	P<0.001
Mean arterial pressure	82.9 (8.8)	92.6 (9.1)	89.2 (9.5)	P<0.001
Third trimester				
Systolic blood pressure	117.4 (11.6)	128.8 (12.9)	124.9 (13.1)	P<0.001
Diastolic blood pressure	68.2 (8.8)	79.1 (9.7)	76.7 (9.4)	P<0.001
Mean arterial pressure	84.6 (8.6)	95.7 (9.5)	92.8 (9.4)	P<0.001

[1]Values are means (standard deviation).
[2]Differences in blood pressure levels between the groups were evaluated using one-way ANOVA tests.

Blood pressure tracking during pregnancy

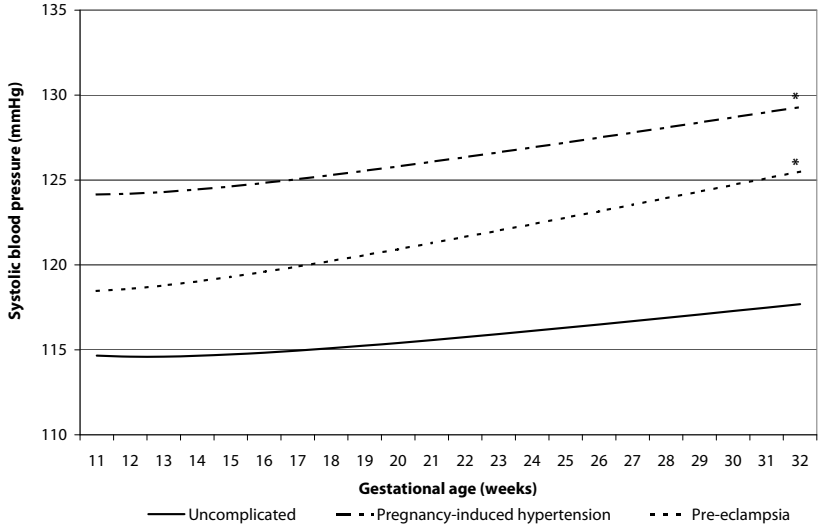
Correlation coefficients between first and third trimester for systolic and diastolic blood pressure and mean arterial pressure were 0.47, 0.46 and 0.49 respectively. The specific scatterplots are given in Supplemental Figure 1.

Table 3 shows that for systolic blood pressure, about 55% of the women, who started in the highest tertile in first trimester remained in the highest tertile in third trimester, while approximately 29% and 15% were in the middle and lowest tertile, respectively. Similar patterns were observed for diastolic blood pressure and mean arterial pressure. The odds ratios for staying in the upper tertile from first to third trimester for systolic blood pressure and diastolic blood pressure were 3.09 (95% CI: 2.73, 3.50) and 3.28 (95% CI: 2.90, 3.69), respectively. A similar trend was observed for tertiles of mean arterial pressure. Blood pressure tracking coefficients were lower in younger, shorter and non-European women and in women with higher gestational weight gain (Table 4). Corresponding correlation coefficients are given in Supplementary Table S2.

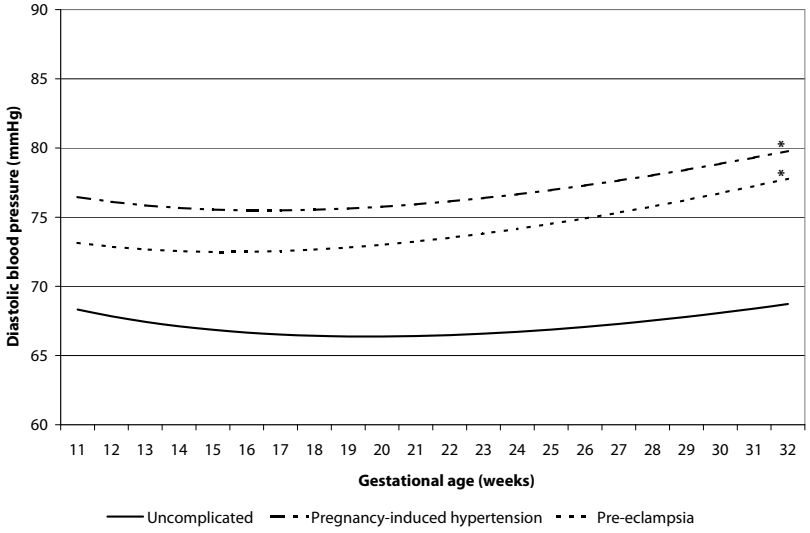
Table 5 shows that systolic and diastolic blood pressure change from first to second trimester was not associated with the risk of pregnancy-induced hypertension. Diastolic blood pressure change from first to second trimester was associated with the risk of preeclampsia (OR, 1.20 (95% CI: 1.01, 1.44) per standard deviation of blood pressure change). Second to third trimester changes in diastolic blood pressure and mean arterial pressure were associated with the risk of pregnancy-induced hypertension (OR, 1.20 (95% CI: 1.06, 1.35) and OR, 1.18 (95% CI: 1.04, 1.33) per standard deviation of blood pressure change, respectively). Second to third trimester changes in systolic

FIGURE 2. Blood pressure patterns in uncomplicated and complicated pregnancies

A. Systolic blood pressure



B. Diastolic blood pressure



Change in blood pressure in mmHg for women with a pregnancy complicated by pregnancy-induced hypertension and women with a pregnancy complicated by preeclampsia compared to women with an uncomplicated pregnancy based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$* hypertensive complication + β_2*gestational age + β_3*gestational age^{-2} + β_4* hypertensive complication *gestational age and diastolic blood pressure = $\beta_0 + \beta_1$*hypertensive complication + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*hypertensive complication*gestational age). P–value reflects the significance level of β_4, which reflects the difference in change in blood pressure per week per pregnancy hypertensive complication, as compared to normal pregnancies. Estimates are given in Supplementary Table S1. *P<0.05

TABLE 3. Blood pressure tracking from first to third trimester (N=6053)[1,2]

Tertiles first trimester	Tertiles third trimester			
	First	**Second**	**Third**	
Systolic blood pressure				n
First	2.73 (2.43, 3.07)** n=1202 (53.9%)	0.90 (0.80, 1.01) n=667 (29.9%)	0.33 (0.28, 0.37)** n=359 (16.1%)	2228
Second	0.92 (0.81, 1.03) n=701 (34.6%)	1.19 (1.06, 1.34)** n=678 (33.4%)	0.94 (0.83, 1.05) n=649 (32.0%)	2028
Third	0.29 (0.25, 0.34)** n=284 (15.8%)	0.92 (0.81, 1.04) n=524 (29.2%)	3.09 (2.73, 3.50)** n=989 (55.0%)	1797
n	2187	1869	1997	6053
Diastolic blood pressure				n
First	3.32 (2.95, 3.72)** n=1 269 (57.4%)	0.80 (0.71, 0.90)** n=609 (27.6%)	0.29 (0.25, 0.33)** n=331 (15.0%)	2209
Second	0.76 (0.67, 0.85)** n=626 (33.6%)	1.42 (1.26, 1.60)** n=658 (35.3%)	0.95 (0.84, 1.07) n=581 (31.2%)	1865
Third	0.32 (0.29, 0.37)** n=371 (18.7%)	0.86 (0.76, 0.98)* n=551 (27.8%)	3.28 (2.90, 3.69)** n=1 057 (53.4%)	1979
n	2266	1818	1969	6053
Mean arterial pressure				n
First	3.44 (3.06, 3.87)** n=1146 (54.7%)	0.73 (0.65, 0.81)** n=650 (31.0%)	0.27(0.23, 0.31)** n=299 (14.3%)	2095
Second	0.67 (0.60, 0.75)** n=587 (30.0%)	1.48 (1.33, 1.66)** n=775 (39.6%)	1.01 (0.89, 1.14) n=595 (30.4%)	1957
Third	0.29 (0.25, 0.34)** n=302 (15.1%)	0.89 (0.79, 1.01) n=595 (29.7%)	3.40 (2.69, 3.50)** n=1104 (55.2%)	2001
n	2035	2020	1998	6053

[1]Values are odds ratio (95% confidence interval) (number and percentage of women that remain in the same tertile) to remain in the same tertiles of systolic blood pressure, diastolic blood pressure and mean arterial pressure. Estimates are from multiple imputed data.

[2]Model is adjusted for gestational age at intake, gestational age, maternal age, educational level, parity, ethnicity, pre-pregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption and caffeine intake.

*P-value<0.05

**P-value<0.01

TABLE 4. Maternal characteristics and blood pressure tracking coefficients[1]

	Systolic blood pressure		**Diastolic blood pressure**		**Mean arterial pressure**	
Maternal characteristics	Regression coefficient (95% CI)	p-value	Regression coefficient (95% CI)	p-value	Regression coefficient (95% CI)	p-value
Age (yrs)						
< 25 years (n=1801)	0.43 (0.38, 0.49)	P<0.001	0.37 (0.31, 0.42)	P<0.001	0.37 (0.31, 0.42)	P<0.001
25-35 years (n=5432)	0.48 (0.45, 0.50)	P<0.001	0.47 (0.45, 0.50)	P<0.001	0.47 (0.45, 0.50)	P<0.001
>35 years (n=1249)	0.41 (0.34, 0.47)	P<0.001	0.47 (0.40, 0.53)	P<0.001	0.47 (0.40, 0.53)	P<0.001
	Interaction P=0.820		*Interaction P<0.001*		*Interaction P=0.027*	
Height (cm)						
< 165 cm (n=3677)	0.42 (0.39, 0.46)	P<0.001	0.42 (0.38, 0.45)	P<0.001	0.44 (0.41, 0.48)	P<0.001
165-175 cm (n=3626)	0.46 (0.42, 0.49)	P<0.001	0.47 (0.44, 0.51	P<0.001	0.50 (0.47, 0.53)	P<0.001
>175 cm (n=1149)	0.44 (0.39, 0.49)	P<0.001	0.48 (0.43, 0.53)	P<0.001	0.50 (0.45, 0.55)	P<0.001
	Interaction P=0.166		*Interaction P<0.001*		*Interaction P=0.001*	
Pre-pregnancy body mass index (kg/m^2)						
Normal (n=4968)	0.44 (0.41, 0.46)	P<0.001	0.43 (0.40, 0.46)	P<0.001	0.46 (0.43, 0.49)	P<0.001
Overweight (n=1298)	0.45 (0.39, 0.51)	P<0.001	0.39 (0.34, 0.45)	P<0.001	0.42 (0.37, 0.48)	P<0.001
Obesity (n=567)	0.44 (0.35, 0.52)	P<0.001	0.48 (0.39, 0.56)	P<0.001	0.50 (0.42, 0.58)	P<0.001
	Interaction P=0.590		*Interaction P=0.715*		*Interaction P=0.592*	
Gestational weight gain (kg)						
< 7 kg (n=1638)	0.47 (0.42, 0.51)	P<0.001	0.48 (0.44, 0.53)	P<0.001	0.50 (0.46, 0.54)	P<0.001
7-11.9 kg (n=2877)	0.44 (0.41, 0.48)	P<0.001	0.46 (0.42, 0.49)	P<0.001	0.48 (0.44, 0.51)	P<0.001
>12 kg (n=2010)	0.45 (0.40, 0.49)	P<0.001	0.43 (0.39, 0.48)	P<0.001	0.47 (0.43, 0.51)	P<0.001
	Interaction P=0.014		*Interaction P<0.001*		*Interaction P=0.005*	
Parity						
Nulliparous (n=4666)	0.45 (0.42, 0.48)	P<0.001	0.43 (0.40, 0.46)	P<0.001	0.46 (0.43, 0.49)	P<0.001
Multiparous (n=3711)	0.46 (0.43, 0.50)	P<0.001	0.47 (0.43, 0.50)	P<0.001	0.50 (0.46, 0.53)	P<0.001
	Interaction P=0.574		*Interaction P=0.099*		*Interaction P=0.115*	
Highest completed education						
Primary (n=896)	0.43 (0.35, 0.51)	P<0.001	0.43 (0.35, 0.51)	P<0.001	0.47 (0.40, 0.55)	P<0.001
Secondary (n=3572)	0.48 (0.44, 0.51)	P<0.001	0.46 (0.43, 0.50)	P<0.001	0.50 (0.46, 0.53)	P<0.001
Higher (n=3244)	0.45 (0.43, 0.48)	P<0.001	0.45 (0.42, 0.49)	P<0.001	0.48 (0.44, 0.51)	P<0.001
	Interaction P=0.693		*Interaction P=0.968*		*Interaction P=0.615*	
Ethnicity						
European (n=4508)	0.45 (0.42, 0.48)	P<0.001	0.49 (0.46, 0.52)	P<0.001	0.51 (0.48, 0.54)	P<0.001
Non-European (n=3335)	0.43 (0.39, 0.47)	P<0.001	0.39 (0.35, 0.43)	P<0.001	0.43 (0.39, 0.47)	P<0.001

TABLE 4. (continued)

	Systolic blood pressure		Diastolic blood pressure		Mean arterial pressure	
Maternal characteristics	Regression coefficient (95% CI)	p-value	Regression coefficient (95% CI)	p-value	Regression coefficient (95% CI)	p-value
	Interaction P=0.448		*Interaction P<0.001*		*Interaction P=0.001*	
Alcohol consumption						
No (n=3620)	0.46 (0.43, 0.50)	P<0.001	0.46 (0.42, 0.49)	P<0.001	0.49 (0.46, 0.52)	P<0.001
Yes (n=3676)	0.45 (0.42, 0.49)	P<0.001	0.45 (0.42, 0.48)	P<0.001	0.48 (0.45, 0.51)	P<0.001
	Interaction P=0.433		*Interaction P=0.666*		*Interaction P=0.553*	
Smoking habits						
None (n=5045)	0.47(0.44, 0.50)	P<0.001	0.47 (0.44, 0.49)	P<0.001	0.50 (0.47, 0.53)	P<0.001
Yes (n=1847)	0.42 (0.37, 0.47)	P<0.001	0.42 (0.37, 0.47)	P<0.001	0.45 (0.40, 0.49)	P<0.001
	Interaction P=0.072		*Interaction P=0.079*		*Interaction P=0.042*	
Caffeine intake						
No (n=359)	0.49 (0.38, 0.60)	P<0.001	0.54 (0.45, 0.64)	P<0.001	0.55 (0.46, 0.65)	P<0.001
Yes (n=7404)	0.46 (0.43, 0.48)	P<0.001	0.45 (0.43, 0.47)	P<0.001	0.48 (0.46, 0.50)	P<0.001
	Interaction P=0.672		*Interaction P=0.550*		*Interaction P=0.504*	

[1]Values are regression coefficients (95% CI) from first to third trimester for systolic blood pressure, diastolic blood pressure and mean arterial pressure.

blood pressure, diastolic blood pressure and mean arterial pressure were associated with the risk of preeclampsia (OR, 1.22 (95% CI: 1.04, 1.43), 1.22 (95% CI: 1.03, 1.43), and 1.26 (95% CI: 1.07, 1.48) per standard deviation of blood pressure change, respectively).

DISCUSSION

Results from this prospective cohort study showed that gestational blood pressure development is different from first trimester onwards between non-hypertensive pregnancies and pregnancies complicated by gestational hypertensive disorders. Systolic and diastolic blood pressure and mean arterial pressure track moderately during pregnancy. This tracking is influenced by maternal characteristics. Systolic and diastolic blood pressure changes from second to third trimester are positively associated with the risk of gestational hypertensive disorders.

Methodological considerations

Some methodological issues need to be considered. One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 8482 participants with 22287 blood pressure measurements. The response rate at baseline for participation in the study was 61%. The non-response would lead to biased effect estimates if

TABLE 5. Blood pressure development and the risks of pregnancy-induced hypertension and preeclampsia (N=8236)[1,2]

Pregnancy period	Pregnancy-induced hypertension[2,3]	Preeclampsia[2,3]
First to second trimester		
Systolic blood pressure	1.06 (0.93, 1.20)	1.00 (0.84, 1.20)
Diastolic blood pressure	1.05 (0.92, 1.20)	1.20 (1.01, 1.44)*
Mean arterial pressure	1.06 (0.93, 1.21)	1.14 (0.95, 1.37)
Second to third trimester		
Systolic blood pressure	1.09 (0.97, 1.23)	1.22 (1.04, 1.43)*
Diastolic blood pressure	1.20 (1.06, 1.35)**	1.22 (1.03, 1.43)*
Mean arterial pressure	1.18 (1.04, 1.33)**	1.26 (1.07, 1.48)**
First to third trimester		
Systolic blood pressure	1.15 (1.01, 1.31)*	1.23 (1.02, 1.47)*
Diastolic blood pressure	1.28 (1.12, 1.46)**	1.42 (1.18, 1.70)**
Mean arterial pressure	1.27 (1.11, 1.45)**	1.40 (1.16, 1.67)**

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risks of pregnancy-induced hypertension and preeclampsia per standard deviation change in blood pressure level between trimesters. Estimates are from multiple imputed data.
[2]Model is adjusted for gestational age at intake, gestational age at each pregnancy period, educational level, maternal age, ethnicity, parity, pre-pregnancy body mass index, gestational weight gain, smoking habits, alcohol consumption and caffeine intake.
*P-value<0.05
**P-value<0.01.

the associations would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from non-response at baseline[15]. Detailed information about a large number of potential confounding factors was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle related determinants. Furthermore, blood pressure has a large within subject-variation and is also liable to measurement error. Measurement error might cause an underestimation of the true tracking correlation of blood pressure[7]. However, when tracking is used to examine the predictive value of early measurements to identify those at risk, measurement error will not bias the results, because measurement error also occurs in real clinical setting.[7] Finally, we had a relative small number of cases of pregnancy-induced hypertension and preeclampsia, which might indicate a selection towards a healthy, low-risk population. It might be of interest to perform a similar analysis in a high risk, hospital based population.

Blood pressure development during pregnancy

Several studies have reported differences in blood pressure development between non-hypertensive complicated pregnancies and pregnancies complicated by pregnancy-induced hypertension or preeclampsia[5-6]. A previous study among 202 primigravid women at high-risk for gestational hypertensive disorders, observed differences in the circadian variability of systolic and diastolic blood pressure between uncomplicated pregnancies and pregnancies complicated by gestational hypertensive disorders. Pregnancies leading to gestational hypertensive disorders had elevated blood pressure levels in first trimester[6]. In the same study, the known second trimester blood pressure dip was not present in complicated pregnancies, and blood pressure increased strongly in complicated pregnancies, particularly in those complicated by preeclampsia. We observed similar differences in the blood pressure patterns using office blood pressure measurements. Although we did not observe an absence of the mid-pregnancy dip in pregnancies complicated by gestational hypertensive disorders, we did observe that the mid-pregnancy dip was smaller and tended to occur earlier in pregnancy. We also observed a larger increase in blood pressure levels from second to third trimester in complicated pregnancies, particularly for pregnancies complicated by preeclampsia. Even though these observed differences in blood pressure development are highly statistically significant, it needs to be considered that both systolic blood pressure and diastolic blood pressure were within the physiological range of blood pressure variability. However, these differences might provide clues on how to earlier identify those women at increased risk of gestational hypertensive disorders.

Blood pressure tracking

Blood pressure tracking in pregnancy might help to early identify those women that are at high risk to develop gestational hypertensive disorders. Our study shows that systolic blood pressure, diastolic blood pressure and mean arterial pressure track moderately from first to third trimester. Several variables have been identified that might influence or predict tracking in studies among children and adults. It has been shown that length of follow-up is inversely associated with the tracking correlation[16-17]. We observed that the tracking correlation for systolic and diastolic blood pressure was stronger between first and second trimester and second and third trimester compared to the tracking correlation between first and third trimester. Also, some studies have suggested that blood pressure tracking is different in different ethnic populations[16,18-19]. Accordingly, we observed differences in tracking coefficients for diastolic blood pressure and mean arterial pressure in European women and non-European women. Furthermore, age, overweight and weight change have been suggested to influence tracking[16,19-20]. A study among men and women showed the tracking correlation for different age categories; for women aged 20 to 24 the tracking correlation for systolic blood pressure was 0.43 and the tracking correlation for diastolic blood pressure was 0.59, while for women aged 35 to 39 the tracking correlation was 0.64 and 0.68, respectively[19]. A study among Australian children reported that tracking of blood pressure, especially systolic blood pressure, was influenced by body mass index and change in body mass

index[20]. Those individuals in the highest quartile of body mass index and those individuals in the highest quartile of weight gain had higher risks of persistence of high blood pressure levels. Similarly, maternal age, pre-pregnancy body mass index and gestational weight gain might influence tracking. We observed that, especially tracking of diastolic blood pressure and mean arterial pressure, were influenced by maternal characteristics such as in older age and lower gestational weight gain.

Finally, systolic blood pressure, diastolic blood pressure and mean arterial pressure tracked equally. However, diastolic blood pressure and mean arterial pressure were more strongly associated with the risks of pregnancy-induced hypertension and preeclampsia as compared to systolic blood pressure. This might indicate that diastolic blood pressure and mean arterial pressure have a higher predictive accuracy for gestational hypertensive disorders than systolic blood pressure.

Conclusion

Blood pressure tracks moderately during pregnancy. Second to third trimester increases in systolic and diastolic blood pressure are associated with the risk of gestational hypertensive disorders. Blood pressure tracking is related to maternal characteristics. Further research is needed focused on factors influencing blood pressure tracking and their associations with gestational hypertensive disorders.

REFERENCES

1. Nijdam ME, Janssen KJ, Moons KG, et al. Prediction model for hypertension in pregnancy in nulliparous women using information obtained at the first antenatal visit. J Hypertens. 2010;28:119-26.
2. Steegers EA, von Dadelszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet. 2010;376:631-44.
3. Cnossen JS, Vollebregt KC, de Vrieze N, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. BMJ. 2008;336:1117-20.
4. Hermida RC, Ayala DE, Iglesias M. Predictable blood pressure variability in healthy and complicated pregnancies. Hypertension. 2001;38:736-41.
5. Poon LC, Kametas NA, Valencia C, et al. Hypertensive Disorders in Pregnancy: Screening by Systolic Diastolic and Mean Arterial Pressure at 11-13 Weeks. Hypertens Pregnancy. 2010;30:93-107.
6. Hermida RC, Ayala DE, Mojon A, et al. Blood pressure patterns in normal pregnancy, gestational hypertension, and preeclampsia. Hypertension. 2000;36:149-58.
7. Hofman A, Valkenburg HA, Maas J, et al. The natural history of blood pressure in childhood. Int J Epidemiol. 1985;14:91-6.
8. Twisk, JWR. Applied longitudinal data analyses for epidemiology: a practical guide. Cambridge University Press: New York, 2003
9. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
10. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010; 25:823-41.
11. El Assaad MA, Topouchian JA, Darne BM, et al. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7:237-41.
12. Coolman M, de Groot CJ, Jaddoe VW, et al. Medical record validation of maternally reported history of preeclampsia. J Clin Epidemiol. 2010;63:932-7.
13. Brown MA, Lindheimer MD, de Swiet M, et al. 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:IX-XIV.
14. Goldstein H. Multilevel statistical methods. 2nd ed. Edward Arnold: London,1995.
15. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
16. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic review and meta-regression analysis. Circulation. 2008;117:3171-80.
17. Clarke WR, Schrott HG, Leaverton PE, et al. Tracking of blood lipids and blood pressures in school age children: the Muscatine study. Circulation. 1978;58:626-34.
18. Manatunga AK, Jones JJ, Pratt JH. Longitudinal assessment of blood pressures in black and white children. Hypertension. 1993;22:84-9.
19. Rosner B, Hennekens CH, Kass EH, et al. Age-specific correlation analysis of longitudinal blood pressure data. Am J Epidemiol. 1977;106:306-13.
20. Burke V, Beilin LJ, Dunbar D. Tracking of blood pressure in Australian children. J Hypertens. 2001;19:1185-92.

SUPPLEMENTARY TABLE S1. Longitudinal associations between pregnancy hypertensive complication and systolic and diastolic blood pressure[1]

			Difference in systolic blood pressure	
Hypertensive complication	Intercept (mmHg (95% CI))	P-value[2]	Slope (mmHg / 10 weeks of gestation (95% CI)	P-value[2]
Uncomplicated	110.6	P<0.001	Reference	
Pregnancy-induced hypertension	118.9	P<0.001	0.10	P=0.020
Preeclampsia	112.3	P=0.273	0.19	P=0.002
			Difference in diastolic blood pressure	
Hypertensive complication	Intercept (mmHg (95% CI))	P-value[2]	Slope (mmHg / 10 weeks of gestation (95% CI))	P-value[2]
Uncomplicated	97.2	P<0.001	Reference	
Pregnancy-induced hypertension	103.8	P<0.001	0.14	P<0.001
Preeclampsia	99.8	P=0.031	0.20	P<0.001

[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per pregnancy hypertensive complication compared to the reference group of women with an uncomplicated pregnancy.
[2]P–value reflects the significance level of the estimate.

SUPPLEMENTARY TABLE S2. Maternal characteristics and blood pressure correlation coefficients[1]

	Systolic blood pressure		**Diastolic blood pressure**		**Mean arterial pressure**	
Maternal characteristics	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
Age (yrs)						
< 25 years (n=1801)	0.41	P<0.001	0.36	P<0.001	0.40	P<0.001
25 -35 years (n=5432)	0.49	P<0.001	0.49	P<0.001	0.53	P<0.001
>35 years (n=1249)	0.41	P<0.001	0.45	P<0.001	0.46	P<0.001
	Interaction P=0.820		*Interaction P<0.001*		*Interaction P=0.027*	
Height (cm)						
< 165 cm (n=3677)	0.43	P<0.001	0.41	P<0.001	0.45	P<0.001
165 -175 cm (n=3626)	0.46	P<0.001	0.48	P<0.001	0.50	P<0.001
>175 cm (n=1149)	0.49	P<0.001	0.51	P<0.001	0.55	P<0.001
	Interaction P=0.166		*Interaction P<0.001*		*Interaction P=0.001*	
Pre-pregnancy body mass index (kg/m^2)						
Normal (n=4968)	0.44	P<0.001	0.42	P<0.001	0.45	P<0.001
Overweight (n=1298)	0.45	P<0.001	0.40	P<0.001	0.43	P<0.001
Obesity (n=567)	0.46	P<0.001	0.50	P<0.001	0.53	P<0.001
	Interaction P=0.590		*Interaction P=0.715*		*Interaction P=0.592*	

SUPPLEMENTARY TABLE S2. (continued)

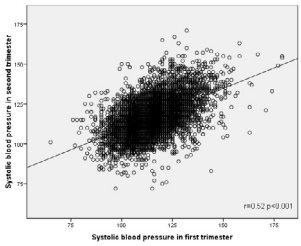
	Systolic blood pressure		Diastolic blood pressure		Mean arterial pressure	
Maternal characteristics	Correlation Coefficient	p-value	Correlation Coefficient	p-value	Correlation Coefficient	p-value
Gestational weight gain (kg)						
< 7 kg (n=1638)	0.51	P<0.001	0.51	P<0.001	0.54	P<0.001
7-11.9 kg (n=2877)	0.45	P<0.001	0.46	P<0.001	0.49	P<0.001
>12 kg (n=2010)	0.45	P<0.001	0.42	P<0.001	0.47	P<0.001
	Interaction P=0.014		*Interaction P<0.001*		*Interaction P=0.005*	
Parity						
Nulliparous (n=4666)	0.46	P<0.001	0.44	P<0.001	0.48	P<0.001
Multiparous (n=3711)	0.46	P<0.001	0.47	P<0.001	0.50	P<0.001
	Interaction P=0.574		*Interaction P=0.099*		*Interaction P=0.115*	
Highest completed education						
Primary (n=896)	0.42	P<0.001	0.42	P<0.001	0.46	P<0.001
Secondary (n=3572)	0.47	P<0.001	0.47	P<0.001	0.49	P<0.001
Higher (n=3244)	0.48	P<0.001	0.46	P<0.001	0.50	P<0.001
	Interaction P=0.693		*Interaction P=0.968*		*Interaction P=0.615*	
Ethnicity						
European (n=4508)	0.47	P<0.001	0.50	P<0.001	0.52	P<0.001
Non-European (n=3335)	0.42	P<0.001	0.39	P<0.001	0.43	P<0.001
	Interaction P=0.448		*Interaction P<0.001*		*Interaction P=0.001*	
Alcohol consumption						
No (n=3620)	0.47	P<0.001	0.46	P<0.001	0.50	P<0.001
Yes (n=3676)	0.46	P<0.001	0.45	P<0.001	0.49	P<0.001
	Interaction P=0.433		*Interaction P=0.666*		*Interaction P=0.553*	
Smoking habits						
None (n=5045)	0.49	P<0.001	0.47	P<0.001	0.51	P<0.001
Yes (n=1847)	0.41	P<0.001	0.42	P<0.001	0.44	P<0.001
	Interaction P=0.072		*Interaction P=0.079*		*Interaction P=0.042*	
Caffeine intake						
No (n=359)	0.47	P<0.001	0.58	P<0.001	0.56	P<0.001
Yes (n=7404)	0.46	P<0.001	0.45	P<0.001	0.49	P<0.001
	Interaction P=0.672		*Interaction P=0.550*		*Interaction P=0.504*	

[1]Values are correlation coefficients from first to third trimester for systolic blood pressure, diastolic blood pressure and mean arterial pressure.

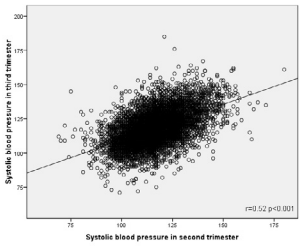
SUPPLEMENTARY FIGURE S1. Correlation of blood pressure between different trimesters of pregnancy

A. Systolic blood pressure

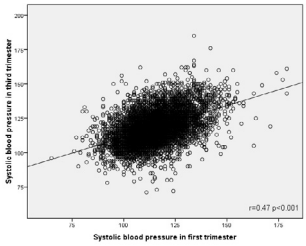
First to second



Second to third

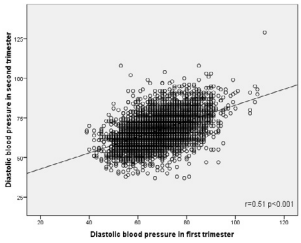


First to third

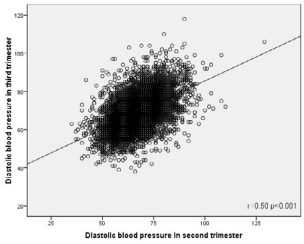


B. Diastolic blood pressure

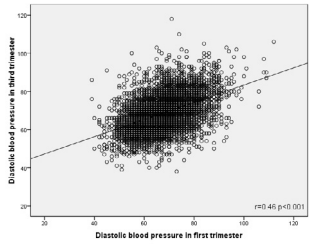
First to second



Second to third

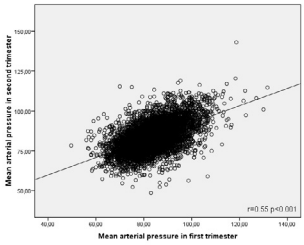


First to third

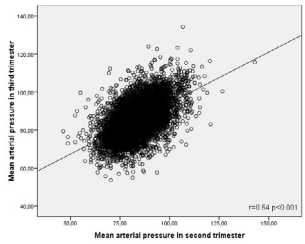


C. Mean arterial pressure

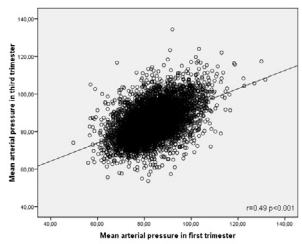
First to second



Second to third



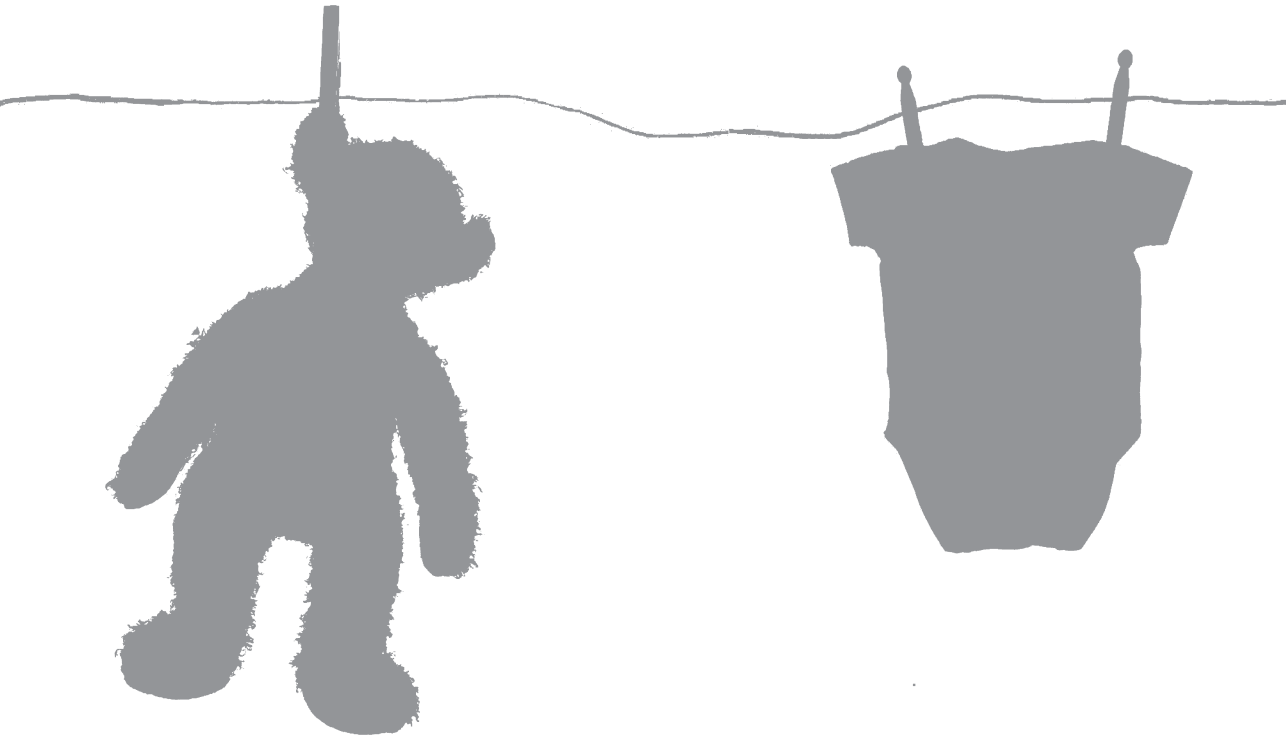
First to third



Chapter 3.2

Maternal age, blood pressure and hypertensive pregnancy complications

Romy Gaillard
Rachel Bakker
Eric AP Steegers
Albert Hofman
Vincent WV Jaddoe
Submitted for publication



ABSTRACT

We hypothesized that hemodynamic adaptations related to pregnancy and ageing might be associated with differences in blood pressure levels during pregnancy between younger and older women. This might partly explain the increased risk of gestational hypertensive disorders with advanced maternal age. We examined the associations of maternal age with systolic and diastolic blood pressure in each trimester of pregnancy and the risks of gestational hypertensive disorders. The study was conducted among 8623 women participating in population-based prospective cohort study from early pregnancy onwards. Age was assessed at enrolment. Blood pressure was measured in each trimester. Information about gestational hypertensive disorders was available from medical records. Maternal age was not associated with first trimester blood pressures. In second and third trimester, older maternal age was associated with lower systolic blood pressure (-0.9 mmHg (95% confidence interval: -1.4, -0.3) and -0.6 mmHg (95% confidence interval: -1.1, -0.02) per additional 10 maternal years, respectively). Older maternal age was associated with higher third trimester diastolic blood pressure (0.5 mmHg (95% confidence interval: 0.04, 0.9) per additional 10 maternal years). Maternal age was associated with pregnancy-induced hypertension among overweight and obese women. Our results suggest that older maternal age is associated with lower second and third trimester systolic blood pressure, but higher third trimester diastolic blood pressure. These small differences in blood pressure levels between younger and older women are within the physiological range of blood pressure variability. Maternal age is not consistently associated with the risk of gestational hypertensive disorders.

INTRODUCTION

Hypertensive disorders during pregnancy complicate about 7% of all pregnancies and are important causes of maternal and perinatal morbidity and mortality worldwide[1-2]. Increased maternal age has been suggested as risk factor for the development of hypertensive disorders during pregnancy[1-4]. The pathway of this association remains unclear. It might be attributable to vascular endothelial changes that occur with ageing, but also confounding factors, such as parity and body mass index, might explain the association[3,5]. Furthermore, not much is known about the relationship between maternal age and development of blood pressure levels during pregnancy within the normal range. During early pregnancy, the systemic vascular resistance and mean arterial blood pressure decline and the cardiac output increases[6-8]. This afterload reduction is partly caused by a higher compliance of the large arteries, which is about 15% higher than in non-pregnant women[9]. In later pregnancy, blood pressure rises again and by term it may reach preconceptional values or higher[10]. A different process occurs with ageing. Older age is associated with a gradual loss of vascular compliance, which subsequently leads to a higher afterload[7]. We hypothesized that differences in hemodynamic adaptations related to pregnancy and ageing might be associated with differences in blood pressure levels during pregnancy. The influence of maternal age on blood pressure levels during pregnancy might thereby partly explain the observed associations between advanced maternal age and the risk of gestational hypertensive disorders.

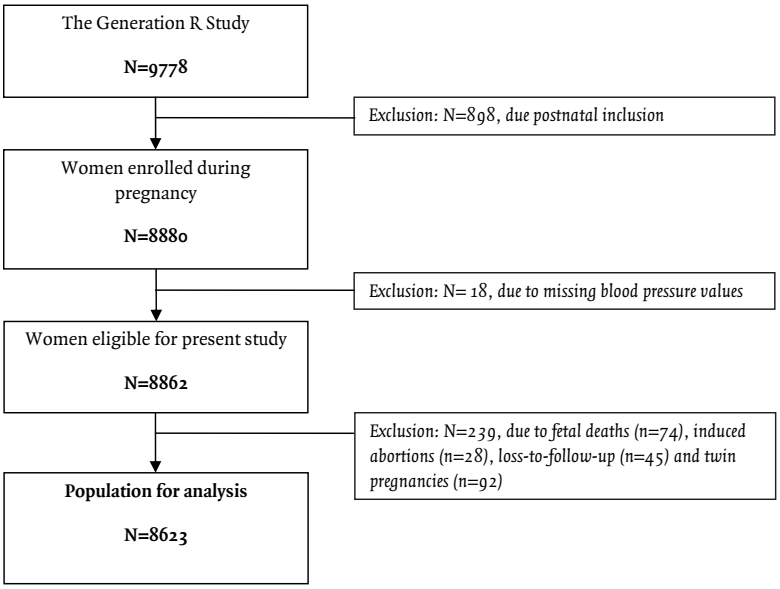
Therefore, we assessed in a population-based prospective cohort study among 8623 pregnant women, the associations of maternal age with systolic and diastolic blood pressure in each trimester of pregnancy and the risks of pregnancy-induced hypertension and preeclampsia.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards based in Rotterdam, the Netherlands[11,12]. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating women[13]. Enrollment was aimed in first trimester, but allowed until delivery[12]. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without blood pressure measurements (n = 18), leading to 8862 women. Also, we excluded pregnancies leading to induced abortions (n = 28), loss to follow up (n = 45), twin pregnancies (n = 92) and fetal death (n = 74). Similar results were found after including fetal death in the analyses. Thus, the cohort for analysis comprised 8623 women (Figure 1).

FIGURE 1. Flow chart of the participants included for analysis



Maternal age

Maternal age was assessed at enrolment in the study. In the analyses, we used maternal age as continuous variable and categorized in 6 groups: younger than 20 years (n = 375); 20 to 24.9 years (n = 1446); 25 to 29.9 years (n = 2348); 30 to 34.9 years (n = 3172); 35 to 39.9 years (n = 1137); 40 years and over (n = 145). Since the median maternal age was the age-group of 30 to 34.9 years, we used this group as reference in all analyses.

Blood pressure

Blood pressure was measured with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands)[14]. All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 centimeters (cm) a larger cuff (32~42 cm) was used. The mean value of 2 blood pressure readings over a 60-second interval was documented for each participant.

Pregnancy-induced hypertension and preeclampsia

Information on pregnancy complications was obtained from medical records or the hospital registries. Women who were reported to have experienced pregnancy-induced hypertension or preeclampsia were selected from the hospital registries. Their individual medical records were subsequently studied[15]. The following criteria were used to identify women with pregnancy-induced

hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia. These criteria were defined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP)[16].

Covariates

Gestational age was established by fetal ultrasound examination during the first ultrasound visit[12]. Information on educational level, ethnicity, parity and folic acid supplementation use was obtained at enrolment. Information about smoking, alcohol consumption and caffeine intake were assessed by questionnaires in each trimester. At enrolment height (cm) and weight (kg) were measured without shoes and heavy clothing. Weight was repeatedly measured during subsequent visits at the research center. Body mass index (kg/m^2) was calculated with these measurements. Maternal distress was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory, which gives a Global Severity Index[17].

Statistical power

Power calculations were performed based on 7000 subjects. For a normally distributed continuous outcome it was possible to detect with a type I error of 5% and a type II error of 20% (power 80%) a difference of 0.11 SD, which corresponds to a difference of 1.3 mmHg and 1.0 mmHg for systolic and diastolic blood pressure, respectively. For preeclampsia and pregnancy-induced hypertension, we were able to detect an odds ratio of 1.39, if 10% of the cohort has the relevant exposure[12]. These differences are smaller or similar as differences that could be detected in previous studies.

Statistical analysis

First, the associations of maternal age with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models.

These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data and are described in detail in the Supplementary Material[18]. For presentation aims we categorized maternal age into three categories in these analyses; ≤24.9 years, 25-34.9 years, and ≥35 years.

Second, the cross-sectional associations of maternal age with blood pressure in first, second and third trimester were assessed using linear regression models. For these models, we examined whether the residuals were normally distributed using normal probability plots, and whether the variance of the residuals was homoscedastic and whether the regression models were linear[19]. Third, the associations of maternal age categories with the risks of pregnancy-induced hypertension and preeclampsia were assessed using multiple logistic regression models. Tests for trend were based on multiple regression models with maternal age as a continuous variable. Exclusion

of women with pre-existent hypertension from these analyses did not change the results. All models were adjusted for gestational age at visit, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, body mass index at each visit and maternal stress. We have tested potential interactions with maternal age[19]. We found that the interaction term with pre-pregnancy body mass index was significant for the association between maternal age and the risk of pregnancy-induced hypertension ($p<0.01$). The interaction term was included in this model. We have also tested the interaction between maternal age and body mass index for the associations with systolic and diastolic blood pressure. We observed only one significant interaction term ($p=0.03$), for the association between maternal age and third trimester systolic blood pressure). However, after adjusting for multiple testing, we considered this as not significant. The percentages of missing values within the population for analysis were lower than 15%, except for folic acid supplement use (26%) and maternal stress (24%). We used multiple imputation for missing values in the covariates. The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc. Gary NC, USA), including the Proc Mixed module for unbalanced repeated measurements.

RESULTS

Subject characteristics

Characteristics of all included women for this analysis according to their age are shown in Table 1. In total, there were 311 cases (3.6 %) of pregnancy-induced hypertension and 171 cases (2.0%) of preeclampsia.

Maternal age and longitudinally measured blood pressure

Figure 2 gives the blood pressure development during pregnancy for women aged ≤24.9 years, 25 to 34.9 years, and ≥35 years. Systolic blood pressure was highest among women aged 25 to 34.9 years. In all age-groups, systolic blood pressure increased throughout pregnancy (Figure 2a). Women aged ≤24.9 and 25 to 34.9 years showed the steepest increase in systolic blood pressure. For all age-groups, diastolic blood pressure showed a mid-pregnancy dip, with an increase afterwards (Figure 2b). Women aged 25 to 34.9 years had the highest diastolic blood pressure throughout pregnancy, but the steepest increase was observed in those aged 35 years and older. The exact regression coefficients for gestational age independent (intercept) and gestational age dependent differences (interaction maternal age and gestational age) are given in the Supplementary Table S1.

Maternal age and blood pressure in different trimesters

Maternal age was not significantly associated with first trimester systolic and diastolic blood pressure (for systolic blood pressure: p-value = 0.15 and for diastolic blood pressure: p-value = 0.20, respectively.) The trend analyses showed that in second and third trimester, older maternal

TABLE 1. Subject characteristics by age-group (N=8623)[1]

Maternal age	<20 yrs n=375	20 to 24.9 yrs n=1446	25 to 29.9 yrs n=2348	30 to 34.9 yrs n=3172	35 to 39.9 yrs n=1137	≥40 yrs n=145	P-value[3]
Age, yrs	18.7 (1.0)	22.7 (1.4)	27.7 (1.5)	32.4 (1.4)	36.8 (1.4)	41.6 (1.3)	P<0.01
Height, cm	165.0 (6.5)	165.3 (7.1)	166.4 (7.4)	168.3 (7.4)	168.2 (7.4)	167.8 (8.2)	P<0.01
Weight, kg	65.1 (13.1)	67.9 (13.9)	69.8 (14.1)	69.7 (12.5)	71.1 (12.1)	72.4 (13.5)	P<0.01
Body mass index, kg/m^2	23.9 (4.5)	24.9 (4.7)	25.2 (4.8)	24.6 (4.3)	25.1 (4.1)	25.7 (4.6)	P<0.01
Parity, % nulliparous	88.3	72.0	59.7	50.4	33.5	34.5	P<0.01
Gestational age at intake, wks[2]	16.8 (10.6-30.9)	14.8 (10.2-29.9)	14.4 (9.6-25.8)	13.9 (10.4-24.2)	14.9 (10.8-30.3)	17.1 (11.3-35.5)	P<0.01
Highest completed education, %							
Primary school	30.5	18.5	12.6	7.2	8.8	14.9	P<0.01
Secundary school	68.6	73.4	52.6	34.0	32.5	25.4	
Higher education	1.0	8.1	34.8	58.8	58.7	56.7	
Ethnicity, %							
European	23.5	30.2	52.6	72.2	68.7	61.3	P<0.01
Non-European	76.5	69.8	47.4	27.8	31.3	38.7	
Maternal stress index[2]	0.38 (0.02-2.2)	0.31 (0.00-2.00)	0.17 (0.00-1.53)	0.13 (0.00-1.07)	0.13 (0.00-1.33)	0.14 (0.00-1.12)	P<0.01
Alcohol consumption, %							
None	64.1	68.4	59.4	38.1	36.0	40.5	P<0.01
First trimester only	17.1	13.7	13.3	14.4	10.8	9.1	
Continued	18.8	17.9	27.3	47.5	53.2	50.4	
Smoking habits, %							
None	59.3	63.5	75.6	79.5	75.9	79.0	P<0.01
First trimester only	9.7	8.4	8.7	8.4	6.8	5.9	
Continued	31.0	28.0	15.8	12.1	17.3	15.1	
Folic acid supplement use, %							
Preconceptional use	7.3	16.4	37.0	51.0	47.4	43.5	P<0.01
First 10 weeks use	23.8	33.3	32.9	30.7	29.4	24.1	
No use	69.0	50.3	30.1	18.3	23.2	32.4	
Caffeine intake, %							
None	7.8	5.4	4.8	4.2	3.9	2.3	P<0.01
<2 units per day	77.5	68.4	62.3	50.1	45.0	42.1	
2-3.9 units per day	12.8	22.3	27.7	36.0	38.6	42.9	
4-5.9 units per day	1.9	3.1	4.3	8.1	9.9	10.5	
≥6 units per day	0	0.9	0.9	1.7	2.5	2.3	
1st trimester SBP, mmHg	113 (12)	115 (12)	116 (12)	116 (12)	115 (12)	116 (12)	P<0.05
2nd trimester SBP, mmHg	116 (12)	116(12)	117 (12)	117 (12)	116 (12)	117 (12)	P=0.07
3rd trimester SBP, mmHg	117 (13)	118(13)	118 (13)	118 (12)	118 (12)	119 (11)	P=0.36

TABLE 1. (continued)

Maternal age	<20 yrs n=375	20 to 24.9 yrs n=1446	25 to 29.9 yrs n=2348	30 to 34.9 yrs n=3172	35 to 39.9 yrs n=1137	≥40 yrs n=145	P-value[3]
1st trimester DBP, mmHg	66 (9)	68 (10)	69 (10)	69 (9)	68 (10)	68 (9)	P<0.01
2nd trimester DBP, mmHg	66 (9)	67 (9)	68 (9)	67 (9)	67 (10)	67 (10)	P<0.01
3rd trimester DBP, mmHg	68 (9)	69 (9)	69 (9)	69 (9)	69 (10)	69 (10)	P=0.52
Preeclampsia, %	2.9	1.7	2.5	1.8	1.4	1.8	P=0.17
Pregnancy-induced hypertension, %	3.5	3.0	3.6	3.8	3.9	2.8	P=0.76

Abbreviations; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[1]Values are means (standard deviation) or percentages.
[2]Median (95% range).
[3]Significant differences (P<0.001) between characteristics in age-categories were tested with one-way ANOVA for continuous variables, and Chi-square test for categorical variables.

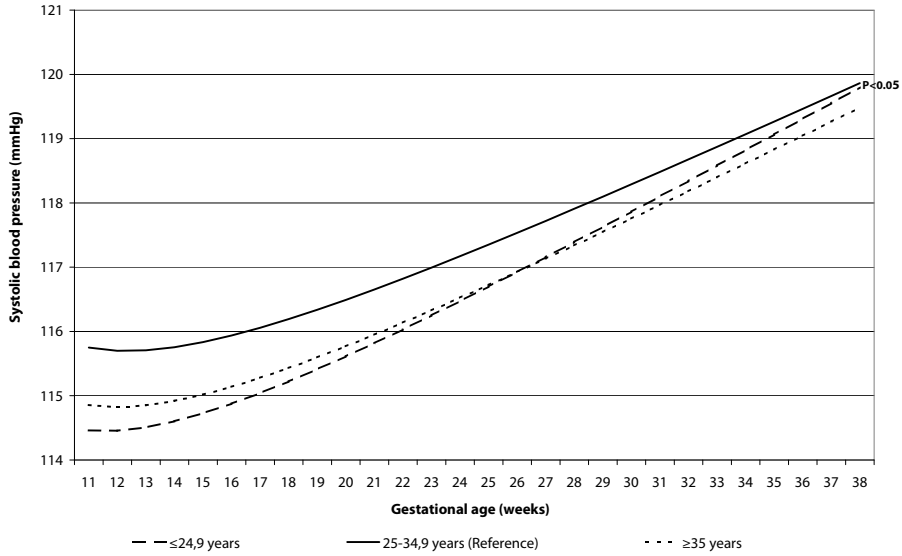
age was associated with lower systolic blood pressure (differences for second and third trimester: -0.9 mmHg (95% confidence interval (CI): -1.4, -0.3) and -0.6 mmHg (95% CI: -1.1,-0.02) per additional 10 maternal years). In second trimester, as compared to women aged 30 to 34.9 years, those younger than 20 years had the highest systolic blood pressure (difference: 1.7 mmHg (95% CI: 0.4, 3.1)). In third trimester, no significant differences in systolic blood pressure were observed between the age-groups (Table 2). Maternal age was not associated with second trimester diastolic blood pressure, but older age was associated with higher third trimester diastolic blood pressure (0.5 mmHg (95% CI: 0.04, 0.9) per additional 10 maternal years) (Table 3).

Maternal age and the risks of pregnancy-induced hypertension and preeclampsia

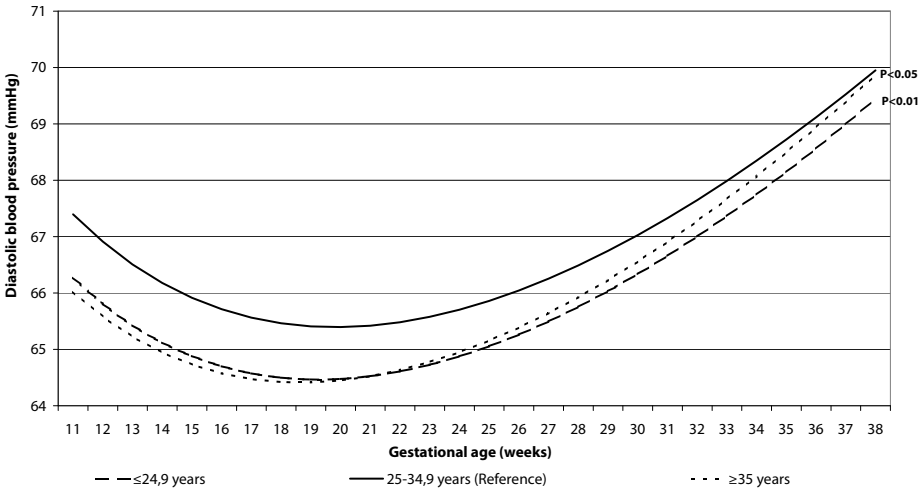
Table 4 shows the associations between maternal age and the risk of gestational hypertensive disorders. We found a significant interaction term between maternal age and pre-pregnancy body mass index for the association with pregnancy-induced hypertension (p<0.01), but not for the association with preeclampsia. Stratified analyses, according to body mass index, showed that among mothers with a normal weight no trend was present (OR, 0.98 (95% CI: 0.94, 1.02) per year). Among mothers with overweight and obesity, we observed a positive trend for the association between maternal age and risk of pregnancy induced hypertension (OR, 1.05 (95% CI: 1.02, 1.08) per year). As compared to women aged 30 to 34.9 years, the risk of preeclampsia tended to be lower among women aged younger (OR, 0.53 (95% CI: 0.32, 0.90)), but the test for trend was not significant.

FIGURE 2. Blood pressure patterns in different maternal age categories

A. Systolic blood pressure



B. Diastolic blood pressure



Change in blood pressure in mmHg for women aged 24.9 years and younger and women aged 35 years and above compared to women aged 25 to 34.9 years based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$*maternal age + β_2*gestational age + β_3*gestational age^{-2} + β_4*maternal age*gestational age and diastolic blood pressure = $\beta_0 + \beta_1$*maternal age + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*maternal age*gestational age). P–value reflects the significance level of β_4, which reflects the difference in change in blood pressure per week per maternal age category. Estimates are given in Supplementary Table S1.

TABLE 2. Cross-sectional associations of maternal age with systolic blood pressure (N=8623)[1]

	Difference in systolic blood pressure (mmHg)		
Maternal age	**First trimester[2]**	**Second trimester[2]**	**Third trimester[2]**
<20 yrs	0.6 (-1.09, 2.19)	1.7 (0.4, 3.1)	0.7 (-0.6, 2.1)
	n=231	n=331	n=340
20 to 24.9 yrs	0.3 (-0.61, 1.25)	0.7 (-0.1, 1.5)	0.8 (-0.03, 1.6)
	n=1049	n=1338	n=1352
25 to 29.9 yrs	0.1 (-0.63, 0.80)	0.5 (-0.2, 1.1)	0.03 (-0.6, 0.7)
	n=1809	n=2194	n=2202
30 to 34.9 yrs	Reference	Reference	Reference
	n=2518	n=3010	n=3014
35 to 39.9 yrs	-0.6 (-1.52, 0.30)	-0.6 (-1.4, 0.2)	-0.3 (-1.1, 0.5)
	n=801	n=1050	n=1078
≥40 yrs	-0.2 (-2.63, 2.31)	0.5 (-1.5, 2.5)	1.1 (-0.8, 3.1)
	n=85	n=125	n=134
Trend[3]	-0.5 (-1.2, 0.2)	-0.9 (-1.4, -0.3)	-0.6 (-1.1, -0.02)
	P_{trend}=0.15	P_{trend}<0.01	P_{trend}=0.04

[1]Values are regression coefficients (95% confidence interval) that reflect the difference in blood pressure in mmHg per maternal age-group compared to the reference group of women aged between 30 and 34.9 years. Estimates are from multiple imputed data.
[2]Models are adjusted for gestational age at visit, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, body mass index at each visit and maternal stress.
[3]Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences per additional 10 maternal years.

DISCUSSION

Results from this prospective population-based cohort study showed that older maternal age is associated with a lower second and third trimester systolic blood pressure, and a higher third trimester diastolic blood pressure. These small differences between younger and older women are within the physiological range of blood pressure variability. Maternal age is not consistently associated with the risk of gestational hypertensive complications. From our results, we cannot conclude that risk differences for gestational hypertensive disorders between younger and older women are present or that they are explained by differences in blood pressure levels.

Methodological considerations

One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 8623 participants with 22661 blood pressure measurements. However, we had a small number of women in the age-group 40 years and older. Therefore, results for women aged 40 years and older should be interpreted with caution. The response

TABLE 3. Cross-sectional associations of maternal age with diastolic blood pressure (N=8623)[1]

	Difference in diastolic blood pressure (mmHg)		
Maternal age	**First trimester[2]**	**Second trimester[2]**	**Third trimester[2]**
<20 yrs	-1.2 (-2.4, 0.1)	-0.7 (-1.8, 0.3)	-0.6 (-1.7, 0.4)
	n=231	n=331	n=340
20 to 24.9 yrs	-0.7 (-1.4, 0.03)	-0.9 (-1.56, -0.31)	-0.5 (-1.1, 0.2)
	n=1049	n=1338	n=1352
25 to 29.9 yrs	-0.2 (-0.7, 0.4)	-0.3 (-0.77, 0.22)	-0.2 (-0.7, 0.3)
	n=1809	n=2194	n=2202
30 to 34.9 yrs	Reference	Reference	Reference
	n=2518	n=3010	n=3014
35 to 39.9 yrs	-0.5 (-1.2, 0.2)	-0.5 (-1.09, 0.13)	0.5 (-0.1, 1.2)
	n=801	n=1050	n=1078
≥40 yrs	-0.4 (-2.3, 1.5)	-0.8 (-2.37, 0.73)	-0.1 (-1.6, 1.5)
	n=85	n=125	n=134
Trend[3]	0.3 (-0.2, 0.9)	0.3 (-0.2, 0.7)	0.5 (0.04, 0.9)
	P_{trend} =0.20	P_{trend} =0.21	P_{trend} =0.03

[1]Values are regression coefficients (95% confidence interval) that reflect the difference in blood pressure in mmHg per maternal age-group compared to the reference group of women aged between 30 and 34.9 years. Estimates are from multiple imputed data.
[2]Models are adjusted for gestational age at visit, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, body mass index at each visit and maternal stress.
[3]Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences per additional 10 maternal years.

rate at baseline for participation in the Generation R Study cohort was 61%. The non-response would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from non-response at baseline[20]. Furthermore, not all women were already enrolled in the study in first trimester. Therefore, we did not have first trimester blood pressure measurements in approximately 25% of the participating women. It seems unlikely that late enrollment has biased our results. We observed only marginal differences in the associations of maternal age with the risk of gestational hypertensive disorders between women who were enrolled during first trimester or later in pregnancy. Detailed information about a large number of potential confounding factors was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle related determinants might still be an issue. In addition, information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle related determinants. Finally, we had relatively small numbers of pregnancy-induced hypertension cases (n = 311) and preeclampsia cases (n = 171), which might have led to lack of power to assess the associations with pregnancy-induced hypertensive complications.

TABLE 4. Associations between maternal age and risk of pregnancy-induced hypertension and preeclampsia[1]

	Pregnancy-induced hypertension[2]		Preeclampsia[2,3]
Maternal age	Normal weight women[4] n=5189	Overweight and obese women[4] n=3367	Entire population n=8623
<20 yrs	1.60 (0.65, 3.95)	0.66 (0.27, 1.62)	0.81 (0.40, 1.66)
	n_{cases}=7	n_{cases}=6	n_{cases}=11
20 to 24.9 yrs	1.34 (0.72, 2.47)	0.52 (0.30, 0.88)	0.53 (0.32, 0.90)
	n_{cases}=21	n_{cases}=21	n_{cases}=25
25 to 29.9 yrs	1.18 (0.75, 187)	0.78 (0.53, 1.14)	1.02 (0.70, 1.50)
	n_{cases}=36	n_{cases}=49	n_{cases}=58
30 to 34.9 yrs	Reference	Reference	Reference
	n_{cases}=46	n_{cases}=73	n_{cases}=58
35 to 39.9 yrs	0.93 (0.48, 1.82)	1.27 (0.82, 1.96)	0.89 (0.51, 1.56)
	n_{cases}=11	n_{cases}=33	n_{cases}=16
≥40 yrs	0.61 (0.13, 6.99)	0.98 (0.30, 3.25)	1.11 (0.34, 3.62)
	n_{cases}=1	n_{cases}=3	n_{cases}=3
Trend[5]	0.98 (0.94, 1.02)	1.05 (1.02, 1.08)	1.02 (0.98, 1.05)
	P_{trend}= 0.29	P_{trend}<0.01	P_{trend}=0.29

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risks of pregnancy-induced hypertension and preeclampsia in different age-groups compared to the reference group of women aged between 30 and 34.9 years. Estimates are from multiple imputed data.
[2]Model is adjusted for educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, maternal stress.
[3]Model is also adjusted for pre-pregnancy body mass index
[4]A significant interaction term with pre-pregnancy body mass index was found for the association between maternal age and the risk of pregnancy-induced hypertension.
[5]Tests for trend were based on multiple logistic regression models with maternal age as a continuous variable.

Maternal age and blood pressure

Studies focused on the association of maternal age and blood pressure development during pregnancy are scarce. A study conducted in 189 Nigerian women, with a mean age of 28 years, reported that maternal age was not associated with systolic blood pressure in any trimester[21]. A positive correlation was reported between maternal age and diastolic blood pressure at 30 to 38 weeks gestation[21]. In our study, we observed a similar association; older maternal age was associated with a higher third trimester diastolic blood pressure. Also, we observed that older maternal age was associated with a lower second and third trimester systolic blood pressure. Differences in our results and the results of the study among the Nigerian women might be explained by a different age distribution (95% range of the Nigerian study population: 27.4 to 29.1 years; and 95% range of our study population: 19.2 to 39.2 years). Also, differences in lifestyle related determinants between the Nigerian and the Dutch population may explain the difference in the observed association.

The mechanisms explaining the differences in age effect on systolic and diastolic blood pressure are not known. It has been suggested that with older age the vascular compliance declines, leading to a higher afterload. This is contradictory to the hemodynamic adaptation during pregnancy, in which the afterload declines. However, this hypothesis is not in line with our observed associations, where older women tend to have a lower systolic blood pressure. The association between older maternal age and a higher diastolic blood pressure could be explained by the fact that during the third trimester blood pressure may reach preconceptional values. The before pregnancy value might be higher in older women compared with younger women, considering that blood pressure increases with age. In the present study, we had no data about pre-pregnancy blood pressure available.

Furthermore, our hypothesis, that differences in blood pressure levels between younger and older women might be part of the underlying mechanism explaining the association between advanced maternal age and hypertensive complications in pregnancy, is not supported by our results. The blood pressure differences between younger and older women appear to be small and the results regarding the association of maternal age with risk of gestational hypertensive disorders are inconsistent.

Maternal age and the risks of pregnancy-induced hypertension and preeclampsia

We did not observe a consistent association between maternal age and the risk of pregnancy-induced hypertension. The stratified analysis showed that among normal weight women no significant association was present, but among overweight and obese women a small positive trend was present. A Swedish population-based cohort study among 10666 nulliparous women aged 34 years or less, reported that maternal age was not associated with pregnancy-induced hypertension[22]. Two smaller studies, one among 400 Iranian women and one among 328 Kuwaitis women, reported that pregnancy-induced hypertension was more prevalent among women 40 years of age and older[3,23]. Several studies assessed the association between maternal age and the risk of preeclampsia. In a French register-based study among 8514 women aged less than 31 years, a lower risk of preeclampsia was observed for 16-year old women[24]. Three other studies did not observe an association between younger maternal age and the risk of preeclampsia[22,25,26]. The largest study was conducted among 854377 Latin- American women[26]. One study, which assessed the risk of pregnancy complications among women aged 40 years or older and compared these with women aged 20 to 29 years, reported that there was an increase in the frequency of preeclampsia among the older women[27]. A similar result was found by the study conducted among 400 Iranian women[3]. We observed inconsistent results regarding the association of maternal age and the risk of preeclampsia. As compared to women aged 30 to 34.9 years, the risk of preeclampsia tended to be lower among women aged younger, but the test for trend was not significant. Also, our crude analyses did not show strong associations between maternal age and the risk of gestational hypertensive disorders (results not shown). In this study, we had a small number of

cases of pregnancy-induced hypertension and preeclampsia, which might not only have caused a lack of power to detect significant differences between the age-groups, but might also indicate a selection towards a healthy population. An association between advanced maternal age and the risk of gestational hypertensive complications might be more apparent in high risk populations. However, this suggested association of advanced maternal age with the risk of gestational hypertensive disorders by studies among more high risk populations might also partly be explained by confounding factors[3,7,22]. Therefore, further research among high risk populations is necessary.

Conclusion

This large population-based cohort study showed that older maternal age is associated with lower second and third trimester systolic blood pressure, and higher third trimester diastolic blood pressure. These differences in blood pressure levels between younger and older women are small and well within the physiological range of blood pressure variability. They are of interest from an etiological perspective rather than from an individual clinical perspective. Maternal age is not consistently associated with the risks of gestational hypertensive disorders. Our results suggest that maternal body mass index might influence the association between maternal age and the risk of pregnancy-induced hypertension. Further studies are needed to explore whether an association is present among high risk populations.

REFERENCES

1. Nijdam ME, Janssen KJ, Moons KG, et al. Prediction model for hypertension in pregnancy in nulliparous women using information obtained at the first antenatal visit. J Hypertens. 2010;28:119-26.
2. Steegers EAP, von Dadelszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet. 2010 doi:10.1016/S0140-6736(10)60279-6
3. Jahromi BN, Husseini Z. Pregnancy outcome at maternal age 40 and older. Taiwan J Obstet Gynecol. 2008;47: 318-21.
4. Poon LC, Kametas NA, Chelemen T, et al. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. J Hum Hypertens. 2010;24:104-10.
5. Bobrowski RA, Bottoms SF. Underappreciated risks of the elderly multipara. Am J Obstet Gynecol. 1995;172: 1764-7.
6. Buchbinder A, Sibai BM, Caritis S, et al; National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild preeclampsia. Am J Obstet Gynecol. 2002;186:66-71.
7. van Katwijk C, Peeters LL. Clinical aspects of pregnancy after the age of 35 years: a review of the literature. Hum Reprod Update. 1998;4:185-94.
8. Gilson GJ, Mosher MD, Conrad KP. Systemic hemodynamics and oxygen transport during pregnancy in chronically instrumented, conscious rats. Am J Physiol. 1992;263:H1911-8.
9. Hart MV, Morton MJ, Hosenpud JD, et al. Aortic function during normal human pregnancy. Am J Obstet Gynecol. 1986;154:887-91.
10. Zhang J, Klebanoff MA. Low blood pressure during pregnancy and poor perinatal outcomes: an obstetric paradox. Am J Epidemiol. 2001;153:642-6.
11. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
12. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010; 25:823-41.
13. World Medical Association I. Declaration of Helsinki. Ethical principles for medical research involving human subjects. J Indian Med Assoc. 2009;107:403-5.
14. El Assaad MA, Topouchian JA, Darne BM, et al. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7:237-41.
15. Coolman M, de Groot CJ, Jaddoe VW, et al. Medical record validation of maternally reported history of preeclampsia. J Clin Epidemiol. 2010;63:932-937.
16. Brown MA, Lindheimer MD, de Swiet M, et al. 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:IX-XIV.
17. Derogatis LR. Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition. Minneapolis, MN. 1993
18. Goldstein H. Multilevel statistical methods. 2nd ed. Edward Arnold: London,1995.
19. D.G. Altman, Practical statistics for medical research, Chapman & Hall, London, UK: 1991
20. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
21. Okonofua FE, Balogun JA, Amiengheme NA, et al. Blood pressure changes during pregnancy in Nigerian women. Int J Cardiol. 1992;37:373-9.
22. Ros HS, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. Am J Epidemiol. 1998;147:1062-70.
23. Diejomaoh MF, Al-Shamali IA, Al-Kandari F, et al. The reproductive performance of women at 40 years and over. Eur J Obstet Gynecol Reprod Biol. 2006;126:33-8.
24. de Vienne CM, Creveuil C, Dreyfus M. Does young maternal age increase the risk of adverse obstetric, fetal and neonatal outcomes: a cohort study. Eur J Obstet Gynecol Reprod Biol. 2009;147:151-6.

25. Jolly MC, Sebire N, Harris J, et al. Obstetric risks of pregnancy in women less than 18 years old. Obstet Gynecol. 2000;96:962-6.
26. Conde-Agudelo A, Belizan JM, Lammers C. Maternal-perinatal morbidity and mortality associated with adolescent pregnancy in Latin America: Cross-sectional study. Am J Obstet Gynecol. 2005;192:342-9.
27. Ziadeh S, Yahaya A. Pregnancy outcome at age 40 and older. Arch Gynecol Obstet. 2001;265:30-3.

SUPPLEMENTARY MATERIAL

Unbalanced repeated measurement regression models

The associations of maternal age with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data[1].Using fractional polynomials of gestational age, the best fitting models were constructed. For this analysis, maternal age was categorized into three groups; ≤24.9 years, 25-34.9 years, and ≥35 years, and included in these models as intercept and as an interaction term with gestational age. These models can be written as:

Systolic blood pressure= β_0 + β_1*maternal age + β_2*gestational age + β_3*gestational age^{-2} + β_4*maternal age*gestational age

Diastolic blood pressure = β_0 + β_1*maternal age + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*maternal age*gestational age

In these models, 'β_0 + β_1 *maternal age' reflects the intercept. The intercept reflects the mean systolic and diastolic blood pressure value for these three maternal age categories. 'β_2*gestational age + β_3*gestational age^{-2}'reflects the slope of change in blood pressure per week for systolic blood pressure, and 'β_2*gestational age + β_3*gestational age$^{0.5}$'reflects the slope of change in blood pressure per week for diastolic blood pressure. Main interest was in the term 'β_4*maternal age*gestational age', which reflects the difference in change in blood pressure per week between the different maternal age categories for systolic and diastolic blood pressure. The exact regression coefficients for gestational age independent (intercept) and gestational age dependent differences (interaction maternal age and gestational age) are given in the Supplementary Table S1.

REFERENCES

1. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28:964–74.

SUPPLEMENTARY TABLE S1. Longitudinal associations between maternal age and systolic and diastolic blood pressure[1]

	Difference in systolic blood pressure			
Maternal age	Intercept	P-value[2]	Slope (mmHg (95% CI))	P-value[2]
≤24.9 yrs	110.08	<0.01	0.05 (0.00, 0.09)	0.03
25-34.9 yrs	111.87	<0.01	Reference	
≥35 yrs	110.77	0.09	0.02 (-0.03, 0.07)	0.44
	Difference in diastolic blood pressure			
Maternal age	Intercept	P-value[2]	Slope (mmHg (95% CI))	P-value[2]
≤24.9 yrs	96.29	<0.01	0.05 (0.02, 0.08)	<0.01
25-34.9 yrs	98.19	<0.01	Reference	
≥35 yrs	96.81	<0.01	0.04 (0.00, 0.08)	0.04

[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mmHg per maternal age category compared to the reference group of women aged between 25 and 34.9 years.

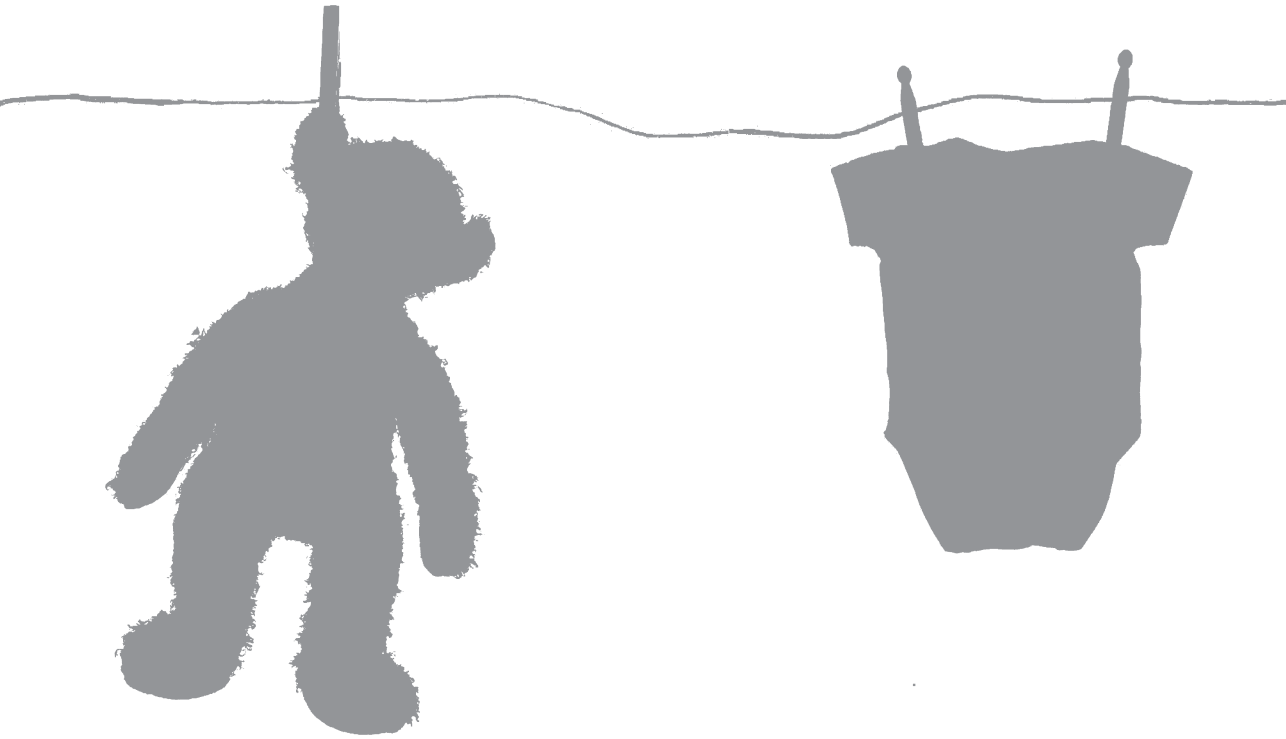
[2]P–value reflects the significance level of the estimate.

Chapter 3.3

Smoking and blood pressure in different trimesters of pregnancy

Rachel Bakker
Eric AP Steegers
Johan P Mackenbach
Albert Hofman
Vincent WV Jaddoe

Adapted from J Hypertens. 2010;28:2210-8.



ABSTRACT

Smoking during pregnancy is a risk factor for various adverse birth outcomes but lowers the risk of preeclampsia. Cardiovascular adaptations might underlie these associations. We examined the associations of smoking in different trimesters of pregnancy with repeatedly measured blood pressure and the risks of preeclampsia and pregnancy-induced hypertension in a low risk population-based cohort of 7106 pregnant women. This study was embedded in a population-based prospective cohort study from early pregnancy onwards. Smoking and systolic and diastolic blood pressures were assessed by questionnaires and physical examinations in each trimester of pregnancy. Information about preeclampsia and pregnancy-induced hypertension was obtained from medical records. Compared to non-smoking women, both first trimester only and continued smoking were associated with a steeper increase for systolic blood pressure and a lowest mid-pregnancy level and steeper increase thereafter for diastolic blood pressure throughout pregnancy. We did not find any significant associations in risk of preeclampsia for first trimester only smoking (odds ratio, 1.28 (95% confidence interval: 0.74, 2.21)) and continued smoking (odds ratio, 0.83 (95% confidence interval: 0.50, 1.36)), respectively. Our results suggest that both first trimester only and continued smoking are associated with persistent maternal cardiovascular adaptations during pregnancy. Strategies for prevention of smoking during pregnancy should be focused on the preconception period. The effects of early and late pregnancy smoking on the risk of preeclampsia should be further explored. Our results should be carefully interpreted to the general population of pregnant women.

INTRODUCTION

Hypertensive disorders during pregnancy are leading causes of maternal and neonatal morbidity worldwide, and include preeclampsia and pregnancy-induced hypertension[1-2]. Preeclampsia occurs in approximately 5% of all pregnant women. Another 6% is complicated by pregnancy-induced hypertension[3]. Risk factors for preeclampsia and pregnancy-induced hypertension include family or own history of preeclampsia, first pregnancy, increased body mass index, higher maternal age, pre-existing diabetes, renal disease, hypertension, and chronic autoimmune disease[4-8]. Smoking during pregnancy is one of the most important risk factors for various adverse birth outcomes, such as low birth weight and preterm birth[9-12], but lowers the risk of preeclampsia[13-17]. Systematic reviews focused on the effects of maternal smoking during pregnancy on the risk of hypertensive disorders showed that smoking reduces the risk of preeclampsia and pregnancy-induced hypertension with 40% to 50%[13,18-21]. The underlying mechanisms for the associations between smoking and hypertensive disorders during pregnancy are not known and information about trimester specific effects of smoking on the risks of hypertensive disorders during pregnancy is limited. It has been suggested that maternal smoking affects early placentation and subsequent maternal cardiovascular adaptation. Differences in blood pressure associated with smoking in pregnancy might be markers of cardiovascular adaptation and the subsequent risk of hypertensive disorders.

This study was designed to identify critical periods and cardiovascular adaptations as mechanisms underlying the associations between smoking and the risks of hypertensive disorders. We examined the associations of smoking in different periods of pregnancy with repeatedly measured blood pressure levels and the risks of preeclampsia and pregnancy-induced hypertension in a low risk population-based prospective cohort study among 7106 pregnant women.

METHODS

Study design

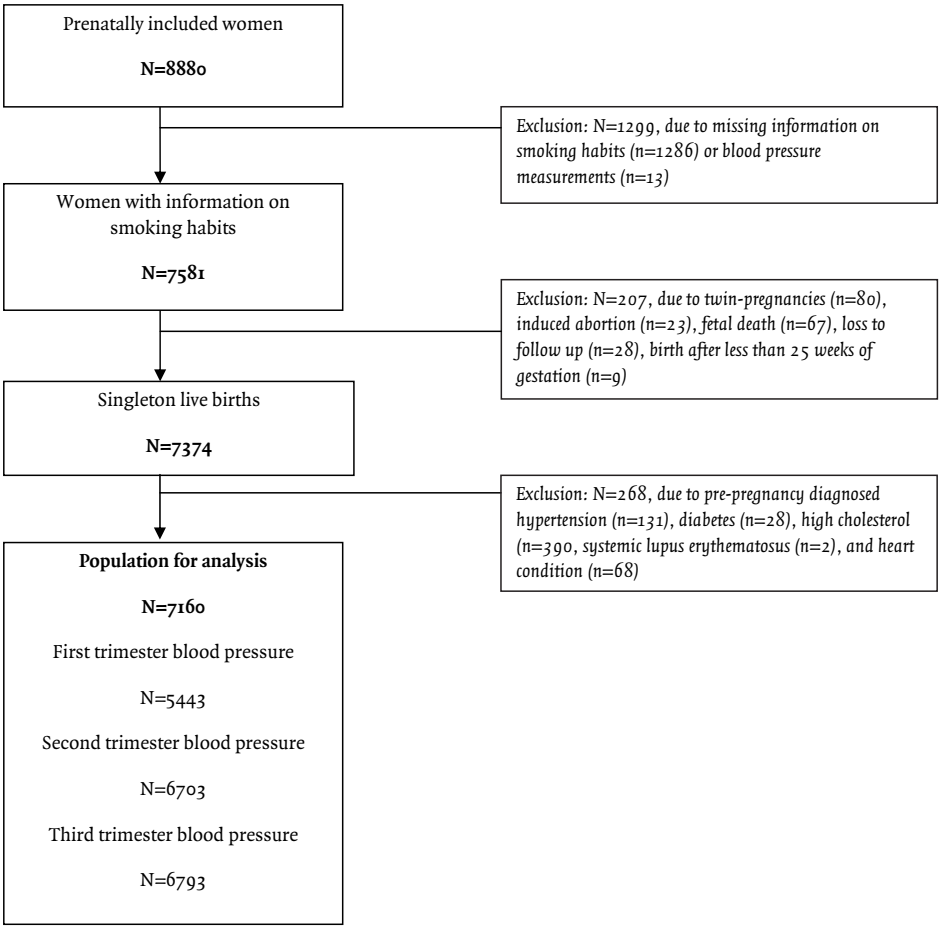
This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards, designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood. Details have been described elsewhere[22-23]. The cohort comprises 9778 women and their children born in Rotterdam, the Netherlands. The response rate of the Generation R Study is 61%. Enrolment was aimed in early pregnancy (gestational age <18 weeks), but was allowed until delivery of the child. In total, 8880 women were enrolled in pregnancy, of which 75% before a gestational age of 18 weeks. All children were born between April 2002 and January 2006. Response rate at birth was 61%. Assessments during pregnancy were planned in early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18-24.9 weeks), and late pregnancy (gestational age ≥25 weeks) and included physical examinations, fetal ultrasounds examinations, and self-administered

questionnaires. These measurements were considered as first, second and third trimester measurements, respectively. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam. Written consent was obtained from all participating parents[24].

Population for analysis

In total, 8880 women were enrolled during pregnancy. For this analysis, we excluded women without information on smoking (n = 1286), and women without blood pressure measurements during pregnancy (n = 13) leading to 7851 women. Since our main interest was in low-risk pregnancies, we subsequently excluded women with twin-pregnancies (n = 80), induced abortion (n = 23), fetal death (n = 67), loss to follow up (n = 28), and delivery after less than 25 weeks of gestation (n = 9). Finally, we also excluded women with diagnosed hypertension before pregnancy (n = 131), diabetes

FIGURE 1. Flow chart of participants included for analysis



(n = 28), high cholesterol (n = 39), systemic lupus erythematosus (n = 2), and heart condition (n = 68). Thus, the cohort for analysis comprised 7106 women (80% of 8880) (Figure 1). Of all included pregnancies, 5.5% were second (n = 387) or third (n = 3) pregnancies in this study. Since there were no differences in results after exclusion of these women, they were included in the analyses. The analyses focused on the effects of first trimester only smoking on pregnancy outcomes, were performed in women who did not continue smoking after first trimester (n = 5882).

Smoking during pregnancy

Information on smoking was obtained by self-administered questionnaires sent in the first, second, and third trimester. Response rates for these questionnaires were 91%, 80%, and 77%, respectively[23]. Smoking at enrolment was assessed in the first questionnaire by asking each woman whether she smoked during pregnancy (categories: no smoking, first trimester only smoking, continued smoking). This questionnaire was sent to all women, regardless of the gestational age at enrolment. To assess smoking habits in second and third trimester, women were asked whether they smoked in the past 2 months (categories: no, yes) in the second and third questionnaire. Women who reported in the first questionnaire that they smoked first trimester only (n = 849), but still reported smoking in the second or third questionnaire (n = 259) were reclassified into the 'continued smoking' category. The same strategy was used for women who reported no smoking in the first questionnaire, but reported smoking in the second or third questionnaire (n = 82). Among women who smoked, the number of cigarettes smoked daily was assessed in six categories: <1, 1-2, 3-4, 5-9, 10-19, and ≥20. To increase the number of subjects per category we combined and reclassified these categories into three previously used categories: <5 cigarettes/day, 5-9 cigarettes/day, and ≥10 cigarettes/day[10].

Blood pressure in different trimesters of pregnancy

Blood pressure was measured with the Omron 907® automated digital oscillometric sphygmanometer, which was validated in non-pregnant adults (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands)[25], but not in pregnant women. This might have led to non-differential measurement errors of the determinant and subsequently might have led to possible underestimation of the results. All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 centimeters (cm) a larger cuff (32~42 cm) was used. The mean value of two blood pressure readings over a 60-second interval was documented for each participant. In total, blood pressure was measured in 5443 women in first trimester (mean, 13.6 weeks of gestation; range, 7.1-17.9), in 6703 women in second trimester (mean, 20.7 weeks of gestation; range, 18.0-24.9) and 6793 women in third trimester (mean, 30.4 weeks of gestation; range, 25.0-39.2). In total, 18939 blood pressure measurements were available for analyses. Three, two and one blood pressure measurements were available for 5063, 1707, and 336 women, respectively.

Pregnancy-induced hypertension and preeclampsia

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere[26]. Briefly, the following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia.

Covariates

Information on maternal age at enrolment (continuous), educational level (primary school; secondary school; higher education), ethnicity (Dutch or other European; Non-European), parity (nulliparous; multiparous), folic acid supplementation use (preconception use; first 10 weeks of pregnancy; none) was obtained from the first questionnaire at enrolment in the study. Height (in cm) and weight (in kg) at enrolment were measured without shoes and heavy clothing. Weight was repeatedly measuring during subsequent visits at the research center. Alcohol consumption (none; first trimester only; continued) was assessed in each questionnaire. Maternal distress (continuous) was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory, which gives a Global Severity Index (GSI). Higher GSI reflects more stress pregnant women experience.

Statistical analyses

First, the associations of smoking habits during pregnancy with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression analysis. These models take into account the correlation between repeated measurements of the same subject, and allow for incomplete outcome data[27]. The best fitting models were constructed using fractional polynomials of gestational age[28]. Maternal smoking during pregnancy (no smoking; first trimester only smoking; continued smoking) was included in these models as intercept and as interaction term with gestational age. The models can be written as:

Systolic blood pressure = $\beta_0 + \beta_1$*smoking + β_2*gestational age + β_3*gestational age^{-2} + β_4*smoking*gestational age

Diastolic blood pressure = $\beta_0 + \beta_1$*smoking + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*smoking*gestational age

In these models, '$\beta_0 + \beta_1$*smoking' reflects the intercept and 'β_2*gestational age + β_3*gestational age^{-2}'reflects the slope of change in blood pressure per week for systolic blood pressure, and 'β_2*gestational age + β_3*gestational age$^{0.5}$, reflects the slope of change in blood pressure per week for diastolic blood pressure. Main interest was in the term 'β_4*smoking*gestational age', which reflects the difference in change in blood pressure per week between the different smoking categories for systolic and diastolic blood pressure. Second, the cross-sectional associations of the number of cigarettes smoked with blood pressure in first, second and third trimester were assessed using multiple linear regression models. Linear regression models in which the smoking categories were included as continuous variables were considered as test for trends. Third, the associations of maternal smoking categories with pregnancy-induced hypertension and preeclampsia were assessed using multiple logistic regression models. All models were adjusted for age, weight at enrolment, height, educational level, parity, ethnicity, maternal alcohol consumption in pregnancy, folic acid supplementation use, and stress. Maternal weight measured at enrolment in the study (median gestational age at enrolment: 14.4 weeks (95% range, 10.4-25.5)) was strongly correlated with pre-pregnancy weight ($r = 0.95$, $p<0.01$). We used maternal weight measured at enrolment in the analyses, because the numbers of missing values were smaller and data quality higher than the pre-pregnancy weight, which was reported in a self-administered questionnaire. Our cross-sectional analyses were also adjusted for gestational age at enrolment. Also, no differences in results were observed when we used maternal weight based on the questionnaire data. We imputed missing data of the covariates with the mean value for continuous variables, and adding a separate category for missing values for categorical variables. The percentages of missing values within the population for analysis were lower than 2%, except for folic acid supplementation use (15%) and maternal stress (21%). These higher percentages were due the large number of women who only partially filled out the questionnaire or were not enrolled in first trimester. No differences in results were observed between analyses with imputed missing data or complete cases only. The repeated measurement analysis was performed using the Statistical Analysis System version 8.2 (SAS, Institute Inc. Gary NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject characteristics

Of all 7106 women, 8.3% (n = 590) reported smoking in first trimester only and 17.2% (n = 1224) continued smoking during pregnancy. Median gestational age at enrolment was 14.4 (90% range, 10.9-22.8) weeks. Mean age at enrolment ranged from 15.3 to 46.3 years with a mean of 29.7 years. Among all women, those who continued smoking during pregnancy were youngest. The overall percentage of women who continued to consume alcohol during pregnancy was 49.3%

TABLE 1. Maternal characteristics according to the category of smoking habits during pregnancy[1,2]

	Smoking during pregnancy (N=7106)		
	No n=5292	**First trimester only n=590**	**Continued n=1224**
Age, yrs	30.1 (5.1)	29.3 (5.2)	28.3 (5.8)
Height, cm	167.3 (7.5)	168.3 (7.1)	167.1 (6.9)
Weight at enrolment, kg	68.9 (12.7)	69.1 (12.6)	69.9 (13.7)
Parity at enrolment ≥ 1, %	44.3	30.1	43.6
Gestational age at enrolment, wks[3]	14.4 (10.5-26.2)	13.6 (9.5-22.5)	14.6 (9.8-29.8)
Education, %			
Primary school	10.0	9.4	18.1
Secondary school	42.6	47.9	63.6
Higher education	47.5	42.7	18.4
Missing	2.1	0.7	2.5
Ethnicity, %			
Dutch, other European	56.9	62.9	56.5
Non-Western	42.6	36.6	43.0
Missing	0.5	0.5	0.5
Alcohol consumption during pregnancy, %			
No	52.7	25.8	46.3
First trimester only	12.0	27.5	13.5
Continued	35.2	46.8	40.2
Missing	0.2	0.7	0.4
Folic acid supplementation use, %			
Preconception use	37.8	28.0	18.5
First 10 weeks of pregnancy	24.3	38.1	30.6
No use	23.8	18.5	33.8
Missing	14.4	15.4	17.1
Maternal stress, index[3]	0.21 (0.00-1.16)	0.25 (0.00-1.41)	0.30 (0.02-1.81)
Mean systolic blood pressure, mmHg			
First trimester	115.3 (12.0)	115.4 (11.7)	115.4 (12.0)
Second trimester	116.3 (12.0)	117.3 (11.6)	117.4 (11.6)
Third trimester	117.7 (11.8)	119.6 (11.4)	119.4 (12.4)
Mean diastolic blood pressure, mmHg			
First trimester	68.3 (9.2)	67.3 (9.0)	66.6 (9.5)
Second trimester	67.2 (9.2)	66.5 (8.9)	66.1 (9.2)
Third trimester	68.9 (9.1)	69.3 (8.7)	68.6 (9.8)
Pregnancy complications, %			
Preeclampsia	2.0	2.8	1.7
Pregnancy-induced hypertension	3.7	4.5	4.1

[1]Characteristics given are woman based.

[2]Values are means with standard deviations or percentages.

[3]Median (95% range).

and 13.5% of women drank alcohol in first trimester only. In total, there were 264 cases (3.7%) of pregnancy-induced hypertension and 140 cases (2.0%) of preeclampsia in this study (Table 1).

Smoking and longitudinally measured blood pressure patterns during pregnancy

Systolic blood pressure increased throughout pregnancy (Figure 2a). Compared to non-smoking, first trimester only and continued smoking showed a steeper increase for systolic blood pressure throughout pregnancy (differences in systolic blood pressure change compared to non-smoking: 0.11 mm Hg per week (95% confidence interval (CI): 0.05, 0.17) and 0.10 mm Hg per week (95% CI: 0.05, 0.14) for first trimester only smoking and continued smoking, respectively). In all groups of women, diastolic blood pressure showed a mid-pregnancy dip, with an increase thereafter. First trimester only and continued smoking showed the lowest mid-pregnancy level and steeper increase thereafter (Figure 2b). Overall differences in diastolic blood pressure change were 0.10 mm Hg per week (95% CI: 0.05, 0.15) and 0.10 mm Hg per week (95% CI: 0.06, 0.14) for first trimester only and continued smoking during pregnancy, respectively. We also performed the repeated-measurement analyses in women with three available blood pressure measurements in pregnancy (n = 5063). Similar results were found (Supplementary Table S1).

Associations of smoking with blood pressure in different trimesters

No consistent associations were observed between first trimester maternal smoking and systolic blood pressure. The number of cigarettes smoked was positively associated with second and third trimester systolic blood pressure (both trend tests P<0.01). In second trimester, compared to non-smoking, smoking 5 to 9 cigarettes per day was associated with a systolic blood pressure increase of 2.38 mm Hg (95% CI: 1.01, 3.75). Similar associations were observed in third trimester (difference compared to non-smoking 1.39 mm Hg (95% CI: 0.05, 2.74) for 5-9 cigarettes per day, and 4.46 mm Hg (95% CI: 2.72, 6.21) for 10 or more cigarettes per day). For diastolic blood pressure, we observed inverse dose-response associations for the number of cigarettes smoked in first and second trimester (both trend tests P<0.01). For smoking less than 5 and 5 to 9 cigarettes, first trimester diastolic blood pressure was -1.83 mm Hg (95% CI: -2.52, -1.14) and -2.40 mm Hg (95% CI: -3.31, -1.48) lower than non-smoking women. Similar results were found in second trimester. In third trimester, women who smoked less than 5 cigarettes per day had a lower diastolic blood pressure (difference: -0.93 mm Hg (95% CI: -1.80, -0.06)) (Table 2).

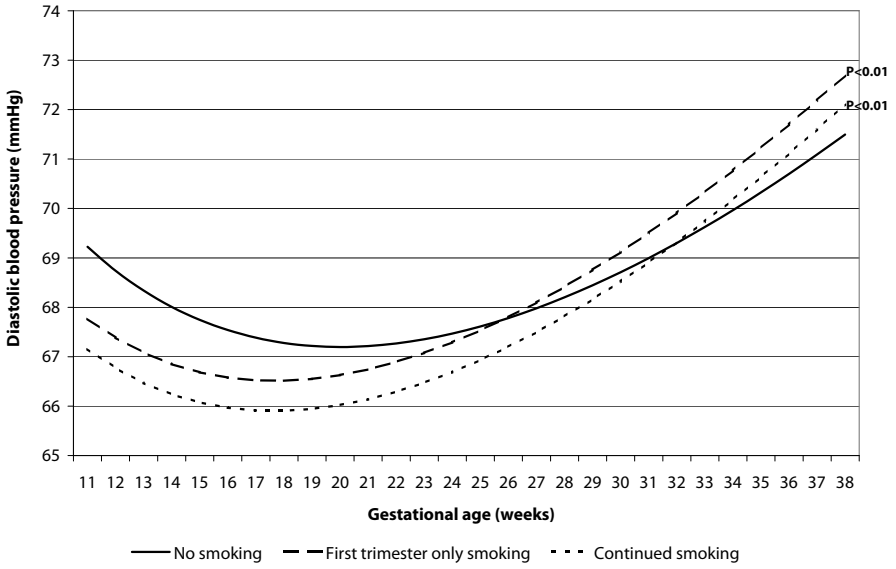
Smoking and the risk of pregnancy-induced hypertension and preeclampsia

No significant associations were found between smoking patterns during pregnancy and the risks of pregnancy-induced hypertension. Similarly, no associations were observed for preeclampsia (Table 3). After repeating these analyses including women with a history of cardiovascular or metabolic problems (hypertension, diabetes, high cholesterol, systemic lupus erythematosus, and heart condition) we found similar results (data not shown).

FIGURE 2. Blood pressure patterns in different smoking categories

A. Systolic blood pressure

B. Diastolic blood pressure



Change in blood pressure in mm Hg per smoking category with non-smokers as reference group based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$*smoking + β_2*gestational age + β_3*gestational age^{-2} + β_4*smoking*gestational age and diastolic blood pressure = $\beta_0 + \beta_1$*smoking + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*smoking*gestational age). P–values reflect the significance level of β_4, which reflects the difference in change in blood pressure per week per smoking category, for first trimester only and continued smoking compared to the non-smokers.

TABLE 2. Cross-sectional associations of smoking with systolic and diastolic blood pressure[1,2]

	First trimester	Second trimester	Third trimester
Smoking	Systolic blood pressure difference (95% CI) (mmHg)	Systolic blood pressure difference (95% CI) (mmHg)	Systolic blood pressure difference (95% CI) (mmHg)
No	Reference	Reference	Reference
	n=4032	n=4344	n=4299
First trimester only	n.a.	0.54 (-0.54, 1.62)	1.17 (0.09, 2.25)*
		n=421	n=423
<5 per day	-0.21 (-1.09, 0.68)	0.34 (-0.71, 1.39)	0.22 (-0.87, 1.32)
	n=729	n=458	n=419
5-9 per day	-0.50 (-1.68, 0.67)	2.38 (1.01, 3.75)**	1.39 (0.05, 2.74)*
	n=380	n=255	n=267
≥10 per day	1.10 (-0.27, 2.46)	1.65 (-0.10, 3.40)	4.46 (2.72, 6.21)*
	n=278	n=153	n=153
	P_{trend}[3] =0.56	P_{trend} <0.01	P_{trend} <0.01
	First trimester	**Second trimester**	**Third trimester**
Smoking	Diastolic blood pressure difference (95% CI) (mmHg)	Diastolic blood pressure difference (95% CI) (mmHg)	Diastolic blood pressure difference (95% CI) (mmHg)
No	Reference	Reference	Reference
	n=4032	n=4344	n=4299
First trimester only	n.a.	-0.38 (-1.24, 0.48)	0.37 (-0.48, 1.23)
		n=421	n=423
<5 per day	-1.83 (-2.52, -1.14)**	-1.20 (-2.03, -0.36)**	-0.93 (-1.80, -0.06)*
	n=729	n=458	n=419
5-9 per day	-2.40 (-3.31, -1.48)**	-1.99 (-3.07, -0.90)**	-0.85 (-1.92, 0.22)
	n=380	n=255	n=267
≥10 per day	-0.56 (-1.63, 0.50)	-0.90 (-2.28, 0.49)	0.88 (-0.51, 2.27)
	n=278	n=153	n=153
	P_{trend} <0.01	P_{trend} <0.01	P_{trend} =0.37

[1]Regression coefficients with 95% confidence interval and reflect the difference in blood pressure in mm Hg to non-smokers.
[2]Models are adjusted for gestational age at visit, maternal age at enrolment, weight, height, educational level, ethnicity, parity, maternal alcohol consumption, folic acid supplementation use and stress in pregnancy.
[3]Tests for trends are based on multiple linear regression models within each trimester.
*P-value<0.05
**P-value<0.01

DISCUSSION

This large scale population-based prospective cohort study from early pregnancy onwards showed that both first trimester only and continued smoking were associated with a steeper rise in systolic and diastolic blood pressure. No significant associations were found between smoking during

TABLE 3. Associations of smoking with pregnancy-induced hypertension and preeclampsia[1,2]

Smoking	Pregnancy-induced hypertension	Preeclampsia
No	Reference	Reference
n=5146	n=189	n=104
First trimester only	1.07 (0.69, 1.65)	1.28 (0.74, 2.21)
n=576	n=26	n=16
Continued	1.06 (0.76, 1.49)	0.83 (0.50, 1.36)
n=1190	n=49	n=20
	P_{trend}[3] =0.69	P_{trend} =0.62

[1]Odds ratios with 95% confidence interval.
[2]Models are adjusted for maternal age at enrolment, weight, height, educational level, ethnicity, parity, maternal alcohol consumption, folic acid supplementation use and stress in pregnancy.
[3]Tests for trends are based on multiple logistic regression models.

pregnancy and the risks of pregnancy-induced hypertension and preeclampsia. Our findings suggest that environmental factors during the first trimester, such as smoking, affect maternal cardiovascular adaptation throughout pregnancy. The differences in maternal blood pressure patterns between smoking categories are not in line with previously demonstrated associations between smoking during pregnancy and the risk of preeclampsia.

Methodological considerations

One of the strengths of this study was the prospective data collection, which started in early pregnancy. In addition, we had a large sample size of 7106 participants with 18939 blood pressure measurements. A wide range of potential confounding factors was available. A potential limitation of our study is that smoking information was not available in all pregnant women, which might have led to loss of power. The associations might be underestimated if among women without information about smoking the percentage of smokers were higher than among women with complete data. However, this seems unlikely since no differences in characteristics between women with and without information about smoking habits was observed. Among women with information about smoking habits, we had a limited loss-to-follow-up. Therefore, we do not expect biased results due to loss-to-follow-up[29].

Second, information about smoking during pregnancy was collected by questionnaires. Although assessing smoking during pregnancy by questionnaire seems to be valid method, misclassification may occur[30]. Underreporting of maternal smoking across the various smoking categories may be present and may lead to misclassification. The estimated difference in blood pressure patterns between non-smoking women and smoking women would be overestimated if this underreporting were selectively present among heavily smoking women who reported less smoking. To overcome these limitations, previous studies have used biomarkers of tobacco exposure, including cotinine, in maternal urine samples[31-32]. However, low correlations between cotinine levels and self-reported smoking habits have been demonstrated[33]. Possible explanations

for these low correlations include inaccurate maternal reporting of smoking during pregnancy, use of categorical rather than continuous variables for assessing the number of cigarettes smoked per day, but also individual differences in inhalation, absorption, and metabolism. It has been demonstrated that use of cotinine levels is not superior to the use of self-reporting questionnaires in studying the effect of maternal smoking in pregnancy on birth weight[34].

Third, the response rate of the study was 61%. Pregnant women who participated were higher educated, more healthy and more frequently of Dutch origin than those who did not participate[23]. This might have led to some selection. However, selection bias is prospective follow up studies primary arises from loss to follow up instead of non-response at baseline. This might explain the small numbers of pregnancy-induced hypertension cases (3.7%) and preeclampsia cases (2.0%), which might have led to a lack of power in our analyses. Our results, in particular the rates of hypertensive disorders during pregnancy, should be carefully interpreted to the general population of pregnant women.

Smoking, blood pressure and the risk of preeclampsia

We observed that both first trimester only and continued smoking were associated with a steeper rise in systolic and diastolic blood pressure compared to non-smoking women. This suggests that first trimester is a critical period for cardiovascular adaptations due to smoking and subsequent blood pressure development. Studies focused on the associations of maternal smoking on blood pressure development during pregnancy are scarce[35-36]. Matkin *et al.* reported that continuing smoking leads to a lower average diastolic blood pressure, but higher systolic blood pressure in normotensive women[35]. Our findings beyond 35 weeks of gestation should be taken with caution because these are based on 23 blood pressure measurements. The mechanisms underlying the associations between smoking and blood pressure during pregnancy are not known. During pregnancy, vasodilatation and uteroplacental circulation lead to a decrease of the total peripheral vascular resistance from early pregnancy onwards, with a mid-pregnancy fall in diastolic blood pressure[37-38]. Mechanisms leading to this vasodilatation are not yet completely understood, but previous findings suggest a decreased vascular responsiveness to the pressor effects of angiotensin II and norepinephrine[39], increased endothelial prostacyclin[40], enhanced nitric oxide production[41], and reduced aortic stiffness[42]. Also, thiocyanate, the metabolic by-product of cyanide which is one of the constituents of cigarette smoke, increases vasodilatation[43]. After the first trimester, we found a steeper increase in first trimester only and continued smokers for both systolic and diastolic blood pressure. For the current study, we only used information about maternal blood pressure as measure of cardiovascular adaptation due to smoking during pregnancy. Studies with information about more maternal or placental cardiovascular measures during pregnancy, such as arterial stiffness, cardiac function, and placental blood flow, might give important additional information. However, these data were not available in this cohort.

We observed no significant associations in risk of preeclampsia in women that smoked first trimester only and continued smoking, respectively. However, our effect estimates in women who

continued smoking seem to be in line with previous studies[15,17,20]. A meta-analysis of Castles *et al.* showed an odds ratio of 0.51 (95% CI: 0.37, 0.63) of smoking on preeclampsia[18]. Not many studies assessed the trimester specific effects of smoking and the risk of preeclampsia. England *et al.* conclude that the evidence whether stopping before pregnancy or in first trimester reduces the risk of preeclampsia is still inconclusive[20]. Based on an in vitro experiment, the mechanism leading from smoking to decreased risk of preeclampsia may be that cigarette smoke reduces fms-like tyrosine kinase-1 (sFlt-1) and increases placental growth factor (PIGF), which are both associated with increased risks of adverse birth outcomes[44]. A recent study of Tranquilli *et al.* hypothesized that pre-conceptional smoking may lead to lower vascularised endometrial tissue, which gives a relatively hypoxic environment. As a response, reactive trophoblast invasion would be more effective, and lead to more angiogenesis, which subsequently leads to lower risk of preeclampsia among periconceptionally smoking mothers[45]. Our findings suggest that the blood pressure patterns among smoking women do not explain the associations between smoking and the risk of preeclampsia. The lower mid-pregnancy diastolic blood pressure among first trimester only and continued smokers is in line with the previously demonstrated association between smoking and a lower risk of preeclampsia. Both mid-diastolic blood pressure and the risk of preeclampsia are lower among women who smoke during pregnancy[13-14,18,20].

Conclusion

Our results suggest that both first trimester only and continued smoking are associated with persistent maternal cardiovascular adaptations during pregnancy. Strategies for prevention of smoking during pregnancy should be focused on the preconception period. The different effects of early and late pregnancy smoking for the risk of preeclampsia should be further explored.

REFERENCES

1. Roberts JM, Lain KY. Recent Insights into the pathogenesis of pre-eclampsia. Placenta. 2002;23:359-72.
2. Zhang J, Zeisler J, Hatch MC, et al. Epidemiology of pregnancy-induced hypertension. Epidemiologic reviews. 1997;19:218-32.
3. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA. 2002;287:3183-6.
4. Hernandez-Diaz S, Toh S, Cnattingius S. Risk of pre-eclampsia in first and subsequent pregnancies: prospective cohort study. BMJ 2009;338:b2255.
5. Kaaja R. Predictors and risk factors of pre-eclampsia. Minerva ginecol. 2008;60:421-9.
6. Magnussen EB, Vatten LJ, Lund-Nilsen TI, et al. Prepregnancy cardiovascular risk factors as predictors of pre-eclampsia: population based cohort study. BMJ 2007;335:978.
7. Poon LC, Kametas NA, Chelemen T, et al. Maternal risk factors for hypertensive disorders in pregnancy: a multivariate approach. J Hum Hypertens. 2009.
8. Ros HS, Cnattingius S, Lipworth L. Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study. Am J Epidemiol. 1998;147:1062-70.
9. Jaddoe VW, Troe EJ, Hofman A, 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;22:162-71.
10. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the Generation R Study. Am J Epidemiol. 2007;165:1207-15.
11. Pipkin FB. Smoking in moderate/severe preeclampsia worsens pregnancy outcome, but smoking cessation limits the damage. Hypertens. 2008;51:1042-6.
12. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gynecol. 2001;97:261-7.
13. Conde-Agudelo A, Althabe F, Belizan JM, et al. Cigarette smoking during pregnancy and risk of preeclampsia: a systematic review. Am J Obstet Gyneacol. 1999;181:1026-35.
14. England LJ, Levine RJ, Qian C, et al. Smoking before pregnancy and risk of gestational hypertension and preeclampsia. Am J Obstet Gyneacol. 2002;186:1035-40.
15. Marcoux S, Brisson J, Fabia J. The effect of cigarette smoking on the risk of preeclampsia and gestational hypertension. Am J Epidemiol. 1989;130:950-7.
16. Ness RB, Zhang J, Bass D, et al. Interactions between smoking and weight in pregnancies complicated by preeclampsia and small-for-gestational-age birth. Am J Epidemiol. 2008;168:427-33.
17. Zhang J, Klebanoff MA, Levine RJ, et al. The puzzling association between smoking and hypertension during pregnancy. Am J Obstet Gyneacol. 1999;181:1407-13.
18. Castles A, Adams EK, Melvin CL, et al. Effects of smoking during pregnancy. Five meta-analyses. Am J Prev Med. 1999;16:208-15.
19. Duffus GM, MacGillivray I. The incidence of pre-eclamptic toxaemia in smokers and non-smokers. Lancet. 1968;1:994-5.
20. England L, Zhang J. Smoking and risk of preeclampsia: a systematic review. Front Biosci. 2007;12:2471-83.
21. Palmgren B, Wahlen T, Wallander B. Toxaemia and cigarette smoking during pregnancy. Prospective consecutive investigation of 3,927 pregnancies. Acta Obstet Gyneacol Scand. 1973;52:183-5.
22. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
23. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
24. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. J Post Med. 2002;48:206-8.
25. El Assaad MA, Topouchian JA, Darne BM, et al. Validation of the Omron HEM-907 device for blood pressure measurement. Blood pressure monitoring. 2002;7:237-41.

26. Silva LM, Steegers EA, Burdorf A, et al. No midpregnancy fall in diastolic blood pressure in women with a low educational level: the Generation R Study. Hypertens. 2008;52:645-51.
27. Goldstein H. Multilevel statistical methods. 2nd ed. London: Edward Arnold. 1995.
28. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28:964-74.
29. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
30. Klebanoff MA, Levine RJ, Morris CD, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol. 2001;15:140-3.
31. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol. 1988;41:483-9.
32. Wang X, Tager IB, Van Vunakis H, et al. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol. 1997;26:978-88.
33. English PB, Eskenazi B, Christianson RE. Black-white differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. Am J Public Health. 1994;84:1439-43.
34. Haddow JE, Knight GJ, Palomaki GE, et al. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gyneacol. 1987;94:678-81.
35. Matkin CC, Britton J, Samuels S, et al. Smoking and blood pressure patterns in normotensive pregnant women. Paediatr Perinat Epidemiol. 1999;13:22-34.
36. Strevens H, Wide-Swensson D, Ingemarsson I. Blood pressure during pregnancy in a Swedish population; impact of parity. Acta Obstet Gyneacol Scand. 2001;80:824-9.
37. Duvekot JJ, Cheriex EC, Pieters FA, et al. Early pregnancy changes in hemodynamics and volume homeostasis are consecutive adjustments triggered by a primary fall in systemic vascular tone. Am J Obstet Gyneacol. 1993; 169:1382-92.
38. Robson SC, Hunter S, Boys RJ, et al. Serial study of factors influencing changes in cardiac output during human pregnancy. Am J Physiol. 1989;256:H1060-5.
39. Curran-Everett D, Morris KG, Jr., Moore LG. Regional circulatory contributions to increased systemic vascular conductance of pregnancy. Am J Physiol. 1991;261:H1842-7.
40. Knock GA, Poston L. Bradykinin-mediated relaxation of isolated maternal resistance arteries in normal pregnancy and preeclampsia. Am J Obstet Gyneacol. 1996;175:1668-74.
41. Weiner CP, Thompson LP. Nitric oxide and pregnancy. Seminars in perinatology. 1997;21:367-80.
42. Edouard DA, Pannier BM, London GM, et al. Venous and arterial behavior during normal pregnancy. Am J Physiol. 1998;274:H1605-12.
43. Alstad KS. Effect of thiocyanate on basal blood pressure. BMJ. 1948;1:250.
44. Mehendale R, Hibbard J, Fazleabas A, et al. Placental angiogenesis markers sFlt-1 and PlGF: response to cigarette smoke. Am J Obstet Gyneacol. 2007;197:363 e1-5.
45. Tranquilli AL, Landi B. The origin of pre-eclampsia: From decidual "hyperoxia" to late hypoxia. Med Hypotheses. 2010;75:38-46.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Associations of smoking habits during pregnancy and longitudinally measured blood pressure levels.

Systolic blood pressure		**β[1]**	**95% CI**	**p-value**
Total dataset (n=7106)				
Smoking habits	None	Reference		
	First trimester only	0.11	0.05, 0.17	<0.01
	Continued	0.10	0.05, 0.14	<0.01
Complete cases (n=5063)				
Smoking habits	None	Reference		
	First trimester only	0.11	0.05, 0.18	<0.01
	Continued	0.09	0.04, 0.14	<0.01
Diastolic blood pressure		**β[1]**	**95% CI**	**p-value**
Total dataset (n=7106)				
Smoking habits	None	Reference		
	First trimester only	0.10	0.05, 0.15	<0.01
	Continued	0.10	0.06, 0.14	<0.01
Complete cases (n=5063)				
Smoking habits	None	Reference		
	First trimester only	0.09396	0.04, 0.15	<0.01
	Continued	0.09706	0.06, 0.14	<0.01

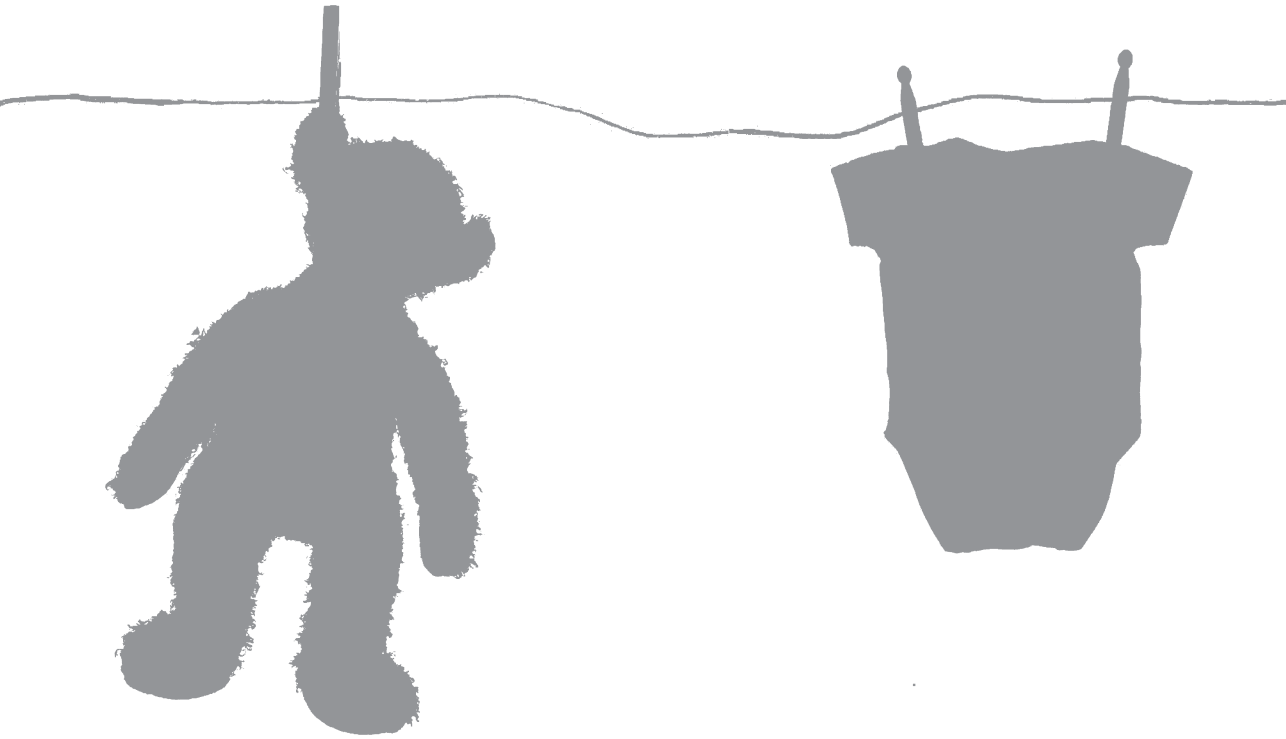
[1]Values are based on repeated non-linear regression models and reflect the change in blood pressure in mm Hg per smoking category with non-smokers as reference group.

Chapter 3.4

Caffeine intake and hypertensive pregnancy complications

Rachel Bakker
Eric AP Steegers
Hein Raat
Albert Hofman
Vincent VW Jaddoe

Adapted from Am J Hypertens. 2010;24:421-8.



ABSTRACT

Caffeine intake has been suggested to be associated with the risk of hypertension. Less is known about the associations of caffeine intake on maternal cardiovascular adaptations during pregnancy. We examined the associations of caffeine intake in different trimesters of pregnancy with repeatedly measured blood pressure and the risks of pregnancy-induced hypertension and preeclampsia in a population-based cohort of 7890 pregnant women. In each trimester caffeine intake and systolic and diastolic blood pressure were assessed by questionnaires and physical examinations, respectively. Information about hypertensive complications was obtained from medical records. Our longitudinal analyses revealed no significant differences for both systolic and diastolic blood pressure. The cross-sectional analyses showed that higher caffeine intake tended to be associated with higher systolic blood pressure in first and third trimester (P_{trend}<0.05), but not in second trimester. Caffeine intake was not consistently associated with diastolic blood pressure levels, or the risk of pregnancy-induced hypertension. As compared to women with caffeine intake of less than 2 units per day, those using 2 to 3.9 units per day had a lower risk of preeclampsia (odds ratio, 0.63 (95% confidence interval: 0.40, 0.96)). Higher caffeine intake during pregnancy seems to be associated with elevated systolic blood pressure levels in first and third trimester, but not with diastolic blood pressure levels. We did not find evidence of significant adverse associations of caffeine intake on maternal cardiovascular adaptations during pregnancy. The unexpected finding of a possible protective association with moderate caffeine intake deserves further investigation.

INTRODUCTION

Caffeine is a frequently used and accepted pharmacologically active substance[1]. Exposure to caffeine is mainly through coffee and tea consumption[2]. Caffeine intake acutely increases blood pressure levels. Habitual caffeine consumption may be associated with chronic blood pressure levels or the risk of hypertension in non-pregnant adults, but results seem inconsistent. Even protective effects of caffeine intake and blood pressure levels have been found previously[3-5]. The mechanisms by which caffeine exposure affect heart rate and blood pressure levels might include increases of catecholamine levels, which might subsequently lead to vasoconstriction[6-7]. Previous studies showed that higher levels of caffeine intake during pregnancy are associated with fetal growth retardation and fetal death, which might both be the result of early vascular placental adaptations[8-10]. However, it is not known whether caffeine intake also affects blood pressure during pregnancy. Differences in blood pressure levels associated with caffeine intake during pregnancy might be markers of subclinical cardiovascular adaptation mechanisms and the subsequent risk of hypertensive complications. Increased blood pressure during pregnancy might lead to hypertensive disorders, such as pregnancy-induced hypertension and preeclampsia, which are leading causes of maternal morbidity during pregnancy and neonatal complications[11-12]. Preeclampsia occurs in approximately 5% of all pregnant women. Another 6% of pregnancies is complicated by pregnancy-induced hypertension[13].

Therefore, we examined the associations of habitual caffeine intake in different periods of pregnancy with repeatedly measured blood pressure levels and the risks of pregnancy-induced hypertension and preeclampsia in a population-based prospective cohort study among 7890 pregnant women.

METHODS

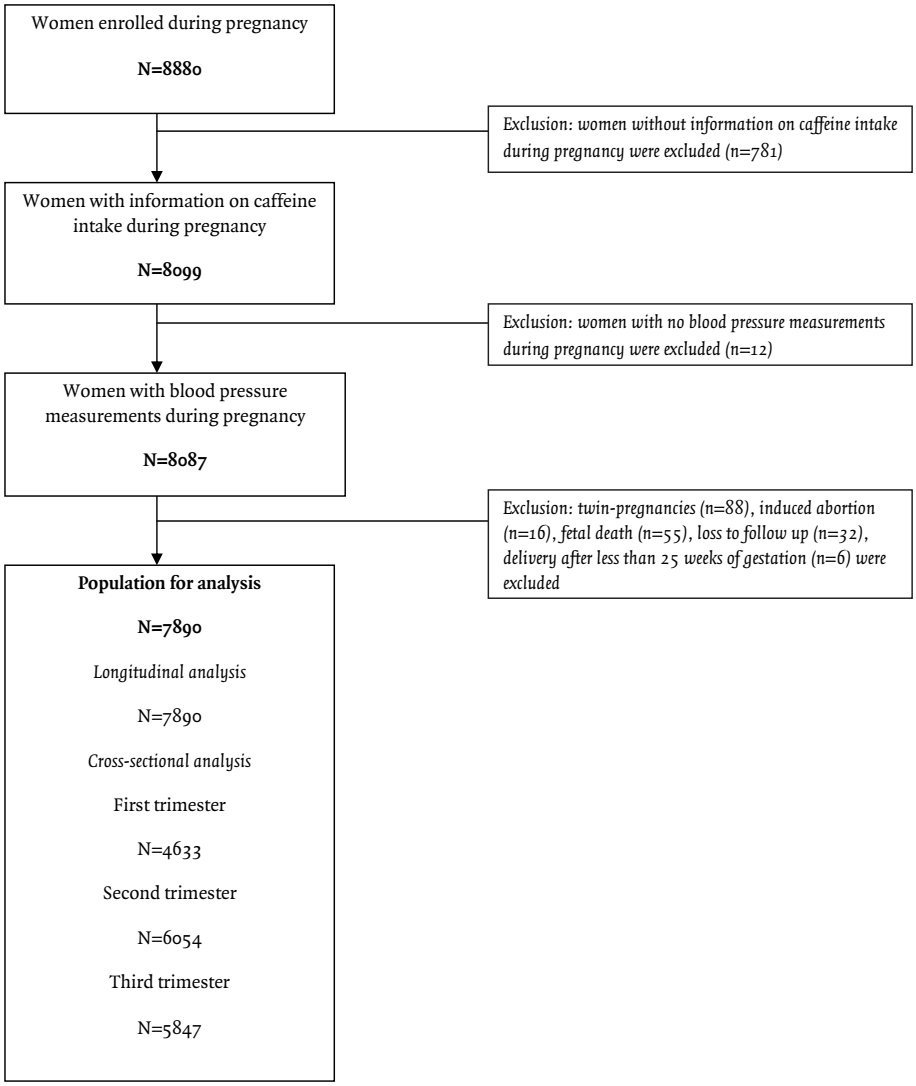
Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards. The cohort includes 9778 women and their children in Rotterdam, the Netherlands. Details have been described elsewhere[14-15]. All pregnant women were enrolled between 2001 and 2005, and all children were born between April 2002 and January 2006. Of all eligible children in the study area, 61% participated at birth[15]. The Medical Ethical Committee of the Erasmus Medical Center in Rotterdam approved the study (MEC 198.782/2001/31). Written consent was obtained from all participating parents. Enrolment was aimed in early pregnancy (gestational age <18.0 weeks) at the routine fetal ultrasound examination but was allowed until birth of the child. In total, 6691 women were enrolled before a gestational age of 18 weeks. Assessments in pregnancy were planned in first, second and third trimester. The individual timing of the assessment depended on the gestational age at enrolment[14-15].

Population for analysis

In total, 8880 women were enrolled during pregnancy. For this analysis, we excluded women without information on caffeine intake in any period of pregnancy (n = 781), and women without blood pressure measurements during pregnancy (n = 12) leading to 8087 women. We subsequently excluded twin-pregnancies (n = 88), induced abortions (n = 16), fetal deaths (n = 55), women who were loss-to-follow-up (n = 32), and deliveries after less than 25 weeks of gestation (n = 6). Similar results were found after including fetal death in the analysis (Supplementary Tables S3 and S4). Thus, the cohort for analysis comprised 7890 women (89% of 8880) (Figure 1). Of all

FIGURE 1. Flow chart of participants included for analysis



pregnancies, 5.9% were second (n = 461) or third (n = 8) pregnancies in the study. Since there were no differences in results after exclusion of these women, they were included in the analyses.

Caffeine intake

Information about maternal caffeine intake was obtained by postal questionnaires in each trimester of pregnancy. Response rates for these questionnaires were 91%, 80%, and 77%, respectively[15]. Women who reported any coffee or tea drinking were asked to categorize their average number of cups of coffee or tea per day, and what type of coffee or tea they consumed (caffeinated, caffeinated and decaffeinated, or decaffeinated). According to standard values for caffeine content, a regular coffee serving (125 ml) in the Netherlands contains about 90 mg of caffeine, decaffeinated coffee contains about 3 mg, and tea contains about 45 mg[16]. To calculate total caffeine intake in each trimester, we weighted the type of coffee or tea (caffeinated coffee=1; caffeinated and decaffeinated coffee=0.5; decaffeinated coffee=0; caffeinated tea=0.5; caffeinated and decaffeinated tea=0.25; decaffeinated tea=0; herbal tea=0; green tea=0.5). Thus, in our analyses each unit of caffeine intake reflects caffeine exposure based on one cup of caffeinated coffee (90 mg caffeine). Daily total caffeine intake was subsequently categorized (less than 2 units; 2 to 3.9 units; 4 to 5.9 units; 6 or more units). Total caffeine intake in first (n = 6062), second (n = 6329), and third (n = 5972) trimester was correlated (Spearman's correlation coefficients ranged from 0.60 to 0.69 (P-value <0.01)). We used the mean caffeine intake during pregnancy to assess the associations with longitudinally measured blood pressure levels and the risks of hypertensive pregnancy complications.

Blood pressure

Blood pressure was measured at our two dedicated research 76 centers in each trimester of pregnancy, with the Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands), which was validated in non-pregnant adults[17]. All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 centimeters (cm) a larger cuff (32~42cm) was used. The mean value of two blood pressure readings over a 60-second interval was documented. In total, blood pressure was measured in 6071 women in first trimester (mean, 13.5 weeks of gestation; range, 4.5-17.9), in 7451 women in second trimester (mean, 20.6 weeks of gestation; range, 18.1-24.9) and 7547 women in third trimester (mean, 30.4 weeks of gestation; range, 25.1-39.2). In total, 21069 blood pressure measurements were collected. Three, two and one blood pressure measurements were available for 5653, 1873, and 364 women, respectively.

Hypertensive pregnancy complications

Information on pregnancy complications was obtained from medical records. Women suspected of pregnancy complications based on these records were cross-checked with the original hospital

charts[18]. The following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia.

Covariates

Information on maternal age at enrolment, highest completed educational level (primary school; secondary school; higher education), ethnicity (European; non-European), parity (nulliparous; multiparous), folic acid supplement use (preconceptional use; first 10 weeks of pregnancy use; no use) was obtained from the first questionnaire at enrolment in the study. Maternal weight (kg) and height (cm) were measured without shoes and heavy clothing at time of enrolment (median gestational age 14.4 weeks (95% range, 10.2-25.3)). Body mass index was subsequently calculated (weight/height^2 (kg/m^2)). We used body mass index measured at enrolment in the analyses, because the numbers of missing values were smaller and data quality higher than values based on questionnaires[19]. Weight at enrolment was strongly correlated with pre-pregnancy weight (Pearson's correlation=0.95, P-value <0.01). Information about alcohol consumption and smoking habits (no; first trimester only; continued) were available from questionnaires. Total daily energy intake was obtained by a food frequency questionnaire at enrolment. Maternal distress was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory, which gives a Global Severity Index; a higher index reflects more stress[20].

Statistical analyses

First, the associations of caffeine intake during pregnancy with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression analyses. These models take into account the correlation between repeated measurements of the same subject, and allow for incomplete outcome data[21]. The best fitting models were constructed using fractional polynomials of gestational age[22]. Mean maternal daily caffeine intake during pregnancy (less than 2 units; 2 to 3.9 units; 4 to 5.9 units; 6 or more units) was included in these models as intercept and as interaction term with gestational age. The models can be written as:

$$\text{Systolic blood pressure} = \beta_0 + \beta_1 * \text{caffeine} + \beta_2 * \text{gestational age} + \beta_3 * \text{gestational age}^{-2} + \beta_4 * \text{caffeine} * \text{gestational age}$$

$$\text{Diastolic blood pressure} = \beta_0 + \beta_1 * \text{caffeine} + \beta_2 * \text{gestational age} + \beta_3 * \text{gestational age}^{0.5} + \beta_4 * \text{caffeine} * \text{gestational age}$$

In these models, '$\beta 0 + \beta 1$ *caffeine' reflects the intercept and '$\beta 2$*gestational age + $\beta 3$*gestational age^{-2}, reflects the slope of change in blood pressure per week for systolic blood pressure, and '$\beta 2$*gestational age + $\beta 3$*gestational age$^{0.5}$, reflects the slope of change in blood pressure per week for diastolic blood pressure. Main interest was in the term '$\beta 4$*caffeine*gestational age', which reflects the difference in change in blood pressure per week between the different caffeine intake categories for either systolic or diastolic blood pressure. Second, the cross-sectional associations of maternal caffeine intake with blood pressure in first, second and third trimester were assessed using multiple linear regression models. Linear regression models in which the caffeine intake categories were included as a continuous variable were considered as test for trend. Third, the associations of maternal caffeine intake with hypertensive pregnancy complications were assessed using multiple logistic regression models. All models were adjusted for gestational age at visit (only in cross-sectional analyses), body mass index, height, maternal age, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and maternal stress. We imputed missing data of the covariates with the mean value for continuous variables, and added a separate category for missing values for categorical variables. The percentages of missing values within the population for analysis were lower than 10%, except for folic acid supplement use (22%), total daily energy intake (24%) and maternal stress (19%). These higher percentages were due the large number of women who were not enrolled in first trimester and therefore did not receive this specific questionnaire. No differences in results were observed between analyses with imputed missing data or complete cases only. The repeated measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS Inc. Gary, NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Maternal characteristics according to their caffeine intake are shown in Table 1. Women with the highest daily caffeine intake (6 or more units) were older and had the highest weight at enrolment. Also, these women were more often of European ethnicity, tended to continue consumption of alcohol and smoking during pregnancy more frequently, had highest percentage of no folic acid supplement use, and the highest stress index score. Women with daily caffeine intake between 4 and 5.9 units were highest educated, taller, and used folic acid supplements preconceptional most often. Mean systolic blood pressure levels in third trimester differed significantly among the caffeine intake categories. Mean diastolic blood pressure levels were similar among all caffeine intake categories. In total, there were 237 cases (3.0%) of pregnancy-induced hypertension and 119 cases (1.5 %) of preeclampsia.

TABLE 1. Maternal characteristics according to caffeine intake during pregnancy[1]

	Caffeine intake during pregnancy (N=7890)				
	<2 units/day n=4833	**2-3.9 units/ day n=2450**	**4-5.9 units/ day n=497**	**≥6 units/day n=110**	**P-value[3]**
Age, yrs	28.9 (5.3)	31.0 (4.8)	31.9 (4.6)	32.1 (4.5)	<0.001
Height, cm	166.6 (7.3)	168.4 (7.2)	169.4 (7.3)	168.0 (7.1)	<0.001
Weight at enrolment, kg	68.9 (13.6)	70.0 (12.3)	71.0 (12.8)	71.5 (12.5)	<0.001
Body mass index at enrolment, kg/m^2	24.8 (4.7)	24.7 (4.2)	24.8 (4.3)	25.4 (4.1)	0.349
Parity, %					<0.001
Nulliparous	59.1	52.2	49.1	41.8	
Multiparous	40.1	47.3	50.5	58.2	
Missing	0.8	0.4	0.4	0.0	
Gestational age at enrolment, wks[2]	14.4 (10.2-24.9)	15.4 (10.2-24.5)	15.6 (10.5-29.7)	14.6 (10.9-29.9)	0.789
Highest completed education, %					<0.001
Primary school	11.6	8.6	7.6	10.0	
Secondary school	47.4	37.5	37.8	53.6	
Higher education	35.0	49.6	51.0	34.6	
Missing	6.0	4.3	3.6	1.8	
Ethnicity, %					<0.001
European	49.2	66.5	75.0	75.5	
Non-European	46.5	30.8	21.8	22.7	
Missing	4.3	2.7	3.2	1.8	
Alcohol consumption, %					<0.001
No	50.0	35.9	32.8	34.5	
First trimester only	12.8	11.0	12.9	8.2	
Continued	27.9	43.4	43.3	50.9	
Missing	9.3	9.7	11.1	6.4	
Smoking habits, %					<0.001
No	70.8	63.1	54.3	39.1	
First trimester only	7.0	8.2	8.2	7.3	
Continued	12.2	18.6	26.0	48.2	
Missing	10.0	0.1	11.5	5.5	
Folic acid supplement use, %					<0.001
Preconception use	28.9	34.7	35.8	23.6	
First 10 weeks	24.2	25.2	25.2	20.9	
No use	23.9	19.8	19.5	28.2	
Missing	23.0	20.3	19.5	27.3	
Total daily energy intake, kcal	1,990 (576)	2,092 (536)	2,203 (545)	2,181 (577)	<0.001
Maternal stress, index[2]	0.19 (0.00-1.46)	0.13 (0.00-1.38)	0.15 (0.00-1.30)	0.21 (0.00-1.58)	<0.001

TABLE 1. (continued)

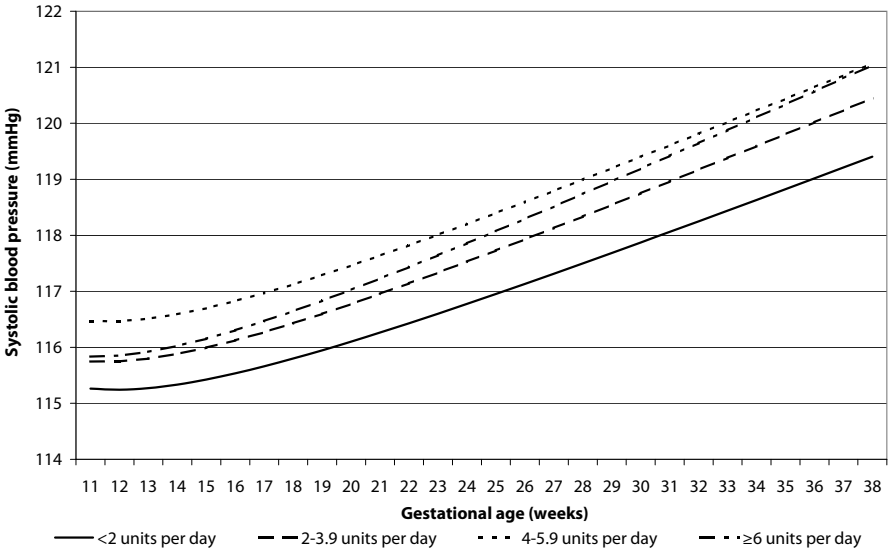
	Caffeine intake during pregnancy (N=7890)				
	<2 units/day n=4833	**2-3.9 units/ day n=2450**	**4-5.9 units/ day n=497**	**≥6 units/day n=110**	**P-value[3]**
First trimester blood pressure					
Gestational age, wks[2]	13.2 (9.7-17.5)	13.2 (9.7-17.7)	13.4 (10.1-17.5)	13.6 (11.1-17.5)	0.423
Systolic blood pressure, mmHg	115.4 (12.4)	115.9 (12.2)	116.8 (12.6)	116.7 (11.6)	0.134
Diastolic blood pressure, mmHg	68.3 (9.7)	68.2 (9.5)	67.7 (9.6)	68.5 (9.6)	0.659
Second trimester blood pressure					
Gestational age, wks[2]	20.4 (18.5-23.8)	20.4 (18.6-23.5)	20.4 (18.6-23.6)	20.5 (18.2-23.9)	0.805
Systolic blood pressure, mmHg	116.4 (12.2)	117.2 (11.9)	117.7 (12.1)	117.5 (11.0)	0.018
Diastolic blood pressure, mmHg	67.3 (9.4)	67.0 (9.2)	66.7 (10.0)	66.0 (9.6)	0.193
Third trimester blood pressure					
Gestational age, wks[2]	30.2 (28.5-32.8)	30.2 (28.4-32.9)	30.2 (28.6-33.1)	30.4 (28.4-31.8)	0.625
Systolic blood pressure, mmHg	117.9 (12.3)	118.7 (11.6)	119.7 (11.1)	119.4 (12.5)	0.002
Diastolic blood pressure, mmHg	69.1 (9.4)	69.0 (9.3)	69.0 (9.1)	68.9 (10.8)	0.937
Pregnancy complications, %					
Preeclampsia	2.2	1.6	3.4	1.0	<0.001
Pregnancy-induced hypertension	4.1	3.7	3.2	3.8	<0.001

[1]Values are means with standard deviations or percentages.
[2]Median (95% range).
[3]Differences in subject characteristics between the age-groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

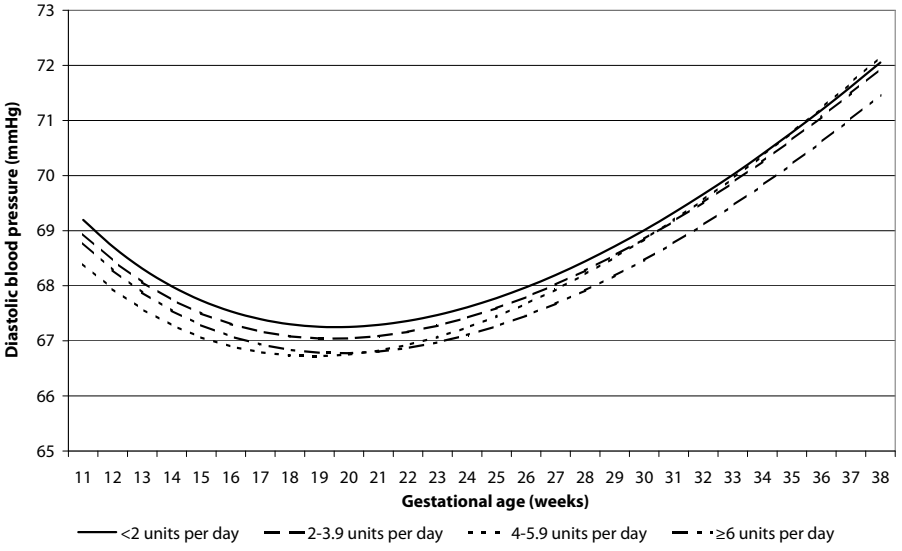
Figure 2a and Figure 2b show the results from our repeated measurement analyses of caffeine intake and systolic and diastolic blood pressure patterns, respectively. As compared to women using less than 2 units per day, we observed no differences in systolic and diastolic blood pressure levels for both the time independent (intercept) and time dependent (change in blood pressure with advancing gestational age) estimates for women who had more caffeine consumptions per day. In the cross-sectional analyses, higher caffeine intake tended to be associated with elevated systolic blood pressure levels in first and third trimester (P_{trend} <0.05), but not in second trimester. We found an increase in systolic blood pressure among the women who consumed between 4 and 5.9 units per day (1.17 mmHg (95% confidence interval (CI): 0.62, 2.81)) in third trimester, compared to women consumed less than 2 units of caffeine per day. Furthermore, no associations or trends were found in the cross-sectional analyses focused on the associations of caffeine intake with diastolic blood pressure (Table 2).

FIGURE 2. Blood pressure patterns in different caffeine intake categories

A. Systolic blood pressure



B. Diastolic blood pressure



Change in systolic blood pressure in mmHg per caffeine intake category with women who consumed less than 2 caffeine containing beverages per day throughout pregnancy as reference group based on repeated measurement analysis (systolic blood pressure = $\beta_0 + \beta_1$*caffeine + β_2*gestational age + β_3*gestational age^{-2} + β_4*caffeine*gestational age and diastolic blood pressure = $\beta_0 + \beta_1$*caffeine + β_2*gestational age + β_3*gestational age$^{0.5}$ + β_4*caffeine*gestational age).

Table 3 shows that we observed no associations of higher caffeine levels with the risk of pregnancy-induced hypertension. As compared to women with caffeine intake of less than 2 units per day, those daily consuming 2 to 3.9 units of caffeine had a lower risk of preeclampsia (Odds Ratio (OR), 0.63 (95% CI: 0.40, 0.96)), but no associations were observed for higher caffeine levels.

TABLE 2. Cross-sectional associations of caffeine intake with systolic and diastolic blood pressure[1]

	Difference in systolic blood pressure (mmHg)[2]		
Caffeine intake	First trimester n=4633	Second trimester n=6054	Third trimester n=5847
<2 units per day	Reference	Reference	Reference
	n=2699	n=3378	n=3124
2-3.9 units per day	0.61 (-0.13, 1.36)	0.33 (-0.29, 0.96)	0.43 (-0.20, 1.06)
	n=1419	n=1981	n=2123
4-5.9 units per day	1.04 (-0.20, 2.28)	0.53 (-0.48, 1.54)	1.71 (0.62, 2.81)*
	n=382	n=554	n=480
≥6 units per day	1.66 (-0.34, 3.66)	-0.47 (-2.34, 1.40)	0.49 (-1.56, 2.53)
	n=133	n=141	n=120
Trend[3]	0.55 (-0.11, 0.96)	0.15 (-0.23, 0.53)	0.54 (0.13, 0.95)
	P=0.014	P=0.441	P=0.009
	Difference in diastolic blood pressure (mmHg)[2]		
Caffeine intake	First trimester n=4633	Second trimester n=6054	Third trimester n=5847
<2 units per day	Reference	Reference	Reference
	n=2699	n=3378	n=3124
2-3.9 units per day	0.31 (-0.27, 0.90)	-0.12 (-0.62, 0.38)	-0.37 (-0.87, 0.13)
	n=1419	n=1981	n=2123
4-5.9 units per day	-0.18 (-1.15, 0.80)	-0.26 (-1.06, 0.54)	-0.45 (-1.32, 0.42)
	n=382	n=554	n=480
≥6 units per day	-0.82 (-2.39, 0.76)	-1.41 (-2.89, 0.07)	0.05 (-1.57, 1.67)
	n=133	n=141	n=120
Trend[3]	-0.07 (-0.41, 0.28)	-0.23 (-0.53, 0.07)	-0.19 (-0.52, 0.13)
	P=0.711	P=0.131	P=0.238

[1]Values are regression coefficients with 95% confidence interval and reflect the difference in blood pressure in mmHg compared to women who did not consume caffeine containing beverages during that particular trimester.
[2]Models are adjusted for gestational age at visit, body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value <0.01.

TABLE 3. Associations of maternal caffeine intake with preeclampsia and pregnancy-induced hypertension[1]

Caffeine intake	Pregnancy-induced hypertension[2] n=237	Preeclampsia[2] n=119	Pregnancy-induced hypertension and preeclampsia[2] n=356
< 2 units per day	Reference	Reference	Reference
	n=130	n=75	n=205
2-3.9 units per day	0.87 (0.65, 1.17)	0.63 (0.40, 0.96)*	0.79 (0.61, 1.01)
	n=81	n=31	n=112
4-5.9 units per day	1.04 (0.63, 1.69)	1.02 (0.51, 2.03)	1.03 (0.69, 1.55)
	n=21	n=10	n=31
≥6 units per day	0.79 (0.31, 2.03)	1.04 (0.32, 3.42)	0.87 (0.41, 1.85)
	n=5	n=3	n=8
Trend[3]	0.95 (0.79, 1.15)	0.88 (0.67, 1.17)	0.93 (0.80, 1.09)
	P=0.593	P=0.379	P=0.382

[1]Values are odds ratios with 95% confidence interval and reflect the difference in risk of pregnancy complications compared to women who did not consume caffeine containing beverages.
[2]Models are adjusted for body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple logistic regression models with caffeine intake as a continuous variable.
*P-value <0.05.

We observed similar results after repeating the analyses with categories of no caffeine intake as reference group, instead of less than 2 units of caffeine per day (Supplementary Tables S1 and S2).

DISCUSSION

Findings from this study suggest that higher caffeine intake during pregnancy is associated with higher systolic blood pressure levels in first and third trimester. Caffeine intake does not seem to be significantly associated with diastolic blood pressure levels. As compared to women with caffeine intake of less than 2 units per day, those with higher caffeine intake had no increased risks of pregnancy-induced hypertension or preeclampsia.

Methodological considerations

One of the strengths of this study was the prospective data collection, which started in early pregnancy. In addition, we had a large sample size of 7890 participants with 21069 blood pressure measurements. The response rate of the study was 61%. Pregnant women who participated were higher educated, more healthy and more frequently of European origin than those who did not

participate[15]. This might have led to some selection. However, selection bias in prospective follow-up studies primary arises from loss to follow up instead of non-response at baseline[23]. Among mothers with information about coffee and tea consumption, we had a limited loss-to-follow-up.

However, the non-response at baseline might explain the small numbers of pregnancy-induced hypertension and preeclampsia cases. Another potential limitation of our study might be the missing data on coffee and tea consumption. The associations may be underestimated if among mothers without caffeine data the percentage of consumers was higher than among mothers without missing data. However, this seems unlikely since no other differences in characteristics between mothers with and without information about coffee and tea consumption were observed. Information on coffee and tea consumption during pregnancy was collected by postal questionnaires. If any, misclassification would most likely be due to underreporting and subsequently lead to underestimation of differences between dosages of caffeine intake[24]. The questionnaires used to measure coffee and tea consumption was not validated in our study, which is another possible limitation of our study. However, many previous studies have used similar questions to assess coffee and tea consumption in their participants. Also, self reported coffee and tea consumption can be validated by comparison with biological samples or a 24 hour recall method. James *et al.* reported reliability of self-reported caffeine consumption by analysing biological samples of their subjects on caffeine metabolites[25]. Finally, we had information about a wide range of potential confounding factors available, but because of the observational design, residual confounding due to socio-demographic and lifestyle factors might still be an issue. Women with high caffeine intake also were more often smokers and had higher alcohol consumption; therefore, differences in lifestyle habits between the caffeine groups can be expected.

Caffeine intake during pregnancy

We have very detailed information about caffeine consumption for coffee and tea. However, caffeine intake from other sources may also be relevant. It has previously been estimated that in adults, coffee and tea consumption comprises 96%[26-27]. Other sources of caffeine (4%) can be found in cacao, chocolate, soft drinks, and caffeine containing medication. Women without tea or coffee consumption, but using large amounts of caffeine intake might be underestimated for the exposure. Although consumption of caffeinated soft drinks is increasing, analyzing only coffee and tea consumption in this study seems sufficient in assessing the effect of caffeine on blood pressure levels during pregnancy[1,23]. We categorized caffeine intake in units instead of calculating the exact milligrams of caffeine per day. Current practice is to advise pregnant women to not consume more than 300 milligrams caffeine. This guideline is based on studies focused on the effects of caffeine consumption on fetal outcomes[9,28]. Accordingly, we should acknowledge the possibility that low intake of caffeine may be a marker of health conscious lifestyle of the participating women. Previous studies showed that higher levels of caffeine intake during pregnancy are associated with fetal growth retardation and fetal death, which might both be the result of early adverse vascular placental adaptations[8-10]. The highest category of caffeine intake in our study (6

or more units) should be considered similar as caffeine intake of more than 540 mg per day. The amount of caffeine per coffee serving was estimated on 90 mg[16]. However, calculations of caffeine intake should be interpreted carefully and might be country specific. European coffee is typically stronger than coffee in the Unites States[1]. A standard coffee serving in the United States contains about 70 mg of caffeine.

Caffeine intake, blood pressure and hypertensive complications

Previous studies have examined the associations between caffeine intake and blood pressure levels and the risk of hypertension[4,29-31]. Thus far, results seem inconsistent. To our knowledge, the effect of caffeine intake during pregnancy on blood pressure levels has not been studied before in a population-based sample. Examining blood pressure levels in different trimester enabled us to identify specific critical periods during pregnancy for caffeine exposure. We found positive associations between caffeine intake and systolic blood pressure levels in first and third trimester of pregnancy. We did not find significant trends in our longitudinal analysis. Also, caffeine intake was not associated with diastolic blood pressure levels. No associations between higher caffeine intake and increased risks of pregnancy-induced hypertension or preeclampsia were observed. Ruder *et al.* suggested that women should limit their caffeine intake to promote fertility and to limit oxidative stress, which might lead to pregnancy complications such as preeclampsia[32]. However, to our knowledge no other studies observed associations of caffeine intake with hypertensive pregnancy complications. We observed a decreased risk of preeclampsia among women who consumed 2 to 3.9 units of caffeine per day. However, this finding was not consistent with the other categories or with previous studies. We cannot explain this lower risk. Residual confounding, due to insufficient or unmeasured lifestyle habits, might be involved. Further studies are needed in larger sample sizes. The mechanisms underlying the associations between caffeine intake and blood pressure during pregnancy are not known. During pregnancy vasodilatation and uteroplacental circulation lead to decreased total peripheral vascular resistance from early pregnancy onwards, with a mid-pregnancy fall in diastolic blood pressure[33-34]. Mechanisms leading to this vasodilatation are not yet completely understood, but previous findings suggest a decreased vascular responsiveness to the pressor effects of angiotensin II and norepinephrine, increased endothelial prostacyclin, enhanced nitric oxide production, and reduced aortic stiffness[35-38]. On the contrary, caffeine increases vasoconstriction, due to increased catecholamine levels[6-7]. This might partly explain the associations in first and third trimester. Furthermore, Apostolakis *et al.* emphasized the importance and difficulties of identifying biomarkers for hypertensive disorders developed during pregnancy. However, they concluded that even though a biomarker whose effect in disease probability is not as strong, it may still give important information on the underlying disease pathophysiology, or help to identify new therapeutic targets[39]. Further studies are needed focused on these underlying mechanisms. In our study, we only used information about maternal blood pressure as measure of cardiovascular adaptation during pregnancy. Studies with information about more maternal or placental cardiovascular measures, such as arterial stiffness, cardiac

function, and placental blood flow might give important additional information. In addition, for this study we focused on caffeine intake during pregnancy based on coffee and tea consumption. However, we assess whether the association are only explained by caffeine intake. Coffee and tea contain other chemical substances which may influence blood pressure levels. Research into the metabolites involved in pathways leading from coffee and tea intake to pregnancy complications is needed.

Conclusion

In conclusion, our results suggest that caffeine intake during pregnancy seems to be associated with higher systolic blood pressure levels in first and third trimester, but not with diastolic blood pressure levels. We did not find evidence of significant adverse associations of caffeine intake on maternal cardiovascular adaptations during pregnancy. The unexpected finding of a possible protective association with moderate caffeine intake deserves further investigation.

REFERENCES

1. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol. 1996;34:119-29.
2. Knight CA, Knight I, Mitchell DC, et al. Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. Food Chem Toxicol. 2004;42:1923-30.
3. Klag MJ, Wang NY, Meoni LA, et al. Coffee intake and risk of hypertension: the Johns Hopkins precursors study. Arch Intern Med. 2002;162:657-62.
4. Winkelmayer WC, Stampfer MJ, Willett WC, et al. Habitual caffeine intake and the risk of hypertension in women. JAMA. 2005;294:2330-5.
5. Noordzij M, Uiterwaal CS, Arends LR, et al. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. J Hypertens. 2005;23:921-8.
6. Smith A, Brice C, Nash J, Rich N, Nutt DJ. Caffeine and central noradrenaline: effects on mood, cognitive performance, eye movements and cardiovascular function. J Psychopharmocol. 2003;17:283-92.
7. Robertson D, Frolich JC, Carr RK, et al. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med. 1978;298:181-6.
8. Bakker R, Steegers EA, Obradov A, et al. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: the Generation R Study. Am J Clin Nutr. 2010;91:1691-8.
9. Cnattingius S, Signorello LB, Anneren G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med. 2000;343:1839-45.
10. Group CS. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ 2008;337:a2332.
11. Roberts JM, Lain KY. Recent Insights into the pathogenesis of pre-eclampsia. Placenta. 2002;23:359-72.
12. Zhang J, Zeisler J, Hatch MC, et al. Epidemiology of pregnancy-induced hypertension. Epidemiol Rev. 1997;19: 218-32.
13. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA. 2002;287:3183-6.
14. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
15. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
16. NEVO-tabel 2006. [Dutch Food Composition Table 2006.] The Hague, Netherlands: Voedingscentrum; [Netherlands Nutrition Center] 2006 (in Dutch).
17. El Assaad MA, Topouchian JA, Darne BM, et al. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Mon. 2002;7:237-41.
18. Silva LM, Steegers EA, Burdorf A, et al. No midpregnancy fall in diastolic blood pressure in women with a low educational level: the Generation R Study. Hypertens. 2008;52:645-51.
19. Ay L, Kruithof CJ, Bakker R, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG. 2009;116:953-63.
20. Derogatis LR. Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition. Minneapolis, MN. 1993.
21. Goldstein H. Multilevel statistical methods. 2nd ed. London: Edward Arnold. 1995.
22. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28:964-74.
23. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
24. Bracken MB, Triche E, Grosso L, et al. Heterogeneity in assessing self-reports of caffeine exposure: implications for studies of health effects. Epidemiology. 2002;13:165-71.
25. James JE, Paull I, Cameron-Traub E, et al. Biochemical validation of self-reported caffeine consumption during caffeine fading. J Behav Med. 1988;11:15-30.

26. Clausson B, Granath F, Ekbom A, et al. Effect of caffeine exposure during pregnancy on birth weight and gestational age. Am J Epidemiol. 2002;155:429-36.
27. International coffee organization (www.ico.org).Historical data sheets/Consumption 2000 to date; http://www.ico.org/historical/2000+/PDF/CONSUMPTION.pdf
28. Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gyneacol. 2008;198:279 e1-8.
29. Geleijnse JM. Habitual coffee consumption and blood pressure: an epidemiological perspective. Vasc Health Risk Manag. 2008;4:963-70.
30. Palatini P, Dorigatti F, Santonastaso M, et al. Association between coffee consumption and risk of hypertension. Ann Med. 2007;39:545-53.
31. Uiterwaal CS, Verschuren WM, Bueno-de-Mesquita HB, et al. Coffee intake and incidence of hypertension. Am J Clin Nutr. 2007;85:718-23.
32. Ruder EH, Hartman TJ, Goldman MB. Impact of oxidative stress on female fertility. Curr Opin Obstet Gynecol. 2009;21:219-22.
33. Duvekot JJ, Cheriex EC, Pieters FA, et al. Early pregnancy changes in hemodynamics and volume homeostasis are consecutive adjustments triggered by a primary fall in systemic vascular tone. Am J Obstet Gyneacol. 1993;169:1382-92.
34. Robson SC, Hunter S, Boys RJ, et al. Serial study of factors influencing changes in cardiac output during human pregnancy. Am J Physiolog. 1989;256:H1060-5.
35. Curran-Everett D, Morris KG, Jr., Moore LG. Regional circulatory contributions to increased systemic vascular conductance of pregnancy. Am J Physiolog. 1991;261:H1842-7.
36. Knock GA, Poston L. Bradykinin-mediated relaxation of isolated maternal resistance arteries in normal pregnancy and preeclampsia. Am J Obstet Gyneacol. 1996;175:1668-74.
37. Weiner CP, Thompson LP. Nitric oxide and pregnancy. Seminars in perinatology. 1997;21:367-80.
38. Edouard DA, Pannier BM, London GM, et al. Venous and arterial behavior during normal pregnancy. Am J Physiolog. 1998;274:H1605-12.
39. Apostolakis S, Shantsila E, Lip GY. Angiopoietins and preeclampsia: new perspectives in the quest for markers. Am J Hypertens. 2009;22:820.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Cross-sectional associations of caffeine intake with systolic and diastolic blood pressure[1]

Caffeine intake	Difference in systolic blood pressure (mmHg)[2]		
	First trimester n=4633	Second trimester n=6054	Third trimester n=5847
None	Reference	Reference	Reference
	n=265	n=485	n=260
>0-1.9 units per day	0.95 (-0.50, 2.40)	-1.18 (-2.24, -0.11)*	0.25 (-1.17, 1.68)
	n=2434	n=2893	n=2864
2-3.9 units per day	1.48 (-0.04, 2.99)	-0.67 (-1.77, 0.43)	0.66 (-0.80, 2.13)
	n=1419	n=1981	n=2123
4-5.9 units per day	1.91 (0.10, 3.72)*	-0.48 (-1.84, 0.88)	1.95 (0.23, 3.67)*
	n=382	n=554	n=480
≥6 units per day	2.53 (0.13, 4.92)*	-1.48 (-3.56, 0.60)	0.72 (-1.72, 3.16)
	n=133	n=141	n=120
Trend[3]	0.56 (0.16, 0.97)	0.02 (-0.32, 0.35)	0.50 (0.12, 0.88)
	P=0.006	P=0.929	P=0.010
Caffeine intake	**Difference in diastolic blood pressure (mmHg)[2]**		
	First trimester n=4633	Second trimester n=6054	Third trimester n=5847
None	Reference	Reference	Reference
	n=265	n=485	n=260
>0-1.9 units per day	0.19 (-0.95, 1.34)	-0.44 (-1.29, 0.40)	-0.01 (-1.14, 1.11)
	n=2434	n=2893	n=2864
2-3.9 units per day	0.49 (-0.70, 1.68)	-0.50 (-1.37, 0.38)	-0.39 (-1.55, 0.78)
	n=1419	n=1981	n=2123
4-5.9 units per day	0.00 (-1.43, 1.43)	-0.64 (-1.72, 0.44)	-0.46 (-1.83, 0.90)
	n=382	n=554	n=480
≥6 units per day	-0.64 (-2.53, 1.25)	-1.79 (-3.44, -0.14)*	0.04 (-1.89, 1.97)
	n=133	n=141	n=120
Trend[3]	-0.04 (-0.35, 0.28)	-0.23 (-0.50, 0.03)	-0.18 (-0.48, 0.12)
	P=0.829	P=0.085	P=0.243

[1]Values are regression coefficients with 95% confidence interval and reflect the difference in blood pressure in mmHg compared to women who did not consume caffeine containing beverages during that particular trimester.
[2]Models are adjusted for gestational age at visit, body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value<0.01

SUPPLEMENTARY TABLE S2. Associations of maternal caffeine intake with preeclampsia and pregnancy-induced hypertension[1]

Caffeine intake	Pregnancy-induced hypertension[2] n=237	Preeclampsia[2] n=119	Pregnancy-induced hypertension and preeclampsia[2] n=356
None	Reference	Reference	Reference
	n=8	n=7	n=15
>0-1.9 units per day	1.51 (0.71, 3.19)	1.06 (0.47, 2.39)	1.25 (0.71, 2.19)
	n=122	n=68	n=190
2-3.9 units per day	1.28 (0.59, 2.76)	0.66 (0.28, 1.56)	0.97 (0.54, 1.73)
	n=81	n=31	n=112
4-5.9 units per day	1.52 (0.64, 3.63)	1.08 (0.39, 2.99)	1.27 (0.65, 2.49)
	n=21	n=10	n=31
≥6 units per day	1.17 (0.36, 3.79)	1.10 (0.27, 4.51)	1.08 (0.43, 2.70)
	n=5	n=3	n=8
Trend[3]	0.98 (0.82, 1.16)	0.90 (0.70, 1.15)	0.95 (0.82, 1.10)
	P=0.803	P=0.393	P=0.502

[1]Values are odds ratios with 95% confidence interval and reflect the difference in risk of pregnancy complications compared to women who did not consume caffeine containing beverages.
[2]Models are adjusted for body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple logistic regression models with caffeine intake as a continuous variable.

SUPPLEMENTARY TABLE S3. Cross-sectional associations of caffeine intake with systolic and diastolic blood pressure[1]

	Difference in systolic blood pressure (mmHg)[2]		
Caffeine intake	First trimester n=4683	Second trimester n=6065	Third trimester n=5852
<2 units per day	Reference	Reference	Reference
	n=2731	n=3385	n=3127
2-3.9 units per day	0.67 (-0.08, 1.41)	0.31 (-0.32, 0.93)	0.45 (-0.18, 1.08)
	n=1434	n=1985	n=2125
4-5.9 units per day	0.99 (-0.25, 2.22)	0.50 (-0.51, 1.52)	1.72 (0.62, 2.81)*
	n=383	n=554	n=480
≥6 units per day	1.67 (-0.32, 3.65)	-0.48 (-2.35, 1.39)	0.50 (-1.54, 2.55)
	n=135	n=141	n=120
Trend[3]	0.56 (0.12, 1.00)	0.14 (-0.24, 0.52)	0.55 (0.14, 0.96)
	P=0.013	P=0.480	P=0.008

SUPPLEMENTARY TABLE S3. (continued)

	Difference in diastolic blood pressure (mmHg)[2]		
Caffeine intake	First trimester n=4683	Second trimester n=6065	Third trimester n=5852
<2 units per day	Reference	Reference	Reference
	n=2731	n=3385	n=3127
2-3.9 units per day	0.35 (-0.24, 0.93)	-0.12 (-0.62, 0.37)	-0.34 (-0.84, 0.16)
	n=1434	n=1985	n=2125
4-5.9 units per day	-0.20 (-1.18, 0.77)	-0.27 (-1.07, 0.54)	-0.44 (-1.31, 0.42)
	n=383	n=554	n=480
≥6 units per day	-0.92 (-2.48, 0.65)	-1.41 (-2.89, 0.08)	0.07 (-1.56, 1.69)
	n=135	n=141	n=120
Trend[3]	-0.07 (-0.42, 0.27)	-0.24 (-0.54, 0.07)	-0.18 (-0.51, 0.14)
	P=0.667	P=0.128	P=0.264

[1]Values are regression coefficients with 95% confidence interval and reflect the difference in blood pressure in mmHg compared to women who consumed less than 2 units of caffeine per day during that particular trimester.
[2]Models are adjusted for gestational age at visit, body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value<0.01

SUPPLEMENTARY TABLE S4. Associations of maternal caffeine intake with preeclampsia and pregnancy-induced hypertension[1]

Caffeine intake	**Pregnancy-induced hypertension[2] n=237**	**Preeclampsia[2] n=119**	**Pregnancy-induced hypertension and preeclampsia[2] n=356**
<2 units per day	Reference	Reference	Reference
	n=130	n=75	n=205
2-3.9 units per day	0.86 (0.64, 1.16)	0.62 (0.40, 0.95)*	0.78 (0.61, 0.99)*
	n=81	n=31	n=112
4-5.9 units per day	0.98 (0.60, 1.59)	0.93 (0.47, 1.84)	0.96 (0.64, 1.43)
	n=21	n=10	n=31
≥6 units per day	0.77 (0.30, 1.96)	0.99 (0.30, 3.24)	0.85 (0.40, 1.79)
	n=5	n=3	n=8
Trend[3]	0.93 (0.77, 1.13)	0.86 (0.65, 1.13)	0.91 (0.78, 1.07)
	P=0.469	P=0.281	P=0.246

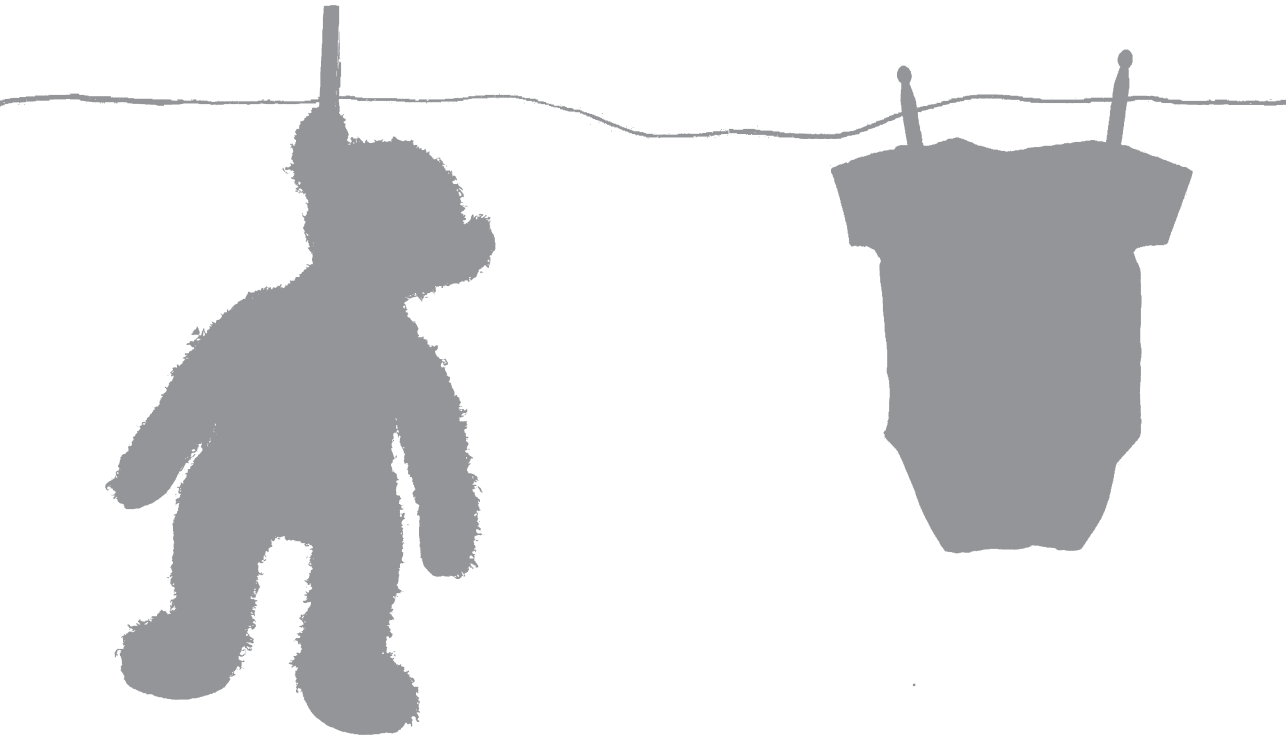
[1]Values are odds ratios with 95% confidence interval and reflect the difference in risk of pregnancy complications compared to women who consumed less than 2 units of caffeine per day.
[2]Models are adjusted for body mass index at enrolment, height, maternal age at enrolment, ethnicity, educational level, parity, alcohol consumption, smoking habits, folic acid supplement use, total daily energy intake and stress in pregnancy.
[3]Tests for trends are based on multiple logistic regression models with caffeine intake as a continuous variable.
*P-value<0.05

Chapter 3.5

Blood pressure in pregnancy, fetal growth and neonatal complications

Rachel Bakker
Eric AP Steegers
Albert Hofman
Vincent WV Jaddoe

Adapted from Am J Epidemiol. 2011 In press



ABSTRACT

Maternal hypertensive disorders during pregnancy have been suggested to affect fetal growth. We examined the associations of systolic and diastolic blood pressure in different trimesters of pregnancy with repeatedly measured fetal growth characteristics and the risks of neonatal complications. This study was performed in 8623 women, participating in a population-based prospective cohort study from fetal life onwards. Blood pressure and fetal growth characteristics were assessed each trimester. Information on hypertensive and neonatal complications was obtained from medical records. Our results suggest that higher blood pressure was not associated with fetal growth characteristics in second trimester, but with smaller fetal head circumference, and femur length and lower fetal weight from third trimester onwards. An increase of blood pressure from second to third trimester was associated with an increased risk of neonatal complications. As compared to non-hypertensive pregnancies, women with preeclampsia had increased risks of preterm (OR, 5.89 (95% CI: 2.63, 13.14)), low birth weight (OR, 8.94 (95% CI: 6.19, 12.90)), and small-size-for-gestational-age (OR, 5.03 (95% CI: 3.31, 7.62)) children. Our results suggest that higher maternal blood pressure is associated with impaired fetal growth during third trimester and increased risk of neonatal complications.

INTRODUCTION

Pregnancy-induced hypertension and preeclampsia are leading causes of maternal morbidity and neonatal complications, and occur in approximately 5% to 6% of all pregnant women[1-4]. Inconsistent results have been observed for the associations of preeclampsia with the risk of birth complications. Some studies reported an increased risks of delivering preterm and small-size-for-gestational-age born children among women who developed gestational hypertension or preeclampsia[3, 5-7], whereas others reported increased risks of delivering large-size-for-gestational-age children among women with preeclampsia[8-9].

It has also been suggested that maternal blood pressure levels are associated with birth weight[10-11]. Waugh *et al*. showed a significant inverse association between diastolic blood pressure in third trimester and birth weight in hypertensive pregnancies[10]. Similar results were reported by Zhang *et al*., who showed lower birth weight and increased risks of low birth weight and small-size-for-gestational-age children among pregnant women with diastolic blood pressure levels above 90 mmHg[11]. Furthermore, Steer *et al*. found an inverse U-shaped association between diastolic blood pressure levels and birth weight in non-hypertensive pregnant women[12]. Less is known about the associations between maternal blood pressure and fetal growth in different periods of pregnancy and the risk of fetal and neonatal complications. This information might be important for identifying critical periods during pregnancy. Diastolic blood pressure levels, rather than systolic blood pressure levels, are believed to contribute to the development of preeclampsia[13]. Also, systolic and diastolic blood pressure might reflect different cardiovascular adaptations and might affect fetal growth.

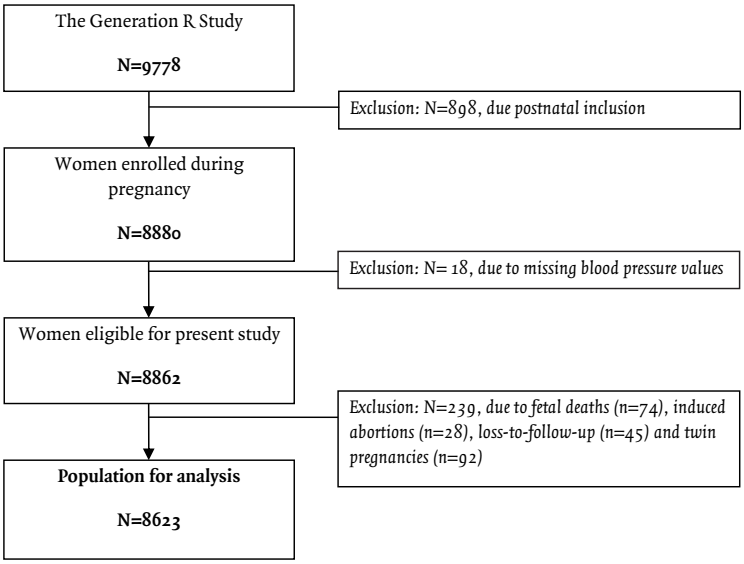
Therefore, we assessed in a population-based prospective cohort study among 8623 pregnant women, the associations of systolic and diastolic blood pressure in different trimesters of pregnancy with repeatedly measured fetal growth characteristics and the risks of neonatal complications, including preterm birth, low birth weight and small-size-for-gestational-age at birth. To identify critical periods during pregnancy of the association of blood pressure levels on fetal growth and neonatal complications, we performed analyses on the changes of blood pressure levels between each trimester of pregnancy. We also examined the associations of pregnancy-induced hypertension and preeclampsia with differences in birth weight and the risks of neonatal complications.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[14-15]. The Generation R Study is a prenatally recruited birth cohort study and therefore response percentage of the children at

FIGURE 1. Flow chart of the participants included for analysis



birth is reported. The study has been approved by the Medical Ethical Committee of the Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). Written consent was obtained from all participating women. All pregnant women were enrolled during pregnancy between 2001 and 2005. Of all eligible children in the study area, 61% participated at birth in the study[15]. Assessments during pregnancy were planned in first, second and third trimester. The individual timing of these assessments depended on the gestational age at enrolment. In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without blood pressure measurements (n = 18), leading to 8862 women. Also, we excluded pregnancies leading to fetal death (n = 74), induced abortions (n = 28), loss-to-follow-up (n = 45) and twin pregnancies (n = 92). Thus, the cohort for analysis comprised 8623 women (Figure 1).

Blood pressure

Blood pressure was measured, at our two dedicated research facilities, with the Omron 907® automated digital oscillometric sphygmanometer, which was validated in non-pregnant adults (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands)[16]. All participants were seated in upright position with back support, and were asked to relax for 5 minutes. A cuff was placed around the non-dominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 centimeters (cm) a larger cuff (32~42cm) was used. The mean value of 2 blood pressure readings over a 60-second interval was documented for each participant. In total, blood pressure was measured in 6493 women in first trimester (mean, 13.2 weeks of gestation; range, 9.8-17.6), in 8046 women in second trimester (mean, 20.4 weeks of gestation; range, 18.5-23.6) and in 8119 women in third

trimester (mean, 30.2 weeks of gestation; range, 28.4-32.9). Three, two and one blood pressure measurements were available for 5959, 2120, and 544 women, respectively.

Gestational hypertensive complications

Information on gestational hypertensive complications was obtained from medical records. Women suspected of hypertensive complications during pregnancy based on these records were cross-checked with the original hospital charts[17]. The following criteria were used to identify women with pregnancy-induced hypertension: development of systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia[18].

Fetal growth

Fetal ultrasound examinations were carried out in two dedicated research centers in each trimester of pregnancy. We established gestational age by first trimester fetal ultrasound examination[19]. In the second and third trimester of pregnancy we measured parameters of head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures[20]. Estimated fetal weight was subsequently calculated by using formula of Hadlock *et al.*[21]. Fetal growth measurements were available in 8068 and 8235 children in second and third trimester, respectively. Standard deviation scores of all fetal growth characteristics were constructed on data from the study group[19].

Neonatal outcomes

Information about offspring sex, gestational age, weight, length, and head circumference was obtained from medical records and hospital registries. Because head circumference and length at birth were not routinely measured at birth fewer measurements were available, n = 4538 and n = 5361, respectively. Gestational-age-adjusted standard deviation (SD) scores for birth weight, length and head circumference were constructed using growth standards from Niklasson *et al.*[22]. Preterm birth was defined as a gestational age of less than 37 weeks at delivery. Since, preterm birth might be a treatment option for severe preeclampsia, analyses with preterm birth as dependent variable were restricted to women who had a spontaneous delivery. Low birth weight was defined as birth weight below 2500 grams. Small-size-for-gestational-age at birth was defined as a gestational-age-adjusted birth weight below 5th percentile in the study cohort (less than 1.78 SD).

Covariates

Information on maternal age (years), educational level (primary school; secondary school; higher education), ethnicity (European; non-European), parity (nulliparous; multiparous) and folic acid supplementation use (preconceptional use; first trimester only; no use) was obtained at enrolment.

Information about smoking (none; first trimester only; continued), alcohol consumption (none; first trimester only; continued) and caffeine intake (none; <2 units per day; 2-5.9 units per day; ≥6 units per day) were assessed by questionnaires in each trimester. At enrolment weight (kg) and height (cm) were measured without shoes and heavy clothing. Weight was repeatedly measured during subsequent visits at the research center. Maternal distress was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory[23]. A higher index reflects more stress pregnant women experience.

Statistical analyses

First, the associations of maternal systolic and diastolic blood pressure in second and third trimester with fetal head circumference (second and third trimester head circumference, and head circumference at birth), length (second and third trimester femur length, and birth length), and weight (second and third trimester estimated fetal weight, and birth weight) were performed by using multiple linear regression models. Third trimester blood pressure levels were used to assess the associations with growth measures at birth. To enable comparison of effect estimates, we used the SD score of systolic and diastolic blood pressure as independent variable and the SD score of each growth characteristic as dependent variable. Second, we assessed the associations of blood pressure change (first-second trimester, second-third trimester, first-third trimester) with birth weight and the risks of neonatal complications (preterm birth, low birth weight, small-size-for-gestational-age at birth) by using multiple linear and logistic regression models. Both analyses were also performed with mean arterial pressure as independent variable[24]. Next, by using similar models, we assessed the associations of maternal hypertensive disorders (pregnancy-induced hypertension and preeclampsia) with birth weight and the risks of neonatal complications. Models were adjusted for gestational age at blood pressure measurement, number of weeks between the measurements for analyses of blood pressure change between trimesters, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height and stress, and fetal sex. Finally, we analyzed the associations of the changes in blood pressure levels per and between different trimesters with fetal growth characteristics, using quartiles of blood pressure. The percentages of missing values within the population for analysis were lower than 15%, except for folic acid supplement use (26%) and maternal stress (24%). These higher percentages were due the large number of women who only partially completed the questionnaire or were not enrolled in first trimester. We used multiple imputations for missing values in the covariates. Five imputed data sets were created and analyzed together. We included all covariates, plus gestational age at birth, gestational age at 20 weeks visit, gestational age at 30 weeks visit, and fetal sex in the imputation model. Furthermore, we added systolic and diastolic blood pressure of first, second and third trimester, and gestational hypertensive complication in the imputation model as prediction variables only, which were not imputed themselves. The pooled standard error was calculated with the average variance of the effect estimate between the imputed sets (variance of the 5 standard errors) and the variance of

TABLE 1. Maternal characteristics[1]

	N=8623
Age, yrs	29.6 (5.3)
Height, cm	167.1 (7.4)
Weight, kg	69.4 (13.2)
Body mass index, kg/m^2	24.9 (4.5)
Parity, %	
Nulliparous	57.1
Multiparous	42.9
Gestational age at intake, wks[2]	14.4 (10.4-28.6)
Highest completed education, %	
Primary school	11.7
Secundary school	46.4
Higher education	41.9
Ethnicity, %	
European	57.5
Non-European	42.5
Maternal stress index[2]	0.17 (0.00-1.46)
Alcohol consumption, %	
None	49.8
First trimester only	13.5
Continued	36.7
Smoking habits, %	
None	74.5
First trimester only	8.3
Continued	17.2
Folic acid supplement use, %	
Preconceptional use	39.5
First 10 weeks use	31.1
No use	29.4
Caffeine intake, %	
None	4.7
<2 units per day	56.6
2-5.9 units per day	37.3
≥6 units per day	1.4
Blood pressure first trimester	
Gestational age, wks[2]	13.2 (9.8-17.6)
Systolic blood pressure, mmHg	116 (12)
Diastolic blood pressure, mmHg	68 (10)
Mean arterial pressure, mmHg	84 (9)

TABLE 1. (continued)

Blood pressure second trimester	
Gestational age, wks[2]	20.4 (18.5-23.6)
Systolic blood pressure, mmHg	117 (12)
Diastolic blood pressure, mmHg	67 (9)
Mean arterial pressure, mmHg	84 (9)
Blood pressure third trimester	
Gestational age, wks[2]	30.2 (28.4-32.9)
Systolic blood pressure, mmHg	118 (12)
Diastolic blood pressure, mmHg	69 (9)
Mean arterial pressure, mmHg	85 (9)
Pregnancy-induced hypertension, %	3.6
Preeclampsia, %	2.0

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

TABLE 2. Fetal characteristics[1]

	N=8623
Second trimester	
Gestational age, wks[2]	20.4 (18.5-23.6)
Head circumference, mm	180 (15)
Femur length, mm	34 (4)
Estimated fetal weight, g	382 (96)
Third trimester	
Gestational age, wks[2]	30.2 (28.4-32.9)
Head circumference, mm	285 (13)
Femur length, mm	57 (3)
Estimated fetal weight, g	1616 (266)
Birth	
Gestational age, wks[2]	40.1 (35.4-42.3)
Head circumference, mm	350 (23)
Length, mm	509 (28)
Weight, g	3411 (562)
Sex, % boys	50.3
Preterm birth, %	5.4
Low birth weight, %	4.8

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

the imputed sets (variance of the 5 effect estimates)[25]. The analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 and 2 present the maternal and fetal characteristics of the participants included for the analysis, respectively. Of all pregnancies, 3.6% (n = 311) led to pregnancy-induced hypertension and 2.0% (n = 171) to preeclampsia. Of all children, 5.4% (n = 433) were born preterm, and 4.8% (n = 400) were born with low birth weight.

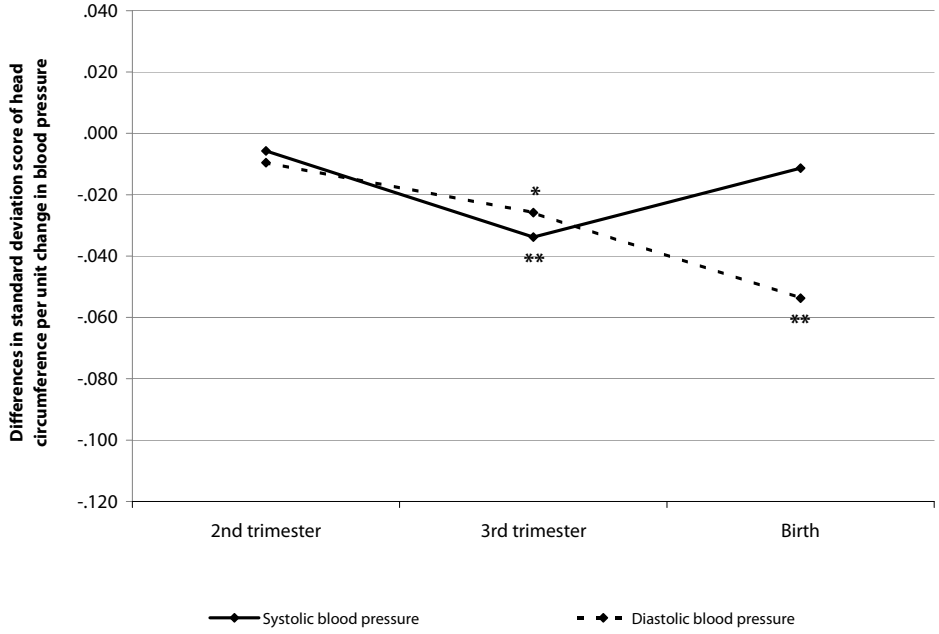
Figures 2a, 2b and 2c show that maternal systolic and diastolic blood pressure were not associated with any second trimester fetal growth characteristic. Figure 2a shows that higher third trimester systolic and diastolic blood pressure were associated with smaller third trimester fetal head circumference (differences of -0.03 SD (-0.39 mm) (95% confidence interval (CI): -0.06, -0.01), and -0.03 SD (-0.39 mm) (95% CI: -0.05, 0), respectively, per 1 SD change in blood pressure), and that higher third trimester diastolic blood pressure was associated with a smaller head circumference at birth (difference of -0.06 SD (-1.38 mm) (95% CI: -0.09, -0.02) per 1 SD change in diastolic blood pressure). Figure 2b shows that higher third trimester diastolic, but not systolic, blood pressure was associated with birth body length (difference of -0.04 SD (-1.12 mm) (95% CI: -0.08, 0) per 1 SD change in diastolic blood pressure). Figure 2c shows that higher third trimester systolic blood pressure was not associated with a lower third trimester estimated fetal weight but with a lower birth weight (difference of -0.03 SD (-16.9 grams) (95% CI: -0.06, -0) per 1 SD change in systolic blood pressure). Higher third trimester diastolic blood pressure was associated with both a lower third trimester estimated fetal weight and lower birth weight (differences of -0.03 SD (-8.0 grams) (95% CI: -0.06, 0) and -0.09 SD (-50.6 grams) (95% CI: -0.12, -0.06), respectively, per 1 SD change in diastolic blood pressure).

For all fetal growth characteristics, we observed larger effect sizes for diastolic than for systolic blood pressure. Also, the effect estimates for the associations between blood pressure and fetal growth characteristics tended to be larger at older gestational age. Similar results were found for mean arterial pressure (All effect estimates are given in the Supplementary Table S1).

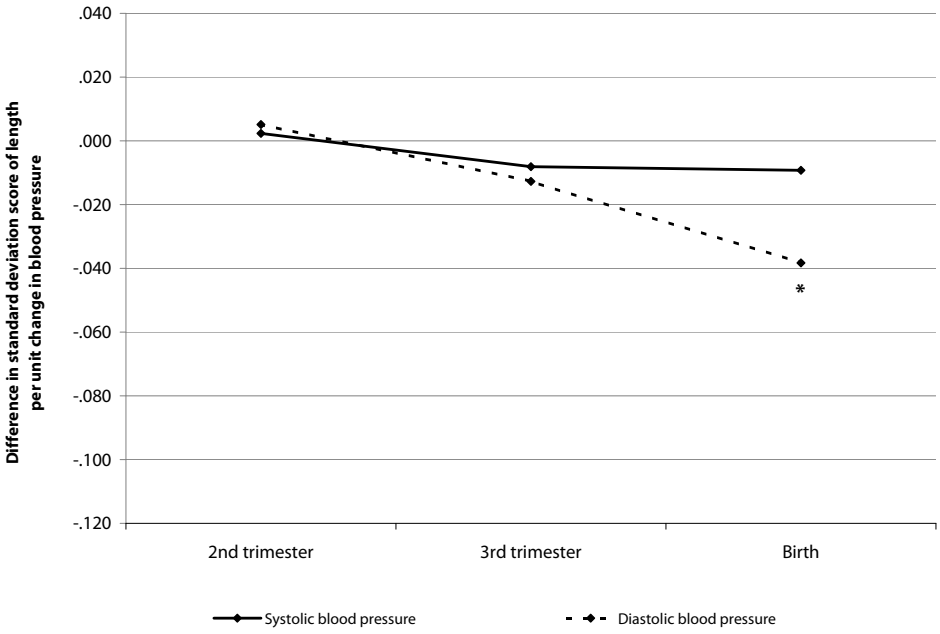
Table 3 shows that first to second trimester change in blood pressure was not associated with birth weight or the risks of neonatal complications. A change in systolic blood pressure from second to third trimester was associated with an increased risk of low birth weight (Odds ratio (OR) of 1.25 (95% CI: 1.12, 1.40)). Also, the change in diastolic blood pressure from second to third trimester was associated with lower birth weight (-11.24 grams (95% CI: -20.63, -1.86)), increased risks of preterm delivery (OR of 1.26 (95% CI: 1.10, 1.44)), low birth weight (OR of 1.49 (95% CI: 1.34, 1.67)), also small-size-for-gestational-age at birth (OR of 1.12 (95% CI: 1.01, 1.24)). For change in mean arterial pressure from second to third trimester similar results were

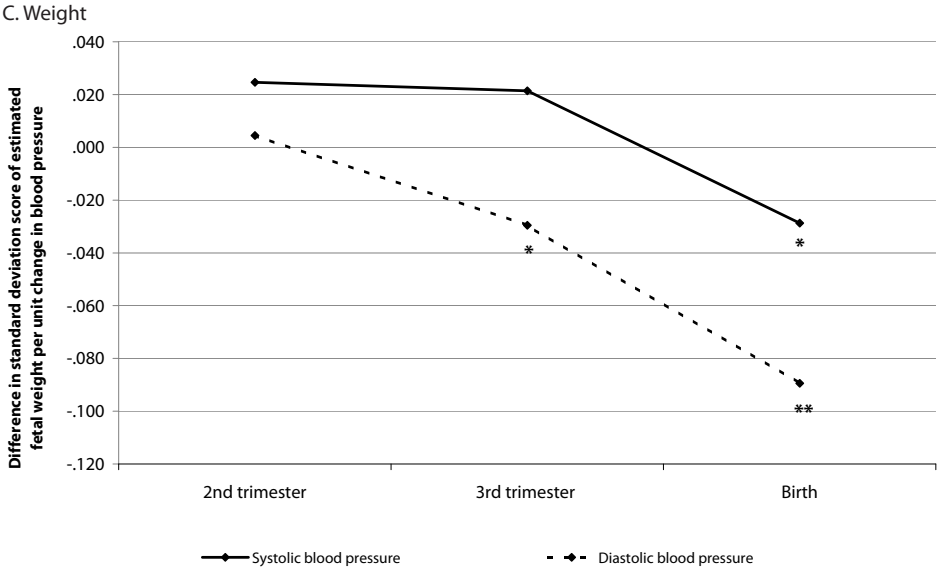
FIGURE 2. Associations of blood pressure per standard deviation during pregnancy with longitudinally measured standard deviation score of growth characteristics

A. Head circumference



B. Length





Adjusted for maternal age, gestational age at the visit, height, weight, ethnicity, educational level, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use, stress and fetal sex. The reference value is an SD-score of 0. *P-value<0.05; **P-value<0.01. Estimates are from multiple imputed data. Total measurements for head circumference: 7880 2nd trimester; 7998 3rd trimester; and 4364 at birth. Total measurements for length: 7903 2nd trimester; 8066 3rd trimester; and 5116 at birth. Total measurements for weight: 7863 2nd trimester; 8036 3rd trimester; and 8070 at birth.

found for preterm birth and low birth weight; however, not for small-size-for-gestational-age (Supplementary Table S2).

Table 4 shows that, as compared to non-hypertensive pregnancies, women with gestational hypertension and preeclampsia delivered children with lower birth weights (-89 grams (95% CI: -137, -41) and -220 grams (95% CI: -294, -165), respectively). Accordingly, among pregnant women who developed preeclampsia we observed increased risks of preterm delivery (OR of 5.89 (95% CI: 2.63, 13.14)), low birth weight children (OR of 8.94 (95% CI: 6.19, 12.90)), and small-size-for-gestational-age at birth children (OR of 5.03 (95% CI: 3.31, 7.62)). Smaller increased risks of adverse birth outcomes were found in women who developed gestational hypertension; however, no differences in risk of preterm delivery were found, compared to non-hypertensive pregnancies.

Supplementary Tables S3 to S6 give the results for the associations of the changes in blood pressure levels between different trimesters with fetal growth characteristics, using quartiles of blood pressure. The results did only show some marginal differences; however, the direction of the effect estimates was similar, and therefore justifies the linear approach in our analyses.

TABLE 3. Associations between change in blood pressure levels during pregnancy, birth weight, and risks of neonatal complications[1,2]

Change in systolic blood pressure	Difference in grams (95% CI) for birth weight[4]	Odds ratio (95% CI) for preterm birth[5, 6]	Odds ratio (95% CI) for low birth weight[5]	Odds ratio (95% CI) for small-size-for-gestational-age[5]
First-second trimester (SD)[3]	6.75 (-3.74, 17.25)	1.04 (0.90, 1.21)	0.91 (0.81, 1.02)	0.95 (0.85, 1.06)
	n=6160	n=3993; n_{cases}=185	n=6160; n_{cases}=297	n=6151; n_{cases}=318
Second-third trimester (SD)[3]	-1.18 (-10.55, 8.19)	1.09 (0.95, 1.25)	1.25 (1.12, 1.40)**	1.00 (0.90, 1.11)
	n=7612	n=4983; n_{cases}=214	n=7612; n_{cases}=317	n=7603; n_{cases}=380
First-third trimester (SD)[3]	4.74 (-5.74, 15.22)	1.12 (0.96, 1.32)	1.13 (0.99, 1.28)	0.95 (0.84, 1.06)
	n=6134	n=3999; n_{cases}=158	n=6134; n_{cases}=254	n=6126; n_{cases}=309
Change in diastolic blood pressure	**Difference in grams (95% CI) for birth weight[4]**	**Odds ratio (95% CI) for preterm birth[5, 6]**	**Odds ratio (95% CI) for low birth weight[5]**	**Odds ratio (95% CI) for small-size-for-gestational-age[5]**
First-second trimester (SD)[3]	3.07 (-7.44, 13.58)	1.05 (0.90, 1.22)	0.91 (0.81, 1.02)	1.02 (0.91, 1.14)
	n=6160	n=3993; n_{cases}=185	n=6160; n_{cases}=297	n=6151; n_{cases}=318
Second-third trimester (SD)[3]	-11.24 (-20.63, -1.86)*	1.26 (1.10, 1.44)**	1.49 (1.34, 1.67)**	1.12 (1.01, 1.24)*
	n=7612	n=4983; n_{cases}=214	n=7612; n_{cases}=317	n=7603; n_{cases}=380
First-third trimester (SD)[3]	-11.13 (-21.64, -0.63)*	1.25 (1.06, 1.46)**	1.33 (1.18, 1.51)**	1.13 (1.01, 1.27)*
	n=6134	n=3999; n_{cases}=158	n=6134; n_{cases}=254	n=6126; n_{cases}=309

Abbreviations: CI, confidence interval

[1]Estimates are from multiple imputed data.

[2]Models are adjusted for gestational age at birth (only in birth weight analyses), number of weeks between measurements, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]A standard deviation change of systolic blood pressure from first to second trimester, from second to third trimester, and from first to third trimester corresponds with change of 12 mmHg. One standard deviation change of diastolic blood pressure from first to second trimester, and from second to third trimester corresponds with change of 9 mmHg, and from first to third trimester with change of 10 mmHg.

[4]Values are differences (95% CI) in birth weight per standard deviation change in blood pressure within the trimesters.

[5]Values are odds ratios (95% CI) that reflect the risks of adverse birth outcomes per standard deviation change in blood pressure within the trimesters.

[6]Analyses of the risk of preterm birth are performed in selection of participants who had spontaneous started delivery.

*P-value<0.05

**P-value<0.01

TABLE 4. Associations between maternal hypertensive disorders, birth weight, and the risks of neonatal complications[1,2]

Blood pressure group	Difference in grams (95% CI) for birth weight (n=8334)[3]	Odds ratio (95% CI) for preterm birth (n=5499; n_{cases}=274)[4,5]	Odds ratio (95% CI) for low birth weight (n=8334; n_{cases}=400)[4]	Odds ratio (95% CI) for small-size-for-gestational-age (n=8324; n_{cases}=424)[4]
Non-hypertensive	Reference	Reference	Reference	Reference
N=7889	n=7857	n_{cases}=255	n_{cases}=330	n_{cases}=364
Pregnancy-induced hypertension	-89 (-137, -41)**	1.42 (0.72, 2.77)	1.85 (1.15, 2.97)*	2.58 (1.67, 3.96)**
N=311	n=310	n_{cases}=10	n_{cases}=21	n_{cases}=27
Preeclampsia	-220 (-294, -165)**	5.89 (2.63, 13.14)**	8.94 (6.19, 12.90)**	5.03 (3.31, 7.62)**
N=171	n=167	n_{cases}=9	n_{cases}=49	n_{cases}=33

Abbreviations: CI, confidence interval

[1]Estimates are from multiple imputed data.

[2]Models are adjusted for gestational age at birth (only in birth weight analyses), maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]Values are differences (95% CI) in birth weight in different blood pressure-groups compared to the reference group of non-hypertensive pregnant women.

[4]Values are odds ratios (95% CI) that reflect the difference in risks of neonatal complications in different blood pressure-groups compared to the reference group of non-hypertensive pregnant women.

[5]Analyses of the risk of preterm birth are performed in selection of participants who had spontaneous started delivery.

*P-value<0.05

**P-value<0.01

DISCUSSION

We observed associations between higher maternal blood pressure and smaller fetal growth characteristics in third trimester and at birth. Overall, stronger associations were observed for diastolic blood pressure levels, and at an older gestational age. The change in blood pressure level from second to third trimester was associated with an increased risk of neonatal complications. As compared to non-hypertensive pregnancies, women with either pregnancy-induced hypertension or preeclampsia had both increased risks of neonatal complications.

Methodological considerations

One of the strengths of this study was the prospective data collection, which started in early pregnancy. In addition, we had a large sample size of 8623 participants with 22658 blood pressure measurements. A wide range of potential confounding factors was available. A potential limitation might be the response rate of 61% in this study. Pregnant women who participated were higher educated, more healthy and more frequently of Dutch origin than those who did not participate[15].

This might have led to some selective participation. Selection bias occurs if participation depends on both the exposure, maternal blood pressure, and the outcome, fetal growth and neonatal complications[26]. However, selection bias in follow-up studies primary arises from loss to follow-up instead of non-response at baseline due to the prospective nature of the study[27].

Blood pressure measurements in third trimester were aimed around week 30 of gestation; therefore, only few measurements are available in late pregnancy. Several different outcomes were studied; fetal growth characteristics, birth weight, preterm delivery, low birth weight, and small-size-for-gestational-age at birth. Since our results are not independent outcomes, we did not perform adjustment for multiple testing.

Blood pressure, gestational hypertensive complications and fetal growth

To our knowledge no previous studies have been performed on blood pressure levels during pregnancy and fetal growth characteristics in different trimesters of pregnancy. Our results are comparable to studies of Waugh et al., Zhang et al. and Churchill et al.[10-11, 28]. Furthermore, Steer et al. showed that even low as high diastolic blood pressure levels were associated with smaller offspring[12].

A change of diastolic blood pressure levels, but not systolic blood pressure levels, from first to third trimester was associated with a lower birth weight, and increased the risks of adverse birth outcomes. Among women who had increased systolic blood pressure levels from second to third trimester we found an increased risk of low birth weight children, while previous studies found associations for diastolic blood pressure levels only. After exclusion of mothers who were treated with medication for high blood pressure during pregnancy, only marginal differences in the effect estimates were found.

Although we have studied fetal growth and neonatal outcomes a review of Cnossen et al. suggested that mean arterial pressure is a better predictor for preeclampsia than systolic and diastolic blood pressure in first or second trimester, and also than the change in blood pressure from first to second trimester[29]. This may also be the case in fetal growth outcomes. Therefore, we have repeated our analyses with mean arterial pressure as independent variable. Similar results were found for fetal growth and the risk of neonatal complications.

Mainly diastolic blood pressure levels are believed to contribute to the development of preeclampsia[13]. Our finding seems to be in line with this hypothesis. The increase in diastolic blood pressure from first to third trimester was much smaller than the increase in systolic blood pressure, which is due to the mid-pregnancy fall in diastolic blood pressure. In non-hypertensive pregnant women, blood pressure, most notably diastolic blood pressure, falls steadily until the middle of gestation and then rises again until delivery[30]. In women who develop preeclampsia, this mid-pregnancy fall in blood pressure does not occur; instead, blood pressure tends to remain stable during the first half of pregnancy and then rise continuously until delivery[30]. Results from an observational study suggest that treatment of hypertensive disorders during early pregnancy may lower the risks of severe maternal hypertensive complications later in pregnancy and the risk

of preterm birth[31]. However, the risk of fetal growth restriction may increase due to non-adequate adjustment of therapy in response to changes in cardiac output or peripheral vascular resistance. On the other hand, a meta-analysis of Abalos et al. reported less clear evidence of adverse associations of treatment of hypertension during pregnancy[32]. Currently, treatment of hypertensive complications should be managed carefully.

Most previous studies that focused on the associations of maternal hypertensive disorders during pregnancy with the risk of preterm birth did not restrict their analyses to spontaneous deliveries. In our study we did made this restriction, since the only effective cure of hypertensive disorders during pregnancy is delivery of the fetus. We observed increased risks on all adverse neonatal outcomes among women with hypertensive complications during pregnancy, as compared to women in the non-hypertensive range. Similar findings are found in previous studies[33-35].

Underlying mechanisms

The mechanisms on how maternal blood pressure levels can affect fetal growth are not yet clear. Higher blood pressure levels and fetal growth retardation may both be the result of placental dysfunction or adverse maternal cardiovascular adaptations on pregnancy. Tranquilli *et al.* proposed that increased maternal blood pressure might be the consequence, rather than the cause, of fetal growth restriction[36]. According to the authors increased blood pressure levels during pregnancy could be compensation for inadequate placental perfusion. Within the non-hypertensive pregnant women with intrauterine growth restriction had significantly higher systolic and diastolic blood pressure. Increased blood pressure levels may affect the development of placental villous tree and lead to reduced functional capacity of the placenta, which may lead to a reduction in fetal growth, and thus lower birth weight[37]. Based on our results we cannot explain the causal mechanism between blood pressure levels and fetal growth. It might be that both blood pressure and fetal growth variation are markers of placental dysfunction.

Conclusion

Our results suggest that higher maternal blood pressure levels are associated with impaired fetal growth from third trimester onwards and increased risks of neonatal complications. Pregnancy-induced hypertension and preeclampsia were associated with strongly increased risks of preterm birth, low birth weight and a small-size-for-gestational-age at birth. The underlying mechanisms for these associations need to be identified.

REFERENCES

1. Khan KS, Wojdyla D, Say L, et al. WHO analysis of causes of maternal death: a systematic review. Lancet 2006; 367:1066–74.
2. Duley L. The global impact of pre-eclampsia and eclampsia. Semin Perinatol 2009;33:130–37.
3. Ray JG, Burrows RF, Burrows EA, et al. MOS HIP: McMaster outcome study of hypertension in pregnancy. Early Human Development 2001;64:129-143.
4. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. JAMA. 2002;287:3183-6.
5. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. Lancet. 2005;365:785-99.
6. Steegers EA, von Dadelszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet. 2010;376:631-44.
7. von Dadelszen P, Ornstein MP, Bull SB, et al. Fall in mean arterial pressure and fetal growth restriction in pregnancy hypertension: a meta-analysis. Lancet. 2000;355:87-92.
8. Xiong X, Demianczuk NN, Buekens P, et al. Association of preeclampsia with high birth weight for gestational age. Am J Obstet Gynecol 2000; 183: 148-55.
9. Eskild A, Romundstad PR, Vatten LJ. Placental weight and birthweight: does the association differ between pregnancies with and without preeclampsia. Am J Obstet Gynecol 2009; 201: 595.e1-5.
10. Waugh J, Perry IJ, Halligan AW, et al. Birth weight and 24-hour ambulatory blood pressure in nonproteinuric hypertensive pregnancy. Am J Obstet Gynecol. 2000;183:633-7.
11. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gyneacol. 2001;97:261-7.
12. Steer PJ, Little MP, Kold-Jensen T, et al. Maternal blood pressure in pregnancy, birth weight, and perinatal mortality in first births: prospective study. BMJ 2004; 329:1312-7.
13. Milne F, Redman C, Walker J, et al. The pre-eclampsia community guideline (PRECOG): how to screen for and detect onset of preeclampsia in the community. BMJ 2005;330: 576–80.
14. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
15. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25:823-841.
16. El Assaad MA, Topouchian JA, Darne BM, et al. Validation of the Omron HEM-907 device for blood pressure measurement. Blood Press Monit. 2002;7:237-41.
17. Coolman M, de Groot CJ, Jaddoe VW, et al. Medical record validation of maternally reported history of pre-eclampsia. J Clin Epidemiol. 2010;63:932-7.
18. Brown MA, Lindheimer MD, de Swiet M, et al. 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:IX-XIV
19. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31: 388-96.
20. Routine ultrasound screening in pregnancy: protocol. London, UK: RCOG Press. 2000.
21. Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985;151:333-7.
22. Niklasson A, Ericson A, Fryer JG, et al. 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;80:756-62.
23. Derogatis LR. Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition. Minneapolis, MN. 1993.
24. Cywinski J. The essentials in pressure monitoring. Boston: Martinus Nijhoff Publishers b.v., 1980:23–4.
25. Schafer JL. Analysis of incomplete multivariate data. Londen: Chapman & Hall; 1997
26. Greenland S. Response and follow-up bias in cohort studies. Am J Epidemiol. 1977;106:184-187.

27. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
28. Churchill D, Perry IJ, Beevers DG. Ambulatory blood pressure in pregnancy and fetal growth. Lancet. 1997;349: 7-10.
29. Cnossen JS, Vollebregt KC, de Vrieze N, et al. Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. BMJ. 2008;336:1117-20.
30. Hermida RC, Ayala DE, Iglesias M. Predictable blood pressure variability in healthy and complicated pregnancies. Hypertension. 2001;38:736-41.
31. Easterling TR, Carr DB, Brateng D, et al. Treatment of hypertension in pregnancy: effect of atenolol on maternal disease, preterm delivery, and fetal growth. Obstet Gynecol. 2001;98:427-33.
32. Abalos E, Duley L, Steyn DW, et al. Antihypertensive drug therapy for mild to moderate hypertension during pregnancy. Cochrane Database Syst Rev. 2007 Jan 24;(1):CD002252.
33. Hauth JC, Ewell MG, Levine RJ, et al. Pregnancy outcomes in healthy nulliparas who developed hypertension. Calcium for Preeclampsia Prevention Study Group. Obstet Gynecol. 2000;95:24-8.
34. Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: opportunities for intervention and screening? BMJ. 2002;325:157-60.
35. Buchbinder A, Sibai BM, Caritis S, et al. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Adverse perinatal outcomes are significantly higher in severe gestational hypertension than in mild preeclampsia. Am J Obstet Gynecol. 2002;186:66-71.
36. Tranquilli AL, Giannubilo SR. Blood pressure is elevated in normotensive pregnant women with intrauterine growth restriction. Eur J Obstet Gynecol Reprod Biol. 2005;122:45-8.
37. Kingdom JC, Kaufmann P. Oxygen and placental villous development: origins of fetal hypoxia. Placenta. 1997; 18:613-21.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Effect estimates of fetal growth characteristics per one standard deviation change in maternal blood and mean arterial pressure[1,2]

	Differences in head circumference (SD) (95% CI)[3]		
Change in blood pressure	Second trimester	Third trimester	Birth
per one SD Systolic	-0.01 (-0.03, 0.02)	-0.03 (-0.06, -0.01)**	-0.01 (-0.05, 0.03)
per one SD Diastolic	-0.01 (-0.03, 0.02)	-0.03 (-0.05, 0)*	-0.06 (-0.09, -0.02)**
per one SD MAP	-0.01 (-0.03, 0.02)	-0.03 (-0.06, -0.01)**	-0.04 (-0.08, -0.01)*
	Differences in length (SD) (95% CI)[3]		
Change in blood pressure	Second trimester	Third trimester	Birth
per one SD Systolic	0 (-0.03, 0.03)	-0.01 (-0.04, 0.02)	-0.01 (-0.05, 0.03)
per one SD Diastolic	-0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.02)	-0.04 (-0.08, 0)*
per one SD MAP	0 (-0.02, 0.03)	-0.03 (-0.05, -0.01)**	-0.05 (-0.08, -0.01)**
	Differences in weight (SD) (95% CI)[3]		
Change in blood pressure	Second trimester	Third trimester	Birth
per one SD Systolic	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.05)	-0.03 (-0.06, 0)*
per one SD Diastolic	0 (-0.03, 0.04)	-0.03 (-0.06, 0)*	-0.09 (-0.12, -0.06)**
per one SD MAP	0.01 (-0.01, 0.04)	-0.02 (-0.05, 0)*	-0.09 (-0.11, -0.06)**

Abbreviations: SD, standard deviation; CI, confidence interval; MAP, mean arterial pressure

[1]Estimates are from multiple imputed data.

[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]Values are differences (95% CI) of standard deviation scores of fetal growth characteristics per one standard deviation change in blood or mean arterial pressure.

*P-value<0.05

**P-value<0.01

SUPPLEMENTARY TABLE S2. Associations between change in mean arterial pressure during pregnancy, birth weight, and risks of neonatal complications[1,2]

Change in mean arterial pressure	**Difference in grams (95% CI) for birth weight[3]**	**Odds ratio (95% CI) for preterm birth[4,5]**	**Odds ratio (95% CI) for low birth weight[4]**	**Odds ratio (95% CI) for small-size-for-gestational-age[4]**
First-second trimester (SD)	5.22 (-5.30, 15.74)	1.05 (0.91, 1.22)	0.90 (0.80, 1.01)	0.99 (0.88, 1.11)
	n=6160	n=3993; n_{cases}=185	n=6160; n_{cases}=297	n=6151; n_{cases}=318
Second-third trimester (SD)	-8.42 (-17.80, 0.96)	1.22 (1.06, 1.39)**	1.46 (1.31, 1.63)**	1.08 (0.98, 1.20)
	n=7612	n=4983; n_{cases}=214	n=7612; n_{cases}=317	n=7603; n_{cases}=380
First-third trimester (SD)	-5.66 (-16.17, 4.86)	1.23 (1.05, 1.44)*	1.29 (1.14, 1.45)**	1.07 (0.95, 1.19)
	n=6134	n=3999; n_{cases}=158	n=6134; n_{cases}=254	n=6126; n_{cases}=309

Abbreviations: CI, confidence interval

[1]Estimates are from multiple imputed data.
[2]Models are adjusted for gestational age at birth (only in birth weight analyses), number of weeks between measurements, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.
[3]Values are differences (95% CI) in birth weight per standard deviation change in mean arterial pressure within the trimesters per standard deviation.
[4]Values are odds ratios (95% CI) that reflect the risks of neonatal complications per standard deviation change in mean arterial pressure within the trimesters.
[5]Analyses of the risk of preterm birth are performed in selection of participants who had spontaneous started delivery.
*P-value<0.05
**P-value<0.01

SUPPLEMENTARY TABLE S3. Associations between change in blood pressure levels in quartiles and birth weight[1,2]

	Difference in grams (95% CI) for birth weight[3]		
Systolic blood pressure	First-second trimester change	Second-third trimester change	First-third trimester change
Linear estimate	6.75 (-3.74, 17.25)	-1.18 (-10.55, 8.19)	4.74 (-5.74, 15.22)
	n=6160	n=7612	n=6134
1st quartile (SD)	Reference	Reference	Reference
	n=1395	n=1955	n=1395
2nd quartile (SD)	35.03 (5.49, 64.57)*	11.73 (-14.93, 38.39)	-1.69 (-30.75, 27.37)
	n=1714	n=1814	n=1823
3rd quartile (SD)	20.15 (-9.84, 50.13)	9.37 (-16.42, 35.16)	18.60 (-11.90, 49.09)
	n=1611	n=2063	n=1496
4th quartile (SD)	30.37 (-0.51, 61.24)	-6.01 (-32.81, 20.80)	8.64 (-22.30, 39.57)
	n=1440	n=1780	n=1420
Diastolic blood pressure	First-second trimester change	Second-third trimester change	First-third trimester change
Linear estimate	3.07 (-7.44, 13.58)	-11.24 (-20.63, -1.86)*	-11.13 (-21.64, -0.63)*
	n=6160	n=7612	n=6134
1st quartile (SD)	Reference	Reference	Reference
	n=1597	n=1975	n=1420
2nd quartile (SD)	-10.55 (-41.12, 20.01)	4.69 (-22.80, 32.18)	14.78 (-14.09, 43.64)
	n=1302	n=1608	n=1822
3rd quartile (SD)	17.95 (-10.72, 46.63)	6.81 (-19.61, 33.24)	-6.17 (-37.78, 25.44)
	n=1672	n=1833	n=1272
4th quartile (SD)	4.65 (-24.55, 33.84)	-25.38 (-50.78, 0.03)	-11.05 (-40.85, 18.74)
	n=1589	n=2196	n=1620

Abbreviations: CI, confidence interval
[1]Estimates are from multiple imputed data.
[2]Models are adjusted for gestational age at birth, number of weeks between measurements, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]Values are differences (95% CI) in birth weight per standard deviation change in blood pressure within the trimesters or per quartile of one standard deviation change in blood pressure.
*P-value<0.05

SUPPLEMENTARY TABLE S4. Associations between change in blood pressure levels during pregnancy in quartiles and head circumference[1,2]

	Difference in head circumference (SD) (95% CI)[3]		
Systolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	-0.01 (-0.03, 0.02)	-0.03 (-0.06, -0.01)**	-0.01 (-0.05, 0.03)
	n=7880	n=7998	n=4364
1st quartile (SD)	Reference	Reference	Reference
	n=2152	n=1823	n=1002
2nd quartile (SD)	0 (-0.06, 0.07)	-0.02 (-0.08, 0.04)	-0.01 (-0.10, 0.09)
	n=1698	n=2200	n=1203
3rd quartile (SD)	-0.01 (-0.07, 0.06)	-0.07 (-0.13, -0.01)*	-0.08 (-0.18, 0.01)
	n=1979	n=2173	n=1232
4th quartile (SD)	-0.01 (-0.07, 0.06)	-0.06 (-0.13, 0)	-0.02 (-0.13, 0.08)
	n=2051	n=1802	n=927
Diastolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	-0.01 (-0.03, 0.02)	-0.03 (-0.05, 0)*	-0.06 (-0.09, -0.02)**
	n=7880	n=7998	n=4364
1st quartile (SD)	Reference	Reference	Reference
	n=1900	n=2278	n=1260
2nd quartile (SD)	0.01 (-0.05, 0.07)	0.01 (-0.05, 0.07)	-0.04 (-0.13, 0.05)
	n=2053	n=2072	n=1229
3rd quartile (SD)	0.01 (-0.05, 0.08)	-0.01 (-0.07, 0.05)	-0.02 (-0.11, 0.08)
	n=1863	n=1799	n=969
4th quartile (SD)	-0.03 (-0.10, 0.04)	-0.07 (-0.13, 0)*	-0.13 (-0.23, -0.03)*
	n=2064	n=1849	n=906

Abbreviations: CI, confidence interval
[1]Estimates are from multiple imputed data.
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.
[3]Values are differences (95% CI) of standard deviation scores of fetal growth characteristics per one standard deviation change in blood pressure or per quartile of one standard deviation change in blood pressure.
*P-value<0.05
**P-value<0.01

SUPPLEMENTARY TABLE S5. Associations between change in blood pressure levels during pregnancy in quartiles and length[1,2]

	Difference in length (SD) (95% CI)[3]		
Systolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	0 (-0.03, 0.03)	-0.01 (-0.04, 0.02)	-0.01 (-0.05, 0.03)
	n=7903	n=8066	n=5116
1st quartile (SD)	Reference	Reference	Reference
	n=2161	n=1837	n=1136
2nd quartile (SD)	0 (-0.07, 0.06)	-0.05 (-0.11, 0.01)	0.02 (-0.07, 0.10)
	N=1709	n=2219	n=1415
3rd quartile (SD)	-0.02 (-0.08, 0.05)	-0.05 (-0.11, 0.01)	0 (-0.09, 0.08)
	n=1981	n=2194	n=1424
4th quartile (SD)	-0.02 (-0.09, 0.05)	-0.08 (-0.14, -0.01)*	-0.04 (-0.14, 0.06)
	n=2052	n=1816	n=1141
Diastolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	-0.01 (-0.03, 0.04)	-0.01 (-0.04, 0.02)	-0.04 (-0.08, 0)*
	n=7903	n=8066	n=5116
1st quartile (SD)	Reference	Reference	Reference
	n=1915	n=2293	n=1447
2nd quartile (SD)	-0.02 (-0.09, 0.04)	0.01 (-0.05, 0.07)	-0.11 (-0.19, -0.03)**
	n=2058	n=2092	n=1395
3rd quartile (SD)	0 (-0.06, 0.07)	-0.01 (-0.07, 0.06)	-0.08 (-0.17, 0)
	n=1864	n=1820	n=1158
4th quartile (SD)	-0.02 (-0.08, 0.05)	-0.07 (-0.13, 0)*	-0.14 (-0.23, -0.05)**
	n=2066	n=1861	n=1116

Abbreviations: CI, confidence interval

[1]Estimates are from multiple imputed data.

[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]Values are differences (95% CI) of standard deviation scores of fetal growth characteristics per one standard deviation change in blood pressure or per quartile of one standard deviation change in blood pressure.

*P-value<0.05

**P-value<0.01

SUPPLEMENTARY TABLE S6. Associations between change in blood pressure levels during pregnancy in quartiles and weight[1,2]

	Difference in weight (SD) (95% CI)[3]		
Systolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	0.03 (-0.01, 0.06)	0.02 (-0.01, 0.05)	-0.03 (-0.06, 0)*
	n=7863	n=8036	n=8070
1st quartile (SD)	Reference	Reference	Reference
	n=2153	n=1827	n=1835
2nd quartile (SD)	0.01 (-0.06, 0.07)	-0.02 (-0.08, 0.04)	-0.01 (-0.07, 0.05)
	n=1698	N=2213	n=2215
3rd quartile (SD)	0.01 (-0.06, 0.07)	0 (-0.06, 0.07)	0.01 (-0.06, 0.07)
	n=1966	N=2189	n=2195
4th quartile (SD)	0 (-0.06, 0.07)	0.01 (-0.06, 0.07)	-0.11 (-0.18, -0.04)**
	n=2046	n=1807	n=1825
Diastolic blood pressure	Second trimester	Third trimester	Birth
Linear estimate	0 (-0.03, 0.04)	-0.03 (-0.06, 0)*	-0.09 (-0.12, -0.06)**
	n=7863	n=8036	n=8070
1st quartile (SD)	Reference	Reference	Reference
	n=1904	n=2283	n=2291
2nd quartile (SD)	-0.01 (-0.07, 0.06)	-0.03 (-0.09, 0.03)	-0.07 (-0.13, -0.01)*
	n=2051	n=2087	n=2098
3rd quartile (SD)	0.02 (-0.05, 0.08)	-0.02 (-0.08, 0.04)	-0.10 (-0.16, -0.04)**
	n=1855	n=1813	n=1816
4th quartile (SD)	0 (-0.07, 0.06)	-0.10 (-0.17, -0.04)**	-0.24 (-0.31, -0.18)**
	n=2053	n=1853	n=1865

Abbreviations: CI, confidence interval

[1]Estimates are from multiple imputed data.

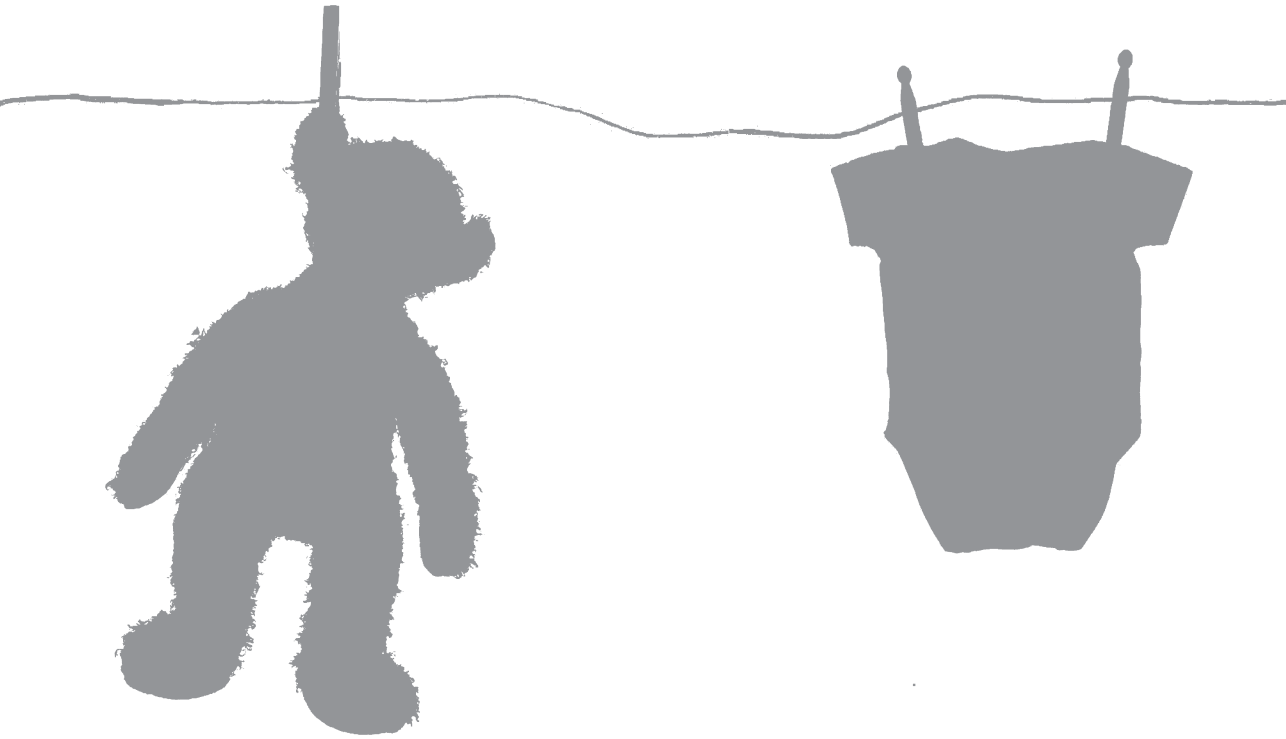
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption, caffeine intake, weight, height, stress, and fetal sex.

[3]Values are differences (95% CI) of standard deviation scores of fetal growth characteristics per one standard deviation change in blood pressure or per quartile of one standard deviation change in blood pressure.

*P-value<0.05

**P-value<0.01

Part 4 | **Fetal and neonatal complications**

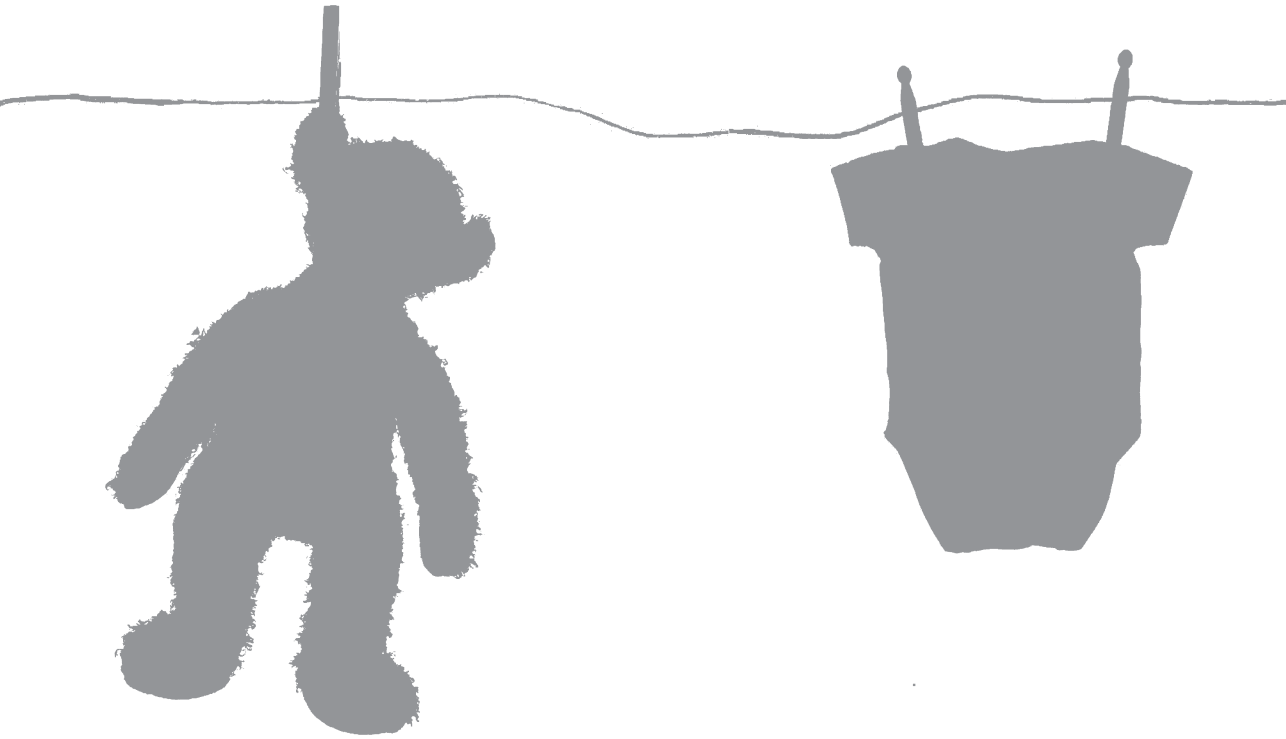


Chapter 4.1

Differences in birth outcomes in relation to maternal age

Rachel Bakker
Eric AP Steegers
Angelique A Biharie
Johan P Mackenbach
Albert Hofman
Vincent WV Jaddoe

Adapted from BJOG. 2011;118:500-9.



ABSTRACT

Previous studies showed lower birth weight among infants from younger mothers, and suggested an inverse U-shaped relationship between maternal age and birth weight. Several mechanisms may explain the association between maternal age and birth weight. We examined the associations of maternal age with birth outcomes and the explaining role of socio-demographic and lifestyle related determinants within 8568 mothers and their children participating in a population-based prospective cohort study from early pregnancy onwards, in Rotterdam, the Netherlands. Maternal age was assessed at enrolment. Information about socio-demographic (height, weight, educational level, ethnicity, parity) and lifestyle related determinants (alcohol consumption, smoking habits, folic acid supplement use, caffeine intake, daily energy intake) and birth outcomes was obtained from questionnaires and hospital records. Multivariate linear and logistic regression analyses were used. The main outcomes measures were birth weight, preterm delivery, small-size-for-gestational-age, and large-size-for-gestational-age. We found that as compared to mothers aged 30 to 34.9 years, no differences in risk of preterm delivery were found. Mothers younger than 20 years had the highest risk of delivering small-size-for-gestational-age children (OR, 1.6 (95% CI: 1.1, 2.5)), however, this increased risk disappeared after adjustment for socio-demographic and lifestyle related determinants. Mothers older than 40 years had the highest risk of delivering large-size-for-gestational-age children (OR, 1.3 (95% CI: 0.8, 2.4)). The associations of maternal age with the risks of delivering large-size-for-gestational-age children could not be explained by socio-demographic and lifestyle related determinants. Our results suggest that as compared to mothers aged of 30 to 34.9 years, younger mothers have increased risk of small-size-for-gestational-age children, whereas older mothers have an increased risk of large-size-for-gestational-age children. Socio-demographic and lifestyle related determinants cannot fully explain these differences.

INTRODUCTION

Birth weight and preterm birth are strong predictors of neonatal morbidity and mortality[1-3]. Also, low birth weight is associated with diseases during adulthood, such as type 2 diabetes and cardiovascular disease, whereas higher birth weight tends to be associated with the risk of obesity in later life[4-6]. Maternal age might be modifiable determinant of weight and gestational age at birth. Preventive strategies focused on having an optimal maternal age for childbearing might be effective. In most Western countries the age at which mothers have their first child is still increasing due to various reasons including prolonged education, commitment to profession, delayed marriage, and other personal reasons[3]. It has been suggested that older maternal age is associated with increased risks of pregnancy complications such as gestational hypertension or diabetes, preterm delivery, fetal malformations and fetal death. A previous study showed lower birth weight among infants from younger mothers, but suggested an inverse U-shaped relationship between maternal age and birth weight[2]. Several mechanisms might explain the associations between maternal age and birth weight. The biological immaturity hypothesis suggests that lower birth weights in infants of younger mothers results from competition of the fetus for nutrients, which are also needed for the still growing mother[7-10]. Disadvantaged social environment and adverse lifestyle habits have also been suggested as underlying mechanisms[11,12].

We assessed in a population-based prospective cohort study among 8568 mothers and their children, the associations of maternal age with birth weight and the risk of preterm delivery, small-size-for-gestational-age, and large-size-for-gestational-age. We also assessed whether socio-demographic and lifestyle related determinants explained these associations.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood[13,14]. The cohort includes 9778 mothers and their children living in Rotterdam, the Netherlands and has been described in detail elsewhere[13,14]. All mothers were enrolled between 2001 and 2005, and all children were born between April 2002 and January 2006. Of all eligible children in the study area, 61% participated at birth in the study[14]. Midwives and obstetricians informed all eligible mothers about the Generation R study at their first prenatal visit in routine care. Inclusion in the study and subsequent measurements were performed in two dedicated research centres[14]. Enrolment was aimed at early pregnancy (gestational age <18 weeks) at the routine fetal ultrasound examination in pregnancy but was allowed until birth of the child. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations, and questionnaires were planned in each trimester of pregnancy. The individual timing of these assessments depended on the gestational age at enrolment[13,14].

Maternal age

Age of the mother was assessed at enrolment and accordingly categorized into 6 groups: younger than 20 years; 20 to 24.9 years; 25 to 29.9 years; 30 to 34.9 years; 35 to 39.9 years; 40 years and over. We used the age-group of 30 to 34.9 years as reference group in all analyses, because the median maternal age was in this group (median, 30.2 years; range, 15.3-46.3).

Birth outcomes

Information about fetal sex, gestational age, and weight at birth was obtained from medical records and hospital registries. Preterm delivery was defined as a gestational age of less than 37 weeks at birth. Small-size-for-gestational-age at birth was defined as a gestational age adjusted birth weight below the 5th percentile in the study cohort (<-1.82 standard deviation score for boys and <-1.74 standard deviation score for girls). Large-size-for-gestational-age at birth was defined as a gestational age adjusted birth weight above the 95th percentile in the study cohort (>1.58 standard deviation score for boys and >1.57 standard deviation score for girls).

Explaining variables

Basic determinants

Gestational age was established by fetal ultrasound examination during the first ultrasound visit[15]. Information on date of birth and fetal sex was obtained from midwife and hospital registries.

Socio-demographic determinants

Maternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment (median gestational age 14.4 weeks (95% range, 10.4-28.9)). Weight at enrolment was strongly correlated with pre-pregnancy weight (Pearson's correlation=0.95, p<0.01). We used maternal weight measured at enrolment in the analyses, because the numbers of missing values were smaller and data quality higher[16]. Information about educational level, ethnicity and parity was obtained by questionnaires. Maternal educational level was assessed by the highest completed education and classified into 3 categories; primary school, secondary school, and higher education. Ethnicity of the mother was classified into 7 groups; Dutch and other European, Surinamese, Turkish, Moroccan, Cape Verdian, Dutch Antilles, and others[17,18]. Parity was classified in 2 categories; nulliparous, and multiparous.

Lifestyle related determinants

Maternal alcohol use and smoking were assessed by questionnaires by asking pregnant women whether they smoked or consumed alcohol during pregnancy (categories; yes, until their pregnancy was known, no)[19,20]. Information about folic acid supplement use was obtained by a questionnaire at enrolment in the study and categorized into; preconceptional use, first 10 weeks of pregnancy use, no use[21]. Information about maternal caffeine intake was obtained by postal questionnaires in first, second, and third trimester of pregnancy. Total caffeine intake was categorized as; none,

<2 units per day, 2 to 3.9 units per day, 4 to 5.9 units per day, ≥6 units per day[22]. First trimester nutritional information (daily energy intake) was obtained by a food frequency questionnaire at enrolment.

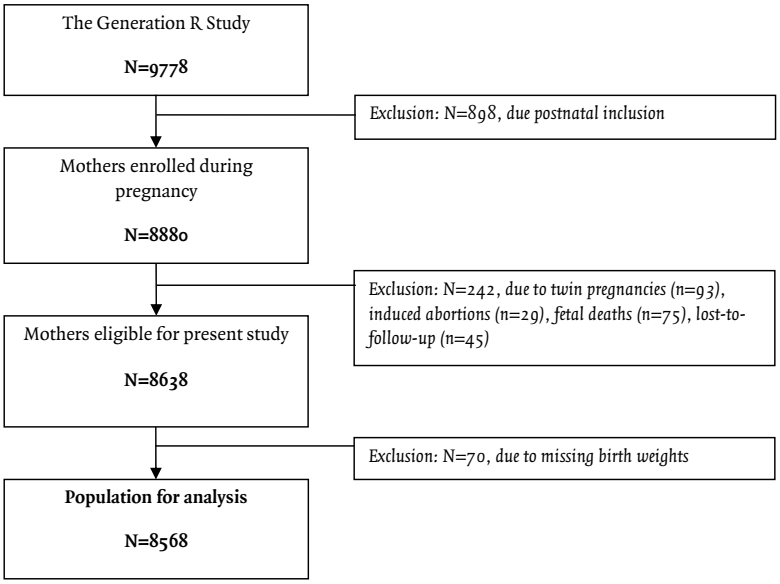
Population for analysis

In total 9778 mothers were enrolled during pregnancy[14]. Those who were enrolled after delivery of their child were excluded from the analyses (9%, n = 898). For the present study, we excluded twin pregnancies (n = 93), induced abortions (n = 29), fetal deaths (n = 75) and participants who were loss-to-follow-up (n = 45), and those with missing birth weights (n = 70). The associations of maternal age with birth outcomes were analyzed in the remaining 8568 mothers (Figure 1).

Statistical analyses

We assessed the associations of maternal age with continuously measured birth weight and the potential role of socio-demographic and lifestyle related determinants using linear regression models. We used a basic model (Model A) with adjustment for gestational age at birth and fetal sex; a socio-demographic determinants model (Model B) with adjustment for maternal height and weight, educational level, ethnicity, and parity and; and a lifestyle related determinants model (Model C) with adjustment for maternal alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake, and a cumulative model (Model D) including all determinants from Model A, B, and C together. Differences in birth weight were presented in grams with the 95% confidence interval (CI) for all age-groups compared to the 30 to 34.9

FIGURE 1. Flow chart of participants included for analysis



years age-group. The associations between maternal age and the risk of preterm delivery, small-size-for-gestational-age, and large-size-for-gestational-age were analyzed with multiple logistic regression analyses. We used similar basic, socio-demographic and lifestyle related, and cumulative determinant models. Models for the associations of maternal age with preterm delivery, small-size-for-gestational-age, and large-size-for-gestational-age were not adjusted for gestational age. Tests for trend were based on multiple non-linear or non-linear logistic regression models with maternal age as a continuous variable, and maternal age^2 as fractional polynomial to describe the non-linear nature of the association (Supplementary Tables S1 and S2). We used the ENTER method to construct our models. This method enters all variables at the same time. The studied covariates were based on prior knowledge from previous studies. The percentages of missing values within the population for analysis were lower than 15%, except for folic acid supplement use data (26%) and daily energy intake data (27%). These higher percentages were due the large number of mothers who only partially completed the food frequency questionnaire or were enrolled later in pregnancy. We used multiple imputation for missing values in the covariates. Five imputed data sets were created and analyzed together. All statistical analyses were performed using Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc. Chicago, IL, USA).

RESULTS

Subject characteristics

Maternal characteristics according to their age are shown in Table 1. Mothers in the youngest age-group were shorter, had the lowest weight and body mass index, were more frequently lower educated, were more frequently of non-Dutch ethnicity, and were more likely to continue smoking during pregnancy. Older mothers tended to be more frequently consuming alcohol and had the highest daily energy intake. Mothers younger than 20 years had children with the lowest mean birth weight and the highest frequencies of children with preterm birth and small-size-for-gestational-age at birth and the lowest frequencies of children with large-size-for-gestational-age. In total, 441 (5.1%) children were born preterm, 427 (5.0%) children were small-size-for-gestational-age, and 425 (4.9%) children were large-size-for-gestational-age.

Maternal age and birth weight

Table 2 shows the associations between maternal age and continuously measured birth weight. As compared to the reference age-group (30 to 34.9 years) lower offspring birth weight was observed for mothers younger than 20 years (difference: -51 grams (95% CI: -100, -2)) and 20 to 24.9 years (difference: -34 grams (95% CI: -64, -4)). Socio-demographic and lifestyle related determinants did explain only part of these associations. We did not observe significant differences in birth weight for the older age-groups (25 to 29.9 years, 35 to 39.9 years, and 40 years and over). For each model a consistent significant positive trend was found (P_{trend} <0.01).

TABLE 1. Subject characteristics by age-group[1]

Maternal age	<20 yrs n=364	20 to 24.9 yrs n=1436	25 to 29.9 yrs n=2339	30 to 34.9 yrs n=3153	35 to 39.9 yrs n=1131	≥40 yrs n=145	P-value[3]
Age, yrs	18.7 (1.1)	22.8 (1.4)	27.7 (1.5)	32.4 (1.4)	36.8 (1.4)	41.6 (1.3)	<0.001
Heigth, cm	165.0 (6.5)	165.2 (7.1)	166.4 (7.4)	168.3 (7.3)	168.2 (7.4)	167.8(8.2)	<0.001
Weight, kg	65.2 (13.3)	67.9(13.9)	69.8 (14.2)	69.7 (12.6)	71.0 (12.0)	72.4 (13.5)	<0.001
Body mass index, kg/m^2	22.5 (4.0)	23.8 (4.7)	24.0 (4.7)	23.5 (4.1)	23.6 (3.8)	23.9(4.4)	<0.001
Parity , % >1							
Nulliparous	74.2	61.6	52.5	45.3	29.7	27.5	<0.001
Multiparous	7.1	22.6	32.5	41.7	57.0	54.5	
Missing	18.7	15.8	15.0	13.0	13.3	17.0	
Education, %							
Primary school	24.7	16.1	11.3	6.6	8.2	16.6	<0.001
Secundary school	56.1	64.6	47.5	31.8	30.6	23.4	
Higher education	0.8	7.2	31.5	54.9	55.5	52.5	
Missing	18.4	12.1	9.7	6.7	5.7	7.5	
Ethnicity, %							
Dutch and other European	20.3	27.0	48.3	68.2	65.7	57.9	<0.001
Surinamese	17.6	13.7	8.9	5.1	6.5	6.2	
Turkish	9.1	16.9	10.8	4.8	4.1	4.8	
Moroccan	5.5	10.8	8.0	3.6	4.4	8.3	
Cape Verdian	11.5	6.4	3.4	2.7	2.7	1.4	
Dutch Antilles	8.5	6.8	3.1	1.6	1.5	1.4	
Other	11.7	8.2	9.6	8.5	10.5	14.5	
Missing	15.8	10.2	7.9	5.5	4.6	5.5	
Alcohol consumption, %							
None	52.2	58.4	51.0	33.4	31.4	33.8	<0.001
First trimester only	14.0	11.6	11.4	12.6	9.5	7.6	
Continued	15.1	15.2	23.4	41.6	46.4	42.1	
Missing	18.7	14.8	14.2	12.4	12.7	16.5	
Smoking habits, %							
None	47.3	53.9	64.5	69.3	65.9	64.8	<0.001
First trimester only	7.7	7.0	7.4	7.3	5.9	4.8	
Continued	25.3	23.6	13.3	10.6	14.9	12.4	
Missing	19.7	15.5	14.8	12.8	13.3	18.0	
Folic acid supplement use, %							
Preconceptional use	29.4	11.4	27.0	39.6	35.9	32.4	<0.001
First 10 weeks use	26.1	23.0	23.9	23.9	22.3	17.9	
No use	24.2	35.0	21.9	14.2	17.3	24.1	
Missing	20.3	30.6	27.2	22.3	24.5	25.6	

TABLE 1. (continued)

Maternal age	<20 yrs n=364	20 to 24.9 yrs n=1436	25 to 29.9 yrs n=2339	30 to 34.9 yrs n=3153	35 to 39.9 yrs n=1131	≥40 yrs n=145	P-value[3]
Daily energy intake, kcal	1986.4 (693.2)	1955.6 (619.2)	2023.7 (581.0)	2069.4 (533.5)	2060.1 (551.0)	2079.3 (557.8)	
Caffeine intake, %							
None	3.3	4.8	4.4	3.9	3.6	2.1	<0.001
<2 units per day	52.7	59.9	56.9	46.8	42.4	38.6	
2-3.9 units per day	29.4	19.8	25.4	33.6	36.3	39.3	
4-5.9 units per day	9.1	2.8	4.0	7.5	9.3	9.7	
≥6 units per day	1.6	0.8	0.8	1.6	2.4	2.1	
Missing	3.9	11.9	8.5	6.6	6.0	8.2	
Gender, % boys	47.5	50.8	50.1	50.5	51.6	50.3	0.85
Birth weight, g	3191 (521)	3309 (530)	3398 (550)	3465 (575)	3483 (552)	3421 (653)	<0.001
Gestational age, wks[2]	39.9 (34.9-42.3)	39.8 (35.4-42.3)	39.8 (35.9-42.4)	39.9 (35.9-42.4)	40.0 (35.9-42.4)	39.8 (34.1-42)	0.003
Preterm delivery, %	8.0	4.9	5.3	4.9	4.6	6.9	0.150
Small-size-for-gestational-age, %	6.9	6.2	5.0	4.3	4.8	4.1	0.064
Large-size-for-gestational-age, %	2.1	2.5	4.0	6.4	4.8	10.1	<0.001

[1]Values are means (standard deviation) or percentages.
[2]Median (95% range).
[3]Differences in subject characteristics between the age-groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.

Maternal age and the risks of adverse birth outcomes

Table 3 shows that mothers younger than 20 years had the highest risk of preterm delivery (Odds ratio (OR), 1.7 (95% CI: 1.1, 2.5)). The overall test for trend was not significant (P_{trend}=0.09). However, this higher risk of preterm delivery was affected after adjustment for socio-demographic and lifestyle related determinants. Older maternal age was not associated with the risk of preterm delivery. Younger mothers tended to have an increased risk of delivering small-size-for-gestational-age children (OR, 1.6 (95% CI: 1.1, 2.5)) for mothers aged younger than 20 years, and 1.5 (95% CI: 1.1, 1.9) for mothers aged between 20 and 24.9 years. Adjustment for socio-demographic and for lifestyle related determinants the associations changed. No significant trends were found for the risk of delivering a small-size-for-gestational-age. The risk of delivering a large-size-for-gestational-age child was reduced in age-groups below 30 years of age (OR, 0.3 (95% CI: 0.2, 0.6)) for mothers younger than 20 years, 0.4 (95% CI: 0.3, 0.5) for mothers aged between 20 and 24.9 years, and 0.6 (95% CI: 0.5, 0.8) for mothers aged 25 to 29.9 years. The reduced risk in mothers younger than 20 years of age was affected by adjusted for socio-demographic determinants. Also, in 35 to 39.9 year-old mothers, a reduced risk (OR, 0.7 (95% CI: 0.5, 1.0) of having a large-size-for-gestational-age

TABLE 2. Associations between maternal age and birth weight (N=8568 mothers)

	Difference in birth weight (grams (CI))[1]			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	-210 (-258, -162)**	-69 (-119, -20)**	-170 (-220, -120)**	-51 (-100, -2)*
n=364				
20 to 24.9 years	-142 (-169, -114)**	-48 (-78, -19)**	-110 (-140, -81)**	-34 (-64, -4)*
n=1436				
25 to 29.9 years	-56 (-79, -32)**	-14 (-37, 9)	-44 (-69, -20)**	-13 (-36, 11)
n=2339				
30 to 34.9 years	Reference	Reference	Reference	Reference
n=3153				
35 to 39.9 years	-0 (-29, 29)	-33 (-62, -4)*	4 (-26, 34)	-28 (-57, 1)
n=1131				
≥40 years	-29 (-67, 8)	-66 (-136, 4)	-26 (-99, 47)	-64 (-134, 6)
n=145				
	P_{trend}[6] <0.01	P_{trend} <0.01	P_{trend} <0.01	P_{trend} <0.01

Abbreviations: CI, confidence interval.

[1]Values reflect the differences (95% confidence interval) in birth weight in grams of children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimated from multiple imputed datasets.

[2]Model A (Basic model) is adjusted for gestational age at birth and fetal sex.

[3]Model B (Basic and socio-demographic model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity and parity.

[4]Model C (Basic and lifestyle related model) is adjusted for gestational age at birth, fetal sex, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[5]Model D (Cumulative model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[6]P-values for trend were based on multiple non-linear regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.

*P-value<0.05

**P-value<0.01

child was found. A tendency toward a higher risk was observed in mothers aged 40 years and over, which was not much affected by adjustment for socio-demographic and lifestyle related determinants. In all models a significant positive trend was found (P_{trend}<0.01).

We have repeated our analyses of maternal age and risk of adverse birth outcomes stratified for parity, results were similar (Supplementary Tables S3, S4, S5, and S6). Additional adjustment for gestational diabetes mellitus did not change our results (data not shown).

TABLE 3. Associations between maternal age and risk of adverse birth outcomes (N=8568 mothers)[1]

	Preterm delivery (Odds ratio (CI))[1] (n=441)			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	1.7 (1.1, 2.5)*	1.1 (0.7, 1.7)	1.4 (0.9, 2.2)	1.0 (0.6, 1.6)
n_{cases}=29				
20 to 24.9 years	1.0 (0.8, 1.3)	0.7 (0.5, 1.0)	0.9 (0.6, 1.2)	0.7 (0.5, 1.0)*
n_{cases}=71				
25 to 29.9 years	1.1 (0.9, 1.4)	0.9 (0.7, 1.2)	1.0 (0.8, 1.3)	0.9 (0.7, 1.2)
n_{cases}=124				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=155				
35 to 39.9 years	0.9 (0.7, 1.3)	1.0 (0.8, 1.4)	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)
n_{cases}=52				
≥40 years	1.4 (0.7, 2.8)	1.6 (0.8, 3.0)	1.4 (0.7, 2.8)	1.6 (0.8, 3.1)
n_{cases}=10				
	P_{trend}[6] =0.09	P_{trend}=0.22	P_{trend}=0.17	P_{trend}=0.29
Maternal age	**Small-size-for-gestational-age (Odds ratio (CI))[1] (n=427)**			
<20 years	1.6 (1.1, 2.5)*	0.9 (0.6, 1.5)	1.3 (0.8, 2.0)	0.8 (0.5, 1.3)
n_{cases}=25				
20 to 24.9 years	1.5 (1.1, 1.9)**	1.0 (0.7, 1.3)	1.2 (0.9, 1.6)	0.9 (0.6, 1.2)
n_{cases}=89				
25 to 29.9 years	1.1 (0.9, 1.5)	1.0 (0.7, 1.2)	1.1 (0.8, 1.4)	0.9 (0.7, 1.2)
n_{cases}=116				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=137				
35 to 39.9 years	1.1 (0.8, 1.5)	1.3 (0.9, 1.8)	1.1 (0.8, 1.5)	1.2 (0.9, 1.7)
n_{cases}=54				
≥40 years	0.9 (0.4, 2.2)	1.1 (0.5, 2.5)	0.9 (0.4, 2.1)	1.1 (0.5, 2.5)
n_{cases}=6				
	P_{trend}=0.13	P_{trend}=0.38	P_{trend}=0.41	P_{trend}=0.67
Maternal age	**Large-size-for-gestational-age (Odds ratio (CI))[1] (n=425)**			
<20 years	0.3 (0.2, 0.6)**	0.7 (0.3, 1.5)	0.4 (0.2, 0.8)*	0.8 (0.4, 1.6)
n_{cases}=8				
20 to 24.9 years	0.4 (0.3, 0.5)**	0.6 (0.4, 0.9)*	0.4 (0.3, 0.7)**	0.7 (0.4, 1.0)*
n_{cases}=38				
25 to 29.9 years	0.6 (0.5, 0.8)**	0.7 (0.5, 0.9)*	0.6 (0.5, 0.8)**	0.7 (0.6, 0.9)*
n_{cases}=96				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=212				
35 to 39.9 years	0.7 (0.5, 1.0)*	0.6 (0.5, 0.8)**	0.7 (0.5, 1.0)*	0.6 (0.5, 0.9)**
n_{cases}=56				

TABLE 3. (continued)

≥40 years	1.6 (0.9, 2.7)	1.3 (0.8, 2.4)	1.6 (0.9, 2.8)	1.3 (0.8, 2.4)
n_{cases}=15				
	P_{trend}=0.01	P_{trend}=0.13	P_{trend}=0.04	P_{trend}=0.21

Abbreviations: CI, confidence interval.
[1]Values are odds ratios (95% confidence interval) that reflect the difference in risk of adverse birth outcomes between children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimated from multiple imputed datasets.
[2]Model A (Basic model) is adjusted fetal sex (only for preterm delivery).
[3]Model B (Basic and socio-demographic model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity and parity.
[4]Model C (Basic and lifestyle related model) is adjusted for fetal sex (only for preterm delivery), alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[5]Model D (Cumulative model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[6]P-values for trend were based on multiple non-linear logistic regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.
*P-value<0.05
**P-value<0.01

DISCUSSION

Our results suggest that younger mothers deliver children with a lower birth weight. Younger maternal age was associated with increased risks of small-size-for-gestational-age children, and lower risks of delivering large-size-for-gestational-age children. Socio-demographic but not lifestyle related determinants explained the associations between maternal age and the risks delivering small-size-for-gestational-age children. The associations of maternal age with the risks of delivering large-size-for-gestational-age children could not be explained by socio-demographic and lifestyle related determinants.

Methodological considerations

One of the strengths of this study is the population-based cohort including a large number of subjects studied from early pregnancy onwards. Furthermore, detailed information about a large number of potential determinants was available in this study. However, because of the observational design, residual confounding due to other socio-demographic and lifestyle related determinants might still be an issue. The response rate at baseline for participation of the children at birth in the Generation R Study cohort was 61%[14]. This non-response would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss-to-follow-up rather than from non-response at baseline[23]. Selection bias occurs if participation depends on both the exposure, maternal age, and the outcome,

birth outcomes[24]. Due to the prospective nature of the study selection on the outcome at baseline is not an issue.

Information on many covariates in this study was self-reported, which may have resulted in underreporting of certain lifestyle related determinants and subsequently have led to underestimation of differences those with and without certain lifestyles. This may occurred most likely in reporting smoking habits and alcohol consumption. Previously, biomarkers have been used to validate tobacco exposure (with cotinine as biomarker) and alcohol consumption (with carbohydrate-deficient transferrin or gamma-glutamyl transferase as biomarker)[25-27]. However, it has been demonstrated that use of cotinine levels is not superior to the use of self-reporting questionnaires[28]. Also, the use of biomarkers of alcohol consumption have a low sensitivity in subjects with light-to-moderate alcohol consumption, which are most of the mothers included[27]. Furthermore, we only had a relatively smaller number (n = 145) of participants in the age-group 40 years and over, which might have led to loss of power in this group.

In total, only four different outcomes were studied; birth weight, preterm delivery, small-size-for-gestational-age at birth, and large-size-for-gestational-age at birth. Since our results are not independent outcomes, we did not perform adjustment for multiple testing. However, if we would apply Bonferroni correction, the associations of maternal age with birth weight would remain significant (P-value<0.01).

Maternal age and birth weight

The inverse U-shaped relationship between maternal age and birth weight found in a previous study of MacLeod and Kiely was consistent with our results[2]. We found a significantly lower birth weight in infants of mothers younger than 20 years and a higher birth weight after 20 years of age following a lower birth weight in the highest age-group of 40 years and over. The mechanisms explaining the associations between maternal age and pregnancy outcomes are not known but might include socio-demographic and lifestyle related determinants. We and others have previously suggested that birth weight is influenced by maternal factors such as ethnicity, educational level, parity and smoking[2,17,20,29,30-32]. Our results show that the associations between maternal age and offspring birth weight could not fully be explained by socio-demographic and lifestyle related determinants. Although, the differences in birth weight were smaller after adjustment for socio-demographic determinants, the tests for trend remained significant. Our findings are consistent with previous studies that showed lower birth weight among the infants of younger mothers and suggested that social environmental factors explain these differences[2,33]. Strobino *et al.* observed a lower offspring birth weight in the maternal age-groups of 14 to 17 years (difference: -133.0 grams, 95% CI: -231.1, -34.9), 18 to 19 years (difference: -54.2 grams, 95% CI: -135.6, 27.2)) and 20 to 22 years (difference:-88.37 grams, 95% CI: -165.4, -11.3)[33]. They concluded that these differences were largely a result of a disadvantaged social environment. A population based study in New York also observed a higher birth weight among older mothers after adjustment for gestational age and parity[2]. This finding is consistent with our results in older mothers. Thus far

studies focused on the association between maternal age and birth weight are not consistent[34,35]. Differences in results might be explained by differences in characteristics of the study populations. Mothers living in the Netherlands, especially compared to non-Western countries, are relatively rich and live in relatively good social environments.

It has been hypothesized that the lower birth weight of children of younger mothers might be explained by a nutrients competition between the mother and the fetus[8]. Also, some suggest that other socio-demographic and psychological factors might be important since childbearing in younger mothers is more often unplanned or established at older gestational age. Adolescent mothers are more likely to book for antenatal care later in pregnancy or fail to use it than older mothers, while it has been demonstrated that mothers who had antenatal care from first trimester onwards have lower frequencies of low birth weight children[36-38].

Maternal age and the risk of adverse birth outcomes

Among the mothers in different age-groups we did not find increased or decreased risks of preterm delivery. Milner *et al.* suggested that mothers of 40 years and over had increased risks of preterm delivery and low birth weight[39]. The authors suggested that these increased risks in preterm delivery and low birth weight in the age-group of 40 years and over is explained by higher parity in this age-group[40]. Studies showed interaction of parity on the association of maternal age and birth weight, and the risk of neonatal and fetal deaths[2,42]. Keily *et al.* found interaction between parity and age such that mothers over 34 years old having their first birth were at especially high risk of neonatal death[41]. After repeating our analyses stratified for parity, no differences in results were found between nulliparous versus multiparous women. Scholl *et al.* observed increased risks of preterm delivery and low birth weight among children from younger mothers, which might be explained by poverty and poor nutrition[42]. Rich-Edwards and Grizzard suggested that chronic exposure to poverty, racism, and insecure neighborhoods may cause stress responses, which results in altered physiologic effects and increase the risk of preterm delivery[43]. Higher stress levels are related to early childbearing, and might be involved in the pathways leading from younger maternal age to higher risk of preterm delivery.

We observed an increased risk of small-size-for-gestational-age in children from mothers younger than 24.9 years, and a reduced risk of large-size-for-gestational-age children in all age-groups compared to the reference group. This increased risk in small-size-for-gestational-age was mainly explained by socio-demographic and lifestyle related determinants. The reduced risk of large-size-for-gestational-age children in mothers younger than 24.9 years of age was coherent to the results on small-size-for-gestational-age. However, the results found in the higher age-groups suggest an optimum birth weight for mothers between the ages of 30 to 34.9 years.

Our results suggest that adverse birth outcomes associated with maternal age are not only to socio-demographic and lifestyle related determinants. Biological factors in mothers younger than 20 years of age and mothers older than 40 years of age may also influence birth outcomes. Lowest birth weights were found among mothers younger than 25 years of age, and highest risks of

delivering a large-size-for-gestational-age child was found among mothers over 39.9 years of age. Future research is needed to identify these biological effects. Also, more studies of other adverse pregnancy outcomes associated with maternal age might be of great interest for healthcare policy makers.

Conclusion

As compared to mothers aged of 30 to 34.9 years, younger mothers have children with a lower birth weight, and increased risks of small-size-for-gestational-age children whereas, older mothers have increased risks of large-size-for-gestational-age children. Socio-demographic and lifestyle related determinants cannot fully explain these differences.

REFERENCES

1. Bacak SJ, Baptiste-Roberts K, Amon E, et al. Risk factors for neonatal mortality among extremely-low-birth-weight infants. Am J Obstet Gynecol. 2005;192:862-7.
2. MacLeod S, Kiely JL. The effects of maternal age and parity on birthweight: a population-based study in New York City. Int J Gynaecol Obstet. 1988;26:11-9.
3. Vohr BR, Tyson JE, Wright LL, et al. Maternal age, multiple birth, and extremely low birth weight infants. J Pediatr. 2009;154:498-503 e2.
4. Barker DJ. Fetal origins of coronary heart disease. BMJ 1995;311:171-4.
5. Curhan GC, Willett WC, Rimm EB, et al. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation. 1996;94:3246-50.
6. Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. BMJ 2001;323:1331-5.
7. Erkan KA, Rimer BA, Stine OC. Juvenile pregnancy: role of physiologic maturity. Md State Med J. 1971;20:50-2.
8. Garn SM, Petzold AS. Characteristics of the mother and child in teenage pregnancy. Am J Dis Child. 1983;137: 365-8.
9. Naeye RL. Teenaged and pre-teenaged pregnancies: consequences of the fetal-maternal competition for nutrients. Pediatrics. 1981;67:146-50.
10. Scholl TO, Decker E, Karp RJ, et al. Early adolescent pregnancy: a comparative study of pregnancy outcome in young adolescents and mature women. J Adolesc Health Care. 1984;5:167-71.
11. Graham D. The obstetric and neonatal consequence of adolescent pregnancy. Birth Defects Orig Artic Ser. 1981; 17:49-67.
12. Zackler J, Andelman SL, Bauer F. The young adolescent as an obstetric risk. Am J Obstet Gynecol. 1969;103: 305-12.
13. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
14. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
15. Verburg BO, Steegers EA, de Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growht: longitudinal data from a population-based cohort study. Ultrasound Obstet Gyneacol. 2008;31: 388-96.
16. Ay L, Kruithof CJ, Bakker R, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG 2009;116:953-63.
17. Troe EJ, Raat H, Jaddoe VW, et al. Explaining differences in birthweight between ethnic populations. The Generation R Study. BJOG 2007;114:1557-65.
18. Statistics Netherlands aSN, 2004a. Statistics Netherlands, 2004a Allochtonen in Nederland 2004 (in Dutch). Voorburg/ Heerlen.
19. Bakker R, Pluimgraaff LE, Steegers EA, et al. Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy: The Generation R Study. Int J Epidemiol. 2010;39:777-89.
20. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the Generation R Study. Am J Epidemiol. 2007;165:1207-15.
21. Timmermans S, Jaddoe VW, Hofman A, et al. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102:777-85.
22. Bakker R, Steegers EA, Obradov A, et al. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: the Generation R Study. Am J Clin Nutr. 2010;91:1691-98.
23. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
24. Greenland S. Response and follow-up bias in cohort studies. Am J Epidemiol. 1977;106:184-187.

25. Hebel JR, Fox NL, Sexton M. Dose-response of birth weight to various measures of maternal smoking during pregnancy. J Clin Epidemiol. 1988;41:483-9.
26. Wang X, Tager IB, Van Vunakis H, et al. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol. 1997;26:978-88.
27. van Pelt J, Leusink GL, van Nierop PW, et al. Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res. 2000;24:176–79.
28. Haddow JE, Knight GJ, Palomaki GE, et al. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gyneacol. 1987;94:678-81.
29. Kovar MG. Variations in birth weight. Legitimate live births. United States-1963. Vital Health Stat 1. 1968;22:1-35.
30. Harper KW. Maternal, infant and social factors associated with birthweight. Ulster Med J. 1976;45:210-5.
31. Jansen PW, Tiemeier H, Looman CW, et al. Explaining educational inequalities in birthweight: the Generation R Study. Paediat Peri Epidemiol. 2009;23:216-28.
32. Lumley J, Correy JF, Newman NM, et al. Cigarette smoking, alcohol consumption and fetal outcome in Tasmania 1981-82. Aust N Z J Obstet Gynaecol. 1985;25:33-40.
33. Strobino DM, Ensminger ME, Kim YJ, et al. Mechanisms for maternal age differences in birth weight. Am J Epidemiol. 1995;142:504-14.
34. Dougherty CR, Jones AD. Th determinants of birth weight. Am J Obstet Gynecol. 1982;144:190-200.
35. Murphy JF, Mulcahy R. The effect of age, parity, and cigarette smoking on baby weight. Am J Obstet Gynecol. 1971;111:22-5.
36. Horon IL, Strobino DM, MacDonald HM. Birth weights among infants born to adolescent and young adult women. Am J Obstet Gynecol. 1983;146:444-9.
37. Gortzak-Uzan L, Hallak M, Press F, et al. Teenage pregnancy: risk factors for adverse perinatal outcome. J Matern Fetal Med. 2001;10:393-7.
38. Haeri S, Guichard I, Saddlemire S. Maternal characteristics and outcomes associated with late enrollment for care in teenage pregnancies. Southern Med J. 2009;102:265-8.
39. Milner M, Barry-Kinsella C, Unwin A, et al. The impact of maternal age on pregnancy and its outcome. Int J Gynaecol Obstet. 1992;38:281-6.
40. Mansfield PK, McCool W. Toward a better understanding of the "advanced maternal age" factor. Health Care Women Int. 1989;10:395-415.
41. Keily JL, Paneth N, Susser M. An assessment of the effects of maternal age and parity in different components of perinatal mortality. Am J Epidemiol. 1986;123:444-54.
42. Scholl TO, Miller LK, Shearer J, et al. Influence of young maternal age and parity on term and preterm low birthweight. Am J Perinatol. 1988;5:101-4.
43. Rich-Edwards JW, Grizzard TA. Psychosocial stress and neuroendocrine mechanisms in preterm delivery. Am J Obstet Gynecol. 2005;192:S30-5.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Effect estimates of non-linear tests for trend of the association between maternal age and birth weight (N=8568)[1]

	Birth weight (grams)		
Term	β	95% confidence interval	P-value
Model A[2]			
Maternal age	52.87	36.91, 68.84	<0.001
(Maternal age)^2	-0.73	-1.00, -0.46	<0.001
Model B[3]			
Maternal age	30.61	15.06, 46.16	<0.001
(Maternal age)^2	-0.50	-0.76, -0.24	<0.001
Model C[4]			
Maternal age	42.02	25.86, 58.17	<0.001
(Maternal age)^2	-0.58	-0.85, -0.31	<0.001
Model D[5]			
Maternal age	24.15	8.59, 39.72	0.002
(Maternal age)^2	-0.41	-0.67, -0.14	0.002

[1]Estimates are pooled estimates from multiple imputed datasets, and are based on multiple non-linear regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.
[2]Model A (Basic model) is adjusted for gestational age at birth and fetal sex.
[3]Model B (Basic and socio-demographic model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity and parity.
[4]Model C (Basic and lifestyle related model) is adjusted for gestational age at birth, fetal sex, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[5]Model D (Cumulative model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

SUPPLEMENTARY TABLE S2. Effect estimates of non-linear tests for trend of the association between maternal age and risk of adverse birth outcomes (N=8568)[1]

	Preterm delivery (Odds ratio)		
Term	β	95% confidence interval	P-value
Model A[2]			
Maternal age	0.87	0.74, 1.01	0.065
(Maternal age)^2	1.00	1.00, 1.00	0.091
Model B[3]			
Maternal age	0.92	0.79, 1.08	0.311
(Maternal age)^2	1.00	1.00, 1.00	0.217
Model C[4]			
Maternal age	0.90	0.77, 1.05	0.167
(Maternal age)^2	1.00	1.00, 1.00	0.170

SUPPLEMENTARY TABLE S2. (continued)

Model D[5]			
Maternal age	0.94	0.80, 1.10	0.427
(Maternal age)[2]	1.00	1.00, 1.00	0.286
	Small-size-for-gestational-age (Odds ratio)		
Term	β	95% confidence interval	P-value
Model A[2]			
Maternal age	0.87	0.74, 1.01	0.068
(Maternal age)[2]	1.00	1.00, 1.00	0.127
Model B[3]			
Maternal age	0.95	0.80, 1.11	0.492
(Maternal age)[2]	1.00	1.00, 1.00	0.376
Model C[4]			
Maternal age	0.93	0.79, 1.09	0.349
(Maternal age)[2]	1.00	1.00, 1.00	0.410
Model D[5]			
Maternal age	0.99	0.84, 1.16	0.877
(Maternal age)[2]	1.00	1.00, 1.00	0.673
	Large-size-for-gestational-age (Odds ratio)		
Term	β	95% confidence interval	P-value
Model A[2]			
Maternal age	1.37	1.12, 1.67	0.002
(Maternal age)[2]	1.00	0.99, 1.00	0.011
Model B[3]			
Maternal age	1.19	0.97, 1.47	0.094
(Maternal age)[2]	1.00	0.99, 1.00	0.127
Model C[4]			
Maternal age	1.29	1.05, 1.57	0.013
(Maternal age)[2]	1.00	0.99, 1.00	0.044
Model D[5]			
Maternal age	1.16	0.94, 1.42	0.171
(Maternal age)[2]	1.00	0.99, 1.00	0.207

[1]Estimates are pooled estimates from multiple imputed datasets, and are based on multiple non-linear logistic regression models; birth weight = maternal age + (maternal age)[2] + covariates per model.
[2]Model A (Basic model) is adjusted fetal sex (only for preterm delivery).
[3]Model B (Basic and socio-demographic model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity and parity.
[4]Model C (Basic and lifestyle related model) is adjusted for fetal sex (only for preterm delivery), alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[5]Model D (Cumulative model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

SUPPLEMENTARY TABLE S3. Associations between maternal age and birth weight in nulliparous mothers (N=4185)

	Difference in birth weight (grams (CI))[1]			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	-145 (-197, -92)**	-71 (-128, -13)*	-86 (-146, -27)**	-56 (-116, 4)
n=270				
20 to 24.9 years	-94 (-129, -59)**	-41 (-82, 1)	-48 (-89, -8)*	-29 (-73, 14)
n=885				
25 to 29.9 years	-15 (-47, 16)	5 (-26, 37)	0 (-33, 32)	4 (-28, 36)
n=1228				
30 to 34.9 years	Reference	Reference	Reference	Reference
n=1426				
35 to 39.9 years	15 (-34, 64)	5 (-42, 53)	17 (-32, 65)	9 (-38, 56)
n=336				
≥40 years	-79 (-209, 50)	-109 (-233, 16)	-75 (-204, 54)	-104 (-228, 21)
n=40				
	P_{trend}[6] <0.01	P_{trend} <0.01	P_{trend} =0.01	P_{trend} =0.05

Abbreviations: CI, confidence interval.

[1]Values reflect the differences (95% confidence interval) in birth weight in grams of children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimates from multiple imputed datasets.

[2]Model A (Basic model) is adjusted for gestational age at birth and fetal sex.

[3]Model B (Basic and socio-demographic model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity and parity.

[4]Model C (Basic and lifestyle related model) is adjusted for gestational age at birth, fetal sex, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[5]Model D (Cumulative model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[6]P-values for trend were based on multiple non-linear regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.

*P-value<0.05

**P-value<0.01

SUPPLEMENTARY TABLE S4. Associations between maternal age and risk of adverse birth outcomes in nulliparous mothers (N=4185)[1]

	Preterm delivery (Odds ratio (CI))[1] (n=260)			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	1.4 (0.9, 2.2)	1.1 (0.7, 1.9)	1.1 (0.6, 1.8)	1.0 (0.6, 1.7)
n_{cases}=25				
20 to 24.9 years	0.9 (0.7, 1.3)	0.8 (0.5, 1.1)	0.7 (0.5, 1.1)	0.7 (0.5, 1.1)
n_{cases}=54				
25 to 29.9 years	1.0 (0.7, 1.4)	0.9 (0.7, 1.3)	0.9 (0.7, 1.3)	0.9 (0.6, 1.3)
n_{cases}=75				

SUPPLEMENTARY TABLE S4. (continued)

30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=86				
35 to 39.9 years	0.9 (0.5, 1.5)	0.9 (0.6, 1.5)	0.9 (0.5, 1.5)	0.9 (0.5, 1.5)
n_{cases}=17				
≥40 years	1.5 (0.5, 4.8)	1.6 (0.5, 5.0)	1.5 (0.5, 4.9)	1.6 (0.5, 5.1)
n_{cases}=3				
	P_{trend}[6] =0.14	P_{trend}=0.26	P_{trend}=0.29	P_{trend}=0.34
Maternal age	**Small-size-for-gestational-age (Odds ratio (CI))[1] (n=254)**			
<20 years	1.4 (0.9, 2.3)	0.9 (0.5, 1.6)	1.0 (0.6, 1.8)	0.8 (0.5, 1.5)
n_{cases}=20				
20 to 24.9 years	1.2 (0.9, 1.7)	0.9 (0.6, 1.3)	0.9 (0.6, 1.4)	0.8 (0.5, 1.3)
n_{cases}=61				
25 to 29.9 years	1.1 (0.8, 1.5)	1.0 (0.7, 1.4)	1.0 (0.7, 1.5)	1.0 (0.7, 1.4)
n_{cases}=70				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=77				
35 to 39.9 years	1.2 (0.7, 1.9)	1.2 (0.8, 2.0)	1.2 (0.7, 1.8)	1.2 (0.7, 1.9)
n_{cases}=22				
≥40 years	1.6 (0.6, 4.7)	1.8 (0.6, 5.3)	1.6 (0.6, 4.5)	1.7 (0.6, 5.0)
n_{cases}=4				
	P_{trend}=0.20	P_{trend}=0.51	P_{trend}=0.60	P_{trend}=0.83
Maternal age	**Large-size-for-gestational-age (Odds ratio (CI))[1] (n=142)**			
<20 years	0.5 (0.2, 1.1)	0.9 (0.4, 2.0)	0.7 (0.3, 1.6)	0.9 (0.4, 2.2)
n_{cases}=6				
20 to 24.9 years	0.5 (0.3, 0.9)**	0.7 (0.4, 1.2)	0.6 (0.4, 1.1)	0.7 (0.4, 1.3)
n_{cases}=20				
25 to 29.9 years	0.7 (0.5, 1.1)	0.8 (0.5, 1.1)	0.8 (0.5, 1.1)	0.8 (0.5, 1.2)
n_{cases}=37				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=62				
35 to 39.9 years	1.1 (0.7, 1.9)	1.0 (0.6, 1.8)	1.1 (0.7, 1.9)	1.0 (0.6, 1.8)
n_{cases}=15				
≥40 years	1.4 (0.4, 5.0)	1.0 (0.3, 4.1)	1.4 (0.4, 5.2)	1.1 (0.3, 4.3)
n_{cases}=2				
	P_{trend}=0.78	P_{trend}=0.87	P_{trend}=0.88	P_{trend}=0.74

Abbreviations: CI, confidence interval.

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risk of adverse birth outcomes between children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimates from multiple imputed datasets.

[2]Model A (Basic model) is adjusted fetal sex (only for preterm delivery).

[3]Model B (Basic and socio-demographic model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity and parity.
[4]Model C (Basic and lifestyle related model) is adjusted for fetal sex (only for preterm delivery), alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[5]Model D (Cumulative model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[6]P-values for trend were based on multiple non-linear logistic regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.
*P-value<0.05
**P-value<0.01

SUPPLEMENTARY TABLE S5. Associations between maternal age and birth weight in multiparous mothers (N=3150)

	Difference in birth weight (grams (CI))[1]			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	-170 (-351, 11)	-66 (-247, 114)	-113 (-282, 56)	-55 (-229, 119)
n=26				
20 to 24.9 years	-139 (-192, -85)**	-62 (-117, -7)*	-87 (-146, -27)**	-45 (-102, 12)
n=324				
25 to 29.9 years	-79 (-117, -40)**	-40 (-78, -2)*	-50 (-91, -8)*	-35 (-73, 4)
n=761				
30 to 34.9 years	Reference	Reference	Reference	Reference
n=1315				
35 to 39.9 years	-53 (-94, -13)**	-62 (-101, -23)**	-53 (-92, -14)**	-57 (-95, -19)**
n=645				
≥40 years	-55 (-150, 40)	-58 (-148, 32)	-54 (-148, 40)	-57 (-147, 33)
n=79				
	P_{trend}[6] <0.01	P_{trend} <0.01	P_{trend} <0.01	P_{trend} <0.01

Abbreviations: CI, confidence interval.
[1]Values reflect the differences (95% confidence interval) in birth weight in grams of children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimates from multiple imputed datasets.
[2]Model A (Basic model) is adjusted for gestational age at birth and fetal sex.
[3]Model B (Basic and socio-demographic model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity and parity.
[4]Model C (Basic and lifestyle related model) is adjusted for gestational age at birth, fetal sex, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[5]Model D (Cumulative model) is adjusted for gestational age at birth, fetal sex, height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.
[6]P-values for trend were based on multiple non-linear regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.
*P-value<0.05
**P-value<0.01

SUPPLEMENTARY TABLE S6. Associations between maternal age and risk of adverse birth outcomes in multiparous mothers (N=3150)[1]

	Preterm delivery (Odds ratio (CI))[1] (n=109)			
Maternal age	Model A[2]	Model B[3]	Model C[4]	Model D[5]
<20 years	1.0 (0.2, 6.7)	0.8 (0.1, 5.5)	0.8 (0.1, 5.6)	0.8 (0.1, 5.4)
n_{cases}=1				
20 to 24.9 years	0.8 (0.4, 1.6)	0.7 (0.4, 1.3)	0.7 (0.4, 1.4)	0.7 (0.3, 1.3)
n_{cases}=11				
25 to 29.9 years	1.1 (0.7, 1.8)	0.9 (0.6, 1.6)	1.0 (0.6, 1.7)	0.9 (0.6, 1.6)
n_{cases}=27				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=43				
35 to 39.9 years	1.1 (0.7, 1.8)	1.2 (0.7, 1.8)	1.2 (0.7, 1.9)	1.2 (0.7, 1.8)
n_{cases}=23				
≥40 years	1.7 (0.6, 4.4)	1.6 (0.6, 4.2)	1.7 (0.6, 4.4)	1.6 (0.6, 4.2)
n_{cases}=4				
	P_{trend}[6] =0.77	P_{trend}=0.99	P_{trend}=0.94	P_{trend}=0.99
Maternal age	**Small-size-for-gestational-age (Odds ratio (CI))[1] (n=102)**			
<20 years	n.a.	n.a.	n.a.	n.a.
n_{cases}=0				
20 to 24.9 years	1.7 (1.0, 3.1)	1.3 (0.7, 2.3)	1.3 (0.7, 2.3)	1.1 (0.6, 1.9)
n_{cases}=18				
25 to 29.9 years	1.0 (0.6, 1.8)	0.8 (0.5, 1.5)	0.8 (0.5, 1.5)	0.8 (0.5, 1.4)
n_{cases}=21				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=39				
35 to 39.9 years	1.2 (0.8, 2.0)	1.3 (0.8, 2.1)	1.3 (0.8, 2.0)	1.3 (0.8, 2.1)
n_{cases}=22				
≥40 years	0.6 (0.2, 2.6)	0.6 (0.1, 2.5)	0.6 (0.2, 2.6)	0.6 (0.1, 2.6)
n_{cases}=2				
	P_{trend}=0.28	P_{trend}=0.50	P_{trend}=0.50	P_{trend}=0.63
Maternal age	**Large-size-for-gestational-age (Odds ratio (CI))[1] (n=226)**			
<20 years	n.a.	n.a.	n.a.	n.a.
n_{cases}=0				
20 to 24.9 years	0.4 (0.2, 0.6)**	0.5 (0.3, 1.0)*	0.5 (0.3, 0.9)*	0.6 (0.3, 1.1)
n_{cases}=11				
25 to 29.9 years	0.6 (0.4, 0.8)**	0.7 (0.5, 1.0)*	0.7 (0.5, 1.0)*	0.7 (0.5, 1.0)
n_{cases}=46				
30 to 34.9 years	Reference	Reference	Reference	Reference
n_{cases}=127				
35 to 39.9 years	0.5 (0.4, 0.8)**	0.5 (0.3, 0.7)**	0.5 (0.3, 0.7)**	0.5 (0.3, 0.7)**

SUPPLEMENTARY TABLE S6. (continued)

n_{cases}=32				
≥40 years	1.4 (0.7, 2.6)	1.4 (0.7, 2.6)	1.4 (0.7, 2.6)	1.4 (0.7, 2.6)
n_{cases}=10				
	P_{trend}<0.01	P_{trend}=0.04	P_{trend}=0.02	P_{trend}=0.06

Abbreviations: CI, confidence interval.

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risk of adverse birth outcomes between children of mothers in different age-groups compared to children of mothers in age-group 30 to 34.9 years. Estimates are pooled estimates from multiple imputed datasets.

[2]Model A (Basic model) is adjusted fetal sex (only for preterm delivery).

[3]Model B (Basic and socio-demographic model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity and parity.

[4]Model C (Basic and lifestyle related model) is adjusted for fetal sex (only for preterm delivery), alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[5]Model D (Cumulative model) is adjusted for fetal sex (only for preterm delivery), height, weight, educational level, ethnicity, parity, alcohol consumption, smoking habits, caffeine intake, folic acid supplement use and daily energy intake.

[6]P-values for trend were based on multiple non-linear logistic regression models; birth weight = maternal age + (maternal age)^2 + covariates per model.

*P-value<0.05

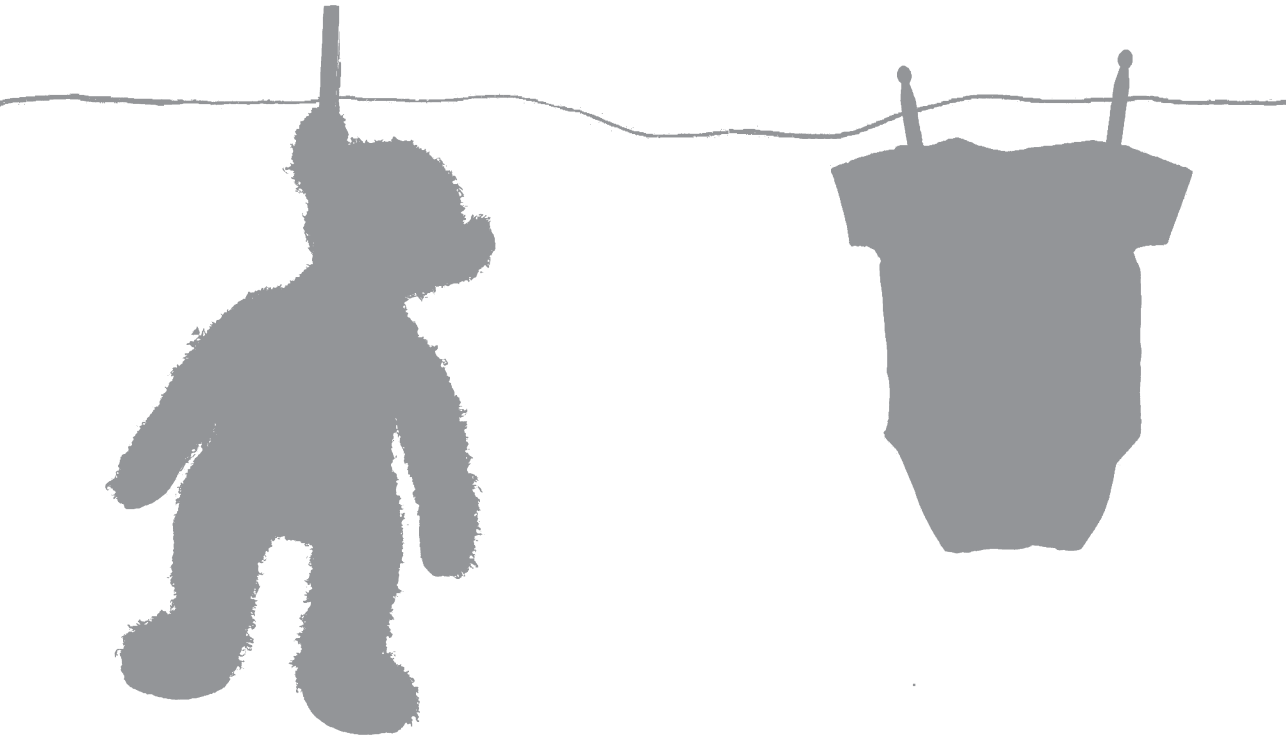
**P-value<0.01

Chapter 4.2

Maternal alcohol consumption and fetal growth

Rachel Bakker
Liane E Pluimgraaff
Eric AP Steegers
Hein Raat
Henning Tiemeier
Albert Hofman
Vincent WV Jaddoe

Adapted from Int J Epidemiol. 2010;39:777-89.



ABSTRACT

Excessive alcohol consumption during pregnancy has adverse effects on fetal growth and development. Less consistent associations have been shown for the associations of light-to-moderate maternal alcohol consumption during pregnancy with health outcomes in the offspring. Therefore, we examined the associations of light-to-moderate maternal alcohol consumption with various fetal growth characteristics measured in different periods of pregnancy. This study was based on 7333 mothers participating in a population-based cohort study. Alcohol consumption habits and fetal growth were assessed in early (gestational age <17.9 weeks), mid- (gestational age 18–24.9 weeks) and late pregnancy (gestational age ≥25 weeks). We assessed the effects of different categories of alcohol consumption (no; less than one drink per week; one to three drinks per week; four to six drinks per week; one drink per day and two to three drinks per day) on repeatedly measured fetal head circumference, abdominal circumference and femur length. In total, 37% of all mothers continued alcohol consumption during pregnancy, of whom the majority used less than three drinks per week. We observed no differences in growth rates of fetal head circumference, abdominal circumference or femur length between mothers with and without continued alcohol consumption during pregnancy. Compared with mothers without alcohol consumption, mothers with continued alcohol consumption during pregnancy had an increased fetal weight gain (difference: 0.61 g (95% confidence interval: 0.18, 1.04) per week). Cross-sectional analyses in mid- and late pregnancy showed no consistent associations between the number of alcoholic consumptions and fetal growth characteristics. All analyses were adjusted for potential confounders. Light-to-moderate maternal alcohol consumption during pregnancy does not adversely affect fetal growth characteristics. Further studies are needed to assess whether moderate alcohol consumption during pregnancy influences organ growth and function in postnatal life.

INTRODUCTION

Excessive maternal alcohol consumption during pregnancy is associated with various pregnancy complications such as multiple birth defects, fetal alcohol syndrome and an increased risk of low birth weight[1-9]. Less is known about the effects of light-to-moderate maternal alcohol consumption[10-12]. In general, light-to-moderate alcohol consumption during pregnancy is considered as one or less alcoholic consumption per day on average.

Previous studies focusing on the effects of light-to-moderate alcohol consumption during pregnancy on birth outcomes have shown inconsistent results[2,3,11-16]. Most of these studies did not take the effect of potential confounders, such as maternal age, smoking habits, weight, height, educational level, ethnicity and parity into account[15,17-20]. Recently, we observed in a population-based cohort among more than 7000 subjects that only an average maternal alcohol consumption of more than one drink per day is associated with the risk of low birth weight[21]. To our knowledge, no previous studies focused on the associations of light and moderate maternal consumption with fetal growth characteristics in different trimesters in a population-based prospective cohort design. Such studies would also be able to identify specific critical periods for fetal growth and development.

Therefore, we examined the associations of light-to-moderate maternal alcohol consumption with fetal growth characteristics measured at different periods of pregnancy. The study was conducted in a population-based prospective cohort among 7333 mothers followed from early pregnancy onwards.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood[22,23]. The cohort includes 9778 mothers and their children of different ethnicities living in Rotterdam, The Netherlands, and has been described in detail previously[22,23]. Our aim was to enrol women at the routine fetal ultrasound examination in early pregnancy (gestational age <17.9 weeks) but enrolment was allowed until birth of the child. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early (gestational age <17.9 weeks), mid- (gestational age 18–24.9 weeks) and late pregnancy (gestational age ≥25 weeks). The individual timing of these assessments depended on the gestational age at enrolment[22,23]. The median gestational ages of the early, mid- and late pregnancy measurements for the present study were 13.4 (95% range, 9.8–17.6), 20.5 (95% range, 18.5–23.6) and 30.4 (95% range, 28.4–32.8) weeks, respectively. All children were born between April 2002 and January 2006. Of the total eligible children born in the study area during the enrolment period, 61% was enrolled in the Generation R Study[23]. The

Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, The Netherlands, has approved the study. Written informed consent was obtained from all participants.

Maternal alcohol consumption

Information about maternal alcohol consumption was obtained by postal questionnaires in early, mid- and late pregnancy[23]. Response rates for these questionnaires were 91, 80 and 77%, respectively. In the first questionnaire, the mothers were asked whether they used any alcoholic drinks in the first 3 months of pregnancy (no; until pregnancy was known; continued after pregnancy was known). This questionnaire was sent to all mothers, including those enrolled after early pregnancy. In the second and third questionnaires, sent in mid- and late pregnancy, respectively, the mothers were asked whether they drank any alcohol in the past 2 months (no; yes). Of the mothers who reported in the first questionnaire drinking only until pregnancy was known (n = 1974), those who reported alcohol consumption in the second or third questionnaire (n = 988), were re-classified into the 'continued after pregnancy was known' category. The same strategy was used for mothers who reported no alcohol use in the first questionnaire (n = 4174) but who reported drinking in the second or third questionnaire (n = 535). This strategy led to the following groups of alcohol consumption; no alcohol consumption (n = 3639), alcohol consumption until the pregnancy was known (n = 986) and continued alcohol consumption (n = 2708). Mothers who reported any drinking were asked to classify their average alcohol consumption into one of the following six categories: less than one per week; one to three per week; four to six per week; one per day; two to three per day; more than three per day. In The Netherlands, the average alcoholic drink (one glass) contains ~12 g of alcohol[24]. Only 12 mothers used more than three alcoholic drinks per day in early pregnancy. In mid- and late pregnancy none of the mothers drank more than three alcoholic drinks per day. In a separate question we asked the mothers to report whether they had drank more than six drinks in 1 day but these numbers were small (n = 23 in mid-pregnancy; n = 22 in late pregnancy).

Fetal growth characteristics

Fetal ultrasound examinations were carried out at the research centers in early, mid- and late pregnancy. These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics[23]. Gestational age was established by fetal ultrasound examination because using the last menstrual period has several limitations, including the large number of women who do not know the exact date of their last menstrual period or have irregular menstrual cycles[25-27]. Pregnancy-dating curves were constructed for subjects with complete data on gestational age measured by ultrasonography and the last menstrual period[28]. Crown–rump length was used for pregnancy dating up to a gestational age of 12 weeks and 5 days (crown–rump length: <65 mm), and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter: >23 mm)[28]. Early-pregnancy measurements were primarily used to establish gestational age and therefore not included in the growth

analyses. Growth characteristics were measured to the nearest millimeter using standardized ultrasound procedures[29]. Estimated fetal weight was calculated using the formula of Hadlock with parameters head circumference, abdominal circumference and femur length[30]. Longitudinal growth curves were constructed for all fetal growth measurements[28].

Covariates

Information about educational level, ethnicity and parity was obtained by a questionnaire at enrolment in the study. Maternal smoking habits were assessed in each questionnaire. Maternal distress was assessed by a questionnaire at 20 weeks of gestation using the Brief Symptom Inventory[31]. Maternal anthropometrics, including height and weight, were measured without shoes and heavy clothing during visits at the research center at enrolment. Date of birth and gender were obtained from midwife and hospital registries.

Population for analysis

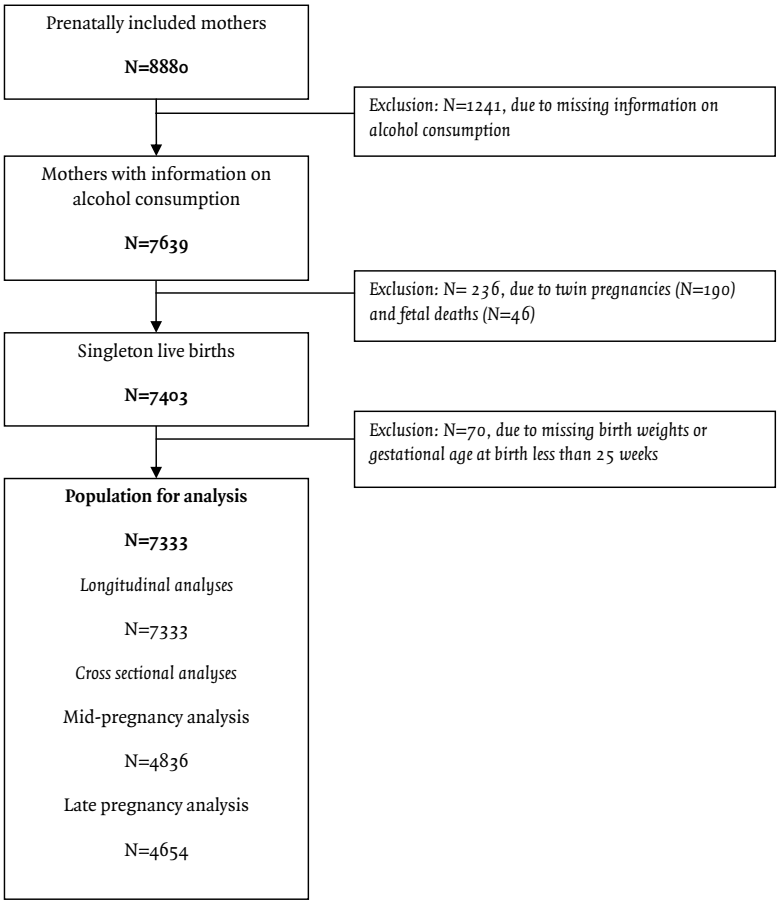
In total, 8880 mothers were enrolled during pregnancy[23]. Those without information about alcohol consumption during pregnancy in the first questionnaire were excluded from the analyses (14%, n = 1241). For the present study, we excluded subjects without fetal growth data in mid- or late pregnancy and twin pregnancies (n = 190). Of the remaining 7449 mothers, those pregnancies leading to fetal deaths (n = 46), missing birth outcomes (n = 69) or with a delivery at <25 weeks of gestation (n = 1) were excluded, since our interest was in low-risk singleton pregnancies. In our population for analysis, we had 397 mothers with two pregnancies, and four mothers with three pregnancies. Since no differences in results were observed between associations with and without these second and third pregnancies, we included them in the analyses. The associations of maternal alcohol consumption during pregnancy with longitudinally measured fetal growth characteristics were analysed in the remaining 7333 mothers (Figure 1). Analyses focused on the effects of alcohol consumption levels in mid- and late pregnancy were performed in mothers who were enrolled in early pregnancy (n = 5612) only, leading to 4836 (86%) and 4654 (83%) subjects for mid- and late pregnancy, respectively.

Statistical analyses

Fetal growth

The associations between alcohol consumption during pregnancy and fetal growth characteristics were analysed using unbalanced repeated-measurements regression analysis assuming random effects for the intercept and slope. These regression models enable studies on repeatedly measured outcomes, taking account for the correlation between measurements and have an optimal use of available data. Both gestational age-independent (difference constant over time) and gestational age-dependent (difference not-constant over time) effects were assessed. We used unstructured covariance models. First, we constructed best-fitting models using second-degree fractional polynomials of gestational age[32]. These models have been described in detail previously[28]. The

FIGURE 1. Flow chart of participants included for analysis



best-fitting fractional polynomial curves were chosen by comparing the deviances and checking the goodness of fit (smallest –2 log likelihood). Subsequently, the correctness of the model was assessed by plotting the standard deviation (SD) scores against gestational age. Secondly, we included alcohol consumption during pregnancy (none; until pregnancy was known; continued after pregnancy was known) to these models as additional intercept as interaction with gestational age. The interactions of alcohol consumption with gestational age were tested with each separate polynomial. Any significant interaction was subsequently included in the final model for analyses. The final models including gestational age and alcohol consumption (alcohol) can be written as:

$$\text{Head circumference} = \beta_0 + \beta_1 * \text{alcohol} + \beta_2 * \text{gestational age}^2 + \beta_3 * \text{alcohol} * \text{gestational age}^2 + \beta_4 * \text{gestational age}^2 * \ln(\text{gestational age}) + \beta_5 * \text{alcohol} * \text{gestational age}$$

Abdominal circumference $=\beta_0 + \beta_1$*alcohol $+ \beta_2$*gestational age$^2 + \beta_3$*gestational age^2*ln(gestational age) $+ \beta_4$*alcohol*gestational age

Femur length $= \beta_0 + \beta_1$*alcohol $+ \beta_2$*gestational age$^3 + \beta_3$*alcohol*gestational age^3

Estimated fetal weight $= \beta_0 + \beta_1$*alcohol $+ \beta_2$* gestational age$^{-2} + \beta_3$* gestational age^{-2}*ln(gestational age) $+ \beta_4$* gestational age^{-2}*ln(gestational age)$^2 + \beta_5$*alcohol*gestational age

In these models, 'β_0' reflects the intercept and 'β_1 * alcohol' tests the difference in intercept between alcohol groups. The other betas (β_2, β_3, β_4, β_5), reflect the linear or non-linear slope (interaction of alcohol consumption with gestational age). These terms test whether the polynomial curves are parallel (whether the groups grow at the same rate as compared with the reference group (no alcohol consumption) between the time points. The main interest for this article is the betas that include an interaction with alcohol consumption. Subsequently, we used these models to estimate the estimated differences in fetal growth characteristics between alcohol categories. For all analyses, the unexposed fetus (no maternal alcohol consumption) was used as reference group. We adjusted the models for the following confounders; maternal age, weight, height and distress (all continuous) and smoking, educational level, ethnicity, parity and infant gender (all categorical)[11]. Analyses were repeated in Dutch and other European mothers only. Since we found similar results, these associations were not presented separately. All levels of association are presented with their 95% CIs. Analyses were performed using the Statistical Analysis System version 8.2 (SAS, Institute Inc., Gary, NC, USA), including the Proc Mixed module for unbalanced repeated-measurements.

Dose–response

Dose–response analyses in mid- and late pregnancy were performed using multiple linear regression models. In these analyses also the unexposed fetus (no maternal alcohol consumption) was used as reference group. We adjusted the models for the following confounders; maternal age, weight, height and distress (all continuous) and smoking, educational level, ethnicity, parity and infant gender (all categorical)[11]. A small proportion of mothers participating in this study had a regular menstrual cycle (28 ± 4 days) and a known and reliable gestational age based on last menstrual period (n = 1796). We repeated the analyses focused on the dose–response associations of maternal alcohol consumption in mid- and late pregnancy in this group of women, to exclude underestimation of the effects by ultrasound dating. Tests for trends were performed by using the alcohol consumption categories as continuous variables in the models. All levels of association are presented with their 95% CIs. Analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

As shown in Table 1, of all mothers, 50% (n = 3639) did not use alcoholic drinks in pregnancy, 13% (n = 986) stopped alcohol consumption after their pregnancy was known and 37% continued alcohol consumption during pregnancy (n = 2708). Age of the mothers in the whole cohort ranged from 15.3 to 46.3 years and was significantly higher in mothers who continued alcohol consumption after pregnancy was known compared with mothers who did not use alcohol during pregnancy. The percentages of highly educated and Dutch or other European mothers were highest among those who continued alcohol consumption. Of all pregnancies, 5.5% (n = 401) were second or third pregnancies in the study. Since no differences in results were observed between associations with and without these second and third pregnancies, we included them in the analyses. Mean birth weight of the children was 3417 g (95% range, 635–5310). Gestational age at birth ranged from 25.3 to 43.6 weeks with a median of 40.1 weeks. Of all singleton live births, 5% were born before a gestational age of 37 weeks. Analyses were repeated in Dutch and other European mothers only. Since we found similar results, these associations were not presented separately.

Fetal growth

The derived fetal growth curves and estimates for the interactions terms of alcohol with each fractional polynomial for the different fetal growth characteristic are given in the supplementary material (Supplementary Figure S1 and Table S1, respectively). Figure 2 shows the associations between maternal alcohol consumption categories with estimated differences in fetal growth characteristics (head circumference, abdominal circumference, femur length, estimated fetal weight) between the gestational ages of 18 and 35 weeks. Except for estimated fetal weight, these associations were not significant after adjusting for potential confounders (Supplementary Table S1). Compared with mothers without alcohol consumption, mothers with continued alcohol consumption during pregnancy had an increased fetal weight gain (difference: 0.61 grams (95% CI: 0.18, 1.04) per week).

Dose–response

Table 2 shows an association between using one to three drinks per week with abdominal circumference (difference: 1.02 mm (95% CI: 0.11, 1.92) compared with no drinking) in mid-pregnancy. Drinking four to six drinks per week was associated with a shorter femur length (difference: –0.63 mm (95% CI: –1.15, –0.11)) in mid-pregnancy. After adjustment for potential confounders, no associations of alcohol consumption with fetal growth characteristics in mid-pregnancy remained significant. In addition, no significant trends were found. Table 3 shows that in late pregnancy, no significant or consistent effects of alcohol consumption during pregnancy on any fetal growth characteristic were observed. We observed no consistent differences between analyses on the whole cohort using ultrasound for pregnancy dating and subgroup with pregnancy dating on last menstrual period (Supplementary Tables S2 and S3).

TABLE 1. Maternal characteristics according to alcohol consumption during pregnancy category (N=7333 mothers)

	No alcohol consumption	Alcohol consumption until the pregnancy was known	Continued alcohol consumption
	n=3639	n=986	n=2708
Age, yrs	28.4 (5.3)	29.5 (5.2)**	31.6 (4.7)**
Height, cm	165.5 (7.3)	168.1 (7.1)**	169.6 (6.9)**
Weight at enrolment, kg	70.0 (14.4)	68.6 (12.9)**	69.0 (11.8)**
Parity ≥ 1, %	46.1	30.6**	43.3*
Education , %			
Primary school	17.8	4.5**	5.2**
Secondary school	56.6	50.2**	32.5**
Higher education	25.6	45.3**	62.3**
Ethnicity, %			
Dutch and other-European	41.1	68.6**	76.0**
Surinamese	10.4	11.1	6.5**
Turkish	16.5	1.7**	1.7**
Moroccan	12.8	0.4**	0.6**
Other	19.2	18.2	15.2**
Smoking, %			
No smoking in pregnancy	79.5	66.2**	71.1**
Smoking until pregnancy was known	4.3	16.7**	10.4**
Continued smoking in pregnancy	16.2	17.1	18.5*
Maternal stress, index[1]	0.19 (0.00-1.25)	0.15 (0.00-1.01)	0.13 (0.00-0.85)
First child of same mother in study, %	96.0	94.3*	92.6**
Enrolment in study in early pregnancy, %	71.0	84.4**	81.1**
Ultrasonography for fetal growth, %			
Mid-pregnancy	93.5	97.4**	96.5**
Late pregnancy	96.5	96.8	97.6**
Birth outcomes			
Birth weight, g	3396 (552)	3354 (571)*	3468 (559)**
Gestational age, wks[1]	40.0 (36.9-42.0)	40.0 (36.5-42.0)	40.3 (37.1-42.1)**
Gender, % boys	49.7	51.5	51.0

[1]Median (90% range).

Values are means (standard deviation) or percentages unless listed otherwise.

Differences in maternal characteristics (compared with the no alcohol consumption category) were evaluated using independent-sample t tests for continuous variables and chi-squared tests for proportions.

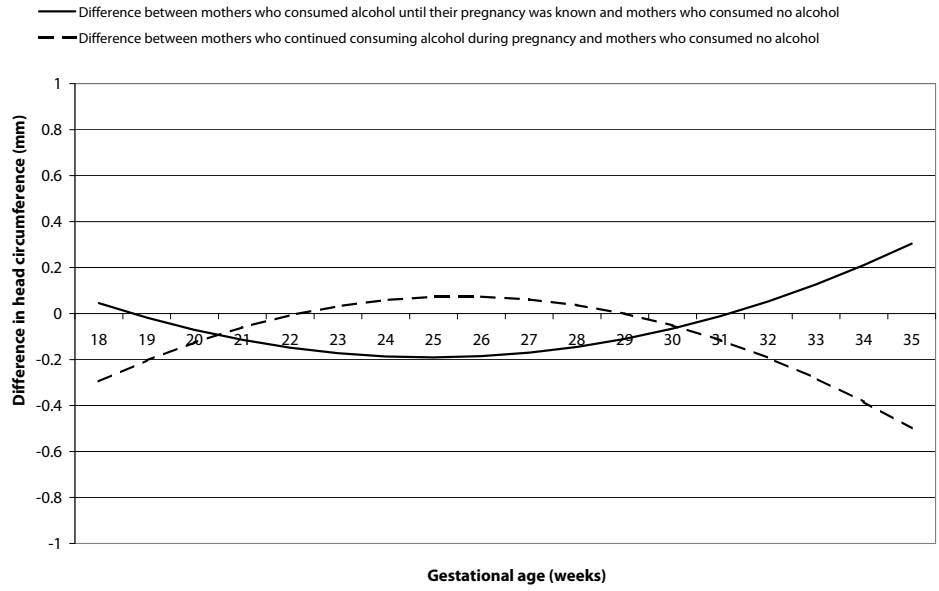
*P-value<0.05

**P-value<0.01

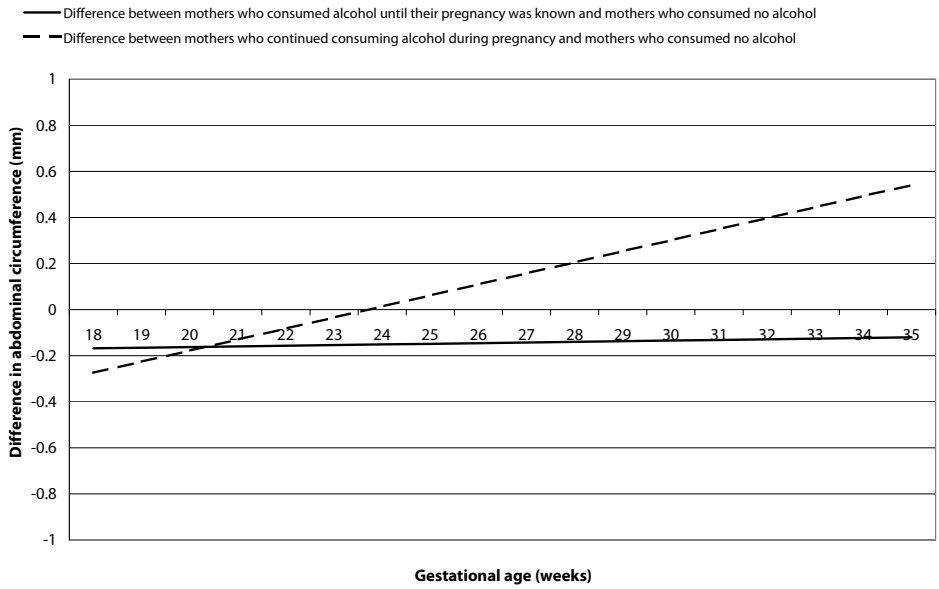
Data were missing on height (n=12), weight (n=28), body mass index (n=39), parity (n=11), educational level (n=151), ethnicity (n=36), smoking (n=65), maternal stress (n=1461), and first child of same mother in study (n=7).

FIGURE 2. Differences in fetal growth measures between alcohol consumption categories

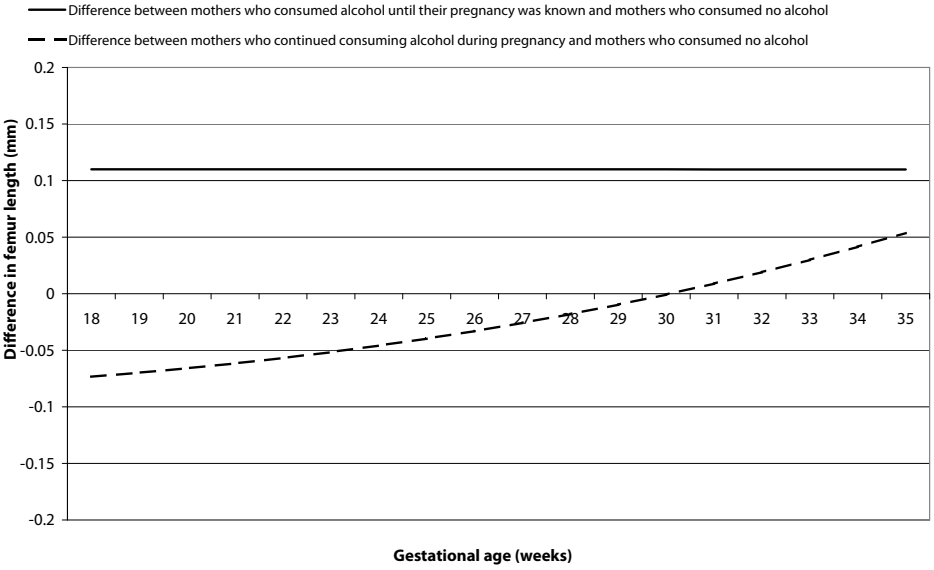
A. Head circumference



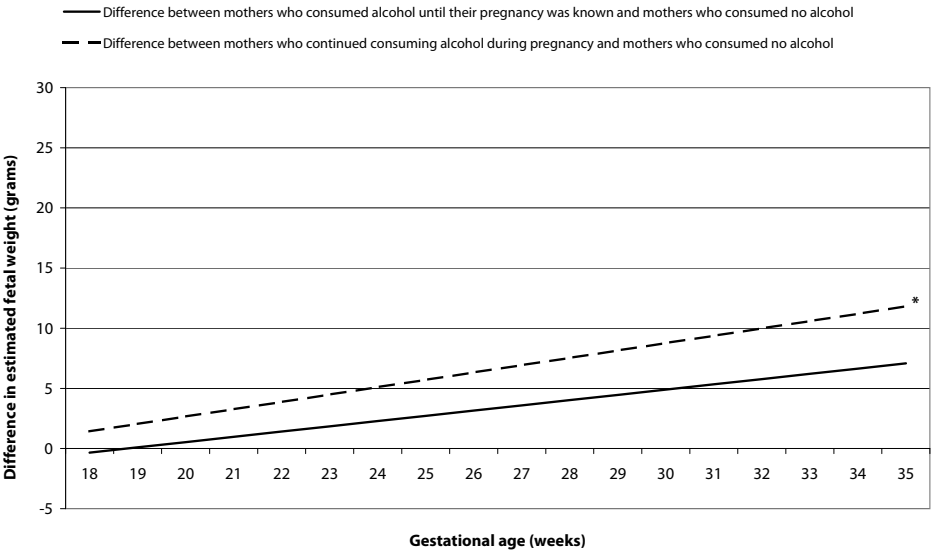
B. Abdominal circumference



C. Femur length



D. Estimated fetal weight



Values reflect difference in fetal growth characteristics of mothers with different alcohol consumption categories and were based on repeated regression models (*P<0.05). The corresponding effect estimates of the differences in the slopes are presented in Supplementary Table S1. All models are adjusted for maternal age at enrolment, weight at enrolment, height, educational level, parity, ethnicity, fetal gender, smoking habits, maternal stress.

TABLE 2. Dose–response associations between maternal alcohol consumption during pregnancy and fetal growth characteristics in mid-pregnancy (18.0-24.9 weeks) (N=5612 mothers)

Alcohol consumption	**Difference in head circumference (mm)**		
	Number of subjects	Crude	Adjusted
None	n=3081	Reference	Reference
< 1 drink/week	n=1235	-0.00 (-0.41, 0.40)	-0.31 (-0.74, 0.12)
1 to 3 drinks/week	n=327	0.50 (-0.20, 1.20)	0.32 (-0.40, 1.04)
4 to 6 drinks/week	n=43	-0.10 (-1.94, 1.74)	-0.08 (-1.98, 1.81)
1 drink/day	n=19	0.92 (-1.84, 3.68)	1.33 (-1.36, 4.02)
2 to 3 drinks/day	n=3	5.14 (-1.79, 12.08)	6.77 (-0.01, 13.55)
		$P_{trend} = 0.21$	$P_{trend} = 0.59$
Alcohol consumption	**Difference in abdominal circumference (mm)**		
	Number of subjects	Crude	Adjusted
None	n=3092	Reference	Reference
< 1 drink/week	n=1237	-0.01 (-0.52, 0.53)	-0.54 (-1.10, 0.02)
1 to 3 drinks/week	n=329	1.02 (0.11, 1.92)*	0.54 (-0.40, 1.48)
4 to 6 drinks/week	n=41	-1.70 (-4.14, 0.75)	-2.11 (-4.64, 0.43)
1 drink/day	n=19	2.29 (-1.29, 5.86)	1.97 (-1.54, 5.48)
2 to 3 drinks/day	n=3	6.02 (-2.96, 15.00)	5.88 (-2.95, 14.71)
		$P_{trend} = 0.13$	$P_{trend} = 0.94$
Alcohol consumption	**Difference in femur length (mm)**		
	Number of subjects	Crude	Adjusted
None	n=3088	Reference	Reference
< 1 drink/week	n=1235	-0.01 (-0.21, 0.02)	-0.03 (-0.15, 0.10)
1 to 3 drinks/week	n=329	-0.05 (-0.25, 0.15)	0.00 (-0.21, 0.21)
4 to 6 drinks/week	n=43	-0.63 (-1.15, -0.11)*	-0.36 (-0.91, 0.19)
1 drink/day	n=19	-0.48 (-1.26, 0.30)	-0.53 (-1.31, 0.25)
2 to 3 drinks/day	n=3	-0.42 (-2.39, 1.55)	-0.26 (-2.22, 1.69)
		$P_{trend} < 0.05$	$P_{trend} = 0.26$
Alcohol consumption	**Difference in estimated fetal weight (grams)**		
	Number of subjects	Crude	Adjusted
None	n=3078	Reference	Reference
< 1 drink/week	n=1228	-0.80 (-3.44, 1.85)	-1.98 (-4.82, 0.86)
1 to 3 drinks/week	n=329	4.17 (-0.38, 8.71)	3.29 (-1.46, 8.04)
4 to 6 drinks/week	n=41	-10.04 (-22.36, 2.28)	-9.78 (-22.59, 3.04)
1 drink/day	n=19	4.94 (-13.09, 22.97)	3.07 (-14.65, 20.78)
2 to 3 drinks/day	n=3	23.98 (-21.31, 69.28)	25.55 (-19.05, 70.14)
		$P_{trend} = 0.54$	$P_{trend} = 0.97$

* P-value<0.05. Values are regression coefficients (95% confidence interval) and reflect the difference for each growth characteristic.

Crude model: adjusted for gestational age. Adjusted model: Crude model + maternal age, weight, height, smoking, distress, educational level, ethnicity, parity, and infant gender.

P-values for trend were based on multiple linear regression models with alcohol consumption categories as a continuous variable.

DISCUSSION

This population-based prospective cohort study showed no associations of light-to-moderate maternal alcohol consumption during pregnancy with longitudinally measured fetal growth characteristics. Mothers who continued alcohol consumption during pregnancy tended to have an increased fetal weight gain. No consistent associations were observed for the associations between number of alcoholic consumptions and any fetal growth characteristics in different periods of pregnancy.

Methodological considerations

The strength of this study is the large number of subjects in a prospectively studied cohort. To our knowledge, this is the largest study to have focused on light-to-moderate alcohol consumption during pregnancy on fetal growth characteristics in different periods of pregnancy. We were also able to control for many possible confounders. A potential limitation of this study is the missing data on alcohol consumption. No differences in maternal age at enrolment, height and birth weight of the offspring were observed between those with and without information about alcohol consumption. Missing information about alcohol consumption might have led to loss of power. The associations might be underestimated if among mothers without alcohol data the percentage of alcohol consumers would be higher than among mothers without missing alcohol data. However, this seems unlikely since no differences in characteristics between those with and without alcohol consumption data were observed. Among mothers with information about alcohol consumption, we had a limited loss-to-follow-up. Therefore, we do not expect biased results due to loss-to-follow-up[33].

Information on alcohol consumption habits during pregnancy was collected by postal questionnaires. If any, misclassification would be most likely due to underreporting and subsequently lead to underestimation of differences between those not using and using alcoholic consumptions. Previous studies suggested use of biomarkers of alcohol consumption such as carbohydrate-deficient transferrin and gamma-glutamyl transferase. However, currently these biomarkers have a low sensitivity in subjects with light-to-moderate alcohol consumption[34]. In our study we had information only about the average number of alcoholic drinks per week, without information on drinking patterns. Our estimation of the amount of alcohol per consumption might be imprecise since both alcohol percentages and serving sizes may differ between mothers.

Gestational age was established by fetal ultrasound examination. This method seems to be superior to use of the last menstrual period[25]. The major disadvantage of establishing gestational age by ultrasonography is that the growth variation of the fetal characteristics used for pregnancy dating is assumed to be zero. Therefore, in our study, crown-rump length and biparietal diameter were used for pregnancy dating but not for assessing fetal growth[25,26]. Since pregnancy dating and growth characteristics are correlated throughout pregnancy, growth variation in head circumference, abdominal circumference and femur length may be reduced by dating the pregnancy on the other fetal characteristics. This may have led to underestimation of our effect estimates. However,

TABLE 3. Dose–response associations between maternal alcohol consumption during pregnancy and fetal growth characteristics in late pregnancy (≥25 weeks) (N=5612 mothers)

Alcohol consumption	**Difference in head circumference (mm)**		
	Number of subjects	Crude	Adjusted
None	n=2890	Reference	Reference
< 1 drink/week	n=1227	0.73 (0.11, 1.34)*	-0.37 (-1.04, 0.30)
1 to 3 drinks/week	n=396	1.48 (0.50, 2.45)**	0.28 (-0.74, 1.30)
4 to 6 drinks/week	n=43	-0.53 (-3.32, 2.27)	-0.76 (-3.65, 2.12)
1 drink/day	n=16	-1.45 (-6.00, 3.11)	-2.74 (-7.23, 1.74)
2 to 3 drinks/day	n=4	1.41 (-7.68, 10.50)	1.64 (-8.33, 11.62)
		P_{trend}< 0.01	P_{trend} = 0.61
Alcohol consumption	**Difference in abdominal circumference (mm)**		
	Number of subjects	Crude	Adjusted
None	n=2904	Reference	Reference
< 1 drink/week	n=1235	0.42 (-0.45, 1.28)	-0.22 (-1.18, 0.75)
1 to 3 drinks/week	n=398	1.37 (0.00, 2.74)	0.03 (-1.44, 1.50)
4 to 6 drinks/week	n=43	-2.97 (-6.90, 0.97)	-3.13 (-7.29, 1.02)
1 drink/day	n=16	-1.13 (-7.54, 5.29)	-2.52 (-8.99, 3.94)
2 to 3 drinks/day	n=4	11.95 (-0.85, 24.74)	10.99 (-3.38, 25.36)
		P_{trend} = 0.16	P_{trend} = 0.56
Alcohol consumption	**Difference in femur length (mm)**		
	Number of subjects	Crude	Adjusted
None	n=2909	Reference	Reference
< 1 drink/week	n=1238	-0.00 (-0.15, 0.15)	-0.07 (-0.23, 0.10)
1 to 3 drinks/week	n=397	-0.07 (-0.30, 0.16)	-0.09 (-0.34, 0.16)
4 to 6 drinks/week	n=43	-0.49 (-1.15, 0.18)	-0.40 (-1.10, 0.31)
1 drink/day	n=16	0.06 (-1.03, 1.14)	-0.28 (-1.38, 0.82)
2 to 3 drinks/day	n=4	-0.54 (-2.71, 1.62)	-0.60 (-3.04, 1.84)
		P_{trend} = 0.36	P_{trend} = 0.18
Alcohol consumption	**Difference in estimated fetal weight (grams)**		
	Number of subjects	Crude	Adjusted
None	n=2899	Reference	Reference
< 1 drink/week	n=1233	3.44 (-8.65, 15.54)	-5.44 (-18.84, 7.97)
1 to 3 drinks/week	n=397	13.65 (-5.39, 32.70)	-1.14 (-21.57, 19.28)
4 to 6 drinks/week	n=43	-47.71 (-102.37, 6.95)	-46.04 (-103.69, 11.61)
1 drink/day	n=16	-12.29 (-101.46, 76.88)	-38.14 (-127.88, 51.59)
2 to 3 drinks/day	n=4	122.26 (-55.71, 300.23)	116.76 (-82.74, 316.26)
		P_{trend} = 0.46	P_{trend} = 0.36

*P-value<0.05, **P-value<0.01. Values are regression coefficients (95% confidence interval) and reflect the difference for each growth characteristic.

Crude model: adjusted for gestational age. Adjusted model: Crude model + maternal age, weight, height, smoking, distress, educational level, ethnicity, parity, and infant gender.

P-values for trend were based on multiple linear regression models with alcohol consumption categories as a continuous variable.

we expect this effect to be small in our results. First, the longitudinal analyses were focused on fetal growth or change in size during pregnancy within individuals. This change in size is unlikely to have been materially affected by our pregnancy-dating method. Secondly, the analyses assessing the associations of maternal alcohol consumption with fetal growth characteristics in mid- and late pregnancy were restricted to mothers who were enrolled and had their pregnancies dated in early pregnancy (76.5% of the population for analysis). Since gestational age and fetal growth were not established concurrently, we believe that we minimized the effect of pregnancy dating on growth variation. Our analysis in a subgroup of mothers with pregnancy dating on their last menstrual period did not show consistent stronger effects. However, results based on the last menstrual period shown in Supplementary Table S2 tended to show larger differences than that in Table 2. This might be due to some underestimation of the main results due to correlation between ultrasound dating and growth characteristics.

Maternal alcohol consumption and fetal growth characteristics

Previous studies focused primarily on the associations between maternal alcohol consumption during pregnancy and birth weight and showed inconsistent associations[2,8,12-18,21]. Smaller studies assessing the effect of maternal alcohol consumption during pregnancy on fetal growth retardation also showed inconsistent results[18,35]. Windham *et al.* showed an association of moderate maternal alcohol consumption with an increased risk of fetal growth retardation (odds ratio (OR), 2.3, 95% CI: 1.2, 4.6) whereas Yang *et al.* showed no effect of moderate alcohol consumption on fetal growth retardation[18,35]. Most studies used low birth weight or fetal growth retardation as outcome measurement and showed conflicting results. Moreover, most of these studies did not adjust for potential confounders[8,15,17-20]. Examining fetal growth characteristics instead of birth weight enables identification of specific critical periods during pregnancy for the exposure on various patterns of fetal growth and development.

In 2006, Handmaker *et al.* examined the effect of maternal alcohol consumption during pregnancy on fetal growth characteristics[36]. This study, comprising 167 subjects, showed an association between heavy drinking and the ratio of head circumference and abdominal circumference. However, no adjustments were made for potential confounders. We did not find any consistent effects of moderate maternal alcohol consumption on fetal growth characteristics. However, studies in larger numbers of subjects might be able to identify smaller effects. Differences in results from the cross-sectional analyses compared with the repeated measurement analysis can be explained by less power and smaller numbers in the alcohol consumption groups in the cross-sectional analyses. From our results, it seems that any trend for positive associations between alcohol consumption during pregnancy and fetal growth characteristics is explained by socio-demographic and lifestyle-related variables. After adjustment, none of the positive associations remained significant. Other potential confounders that might be important include dietary habits and folic acid supplementation[37]. However, including maternal folic acid use in early pregnancy into the current analyses did not have an effect on our findings (data not shown).

Previous studies also suggested effects of maternal alcohol consumption on postnatal growth and development. It was shown that the rate of postnatal growth is reduced in children who were prenatally exposed to alcohol. Weight, length and head circumference were negatively affected by alcohol exposure during pregnancy[38]. However, a more recent study showed that moderate maternal alcohol consumption during pregnancy was not associated with either weight or head circumference at the age of 5 years[20]. Furthermore, inconsistent results were found on behavioural development and cognitive processing in children prenatally exposed to alcohol[39,40]. A study of Faden *et al.* showed a higher activity level, a greater difficulty in following instructions and eating problems among offspring exposed to alcohol during pregnancy[41]. Furthermore, binge drinking during pregnancy was shown to be associated with increased odds for the appearance of psychiatric disorders[1]. Our study was focused on the effects of light and moderate maternal alcohol consumption. We also asked the mothers if they drank occasionally more than six glasses of alcohol in 1 day (yes; no), which is considered as binge drinking. In total, 23 mothers in mid-pregnancy and 22 mothers in late pregnancy reported that they drank occasionally more than six glasses of alcohol in 1 day. No differences in fetal growth were observed between binge drinking and non-drinking mothers (data not shown); however, future studies with more power are needed for this analysis. Although, previous studies did not even show consistent associations of binge drinking during pregnancy with several outcomes, except for neurodevelopmental outcomes[8], future studies with larger numbers are needed. Whether light-to-moderate alcohol consumption in this or any other cohort is related to postnatal growth and development needs to be examined in follow-up studies. The effects of alcohol consumption are dependent of the absorption and metabolism in the mother and fetus. This may be partially genetically determined. Therefore, the effects of alcohol consumption in specific groups of women should still be studied.

Conclusion

Our results suggest that, in a Western population-based cohort, moderate maternal alcohol consumption during pregnancy does not adversely affect fetal growth. Future studies are needed to examine whether the effects differ among subjects with different absorption and metabolism patterns and to examine the effects on organ growth and function in postnatal life. Furthermore, studies are needed in developing countries or populations with different nutritional status. In addition, we had small numbers of women using more than two alcoholic consumptions per day. Results for these groups should be interpreted carefully.

REFERENCES

1. Barr HM, Bookstein FL, O'Malley KD, et al. Binge drinking during pregnancy as a predictor of psychiatric disorders on the Structured Clinical Interview for DSM-IV in young adult offspring. Am J Psychiatry. 2006;163: 1061–65.
2. Lazzaroni F, Bonassi S, Magnani M, et al. Moderate maternal drinking and outcome of pregnancy. Eur J Epidemiol. 1993;9:599–606.
3. Little RE, Wendt JK. The effects of maternal drinking in the reproductive period: an epidemiologic review. J Subst Abuse. 1991;3:187–204.
4. O'Leary CM, Nassar N, Kurinczuk JJ, et al. The effect of maternal alcohol consumption on fetal growth and preterm birth. BJOG. 2009;116:390–400.
5. Warren KR, Bast RJ. Alcohol-related birth defects: an update. Public Health Rep 1988:103;638–42.
6. Chiriboga CA. Fetal alcohol and drug effects. Neurologist. 2003;9:267–79.
7. Sokol RJ, Delaney-Black V, Nordstrom B. Fetal alcohol spectrum disorder. JAMA. 2003;290:2996–99.
8. Henderson J, Kesmodel U, Gray R. Systematic review of the fetal effects of prenatal binge-drinking. J Epidemiol Community Health. 2007;61:1069–73.
9. Henderson J, Gray R, Brocklehurst P. Systematic review of effects of low-moderate prenatal alcohol exposure on pregnancy outcome. BJOG. 2007;114:243–52.
10. Hanson JW, Streissguth AP, Smith DW. The effects of moderate alcohol consumption during pregnancy on fetal growth and morphogenesis. J Pediatr. 1978;92:457–60.
11. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65:663–737.
12. Little RE. Moderate alcohol use during pregnancy and decreased infant birth weight. Am J Public Health. 1977; 67:1154–56.
13. Day NL, Jasperse D, Richardson G, et al. Prenatal exposure to alcohol: effect on infant growth and morphologic characteristics. Pediatrics. 1989;84:536–41.
14. Lundsberg LS, Bracken MB, Saftlas AF. Low-to-moderate gestational alcohol use and intrauterine growth retardation, low birthweight, and preterm delivery. Ann Epidemiol. 1997;7:498–508.
15. Mariscal M, Palma S, Llorca J, et al. Pattern of alcohol consumption during pregnancy and risk for low birth weight. Ann Epidemiol. 2006;16:432–38.
16. Passaro KT, Little RE, Savitz DA, et al. The effect of maternal drinking before conception and in early pregnancy on infant birthweight. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Epidemiology. 1996;7:377–83.
17. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and prematurity. Am J Public Health. 1992;82:87–90.
18. Windham GC, Fenster L, Hopkins B, et al. The association of moderate maternal and paternal alcohol consumption with birthweight and gestational age. Epidemiology. 1995;6:591–97.
19. Virji SK. The relationship between alcohol consumption during pregnancy and infant birthweight. An epidemiologic study. Acta Obstet Gynecol Scand. 1991;70:303–8.
20. O'Callaghan FV, O'Callaghan M, Najman JM, etal. Maternal alcohol consumption during pregnancy and physical outcomes up to 5 years of age: a longitudinal study. Early Human Dev. 2003;71:137–48.
21. Jaddoe VW, Bakker R, Hofman A, et al. Moderate alcohol consumption during pregnancy and the risk of low birth weight and preterm birth. The Generation R Study. Ann Epidemiol. 2007;17:834–40.
22. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917–23.
23. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
24. NEVO tabel 2006. Nederlands Voedingsstoffenbestand 2006/Stichting Nederlands Voedingsstoffenbestand. (2006) Den Haag: Voedingscentrum.

25. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol. 1996;8:178–85.
26. Altman DG, Chitty LS. New charts for ultrasound dating of pregnancy. Ultrasound Obstet Gynecol. 1997;10: 174–91.
27. Robinson HP, Sweet EM, Adam AH. The accuracy of radiological estimates of gestational age using early fetal crown-rump length measurements by ultrasound as a basis for comparison. Br J Obstet Gynaecol. 1979;86: 525–28.
28. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31: 388–96.
29. Routine ultrasound screening in pregnancy: Protocol (2000) London, UK: RCOG Press.
30. Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements—a prospective study. Am J Obstet Gynecol. 1985;151:333–37.
31. Derogatis LR. Brief Symptom Inventory (BSI): Administration, Scoring and Procedures. Manual (1993) 3rd. Minneapolis, MN: National Computer Systems.
32. Royston P, Ambler G, Sauerbrei W. The use of fractional polynomials to model continuous risk variables in epidemiology. Int J Epidemiol. 1999;28:964–74.
33. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413–18.
34. van Pelt J, Leusink GL, van Nierop PW, et al. Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res. 2000;24:176–79.
35. Yang Q, Witkiewicz BB, Olney RS, et al. A case-control study of maternal alcohol consumption and intrauterine growth retardation. Ann Epidemiol 2001;11:497–503.
36. Handmaker NS, Rayburn WF, Meng C, et al. Impact of alcohol exposure after pregnancy recognition on ultrasonographic fetal growth measures. Alcohol Clin Exp Res. 2006;30:892–98.
37. Knudsen VK, Orozova-Bekkevold IM, Mikkelsen TB, et al. Major dietary patterns in pregnancy and fetal growth. Eur J Clin Nutr. 2008;62:463–70.
38. Day NL, Richardson G, Robles N, et al. Effect of prenatal alcohol exposure on growth and morphology of offspring at 8 months of age. Pediatrics. 1990;85:748–52.
39. Jacobson JL, Jacobson SW, Sokol RJ, et al. Effects of alcohol use, smoking, and illicit drug use on fetal growth in black infants. J Pediatr. 1994;124:757–64.
40. Kelly Y, Sacker A, Gray R, et al. Light drinking in pregnancy, a risk for behavioural problems and cognitive deficits at 3 years of age? Int J Epidemiol. 2009;38:129–40.
41. Faden VB, Graubard BI. Maternal substance use during pregnancy and developmental outcome at age three. J Subst Abuse. 2000;12:329–40.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Estimates of interactions terms of alcohol with fractional polynomials for each fetal growth characteristic in fully adjusted models.

	Fractional polynomials (95% CI)					
	GA - crude	**GA - adjusted**	**GA^2 - crude**	**GA^2 - adjusted**	**GA^3 - crude**	**GA^3 - adjusted**
Head circumference						
No alcohol	Reference	Reference	Reference	Reference	-	-
Until pregnancy was known	0.14 (-0.30, 0.58)	-0.25 (-0.73, 0.24)	-0.00 (-0.01, 0.01)	0.01 (-0.01, 0.02)	-	-
Continued	0.50 (0.17, 0.83)	0.33 (-0.03, 0.69)	-0.01 (-0.02, -0.00)	-0.01 (-0.01, 0.00)	-	-
Abdominal circumference						
No alchol	Reference	Reference	-	-	-	-
Until pregnancy was known	-0.01 (-0.09, 0.06)	0.00 (-0.08, 0.09)	-	-	-	-
Continued	0.05 (0.00, 0.11)	0.05 (-0.01, 0.11)	-	-	-	-
Femur length						
No alcohol	-	-	-	-	Reference	Reference
Until pregnancy was known	-	-	-	-	$2.96*10^{-6}$ ($-5.94*10^{-6}$, $1.20*10^{-5}$)	$3.82*10^{-9}$ ($-9.85*10^{-6}$, $9.86*10^{-6}$)
Continued	-	-	-	-	$6.15*10^{-6}$ ($-1.43*10^{-7}$, $1.20*10^{-5}$)	$3.43*10^{-6}$ ($-3.44*10^{-6}$, $1.00*10^{-5}$)
Estimated fetal weight						
No alcohol	Reference	Reference	-	-	-	-
Until pregnancy was known	0.77 (0.19, 0.35)	0.44 (-0.16, 1.03)	-	-	-	-
Continued	0.62 (0.20, 1.04)	0.61 (0.18, 1.04)	-	-	-	-

GA = gestational age in weeks; Head circumference, abdominal circumference, and femur length estimates in mm; estimated fetal weight estimates in grams. Models are adjusted for maternal age at enrolment, weight at enrolment, height, educational level, parity, ethnicity, fetal gender, smoking habits, and maternal stress.

SUPPLEMENTARY TABLE S2. Associations between maternal alcohol consumption during pregnancy and fetal growth characteristics in mid-pregnancy (18 – 24.9 weeks) in mothers with gestational age at visit based on their last menstrual period (N=1254 mothers)

	Difference in head circumference (mm)	
Alcohol consumption	Crude	Adjusted
None (n=859)	Reference	Reference
< 1 drink/week (n=383)	0.09 (-0.93, 1.12)	-0.72 (-1.77, 0.34)
1 to 3 drinks/week (n=109)	0.35 (-1.35, 2.04)	0.18 (-1.56, 1.91)
4 to 6 drinks/week (n=16)	-1.90 (-6.08, 2.9)	-0.71 (-5.34, 3.92)
1 or more drinks/day (n=10)	4.17 (-1.09, 9.44)	6.38 (1.32, 11.44)*
	P_{trend} = 0.54	P_{trend} = 0.58
	Difference in abdominal circumference (mm)	
Alcohol consumption	Crude	Adjusted
None (n=859)	Reference	Reference
< 1 drink/week (n=383)	-0.21 (-1.16, 1.12)	-1.19 (-2.39, 0.01)
1 to 3 drinks/week (n=109)	0.55 (-1.32, 2.42)	0.04 (-1.91, 2.00)
4 to 6 drinks/week (n=16)	-4.72 (-9.52, 0.09)	-3.99 (-9.46, 1.49)
1 or more drinks/day (n=10)	4.28 (-1.57, 10.14)	5.43 (-0.32, 11.17
	P_{trend} = 0.82	P_{trend} = 0.69
	Difference in femur length (mm)	
Alcohol consumption	Crude	Adjusted
None (n=859)	Reference	Reference
< 1 drink/week (n=383)	-0.23 (-0.48, 0.03)	-0.32 (-0.59, -0.04)*
1 to 3 drinks/week (n=109)	-0.16 (-0.58, 0.27)	-0.16 (-0.61, 0.29)
4 to 6 drinks/week (n=16)	-1.46 (-2.51, -0.41)**	-0.88 (-2.09, 0.33)
1 or more drinks/day (n=10)	-0.01 (-1.33, 1.31)	0.15 (-1.17, 1.47)
	P_{trend} = 0.03	P_{trend} = 0.10
	Difference in estimated fetal weight (grams)	
Alcohol consumption	Crude	Adjusted
None (n=859)	Reference	Reference
< 1 drink/week (n=383)	-2.20 (-8.31, 3.91)	-7.61 (-14.07, -1.14)*
1 to 3 drinks/week (n=109)	-0.36 (-10.40, 9.68)	-2.27 (-12.81, 8.27)
4 to 6 drinks/week (n=16)	-27.85 (-53.60, -2.11)*	-21.88 (-51.33, 7.58)
1 or more drinks/day (n=10)	18.78 (-12.61, 50.17)	23.29 (-7.53, 54.31)
	P_{trend} = 0.54	P_{trend} = 0.33

*P-value<0.05; **P-value<0.01. Values are regression coefficients (95% confidence interval) and reflect the difference for each growth characteristic. Crude model: adjusted for gestational age. Adjusted model: Crude model + maternal age, weight, height, smoking, distress, educational level, ethnicity, parity, and infant gender.

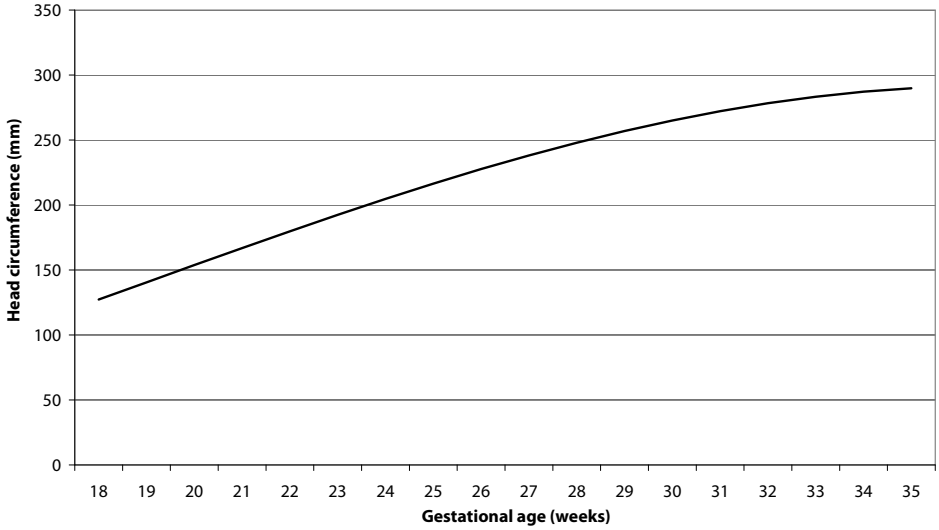
SUPPLEMENTARY TABLE S3. Associations between maternal alcohol consumption during pregnancy and fetal growth characteristics in late pregnancy (≥25 weeks) in mothers with gestational age at visit based on their last menstrual period (N=1354 mothers)

	Difference in head circumference (mm)	
Alcohol consumption	Crude	Adjusted
None (n=804)	Reference	Reference
< 1 drink/week (n=377)	0.54 (-0.71, 1.80)	-0.27 (-1.58, 1.05)
1 to 3 drinks/week (n=153)	1.14 (-0.63, 2.91)	0.46 (-1.40, 2.32)
4 to 6 drinks/week (n=14)	-3.87 (-9.26, 1.51)	-3.25 (-9.28, 2.78)
1 or more drinks/day (n=6)	0.56 (-7.64, 8.76)	3.63 (-4.96, 12.22)
	P_{trend} = 0.44	P_{trend} = 0.90
	Difference in abdominal circumference (mm)	
Alcohol consumption	Crude	Adjusted
None (n=804)	Reference	Reference
< 1 drink/week (n=377)	0.43 (-1.29, 2.14)	-0.10 (-1.96, 1.75)
1 to 3 drinks/week (n=153)	0.08 (-2.34, 2.49)	-1.09 (-3.71, 1.53)
4 to 6 drinks/week (n=14)	-5.41 (-12.78, 1.96)	-3.92 (-12.45, 4.61)
1 or more drinks/day (n=6)	7.06 (-4.16, 18.28)	6.05 (-6.11, 18.20)
	P_{trend} = 0.90	P_{trend} = 0.55
	Difference in femur length (mm)	
Alcohol consumption	Crude	Adjusted
None (n=804)	Reference	Reference
< 1 drink/week (n=377)	-0.21 (-0.50, 0.08)	-0.17 (-0.49, 0.14)
1 to 3 drinks/week (n=153)	-0.17 (-0.58, 0.24)	-0.07 (-0.52, 0.38)
4 to 6 drinks/week (n=14)	-1.85 (-3.10, -0.60)**	-1.31 (-2.76, 0.14)
1 or more drinks/day (n=6)	0.43 (-1.47, 2.33)	0.98 (-1.09, 3.05)
	P_{trend} = 0.06	P_{trend} = 0.41
	Difference in estimated fetal weight (grams)	
Alcohol consumption	Crude	Adjusted
None (n=804)	Reference	Reference
< 1 drink/week (n=377)	-2.63 (-27.20, 21.94)	-7.53 (-34.11, 19.04)
1 to 3 drinks/week (n=153)	-1.76 (-36.49, 32.96)	-9.99 (-47.55, 27.57)
4 to 6 drinks/week (n=14)	-122.84 (-228.61, -17.09)*	-92.06 (-214.01, 29.89)
1 or more drinks/day (n=6)	104.89 (-56.08, 265.86)	116.40 (-57.35, 290.14)
	P_{trend} = 0.61	P_{trend} = 0.53

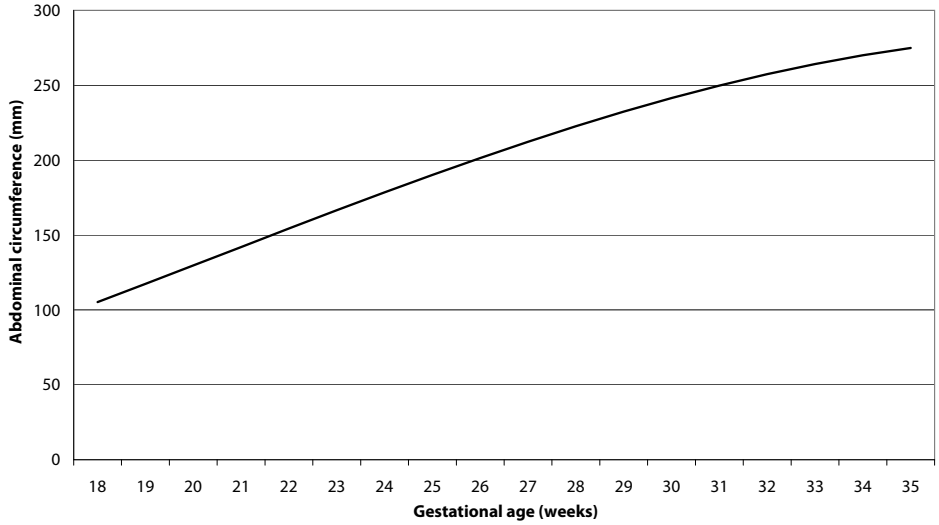
*P-value<0.05; **P-value<0.01. Values are regression coefficients (95% confidence interval) and reflect the difference for each growth characteristic. Crude model: adjusted for gestational age. Adjusted model: Crude model + maternal age, weight, height, smoking, distress, educational level, ethnicity, parity, and infant gender.

SUPPLEMENTARY FIGURE S1. Derived fetal growth curves

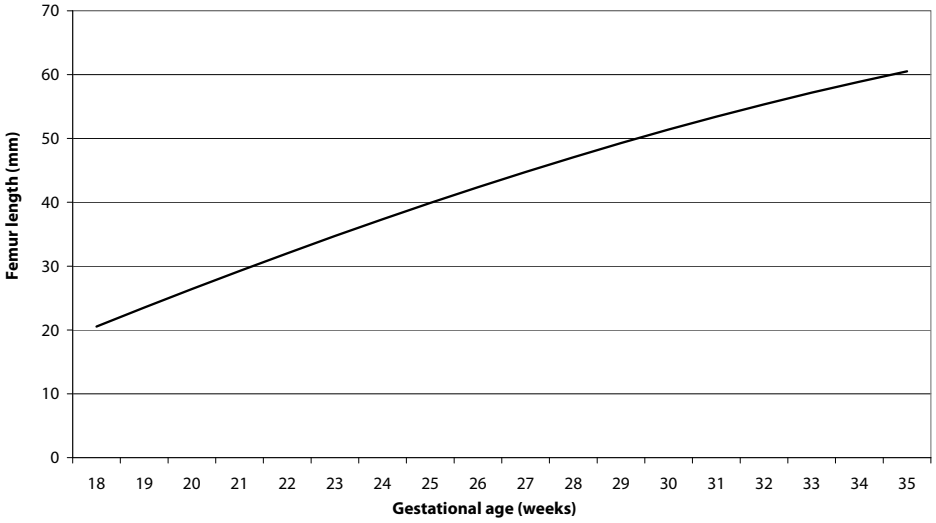
A. Head circumference



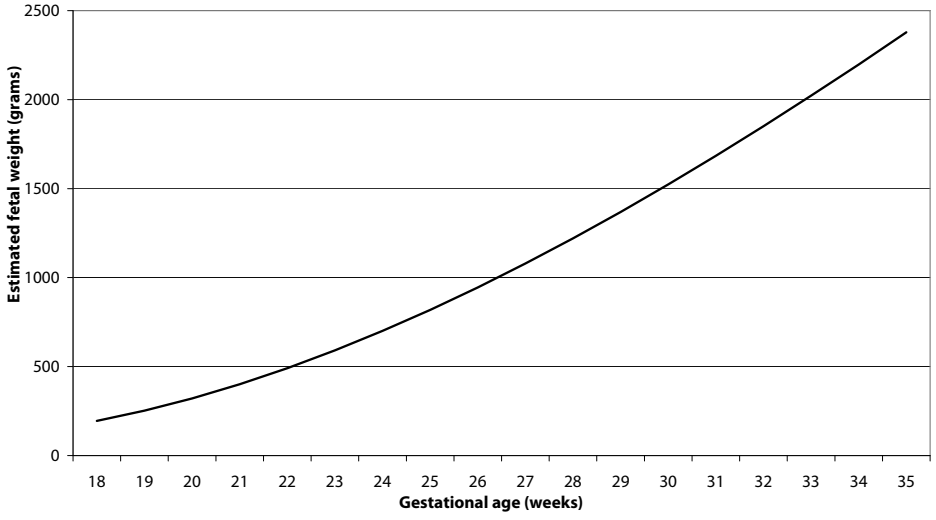
B. Abdominal circumference



C. Femur length



D. Estimated fetal weight



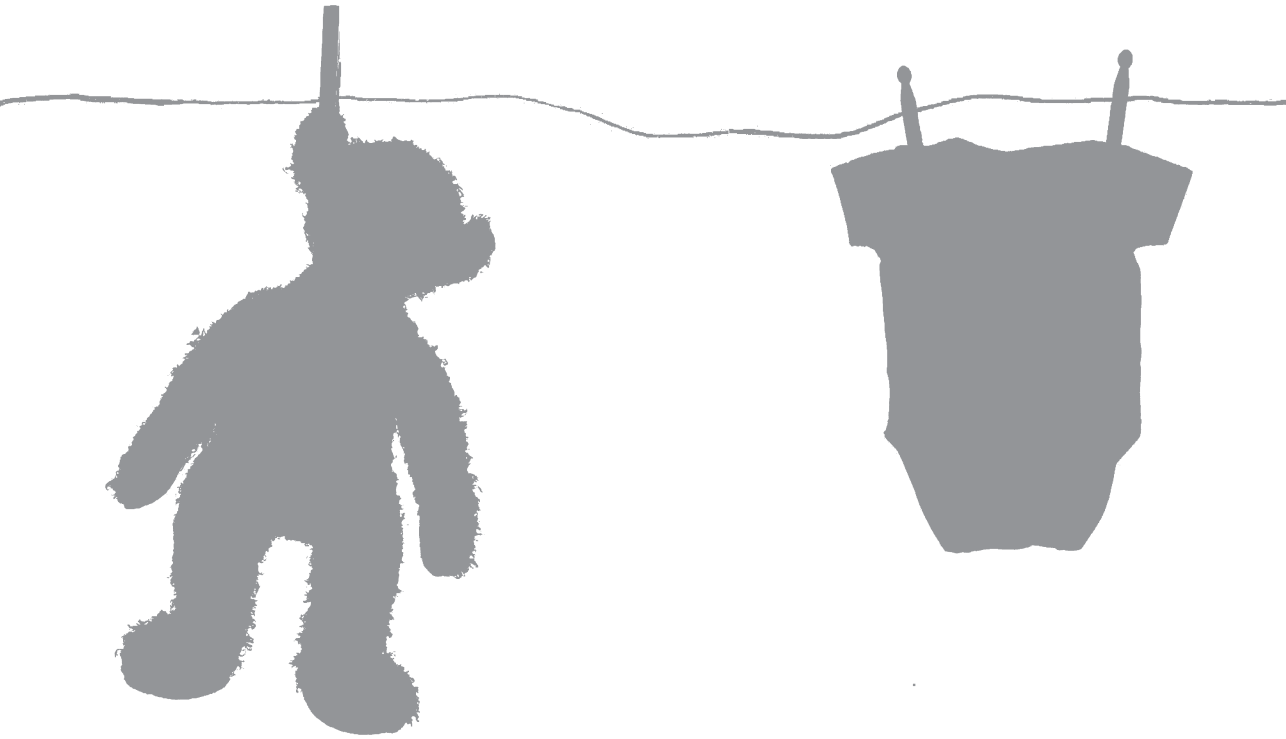
Growth models were constructed using repeated regression models. Values represent fetal growth characteristics of mothers with different alcohol consumption categories. All models are adjusted for maternal age at enrolment, weight at enrolment, height, educational level, parity, ethnicity, fetal gender, smoking habits, and maternal stress.

Chapter 4.3

Moderate alcohol consumption, low birth weight and preterm birth

Vincent WV Jaddoe
Rachel Bakker
Albert Hofman
Johan P Mackenbach
Henriette A Moll
Eric A Steegers
Jacqueline C Witteman

Adapted from Ann Epidemiol. 2007;17:834-40.



ABSTRACT

Previous studies examining the effect of low or moderate alcohol consumption during pregnancy on birth outcomes showed inconsistent results. We examined the associations of alcohol consumption in different periods of pregnancy with the risks of low birth weight and preterm birth. This study was based on 7141 subjects participating in a population-based prospective cohort study from early pregnancy. Alcohol consumption was assessed in early-, mid- and late pregnancy. Birth outcomes were birth weight in grams, low birth weight (< 2500 grams), small-size-for-gestational-age at birth (< -2 standard deviation scores) and preterm birth (gestational age < 37 weeks). Overall, alcohol consumption during pregnancy was not associated with adverse birth outcomes. However, dose-response analyses showed tendencies towards adverse effects of average consumption of ≥1 alcoholic drink per day in early pregnancy on birth weight (difference: -129 (95% confidence interval (CI): -271, 12) grams), low birth weight (adjusted odds ratio (aOR), 4.81 (95% CI: 1.10, 21.08)), small-size-for-gestational-age at birth (aOR, 1.45 (95% CI: 0.33, 6.44)) and preterm birth (aOR, 2.51 (95% CI: 0.92, 6.81). Similar effects were found in late pregnancy. Average consumption of ≥1 but not <1 alcoholic drink per day in early or late pregnancy seems to be associated with adverse birth outcomes in the offspring.

INTRODUCTION

Excessive alcohol consumption during pregnancy is associated with various pregnancy complications including low birth weight, preterm birth, congenital anomalies, fetal alcohol syndrome and perinatal death[1-3]. Excessive maternal alcohol consumption during pregnancy is also associated with an adverse postnatal behavioural development[4].

The effect of excessive alcohol consumption during pregnancy on prenatal growth and development cannot easily be extrapolated to lower levels of alcohol consumption. Previous studies examining the effect of low or moderate alcohol consumption during pregnancy (<1 alcoholic drink per day on average) on birth outcomes showed inconsistent results. Several studies found adverse effects, whereas others did not find any effect or even reported beneficial effects on weight and gestational age at birth[5-9]. These inconsistent results may be due to differences in study design and in timing and methods of assessment of alcohol consumption. Several studies used only dichotomized outcomes including low birth weight (generally defined as less than 2500 grams) and preterm birth[7,10]. However, alcohol consumption may affect the entire birth weight range, not only the risk of low birth weight. Most studies did not examine the effects of alcohol consumption in different periods of pregnancy. This may be important for identifying specific critical periods for exposure to alcohol during fetal life.

In a population-based cohort study among pregnant women and their children, we examined the associations of moderate maternal alcohol consumption in different periods of pregnancy with birth weight as continuous measure, and the risks of low birth weight, small-size-for-gestational-age at birth and preterm birth in the offspring.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described in detail elsewhere[11,12]. Briefly, the cohort includes 9778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrollment was aimed at early pregnancy (gestational age < 18 weeks) at the routine fetal ultrasound examination in pregnancy but was allowed until birth of the child. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early pregnancy (gestational age <18.0 weeks), mid-pregnancy (gestational age 18.0 – 24.9 weeks) and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy

(3%) had one assessment (in late pregnancy) planned. The individual timing of these assessments depended on the gestational age at enrollment[12]. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Of all eligible children in the study area, 61% participated at birth in the study[12]. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, has approved the study. Written informed consent was obtained from all participants.

Maternal alcohol consumption

Information about maternal alcohol consumption was obtained by postal questionnaires in early, mid- and late pregnancy[12]. Response rates for these questionnaires were 91%, 80% and 77% respectively. In the first questionnaire, the mothers were asked whether they used any alcoholic drinks in the first 3 months of pregnancy (with response categories: no; until pregnancy was known; after pregnancy was known). This questionnaire was sent to all mothers, including those enrolled after early pregnancy. In the second and third questionnaires, sent in mid- and late pregnancy respectively, the mothers were asked whether they drank any alcohol in the past 2 months (no; yes). Mothers who reported in the first questionnaire drinking only until pregnancy was known (n = 2061), but who reported alcohol consumption in the second or third questionnaire (n = 1106), were reclassified into the 'after pregnancy was known' category. The same strategy was used for mothers who claimed no alcohol use in the first questionnaire (n = 4072) but who reported drinking in the second or third questionnaire (n = 549). Mothers who reported any drinking were asked to classify their average alcohol consumption into one of the following six categories: < 1 drink per week; 1-3 per week; 4-6 per week; 1 per day; 2-3 per day; >3 per day. For each questionnaire, this information was combined and reclassified into the following categories of maternal alcohol consumption: never; <1 drink per week; 1-6 drinks per week; ≥1 drinks per day. The numbers of subjects having more than 3 alcoholic drinks per day were n = 12 in early pregnancy, n = 8 in mid-pregnancy and n = 6 in late pregnancy. In the Netherlands, an average alcoholic drink contains about 12 grams of alcohol.

Covariates

Information about educational level, ethnicity and parity was obtained by questionnaire at enrollment in the study. Maternal smoking habits were assessed in each questionnaire. Maternal anthropometrics, including height and weight, were measured without shoes and heavy clothing. Body mass index was calculated (weight/height^2 (kg/m^2)) in early, mid- and late pregnancy during visits at the research center.

Birth outcomes

Date of birth, birth weight and gender were obtained from midwife and hospital registries. Gestational age was established by fetal ultrasound examination because using last menstrual period has several limitations, including the large number of women who do not know their exact last

menstrual period date or have irregular cycles[13]. Main outcomes were birth weight in grams, low birth weight (weight < 2500 grams), small-size-for-gestational-age at birth (weight < -2 standard deviation scores (SDS)) and preterm birth (gestational age < 37 weeks). Gestational age adjusted standard deviation birth weight scores were based on published reference charts from a large North-European birth cohort[14].

Population for analysis

Of the total of 9778 mothers, 91% (n = 8880) were enrolled during pregnancy[12]. Those without information about alcohol consumption during pregnancy in the first questionnaire were excluded from the analyses (14%, n = 1204). Of the remaining 7676 mothers, those with twin pregnancies (n = 81), fetal deaths (n = 100) or missing birth outcomes (n = 354) were excluded. The associations of maternal alcohol consumption during pregnancy with birth outcomes were analyzed in the remaining 7141 mothers. Of these mothers, 4% (n = 292) were second or third pregnancies in the study. Since there were no differences in results after exclusion of these subjects, they were included in the analyses. Analyses focused on alcohol consumption in different periods of pregnancy were restricted to mothers enrolled in early pregnancy to minimize misclassification of alcohol consumption during pregnancy period. Since we did not have enough mothers to assess the separate effects of each pregnancy period, analyses were focused on early pregnancy (alcohol consumption until pregnancy was known) and late pregnancy. Among mothers enrolled in early pregnancy (n = 5533), the associations of maternal drinking in early pregnancy with birth outcomes were assessed in those for whom the level of alcohol consumption was available and who did not drink after pregnancy was known (n = 3387). Among mothers enrolled in early pregnancy, information about the average number of alcoholic drinks in late pregnancy was available in 83% (n = 4589).

Statistical analyses

The associations of alcohol consumption habits at any time during pregnancy with birth weight in grams were assessed using multiple linear regression models and with low birth weight (weight < 2500 grams), small-size-for-gestational-age at birth (weight <-2 SDS) and preterm birth (gestational age < 37 weeks) using multiple logistic regression models. These models were adjusted for maternal age, body mass index, smoking status and educational level at the time of enrollment as well as for maternal ethnicity, parity and infant gender. All models with birth weight in grams and low birth weight as dependent variables were additionally adjusted for gestational age. The same models were used to assess the associations of average alcohol consumption categories in separate periods (until pregnancy was known, in late pregnancy) with continuously measured birth weight, low birth weight, small-size-for-gestational-age at birth and preterm birth. All measures of association are presented with their 95% confidence intervals (CI).

RESULTS

Characteristics of the mothers are presented in Table 1. Of all mothers, 51% (n = 3618) used alcoholic drinks in early pregnancy and 37% (n = 2663) continued to use alcoholic drinks after pregnancy was known. In the total group, the age of mothers ranged from 15.3 to 43.3 years with a mean age of 29.8 years. The percentage of mothers with a higher educational level was highest

TABLE 1. Maternal characteristics according to their alcohol consumption habits in pregnancy (N=7141)[1]

	Alcohol consumption in pregnancy		
	Never in pregnancy n = 3523	**Until pregnancy was known n = 955**	**Continued after pregnancy was known n = 2663**
Age, yrs	28.4 (5.3)	29.5 (5.2)	31.6 (4.7)
Height, cm	165.5 (7.3)	168.1 (7.1)	169.6 (6.9)
Weight, kg	70.0 (14.4)	68.6 (12.9)	69.0 (11.8)
Body mass index, kg/m^2	25.5 (4.9)	24.3 (4.3)	24.0 (3.8)
Parity ≥ 1, %	47.8	31.4	44.1
Smoking in pregnancy, %			
No	79.4	65.8	71.3
Until pregnancy was known	4.3	17.0	10.4
Continued	16.2	17.2	18.2
Education, %			
Primary school	19.4	6.7	5.8
Secondary school	55.0	47.6	31.5
Higher education	25.5	45.7	62.8
Ethnicity, %			
Dutch, other-European	41.1	68.4	76.0
Surinamese	10.5	11.2	6.5
Turkish	16.7	1.8	1.7
Moroccan	12.8	0.3	0.6
Cape Verdian	4.3	5.4	3.2
Antillean	3.9	3.8	2.7
Others	10.8	9.0	9.3
1st Child of the same mother in study, %	95.6	95.0	96.5
Enrollment in study in early pregnancy, %	72.2	85.4	81.7
Birth outcomes			
Birth weight, grams	3395 (552)	3351 (573)	3470 (556)
Gestational age, weeks[2]	40.0 (36.7–42.0)	39.9 (36.4–41.9)	40.1 (37.0–42.0)

[1]Values are means (standard deviation) or percentages.
[2]Median (90% range).
Data were missing on height (n=11), weight (n=27), body mass index (n=38), parity (n=419), smoking in pregnancy (n=62), educational level (n=223) and ethnicity (n=61).

among those who continued to use alcoholic drinks after pregnancy was known. In the total cohort, the largest ethnic groups were the Dutch and other-European (57.8%), Surinamese (9.1%), Turkish (9.1%) and Moroccan (6.5%). Among the mothers who continued to use alcoholic drinks after pregnancy was known, the percentage of Dutch mothers was higher than among those who did not drink during pregnancy. Mean birth weight of the children was 3417 (range, 670-5310) grams. Among the children born with a gestational age of more than 37 weeks (n = 6727), 10.2% were defined as low birth weight. Gestational age at birth ranged from 25.4 to 43.1 weeks with a median of 40.0 weeks. Of all singleton live births, 5.8% were born before a gestational age of 37 weeks.

Table 2 demonstrates the effect estimates for alcohol consumption until pregnancy was known and after pregnancy was known for all birth outcomes compared to no alcohol consumption. After adjustment, no significant adverse effects were found for any of these birth outcomes.

Table 3 shows that among mothers having alcoholic drinks until pregnancy was known, no adverse effects were found for having <1 alcoholic drink per day on average, compared to no alcohol consumption. Tendencies towards adverse effects on all birth outcomes were found for

TABLE 2. Associations of alcohol consumption habits during pregnancy with birth outcomes (N=7141)

	Difference (95% CI) in birth weight in grams	
Alcohol consumption[1,2]	Unadjusted	Adjusted[3]
Until pregnancy was known	-16 (-49, 16)	-22 (-54, 11)
After pregnancy was known	46 (24, 69)**	2 (-23, 27)
	Odds ratio (95% CI) for low birth weight (n=344)	
Alcohol consumption[1,2]	Unadjusted	Adjusted[3]
Until pregnancy was known	1.23 (0.82, 1.85)	1.37 (0.87, 2.16)
After pregnancy was known	1.16 (0.86, 1.58)	1.38 (0.95, 2.01)
	Odds ratio (95% CI) for small-size-for-gestational-age (n=241)	
Alcohol consumption[1,2]	Unadjusted	Adjusted[3]
Until pregnancy was known	1.12 (0.77, 1.64)	1.04 (0.68, 1.58)
After pregnancy was known	0.96 (0.72, 1.27)	1.00 (0.71, 1.41)
	Odds ratio (95% CI) for preterm birth (n=414)	
Alcohol consumption[1,2]	Unadjusted	Adjusted[3]
Until pregnancy was known	1.01 (0.75, 1.36)	0.87 (0.63, 1.21)
After pregnancy was known	0.85 (0.69, 1.06)	0.90 (0.69, 1.16)

Abbreviations: CI, confidence interval.

[1]Reference group is no alcohol consumption.

[2]Number of subjects per category: no alcohol consumption: n=3523; until pregnancy was known: n=955; after pregnancy was known: n=2663.

[3]Adjusted model: controlled for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender; birth weight and low birth weight models also controlled for gestational age.

**P-value<0.01

TABLE 3. Associations of alcohol consumption categories until pregnancy was known with birth outcomes (N=3387)

Alcohol consumption until pregnancy was known[1,2]	Difference in birth weight in grams (95% CI)	
	Unadjusted	Adjusted[3]
< 1 drink per week	-26 (-70, 17)	-26 (-69, 17)
1 - 6 drinks per week	18 (-35, 72)	6 (-48, 59)
≥ 1 drink per day	-68 (-216, 81)	-129 (-271, 12)
Alcohol consumption until pregnancy was known[1,2]	**Odds ratio (95% CI) for low birth weight (n=177)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	1.27 (0.74, 2.20)	1.31 (0.87, 2.44)
1 - 6 drinks per week	1.15 (0.58, 2.27)	1.38 (0.66, 2.90)
≥ 1 drink per day	3.16 (0.77, 12.96)	4.81 (1.10, 21.08)*
Alcohol consumption until pregnancy was known[1,2]	**Odds ratio (95% CI) for small-size-for-gestational-age (n=177)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	1.11 (0.68, 1.85)	1.04 (0.59, 1.84)
1 - 6 drinks per week	0.65 (0.30, 1.41)	0.68 (0.30, 1.52)
≥ 1 drink per day	1.63 (0.39, 6.89)	1.45 (0.33, 6.44)
Alcohol consumption until pregnancy was known[1,2]	**Odds ratio (95% CI) for preterm birth (n=208)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	0.89 (0.59, 1.36)	0.79 (0.50, 1.24)
1 - 6 drinks per week	0.96 (0.58, 1.58)	0.80 (0.46, 1.40)
≥ 1 drink per day	2.46 (0.95, 6.42)	2.51 (0.92, 6.81)

Abbreviations: CI, confidence interval.
[1]Reference group is no alcohol consumption.
[2]Number of subjects per category: no alcohol consumption: n=2555; < 1 drink per week: n=490; 1 - 6 drinks per week: n=306; ≥ 1 drink per day: n=36.
[3]Adjusted model: controlled for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender; birth weight and low birth weight models also controlled for gestational age.
*P-value<0.05

having ≥1 alcoholic drink per day until pregnancy was known. Birth weight averaged 129 (95% CI: -12, 271) grams lower in offspring of mothers who used ≥1 alcoholic drink per day until pregnancy was known. An increased risk was found for low birth weight (aOR, 4.81 (95% CI: 1.10, 21.08)). The aOR for small-size-for-gestational-age at birth was 1.45 (95% CI: 0.33, 6.44) and for preterm birth it was 2.51 (95% CI: 0.92, 6.81).

Table 4 gives the associations for the effects of each alcohol category in late pregnancy on all birth outcomes compared to no alcohol consumption. As was the case with alcohol consumption until pregnancy was known, no adverse effects were found for having <1 alcoholic drink per day in late pregnancy. Non-significant tendencies towards adverse effects were found for having ≥1 alcoholic drink per day in late pregnancy on birth weight (decrease in weight of 118 (95% CI: -65, 300) grams) and the risks of low birth weight (aOR, 5.25 (95% CI: 0.57, 48.05)) and

TABLE 4. Associations of alcohol consumption categories in late pregnancy with birth outcomes (N=4589)

Alcohol consumption in late pregnancy[1,2]	**Difference in birth weight in grams (95% CI)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	53 (23, 83)**	-6 (-36, 25)
1 - 6 drinks per week	74 (28, 119)**	5 (-40, 49)
≥ 1 drink per day	-72 (-266, 122)	-118 (-300, 65)
Alcohol consumption in late pregnancy[1,2]	**Odds ratio (95% CI) for low birth weight (n=266)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	0.88 (0.58, 1.34)	0.98 (0.61, 1.59)
1 - 6 drinks per week	1.22 (0.62, 2.40)	1.74 (0.83, 3.64)
≥ 1 drink per day	3.28 (0.41, 26.29)	5.25 (0.57, 48.05)
Alcohol consumption in late pregnancy[1,2]	**Odds ratio (95% CI) for small-size-for-gestational-age (n=184)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	0.77 (0.51, 1.15)	0.94 (0.60, 1.48)
1 - 6 drinks per week	0.95 (0.54, 1.68)	1.07 (0.57, 2.01)
≥ 1 drink per day	3.01 (0.69, 13.12)	2.09 (0.26, 16.67)
Alcohol consumption in late pregnancy[1,2]	**Odds ratio (95% CI) for preterm birth (n=312)**	
	Unadjusted	Adjusted[3]
< 1 drink per week	0.93 (0.69, 1.25)	1.09 (0.78, 1.52)
1 - 6 drinks per week	0.44 (0.24, 0.81)**	0.57 (0.30, 1.08)
≥ 1 drink per day	0.85 (0.11, 6.34)	1.20 (0.16, 9.17)

Abbreviations: CI, confidence interval.
[1]Reference group is no alcohol consumption.
[2]Number of subjects per category: no alcohol consumption: n=2903; < 1 drink per week: n=1229; 1 – 6 drinks per week: n=436; ≥ 1 drink per day: n=21.
[3]Adjusted model: controlled for maternal body mass index, smoking, educational level, height, ethnicity, parity and age and infant gender; birth weight and low birth weight models also controlled for gestational age.
**P-value<0.01

small-size-for-gestational-age at birth (aOR, 2.09 (95% CI: 0.26, 16.67)). The aOR for preterm birth was 1.20 (95% CI: 0.16, 9.17).

DISCUSSION

This population-based prospective cohort study suggested that average maternal alcohol consumption of <1 drink per day was not associated with adverse effects on weight and gestational age at birth. Tendencies towards adverse effects were found for alcohol consumption of ≥1 drinks per day on average in early and late pregnancy.

Methodological considerations

The strength of this study is the large number of subjects prospectively studied from early pregnancy and the ability to control for a large number of potential confounders. A potential limitation of this study is that information about maternal alcohol consumption was missing in 14% of all mothers that were enrolled during pregnancy in the cohort. Birth weight was slightly lower in the offspring of mothers without information about alcohol consumption during pregnancy (difference: -35 (95% CI: -71, 1) grams). Of all mothers enrolled during pregnancy, information at birth was available for 93%. Categories of maternal alcohol consumption at baseline were similarly distributed among those with singleton live birth as pregnancy outcome and those lost to follow-up. Selection bias due to non-response or loss to follow-up would be present if the associations of maternal alcohol consumption during pregnancy with the birth outcomes differ between those with and without complete data. This seems unlikely but can not be ruled out. Biased estimates in large cohort studies primarily arise from loss to follow-up rather than from non-response at baseline[15].

Information about maternal alcohol consumption during pregnancy was prospectively collected by postal questionnaires without reference to the birth outcomes. Using self-reported alcohol consumption may have introduced misclassification mainly because of underreporting of alcohol consumption during pregnancy[16]. If this underreporting were present across all categories of alcohol consumption, the effect estimates would be underestimated. However, if mothers with heavy alcohol consumption selectively underreport the average number of drinks, the differences between no alcohol consumption and the lower categories of alcohol consumption would have been overestimated. This misclassification may be prevented by using objective measures of alcohol consumption. However, current available biomarkers of alcohol consumption, including carbohydrate-deficient transferrin and gamma-glutamyl transferase, seem to be inappropriate for assessment of light to moderate alcohol consumption[17]. A potential limitation in our study is that we use the average number of alcoholic drinks per week without taking into account the patterns of alcohol consumption. However, in our study group, the number of subjects having used ≥6 per day in early and late pregnancy was 6 and 0 respectively. Thus, we do not expect that binge drinking has led to biased effect estimates.

Birth outcomes

In our study, we examined the effects of maternal alcohol consumption during pregnancy on birth weight as a continuous measure, low birth weight (weight < 2500 grams), small-size-for-gestational-age at birth (weight < -2SDS) and preterm birth (gestational age < 37 weeks). Dichotomization of birth weight and gestational age was performed according current obstetrics guidelines for low birth weight, small size at birth adjusted for gestational age and preterm birth. These definitions enable us to compare our results to previous studies.

A recent systematic review focused on the effects of low to moderate alcohol consumption during pregnancy showed no evidence for adverse or positive effects on weight or gestational age at

birth[18]. Inconsistent results from studies assessing the associations of low to moderate maternal alcohol consumption during pregnancy with birth weight may be explained by methodological issues including inappropriate assessments of maternal alcohol consumption habits, the inability to examine the effect of alcohol consumption in different periods of pregnancy and the inability to adjust for potential confounders. Results from a prospective cohort study from fetal life among 10539 subjects suggested that using ≥1 alcoholic drinks per day leads to a decrease in birth weight of 150 grams[6]. Other cohort studies found small beneficial effects of low to moderate alcohol consumption on birth weight and on the risk of low birth weight[9,19-20]. Similarly, studies examining the associations between moderate alcohol consumption and preterm birth are not conclusive[18]. A recent study among 40,892 participants in the Danish National Birth Cohort Study, suggests that using 4 to 6 and ≥7 alcoholic drinks per week during pregnancy was associated with increased risks of preterm birth of 1.15 (95% CI: 0.84, 1.57) and 1.77 (95% CI: 0.94, 3.31), respectively[21].

It seems that for moderate alcohol consumption a cut off value of the average number of alcoholic drinks may exist at which alcohol consumption has adverse effects on birth weight and preterm birth. Both our study and previous studies suggest that this cut off lies between 4 - 7 alcoholic drinks per week, which means approximately 6 - 12 grams of alcohol per day. Lundsberg *et al.* demonstrated a beneficial effect of low to moderate alcohol consumption on fetal growth retardation and suggested a J-shaped relation between maternal alcohol consumption and fetal growth retardation[7]. In our study, a positive association was found between average alcohol consumption of less than one drink per day in late pregnancy and birth weight in the unadjusted models (Table 4). However, these effects were fully explained by life style and socio-economic related factors. Previously suggested beneficial effects may be explained by residual confounding due to unmeasured life style and socio-economic related factors. Another explanation for the beneficial effect of low alcohol consumption may be a healthy drinkers effect in which women with a poor obstetric history or prognosis are more likely to abstain from having alcoholic drinks during pregnancy. Low alcohol consumption might also have genuine biologically beneficial effects on fetal growth and development. This latter hypothesis should be rigorously tested in large prospectively cohort studies in which information about potential confounders are available[18].

Conclusion

Although our outcomes are important from an obstetric perspective, they are rather crude measures of fetal growth and development. Alcohol consumption may affect fetal organ development without any effect on birth weight. Postnatal follow-up studies have suggested that low to moderate alcohol consumption is not associated with anthropometric measures in childhood but adversely influences behaviour and emotional development in childhood[22-24]. Thus, before developing new public health strategies focused on alcohol consumption during pregnancy, studies are needed to assess the associations of low to moderate alcohol consumption with postnatal growth and development.

REFERENCES

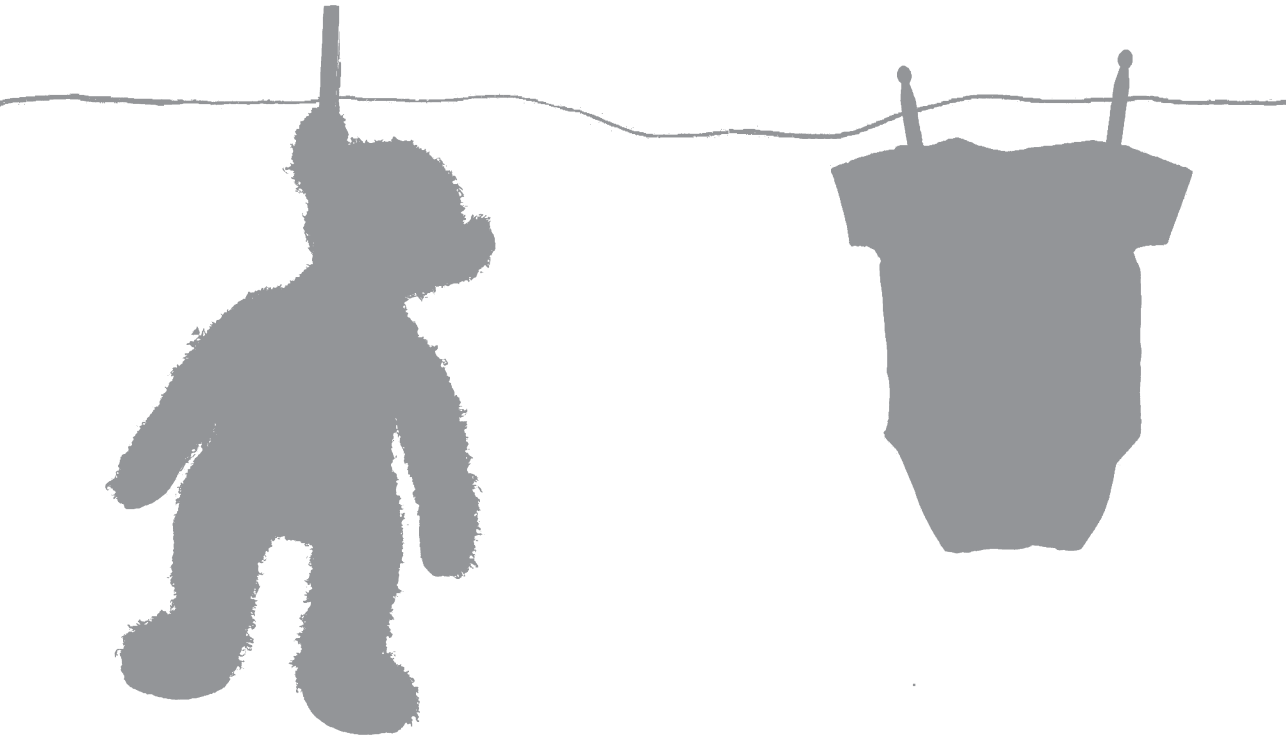
1. Little RE, Wendt JK. The effects of maternal drinking in the reproductive period: an epidemiologic review. J Subst Abuse.1991;3:187-204.
2. Ouellette EM, Rosett HL, Rosman NP, et al. Adverse effects on offspring of maternal alcohol abuse during pregnancy. N Engl J Med. 1977;297:528-30.
3. Sokol RJ, Delaney-Black V, Nordstrom B. Fetal alcohol spectrum disorder. JAMA. 2003;290:2996-9.
4. Barr HM, Bookstein FL, O'Malley KD, et al. Binge drinking during pregnancy as a predictor of psychiatric disorders on the Structured Clinical Interview for DSM-IV in young adult offspring. Am J Psychiatry. 2006;163: 1061-5.
5. Lazzaroni F, Bonassi S, Magnani M, et al. Moderate maternal drinking and outcome of pregnancy. Eur J Epidemiol. 1993;9:599-606.
6. Passaro KT, Little RE, Savitz DA, et al. The effect of maternal drinking before conception and in early pregnancy on infant birthweight. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Epidemiology. 1996;7:377-83.
7. Lundsberg LS, Bracken MB, Saftlas AF. Low-to-moderate gestational alcohol use and intrauterine growth retardation, low birthweight, and preterm delivery. Ann Epidemiol. 1997;7:498-508.
8. Parazzini F, Chatenoud L, Surace M, et al. Moderate alcohol drinking and risk of preterm birth. Eur J Clin Nutr. 2003;57:1345-9.
9. Whitehead N, Lipscomb L. Patterns of alcohol use before and during pregnancy and the risk of small-for-gestational-age birth. Am J Epidemiol. 2003;158:654-62.
10. Mariscal M, Palma S, Llorca J, et al. Pattern of alcohol consumption during pregnancy and risk for low birth weight. Ann Epidemiol. 2006;16:432-8
11. Hofman A, Jaddoe VW, Mackenbach JP, et al. Growth, development and health from early fetal life until young adulthood: the Generation R Study. Paediatr Perinat Epidemiol. 2004;18:61-72.
12. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.
13. Tunon K, Eik-Nes SH, Grottum P. A comparison between ultrasound and a reliable last menstrual period as predictors of the day of delivery in 15,000 examinations. Ultrasound Obstet Gynecol. 1996;8:178-85.
14. Niklasson A EA, Fryer JG, Karlberg J, et al. 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;80:756-62.
15. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
16. da Costa Pereira A, Olsen J, Ogston S. Variability of self reported measures of alcohol consumption: implications for the association between drinking in pregnancy and birth weight. J Epidemiol Community Health. 1993;47:326-30.
17. van Pelt J, Leusink GL, van Nierop PW, et al. Test characteristics of carbohydrate-deficient transferrin and gamma-glutamyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res. 2000;24: 176-9.
18. Henderson J, Gray R, Brocklehurst P. Systematic review of effects of low-moderate prenatal alcohol exposure on pregnancy outcome. BJOG. 2007;114:243–52
19. Bell R, Lumley J. Alcohol consumption, cigarette smoking and fetal outcome in Victoria, 1985. Community Health Stud. 1989;13:484-91.
20. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and prematurity. Am J Public Health. 1992;82:87-90.
21. Albertsen K, Andersen AM, Olsen J, et al. Alcohol consumption during pregnancy and the risk of preterm delivery. Am J Epidemiol. 2004;159:155-61.
22. O'Callaghan FV, O'Callaghan M, Najman JM, et al. Maternal alcohol consumption during pregnancy and physical outcomes up to 5 years of age: a longitudinal study. Early Hum Dev. 2003;71:137-48.

23. Sood B, Delaney-Black V, Covington C, et al. Prenatal alcohol exposure and childhood behavior at age 6 to 7 years: I. dose-response effect. Pediatrics. 2001;108:e34.
24. Sayal K, Heron J, Golding J, et al. Prenatal alcohol exposure and gender differences in childhood mental health problems: a longitudinal population-based study. Pediatrics. 2007;119:e426-34.

Chapter 4.4

Smoking, folic acid supplement use and fetal complications

Rachel Bakker
Sarah Timmermans
Eric AP Steegers
Albert Hofman
Vincent WV Jaddoe
Submitted for publication



ABSTRACT

Maternal smoking during pregnancy leads to increased risks of neonatal complications. Use of folic acid supplements might reduce the adverse effects of smoking. We examined whether folic acid supplement use modifies the associations of maternal smoking with first trimester homocysteine levels, fetal growth characteristics and risks of neonatal complications. The associations were studied in 6294 mothers participating in a prospective population-based cohort study in the Netherlands. Main outcomes measurements were first trimester homocysteine levels, fetal growth characteristics and neonatal complications, including preterm birth, low birth weight and small-size-for-gestational-age. Continued maternal smoking was associated with higher first trimester homocysteine levels (difference: 0.52 μmol/l (95% range, 0.20-2.14)), lower third trimester fetal weight (difference: -44 grams (95% confidence interval (CI): -58, -31)) and birth weight (difference: -152 grams (95% CI: -183, -122)). Periconceptional folic acid supplement use decreased these differences ($P_{interaction}$ <0.001). Among mothers who continued smoking during pregnancy, those who did not use folic acid supplements, tended to have the highest risks of preterm birth (OR, 1.82 (95% CI: 0.82, 4.02)), low birth weight (OR, 3.45 (95% CI: 1.25, 9.54)) and small-size-for-gestational-age at birth (OR, 1.29 (95% CI: 0.69, 2.42)), as compared to those who did use periconceptional folic acid supplements. Our results suggest that the adverse effects of maternal smoking on first trimester homocysteine levels, fetal growth and risks of neonatal complications might be reduced by the use of folic acid supplements. Because of the observational design, our findings should be considered as hypothesis generating.

INTRODUCTION

Impaired fetal growth is associated with neonatal morbidity and mortality[1] and the risks of common diseases in later life such as type 2 diabetes and cardiovascular disease[2-4]. Maternal smoking is one of the most important causes of fetal growth retardation in Western countries. Previous studies showed that maternal smoking is associated with impaired fetal growth from early pregnancy onwards and increased risks of neonatal complications[5-7]. The mechanisms by which maternal smoking affects fetal growth include placental dysfunction, vasoconstriction and hypoxia. Recently, it has been shown that children exposed to maternal smoking have differences in global and gene-specific DNA-methylation, suggesting that smoking during pregnancy might also lead to epigenetic modifications in the offspring[8]. These gene-specific DNA-methylation alterations might lead to fetal and postnatal growth and development adaptations[9]. Use of folic acid supplements might be beneficial for the adverse effects of smoking on DNA-methylation, since folate provides methyl groups for the synthesis of methionine. The derivate of methionine is an important methyl donor in the human body for DNA-methylation[10-12]. We have previously shown that folic acid supplement use is associated with increased fetal growth rates resulting in higher birth weight in the offspring[13]. Folate plays a critical role in homocysteine metabolism. The folate-dependent homocysteine pathway is important for protein, lipid and DNA synthesis. Studies have shown that homocysteine levels are higher among smokers and lower among folic acid supplement users[14-17]. Increased homocysteine levels are associated with alterations in birth weight[18-21]. Based on these studies, we hypothesized that folic acid supplement use modifies the association between maternal smoking and fetal growth, potentially through altered homocysteine levels.

Therefore, we examined in an observational population-based prospective cohort study among 6294 mothers and their children, whether folic acid supplement use modifies the associations of maternal smoking with first trimester homocysteine levels, fetal growth characteristics and the risks of neonatal complications, including preterm birth, low birth weight, and small-size-for-gestational-age at birth.

METHODS

Design and cohort

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[22-23]. All mothers were enrolled between 2001 and 2005. Of all eligible children in the study area, 61% participated at birth in the study[23]. The Medical Ethical Committee of the Erasmus Medical Center in Rotterdam approved the study (MEC 198.782/2001/31). Written consent was obtained from all participating parents. In total, 8880 mothers were enrolled during pregnancy. For this analysis, we excluded mothers

without information on smoking or folic acid supplements use (n = 2396), and those without fetal ultrasound measurements (n = 84). Of the remaining 6400 mothers, we subsequently excluded those with induced abortion (n = 20), fetal death (n = 56), loss-to-follow-up (n = 21), and delivery after less than 25 weeks of gestation (n = 9). Thus, the total cohort for analysis comprised 6294 mothers (71% of 8880). A participant flow chart is given in the Supplementary Figure S1.

Smoking during pregnancy

Information on smoking was obtained by self-administered questionnaires sent in the first, second, and third trimester. Response rates for these questionnaires were 91%, 80%, and 77%, respectively[23]. Smoking at enrolment was assessed in the first questionnaire by asking each mother whether she smoked during pregnancy (no smoking; first trimester only smoking; continued smoking). This questionnaire was sent to all mothers, regardless of the gestational age at enrolment. To assess smoking habits in second and third trimester, mothers were asked whether they smoked in the past 2 months (no; yes) in the second and third questionnaire. Mothers who reported in the first questionnaire that they smoked first trimester only, but still reported smoking in the second or third questionnaire were reclassified into the 'continued smoking' category. The same strategy was used for mothers who reported no smoking in the first questionnaire, but reported smoking in the second or third questionnaire[5].

Folic acid supplement use

All mothers were asked by questionnaire at enrolment whether they had used a folic acid supplement periconceptionally (folic acid dosage of 0.4–0.5 mg/day, according to the advice of the Health Council of the Netherlands), and when supplement use was started[24]. Folic acid supplement use was categorized into three groups: periconception start; start during first trimester (defined as before 8th week of pregnancy); no use. About 15% of the mothers reported to have used folic acid as part of a multivitamin supplement regimen. Self-reported folic acid supplement use was validated by serum folate levels in a small subsample of the present study (n = 272). Blood samples were collected between 7 and 12 weeks of gestation. Within mothers who reported using folic acid supplements (n = 204; 75%), the median of serum folate was 23.5 nmol/l (95% confidence interval (CI): 4.3, 45.3), whereas the median serum folate level within mothers who did not use folic acid supplements (n = 68; 25%) was 11.1 nmol/l (95% CI: 4.7, 29.6). The difference in distribution function (Mann–Whitney test) is statistically significant (P <0.001).

Homocysteine levels

At enrolment in first trimester, venous blood serum and plasma samples were drawn and thereafter stored for future purpose at -80^{o}C[22]. Homocysteine (tHcy) concentrations were analyzed using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands) at the Department of Clinical Chemistry of the Erasmus Medical Center, Rotterdam in 2008. The between-run coefficients of variation for plasma tHcy were 3.1%

at 7.6 µmol/l, 3.1% at 13.7 µmol/l, and 2.1% at 26.1 µmol/l. Homocysteine concentrations in early pregnancy were available in 70% (n = 4432) of the study population. No differences in available smoking information were observed between mothers with and without known homocysteine concentrations.

Fetal growth characteristics

Fetal ultrasound examinations were carried out in two dedicated research centers in each trimester of pregnancy. We established gestational age by first trimester fetal ultrasound examination[25]. In the second and third trimesters of pregnancy we measured parameters of head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures[26]. Estimated fetal weight was subsequently calculated using the formula by Hadlock *et al.*[27]. Gestational age-adjusted standard deviation (SD) scores were constructed for all fetal growth measurements. These gestational age-adjusted SD scores were based on reference growth curves from the complete study population, and represent the equivalent z-scores[25].

Neonatal complications

Low birth weight was defined as birth weight below 2500 grams. Small-size-for-gestational-age at birth was defined as a gestational age adjusted birth weight below the 5th percentile in the study cohort, and preterm birth was defined as gestational age of less than 37 weeks at delivery.

Covariates

Information on maternal age at enrolment (continuous), highest completed educational level (primary school; secondary school; higher education), ethnicity (European; Non-European), parity (nulliparous; multiparous) was obtained from the first questionnaire at enrolment in the study. Height (cm) and weight (kg) at enrolment were measured without shoes and heavy clothing. Weight was repeatedly measured during subsequent visits at the research center. Body mass index at early gestation was calculated from maternal weight and maternal height (weight/height^2 (kg/m^2)). Maternal weight measured at enrolment in the study was strongly correlated with pre-pregnancy weight ($r=0.95$, $p<0.01$). We used maternal weight measured at enrolment to calculate maternal body mass index, because the numbers of missing values were smaller and data quality higher than the pre-pregnancy weight, which was reported by the mother in a self-administered questionnaire[28]. Alcohol consumption (none; first trimester only; continued) and caffeine intake (<2 units per day; 2 to 3.9 units per day; >4 units per day) were assessed in each questionnaire. First trimester total daily energy intake was obtained by a food frequency questionnaire at enrolment. Maternal distress (continuous) was measured by questionnaire at 20 weeks of gestation using the Brief Symptom Inventory, which gives a Global Severity Index[29]. Higher Global Severity Index reflects more stress mothers experience.

Statistical analyses

Differences in maternal characteristics between maternal smoking and folic acid supplement use groups were tested using one-way ANOVA for continuous variables and Chi^2 tests for categorical variables. First, the associations of smoking habits and folic acid supplement use with first trimester homocysteine levels were analyzed using multiple linear regression models. We applied log transformation and presented effect estimates as geometric means since homocysteine levels were not normally distributed. We combined first trimester only smoking and continued smoking mothers into one group of mothers who smoking in first trimester. Second, we analyzed the associations of smoking habits and folic acid supplement use with second and third trimester measured estimated fetal weight and birth weight using multiple linear regression models. Linear regression models, in which the smoking and folic acid supplement categories were included as a continuous variable, were considered as tests for trend. Third, the associations of smoking and folic acid supplement categories with neonatal complications (preterm birth, low birth weight, small-size-for-gestational-age at birth) were assessed using multiple logistic regression models. For all analyses, we tested the interaction between smoking and folic acid supplement use. All models were adjusted for gestational age at the visit or at birth, maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex. Covariates were included based on previous studies, their strong association with the outcome, or change in effect estimates. The percentages of missing values within the population for analysis were lower than 4%, except for total energy intake (23%), and maternal stress (20%). These higher percentages were due the large number of mothers who only partially completed the food frequency questionnaire or were enrolled later in pregnancy. Missing data in the covariates were imputed using the multiple imputation procedure, which is used to select the most likely value for a missing response[30]. Five imputed data sets were created and analyzed together. No differences in results were observed between analyses with imputed missing data or complete cases only. We only present the results based on imputed datasets. The analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Of the total of 6294 mothers, 16.8% (n = 1059) continued smoking during pregnancy and 29.3% (n = 1846) did not use folic acid supplements (Table 1). Children of smoking mothers and mothers who did not use folic acid supplements were born more frequently with a low birth weight and small-size-for-gestational-age at birth (Supplementary Tables S1 and S2).

Mothers who smoked during pregnancy had higher first trimester homocysteine levels as compared to those who did not smoke (geometric means of 7.44 µmol/l (95% range, 4.80-13.80) and 6.92 µmol/l (95% range, 4.60-11.66), respectively) (Supplementary Table S3). Among both

TABLE 1. Subject characteristics[1]

	N=6294
Age, yrs	29.9 (5.2)
Height, cm	167.6 (7.3)
Weight at enrolment, kg	69.6 (13.3)
Body mass index at enrolment, kg/m^2	24.8 (4.5)
Gestational age at enrolment , wks[2]	14.4 (10.2-24.9)
Parity , %	
0	55.9
≥1	42.7
Missing	1.4
Highest educational level, %	
Primary school	10.6
Secondary school	44.9
Higher education	42.5
Missing	2.0
Ethnicity, %	
European	59.2
Non-European	40.4
Missing	0.4
Smoking habits, %	
None	75.0
1st trimester only	8.2
Continued	16.8
Alcohol consumption, %	
None	49.3
1st trimester only	13.4
Continued	37.0
Missing	0.2
Caffeine intake, %	
<2 units per day	58.4
2-3.9 units per day	30.7
≥4 units per day	6.3
Missing	3.4
Total energy intake, kcal	2045 (565.1)
Maternal stress index[2]	0.15 (0.00-1.37)
Folic acid supplementation use, %	
Periconception	39.6
1st trimester	31.1
No	29.3
Homocysteine level, µmol/l[2]	6.9 (4.6-12.2)

TABLE 1. (continued)

Fetal and birth characteristics	
2nd trimester	n=5954
Gestational age, wks[2]	20.5 (18.6-23.4)
Estimated fetal weight, g	381.0 (93.3)
3rd trimester	n=6090
Gestational age, wks[2]	30.4 (28.4-33.0)
Estimated fetal weight, g	1619.4 (262.9)
Birth	n=6294
Gestational age at birth, wks[2]	40.1 (35.6-42.3)
Birth weight, g	3424 (560)
Sex, % boys	50.1
Preterm birth, %	5.2
Low birth weight, %	4.6
Small-size-for-gestational-age, %	5.0

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

TABLE 2. Associations of maternal smoking with first trimester homocysteine levels per folic acid supplement use group (N=4432)

	Homocysteine level (µmol/l)			
	n	Geometric mean[1] (µmol/l (95% range)	Differences within the total group[2] (ln (µmol//l) (95% CI))	Differences within smoking strata[2] (ln (µmol/l) (95% CI))
Non-smoking				
Periconception FA supplement use	1602	6.74 (4.60, 10.40)	Reference	Reference
1st trimester FA supplement use	933	6.86 (4.60, 10.70)	0.03 (0.01, 0.05)*	0.03 (0.01, 0.05)*
No FA supplement use	759	7.39 (4.60, 14.80)	0.13 (0.10, 0.15)*	0.13 (0.10, 0.15)*
Smoking				
Periconception FA supplement use	319	6.88 (4.70, 10.80)	0.02 (-0.01, 0.04)	Reference
1st trimester FA supplement use	479	7.29 (4.70, 12.60)	0.08 (0.05, 0.10)*	0.06 (0.03, 0.10)*
No FA supplement use	340	8.25 (5.35, 15.15)	0.22 (0.19, 0.25)*	0.19 (0.15, 0.24)*
Modification term[3] P <0.001				

Abbreviations: CI, confidence interval; FA, folic acid.
[1]Values are geometric means (95% range) after back transformation from the lognormal distribution of homocysteine levels.
[2]Models are adjusted for maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.
[3]Modification term=smoking x FA supplement use.
*P-value <0.01

non-smoking and smoking mothers, no folic acid supplement use or use in first trimester only was associated with higher homocysteine levels (all P-values <0.01), but the difference in effect estimate was larger among smoking mothers ($P_{interaction}$ (smoking x folic acid supplement use) <0.001) (Table 2).

Associations between maternal smoking and fetal growth are given in Supplementary Table S4. Folic acid supplement use did modify the association of maternal smoking with second trimester estimated fetal weight ($P_{interaction}$=0.040). However, we did find stronger effect modification by folic acid supplement use of the associations of maternal smoking with third trimester estimated fetal weight and birth weight (both $P_{interaction}$ <0.001). Table 3 shows that within each smoking stratum, we observed lower birth weight among mothers who did not use folic acid supplements, as compared to mothers who used folic acid supplements during the periconception period. The largest effect estimates for folic acid supplement use on birth weight were observed among first trimester only and continued smoking mothers (difference: -115 grams (95% CI: -233, 3) among first trimester only smoking mothers, and -112 grams (95% CI: -187, -37) among continued smoking mothers. Figure 1 shows that mothers who continued smoking during pregnancy and did not use folic acid supplements use had the largest decrease in third trimester estimated fetal weight and birth weight compared to mothers who did not smoke during pregnancy and used periconceptional folic acid supplements.

Continued maternal smoking was associated with increased risks of preterm birth, low birth weight and small-size-for-gestational-age at birth (Supplementary Table S5). Table 4 shows that for all three outcomes, these associations were modified by folic acid supplement use ($P_{interaction}$=0.023 for preterm birth; $P_{interaction}$=0.004 for both low birth weight; and $P_{interaction}$<0.001 for small-size-for-gestational-age at birth). Among mothers who continued smoking during pregnancy, those who did not use folic acid supplements, tended to have the highest risks of preterm birth (Odds ratio (OR), 1.82 (95% CI: 0.82, 4.02)), low birth weight (OR, 3.45 (95% CI: 1.25, 9.54)) and small-size-for-gestational-age at birth (OR, 1.29 (95% CI: 0.69, 2.42)), as compared to those who did use periconceptional folic acid supplements. Overall, mothers who continued smoking and did not use folic acid supplements, had the highest risks of preterm birth (OR, 1.54 (95% CI: 0.97, 2.43)), low birth weight (OR, 3.24 (95% CI: 1.71, 6.13)) and small-size-for-gestational-age at birth (OR, 3.19 (95% CI: 2.02, 5.04)).

DISCUSSION

Results from this observational prospective population-based cohort study suggest that periconceptional folic acid supplement use may reduce the adverse effects of maternal smoking during pregnancy on fetal growth throughout pregnancy. As compared to non-smoking mothers who used periconceptional folic acid supplements, those who continued smoking and did

TABLE 3. Associations of maternal smoking with fetal weight and birth weight per folic acid supplement use group[1,2]

	Difference in 2nd trimester estimated fetal weight (gram (95% CI))			Difference in 3rd trimester estimated fetal weight (gram (95% CI))			Difference in birth weight (gram (95% CI))		
	n= 5927	Within total group	Within smoking strata	n= 6070	Within total group	Within smoking strata	n= 6260	Within total group	Within smoking strata
Non-smoking									
Periconception FA supplement use	1992	Reference	Reference	2021	Reference	Reference	2078	Reference	Reference
1st trimester FA supplement use	1272	0 (-3, 3)	0 (-3, 4)	1295	-14 (-27, -1)*	-12 (-25, 1)	1329	-19 (-48, 11)	-12 (-42, 17)
No FA supplement use	1179	3 (-1, 6)	3 (-1, 7)	1238	-16 (-30, -1)*	-13 (-29, 3)	1295	-69 (-103, -35)**	-58 (-94, -23)**
1st trimester smoking									
Periconception FA supplement use	168	-1 (-8, 6)	Reference	168	1 (-27, 30)	Reference	172	37 (-29, 104)	Reference
1st trimester FA supplement use	222	4 (-2, 10)	4 (-4, 12)	226	15 (-10, 40)	6 (-30, 42)	230	9 (-49, 67)	-42 (-129, 45)
No FA supplement use	102	3 (-6, 12)	2 (-8, 13)	106	-26 (-63, 10)	-35 (-83, 14)	109	-85 (-168, -1)*	-115 (-233, 3)
Continued smoking									
Periconception FA supplement use	221	-5 (-12, 1)	Reference	233	-49 (-72, -22)**	Reference	235	-138 (-196, -79)**	Reference
1st trimester FA supplement use	368	-3 (-8, 2)	2 (-5, 10)	374	-54 (-75, -34)**	-8 (-37, 21)	387	-176 (-224, -127)**	-52 (-122, 16)
No FA supplement use	403	-4 (-9, 1)	1 (-7, 9)	409	-58 (-79, -37)**	-17 (-49, 15)	425	-215 (-264, -166)**	-112 (-187, -37)**
Modification term[3]	*P=0.040*			*P <0.001*			*P <0.001*		

Abbreviations: CI, confidence interval; FA, folic acid.

[1]Values are differences in estimated fetal weight and birth weight in grams (95% confidence interval).

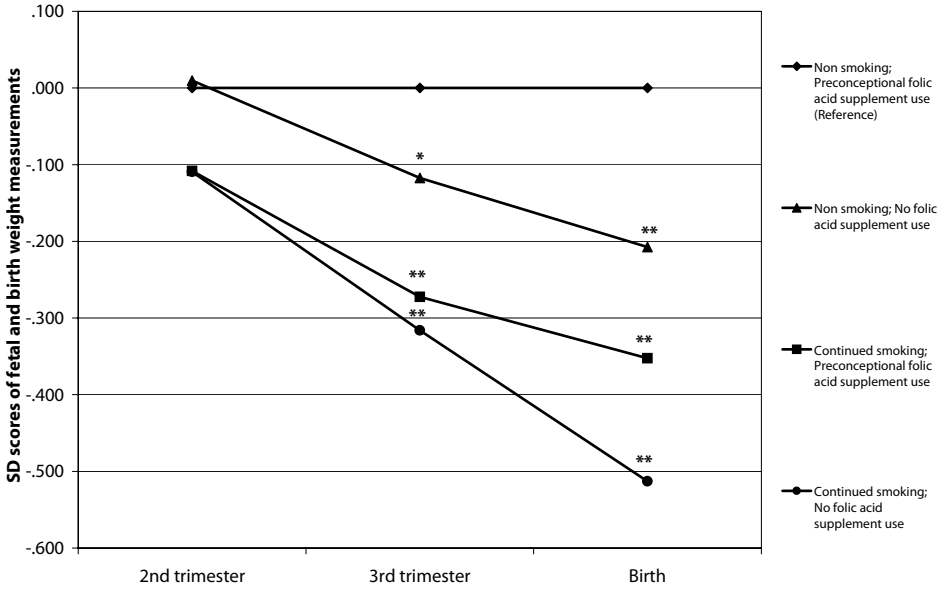
[2]Models are adjusted for gestational age at the visit or at birth, maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.

[3]Modification term=smoking x FA supplement use.

*P-value <0.05

**P-value <0.01

FIGURE 1. Relative growth in fetal weight and birth weight per folic acid supplement use group (periconceptional or no use) in non-smoking and continued smoking mothers (N=4055)



Curves are based on standard deviation (SD) scores of 2nd trimester estimated fetal weight (n=3795), 3rd trimester estimated fetal weight (n=3901), and birth weight (n=4031). Mothers who smoked in first trimester only and mothers who used folic acid supplements in first trimester only were excluded from this analysis. Estimates are given for mothers who did not smoke and did not use folic acid supplements, and mothers who continued smoking and used periconceptional folic acid supplements or no folic acid supplements. The reference group consists of mothers who did not smoke but did use periconceptional folic acid supplements. Estimates are adjusted for gestational age at the visit or at birth, maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, number of cigarettes smoked per day, total energy intake, maternal stress and fetal sex. *P-value<0.05;**P-value<0.01.

not use folic acid supplements, had the highest risks of preterm birth, low birth weight and small-size-for-gestational-age at birth.

Methodological considerations

This study is based on a prospective data collection, which started in early pregnancy. We had a large sample size of 6294 participants and a wide range of potential confounding factors available. A potential limitation of our study is that smoking and folic acid supplement use information was not available in all pregnant mothers, which might have led to loss of power. However, no differences in characteristics between mothers with and without information about smoking habits and folic acid supplement use were observed. Among mothers with information about smoking habits and folic acid supplements use, we had a limited loss-to-follow-up. Therefore, we do not expect biased results due to loss-to-follow-up[31]. Another possible limitation is that information on smoking and folic acid supplement use during pregnancy was collected by questionnaires.

TABLE 4. Associations of maternal smoking with the risk of neonatal complications per folic acid supplement use group

			Preterm birth (Odds ratio (95% CI))	
	n_{total} =6294	n_{cases} =325	Total group	Within smoking strata
Non-smoking				
Periconception FA supplement use	2084	104	Reference	Reference
1st trimester FA supplement use	1335	54	0.76 (0.54, 1.06)	0.77 (0.54, 1.08)
No FA supplement use	1303	68	0.93 (0.64, 1.33)	1.00 (0.68, 1.47)
1st trimester Smoking				
Periconception FA supplement use	172	7	0.80 (0.36, 1.75)	Reference
1st trimester FA supplement use	230	13	1.04 (0.57, 1.89)	1.38 (0.52, 3.67)
No FA supplement use	111	6	0.96 (0.40, 2.28)	0.95 (0.26, 3.43)
Continued Smoking				
Periconception FA supplement use	236	10	0.82 (0.42, 1.61)	Reference
1st trimester FA supplement use	391	28	1.37 (0.86, 2.16)	1.72 (0.81, 3.64)
No FA supplement use	432	35	1.54 (0.97, 2.43)	1.82 (0.82, 4.02)
Modification term[3]: P=0.023				
			Low birth weight (Odds ratio (95% CI))	
	n_{total} =6260	n_{cases} =292	Total group	Within smoking strata
Non-smoking				
Periconception FA supplement use	2078	77	Reference	Reference
1st trimester FA supplement use	1329	52	1.17 (0.71, 1.92)	1.08 (0.66, 1.79)
No FA supplement use	1295	67	2.09 (1.25, 3.48)**	1.79 (1.03, 3.11)*
1st trimester Smoking				
Periconception FA supplement use	172	4	0.46 (0.12, 1.72)	Reference
1st trimester FA supplement use	230	10	1.35 (0.56, 3.25)	2.64 (0.44, 15.55)
No FA supplement use	109	7	2.46 (0.81, 7.37)	4.79 (0.50, 45.21)
Continued Smoking				
Periconception FA supplement use	235	11	1.24 (0.52, 2.97)	Reference
1st trimester FA supplement use	387	26	1.96 (1.02, 3.75)*	1.73 (0.66, 4.53)
No FA supplement use	425	38	3.24 (1.71, 6.13)**	3.45 (1.25, 9.54)*
Modification term[3]: P =0.004				
			Small-size-for-gestational-age (Odds ratio (95% CI))	
	n_{total} =6256	n_{cases} =310	Total group	Within smoking strata
Non-smoking				
Periconception FA supplement use	2077	67	Reference	Reference
1st trimester FA supplement use	1327	64	1.44 (1.01, 2.06)**	1.39 (0.97, 1.99)

TABLE 4. (contined)

No FA supplement use	1295	64	1.41 (0.94, 2.10)	1.27 (0.83, 1.93)
1st trimester smoking				
Periconception FA supplement use	172	6	1.00 (0.42, 2.34)	Reference
1st trimester FA supplement use	230	7	0.80 (0.36, 1.76)	0.96 (0.31, 2.98)
No FA supplement use	109	8	2.05 (0.93, 4.49)	2.56 (0.74, 8.81)
Continued smoking				
Periconception FA supplement use	235	20	2.55 (1.49, 4.36)**	Reference
1st trimester FA supplement use	387	30	2.20 (1.37, 3.53)**	0.87 (0.48, 1.59)
No FA supplement use	424	44	3.19 (2.02, 5.04)**	1.29 (0.69, 2.42)
Modification term3: P <0.001				

Abbreviations: CI, confidence interval; FA, folic acid.
[1]Values are odds ratios (95% confidence interval).
[2]Models are adjusted for gestational age at birth (only at low birth weight analyses), maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.
[3]Modification term=smoking x FA supplement use.
*P-value <0.05
**P-value <0.01

Although assessing smoking and folic acid supplement use during pregnancy by questionnaire seems to be valid method, misclassification may have occurred[32-33]. Most importantly, we used data from an observational design. As in all observational studies, especially focused on an intervention, residual confounding might be an important issue. Although we were able to adjust for multiple potential confounders, our results should be considered as hypothesis generating and ideally need confirmation from randomized controlled trials. Since folic acid supplement use is strongly advised to all pregnant women in the Netherlands, we cannot perform such a study in the Netherlands.

Modification of folic acid supplement use

We hypothesized that folic acid supplement use modifies the association between maternal smoking and fetal growth. Many studies have suggested that maternal smoking is associated with impaired fetal growth and increased risks of neonatal complications[6,34-36]. Birth weight is 150-250 grams lower among children of mothers who continued smoking during pregnancy[6,36]. We previously found reduced fetal growth in different growth parameters throughout pregnancy, and increased risks of low birth weight and preterm birth among mothers who continued smoking during pregnancy[5-37]. Furthermore, fetal growth retardation due to smoking of the mother is suggested to already occur in first trimester[7]. It has been suggested that increased nicotine concentration in the blood of the smoking mother leads to constriction of the uterine blood vessels, and thereby decreases the blood flow to the placenta, which may lead to impaired feto-placental perfusion and decreased oxygen and nutrient transfer across the placenta, resulting in fetal growth

retardation[38-40]. Recently, Breton *et al.* have shown that children exposed to smoking have differences in global and gene-specific DNA-methylation[8]. This suggests that maternal smoking might also lead to epigenetic modifications in the offspring starting in utero. These gene-specific DNA-methylation alterations may lead to alterations of fetal and postnatal growth and development[9]. The adverse effects of maternal smoking on DNA-methylation in the offspring might be influenced by folic acid supplement use. Folate provides methyl groups for the methionine synthesis. The derivate of methionine is an important methyl donor for DNA-methylation[10-12]. Accordingly, we have previously shown that folic acid supplement use is associated with increased fetal growth rates resulting in higher birth weight in the offspring[13]. Furthermore, folate plays a critical role in homocysteine metabolism. The folate-dependent homocysteine pathway is important for protein, lipid and DNA synthesis. Previous studies and also this study show that homocysteine levels are higher among smokers and lower among folic acid supplement users[14-17]. Our results show lower levels of homocysteine among smoking mothers who did use folic acid supplements compared to smoking mothers who did not use folic acid supplements.

We did find reduced effects of maternal smoking on estimated fetal weight in second and third trimester and at birth among mothers who did use folic acid supplements. Pregnant women are advised to use folic acid supplements until the twelfth week of their pregnancy, but we are not sure when exactly they ended the supplementation. Our stronger results found in third trimester and at birth may possibly be the effect of folic acid supplementation on embryonic growth or this may be due to successful early placentation[7,41]. Also, similar modifying effects of folic acid supplement use were found for the associations between maternal smoking and neonatal complications such as preterm birth, low birth weight, and small-size-for-gestational-age at birth. Overall, the beneficial effects of folic acid supplement use on fetal growth and neonatal outcomes were stronger among mothers who smoked during pregnancy as compared to those who did not smoke. Recently, Mook-Kanamori *et al.* showed similar results in early pregnancy[7]. Furthermore, a study of Suter *et al.* emphasized that not all fetuses experience similar adverse outcomes as a result of environmental exposures such as tobacco. There might to be a potential genetic and metabolic susceptibility for the environmental exposures on adverse birth outcomes[42]. Folic acid may play an important role in altering this genetic susceptibility for smoking exposure of the fetus on subsequent impaired growth in utero.

Conclusion

Our results suggest that the adverse effect of maternal smoking on fetal growth and neonatal complications might be reduced by use of folic acid supplements during the periconceptional period. Although our findings might be of important public health relevance, they should be considered as hypothesis generating because of the observational design.

REFERENCES

1. Bacak SJ, Baptiste-Roberts K, Amon E, et al. Risk factors for neonatal mortality among extremely-low-birth-weight infants. Am J Obstet Gynecol. 2005;192:862-7.
2. Gluckman PD, Hanson MA, Cooper C, et al. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61-73.
3. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev. 2006;82:485-91.
4. Whincup PH, Kaye SJ, Owen CG, et al. Birth weight and risk of type 2 diabetes: a systematic review. JAMA. 2008; 300:2886-97.
5. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R study. Am J Epidemiol. 2007;165:1207-15.
6. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65:663-737.
7. Mook-Kanamori DO, Steegers EA, Eilers PH, et al. Risk factors and outcomes associated with first-trimester fetal growth restriction. JAMA. 2010;303:527-34.
8. Breton CV, Byun HM, Wenten M, et al. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. Am J Respir Crit Care Med. 2009;180:462-7.
9. Steegers-Theunissen RP, Obermann-Borst SA, Kremer D, et al. Periconceptional maternal folic acid use of 400 microg per day is related to increased methylation of the IGF2 gene in the very young child. PLoS One. 2009;4: e7845.
10. Bailey LB, Gregory JF, 3rd. Folate metabolism and requirements. J Nutr. 1999;129:779-82.
11. McDonald SD, Perkins SL, Jodouin CA, et al. Folate levels in pregnant women who smoke: an important gene/environment interaction. Am J Obstet Gynecol. 2002;187:620-5.
12. Tamura T, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83:993-1016.
13. Timmermans S, Jaddoe VW, Hofman A, et al. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102:777-85.
14. Nygard O, Vollset SE, Refsum H, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. JAMA. 1995;274:1526-33.
15. Venn BJ, Mann JI, Williams SM, et al. Assessment of three levels of folic acid on serum folate and plasma homocysteine: a randomised placebo-controlled double-blind dietary intervention trial. Eur J Clin Nutr. 2002; 56:748-54.
16. Ozerol E, Ozerol I, Gokdeniz R, et al. Effect of smoking on serum concentrations of total homocysteine, folate, vitamin B12, and nitric oxide in pregnancy: a preliminary study. Fetal Diagn Ther. 2004;19:145-8.
17. van Wersch JW, Janssens Y, Zandvoort JA. Folic acid, Vitamin B(12), and homocysteine in smoking and non-smoking pregnant women. Eur J Obstet Gynecol Reprod Biol. 2002;103:18-21.
18. Jauniaux E, Johns J, Gulbis B, et al. Transfer of folic acid inside the first-trimester gestational sac and the effect of maternal smoking. Am J Obstet Gynecol. 2007;197:58 e1-6.
19. Ronnenberg AG, Goldman MB, Chen D, et al. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. Am J Clin Nutr. 2002;76:1385-91.
20. Murphy MM, Scott JM, Arija V, et al. Maternal homocysteine before conception and throughout pregnancy predicts fetal homocysteine and birth weight. Clin Chem. 2004;50:1406-12.
21. Lindblad B, Zaman S, Malik A, et al. Folate, vitamin B12, and homocysteine levels in South Asian women with growth-retarded fetuses. Acta Obstet Gynecol Scand. 2005;84:1055-61.
22. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
23. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25:823–841.
24. Health Council of the Netherlands. Towards an optimal use of folic acid. The Hague: Health Council of the Netherlands. 2008.

25. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31: 388-96.
26. Routine ultrasound screening in pregnancy: protocol. London, UK: RCOG Press. 2000.
27. Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gyneacol. 1985;151:333-7.
28. Ay L, Kruithof CJ, Bakker R, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. BJOG. 2009;116:953-63.
29. Derogatis LR. Brief Symptom Inventory (BSI): Administration, scoring and procedures. Manual, third edition. Minneapolis, MN. 1993.
30. Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009;338:b2393.
31. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
32. Klebanoff MA, Levine RJ, Morris CD, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol. 2001;15:140-3.
33. Brantsaeter AL, Haugen M, Hagve TA, et al. Self-reported dietary supplement use is confirmed by biological markers in the Norwegian Mother and Child Cohort Study (MoBa). Ann Nutr Metab. 2007;51:146-54.
34. Vik T, Jacobsen G, Vatten L, et al. Pre- and post-natal growth in children of women who smoked in pregnancy. Early Hum Dev. 1996;45:245-55.
35. Bernstein IM, Plociennik K, Stahle S, et al. Impact of maternal cigarette smoking on fetal growth and body composition. Am J Obstet Gynecol. 2000;183:883-6.
36. Abel EL. Smoking during pregnancy: a review of effects on growth and development of offspring. Hum Biol. 1980;52:593-625.
37. Jaddoe VW, Troe EJ, Hofman A, 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;22:162-71.
38. Khosla S, Laddu A, Ehrenpreis S, et al. Cardiovascular effects of nicotine: relation to deleterious effects of cigarette smoking. Am Heart J. 1994;127:1669-72.
39. Lambers DS, Clark KE. The maternal and fetal physiologic effects of nicotine. Semin Perinatol. 1996;20:115-26.
40. Bainbridge SA, Smith GN. The effect of nicotine on in vitro placental perfusion pressure. Can J Physiol Pharmacol. 2006;84:953-7.
41. Steegers-Theunissen RP, Smith SC, Steegers EA, et al. Folate affects apoptosis in human trophoblastic cells. BJOG. 2000;107:1513-5.
42. Suter MA, Aagaard-Tillery KM. Environmental influences on epigenetic profiles. Semin Reprod Med. 2009;27: 380-90.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Maternal, fetal and child characteristics according to maternal smoking group (N=6294)[1]

	Maternal smoking			
	None n=4722	**1st trimester only n=513**	**Continued n=1059**	**P-value[3]**
Age, yrs	30.2 (5.0)	29.6 (5.1)	28.5 (5.8)	<0.001
Height, cm	167.6 (7.4)	168.5 (7.0)	167.1 (7.0)	0.002
Weight at enrolment, kg	69.5 (13.1)	69.7 (12.5)	70.4 (14.5)	0.098
Body mass index at enrolment, kg/m^2	24.7 (4.5)	24.6 (4.3)	25.2 (4.9)	0.004
Gestational age at enrolment, wks[2]	14.4 (10.5-25.5)	13.6 (9.5-22.0)	14.6 (9.7-29.9)	<0.001
Parity, %				<0.001
0	55.1	67.1	54.3	
≥1	43.8	31.4	43.5	
Missing	1.2	1.6	2.2	
Highest educational level, %				<0.001
Primary school	9.2	9.7	17.7	
Secondary school	41.1	46.0	61.0	
Higher education	47.8	43.5	18.6	
Missing	1.9	0.8	2.7	
Ethnicity, %				0.118
European	59.2	62.9	57.3	
Non-European	40.4	36.5	42.4	
Missing	0.4	0.6	0.3	
Alcohol consumption, %				<0.001
None	52.6	25.5	46.1	
1st trimester only	12.0	26.1	13.7	
Continued	35.2	47.8	39.8	
Missing	0.2	0.6	0.4	
Caffeine intake				<0.001
<2 units per day	61.7	54.9	44.7	
2-3.9 units per day	28.9	33.3	37.5	
>4 units per day	5.8	8.6	15.1	
Missing	3.5	3.1	2.8	
Total energy intake, kcal	2031 (557)	2089 (559)	2096 (601)	0.001
Maternal stress, index[2]	0.13 (0.00-1.17)	0.17 (0.00-1.64)	0.25 (0.00-1.98)	<0.001
Folic acid supplementation use, %				<0.001
Periconception use	44.1	33.5	22.3	
1st trimester	28.3	44.8	36.9	
No use	27.6	21.6	40.8	

SUPPLEMENTARY TABLE S1. (continued)

	Maternal smoking			
	None n=4722	**1st trimester only n=513**	**Continued n=1059**	**P-value[3]**
Homocysteine level, µmol/l[2]	6.9 (4.6-11.7)	7.0 (4.5-12.2)	7.3 (4.9-13.9)	<0.001
1st trimester[4]				
Gestational age, wks[2]	12.3 (10.6, 13.9)	12.4 (10.6, 13.9)	12.3 (10.8, 13.9)	0.296
Crown-rump length, mm	60.9 (11.4)	61.4 (11.2)	59.1 (11.6)	0.115
2nd trimester[4]				
Gestational age, wks[2]	20.5 (18.5-23.5)	20.2 (18.6-23.2)	20.4 (18.4-23.5)	0.012
Estimated fetal weight, g	383.4 (94.5)	371.9 (86.8)	374.7 (90.3)	0.002
3rd trimester[4]				
Gestational age, wks[2]	30.2 (28.5-32.9)	30.2 (28.6-32.6)	30.2 (28.4-32.6)	0.033
Estimated fetal weight, g	1630.5 (268.0)	1623.2 (245.9)	1567.5 (240.9)	<0.001
Gestational age at birth, wks[2]	40.1 (35.7-42.4)	40.1 (35.7-42.1)	39.9 (34.6-42.3)	<0.001
Birth weight, g	3459 (555)	3457 (560)	3249 (551)	<0.001
Sex, % boys	49.5	49.4	53.3	0.086
Preterm birth, %	4.8	5.1	6.9	0.020
Low birth weight, %	4.2	4.1	7.1	<0.001
Small-size-for-gestational-age, %	4.1	4.1	8.9	<0.001

[1]Values are means (standard deviation) or percentages.
[2]Median (95% range).
[3]Differences in maternal characteristics between the smoking groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.
[4]Number of 1st trimester measurements=1285; Number of 2nd trimester measurements=5954; Number for 3rd trimester measurements=6090.

SUPPLEMENTARY TABLE S2. Maternal, fetal and child characteristics according to maternal folic acid supplement use group (N=6294)[1]

	Folic acid supplement use			
	Periconception n=2492	**1st trimester n=1956**	**None n=1846**	**P-value[3]**
Age, yrs	31.5 (4.2)	29.8 (5.0)	27.8 (5.9)	<0.001
Height, cm	170.0 (7.0)	168.2 (7.0)	164.4 (7.0)	<0.001
Weight at enrolment, kg	70.0 (12.4)	69.3 (13.0)	69.5 (14.8)	0.257
Body mass index at enrolment, kg/m^2	24.3 (4.1)	24.5 (4.4)	25.7 (5.1)	<0.001
Gestational age at enrolment, wks[2]	13.5 (10.2-23.1)	14.1 (10.2-23.4)	15.9 (10.5-30.4)	<0.001
Parity, %				<0.001
0	60.7	61.9	43.2	
≥1	38.6	37.0	54.4	
Missing	0.7	1.2	2.4	
Highest educational level, %				<0.001

SUPPLEMENTARY TABLE S2. (continued)

Primary school	3.1	6.7	25.1	
Secondary school	36.8	46.8	53.6	
Higher education	59.6	45.3	16.4	
Missing	0.5	1.1	4.9	
Ethnicity, %				<0.001
European	79.6	64.0	26.7	
Non-European	20.3	35.7	72.5	
Missing	0.1	0.3	0.8	
Alcohol consumption, %				<0.001
None	41.7	41.8	67.7	
1st trimester only	14.8	14.6	10.3	
Continued	43.3	43.5	21.6	
Missing	0.1	0.2	0.4	
Caffeine intake				<0.001
<2 units per day	55.2	59.1	61.7	
2-3.9 units per day	33.9	31.0	26.0	
>4 units per day	8.1	7.6	6.8	
Missing	2.7	2.4	5.4	
Total energy intake, kcal	2103 (518)	2069 (565)	1912 (622)	<0.001
Maternal stress, index[2]	0.12 (0.00-0.85)	0.17 (0.00-1.46)	0.27 (0.00-1.96)	<0.001
Smoking habits, %				<0.001
None	83.6	68.3	70.6	
1st trimester only	6.9	11.8	6.0	
Continued	9.5	20.0	23.4	
Homocysteine level, µmol/l[2]	6.7 (4.6-10.4)	6.9 (4.6-11.1)	7.4 (4.7-14.7)	<0.001
1st trimester[4]				
Gestational age, wks[2]	12.3 (10.6, 13.9)	12.3 (10.7, 13.9)	12.4 (10.7, 13.9)	0.055
Crown-rump length, mm	60.5 (11.1)	60.9 (11.3)	61.2 (12.6)	0.686
2nd trimester[4]				
Gestational age, wks[2]	20.4 (18.6-23.0)	20.4 (18.5-23.2)	20.5 (18.5-24.1)	<0.001
Estimated fetal weight, g	377.1 (84.4)	377.6 (89.3)	390.3 (107.8)	<0.001
3rd trimester[4]				
Gestational age, wks[2]	30.2 (28.6-32.6)	30.2 (28.5-32.6)	30.2 (28.1-33.2)	0.758
Estimated fetal weight, g	1636.0 (254.5)	1610.6 (259.5)	1605.8 (276.5)	<0.001
Gestational age at birth, wks[2]	40.3 (35.6-42.4)	40.1 (35.7-42.4)	40.0 (35.6-42.3)	<0.001
Birth weight, g	3483 (551)	3428 (557)	3339 (564)	<0.001
Sex, % boys	51.4	50.8	51.4	0.105
Preterm birth, %	4.9	4.9	5.9	0.231
Low birth weight, %	3.7	4.5	6.1	0.001
Small-size-for-gestational-age, %	3.7	5.2	6.3	<0.001

[1]Values are means (standard deviation) or percentages.
[2]Median (95% range).
[3]Differences in maternal characteristics between the folic acid supplement use groups were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for proportions.
[4]Number of 1st trimester measurements=1285; Number of 2nd trimester measurements=5954; Number for 3rd trimester measurements=6090.

SUPPLEMENTARY TABLE S3. Associations of maternal smoking with first trimester homocysteine levels

Maternal smoking	n=4432	Geometric mean[1] (µmol/l (95% range)	Differences in homocysteine level[2] ln(µmol/l) (95% CI)
Non-smoking	3294	6.92 (4.60, 11.66)	Reference
Smoking	1138	7.44 (4.80, 13.80)	0.06 (0.04, 0.07)*

Abbreviations: CI, confidence interval.
[1]Values are geometric means (95% range) after back transformation from the lognormal distribution of homocysteine levels.
[2]Models are adjusted for maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.
*P-value <0.01

SUPPLEMENTARY TABLE S4. Associations of maternal smoking with fetal weight and birth weight[1]

Maternal smoking	Difference in 2nd trimester estimated fetal weight (gram (95% CI))	Difference in 3rd trimester estimated fetal weight (gram (95% CI))	Difference in birth weight (gram (95% CI))
Non-smoking n=5927	Reference n=4443	Reference n=4554	Reference n=4702
1st trimester smoking n=6070	1 (-3, 5) n=492	9 (-7, 27) n=500	22 (-17, 62) n=511
Continued smoking n=6260	-5 (-8, -2)* n=992	-44 (-58, -31)* n=1016	-152 (-183, -122)* n=1047

Abbreviations: CI, confidence interval.
[1]Models are adjusted for gestational age at the visit or at birth, maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.
*P-value <0.01

SUPPLEMENTARY TABLE S5. Associations of maternal smoking with the risk of neonatal complications1

Maternal smoking	Preterm birth (n_{cases}=325) (Odds ratio (95% CI))
Non-smoking n=4722	Reference n=226
1st trimester smoking n=513	1.04 (0.68, 1.59) n=26
Continued smoking n=1059	1.44 (1.07, 1.94)* n=73

SUPPLEMENTARY TABLE S5. (continued)

Maternal smoking	**Low birth weight (n_{cases}=292) (Odds ratio (95% CI))**
Non-smoking n=4702	Reference n=196
1st trimester smoking n=511	0.90 (0.49, 1.64) n=21
Continued smoking n=1047	1.56 (1.04, 2.33)* n=75
Maternal smoking	**Small-size-for-gestational-age (n_{cases}=310) (Odds ratio (95% CI))**
Non-smoking n=4699	Reference n=195
1st trimester smoking n=511	0.91 (0.57, 1.44) n=21
Continued smoking n=1046	2.09 (1.57, 2.76)** n=94

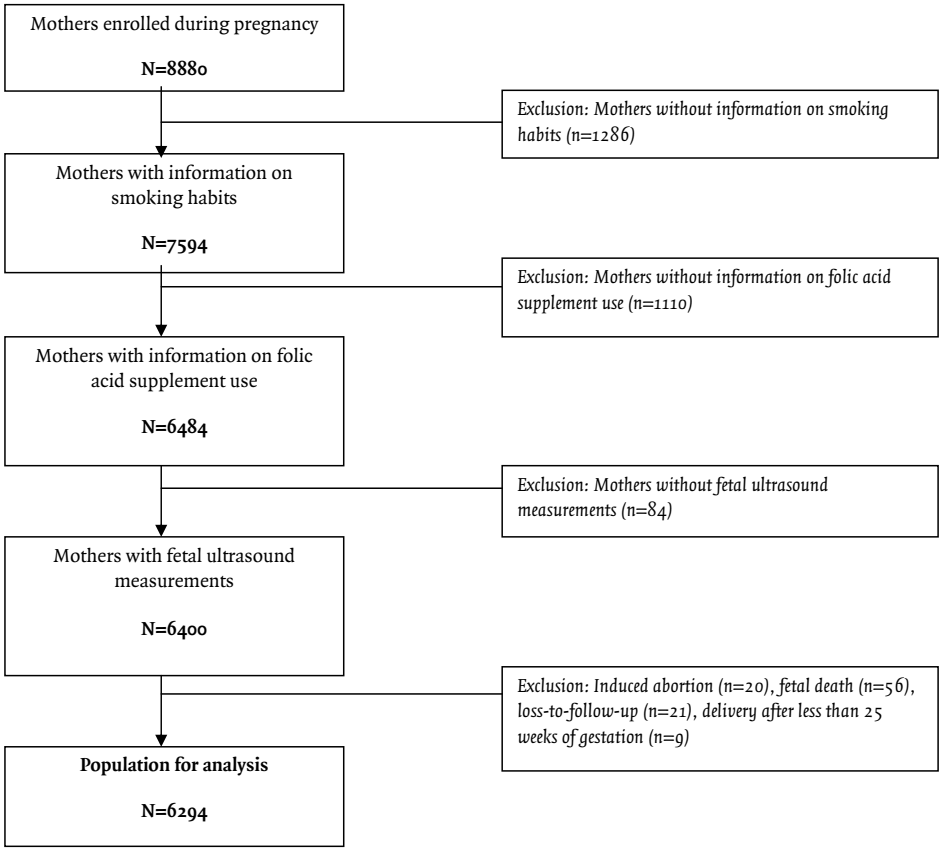
Abbreviations: CI, confidence interval.

[1]Models are adjusted for gestational age at birth (only at low birth weight analyses), maternal age, body mass index at enrolment, educational level, parity, ethnicity, alcohol consumption, caffeine intake, total energy intake, maternal stress and fetal sex.

*P-value<0.05

**P-value<0.01

SUPPLEMENTARY FIGURE S1. Flow chart of participants included for analysis

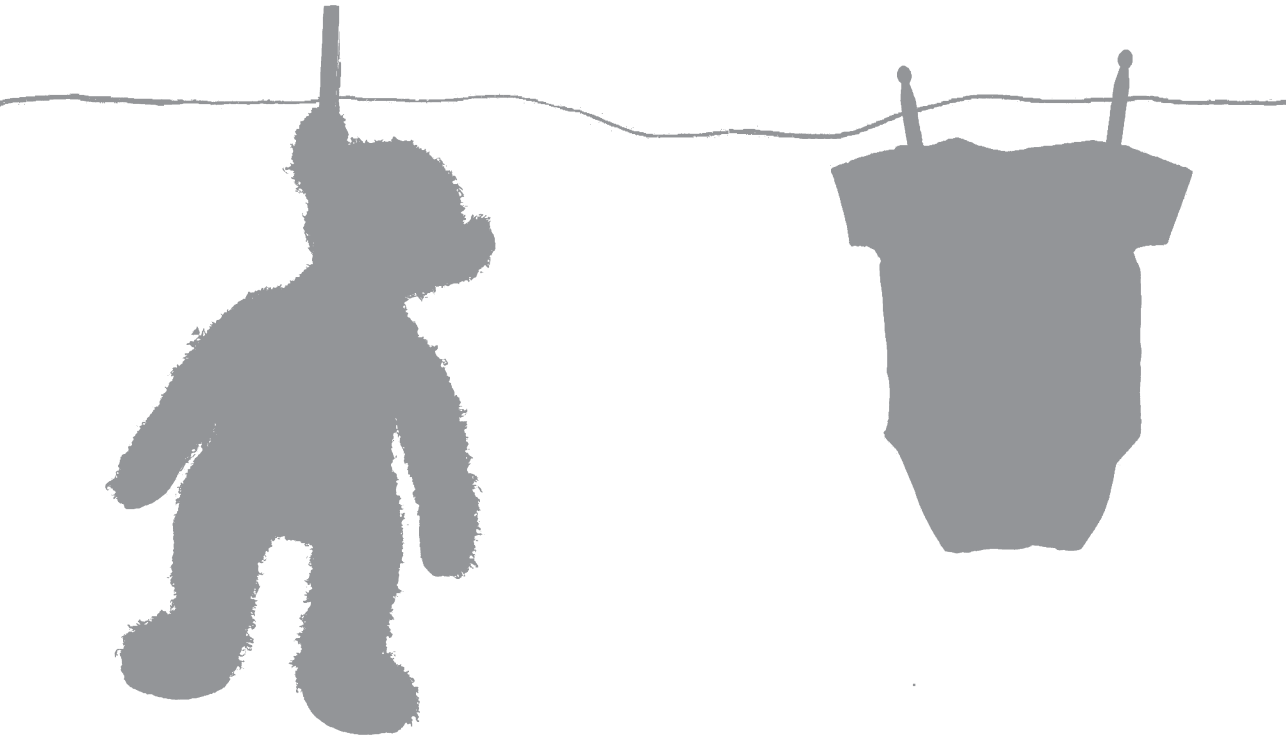


Chapter 4.5

Caffeine intake, fetal growth and birth outcomes

Rachel Bakker
Eric AP Steegers
Aleksandra Obradov
Hein Raat
Albert Hofman
Vincent VW Jaddoe

Adapted from Am J of Clin Nutr. 2010;91:1691-98.



ABSTRACT

Caffeine is a widely used and accepted pharmacologically active substance. The impact of caffeine intake during pregnancy on fetal growth and development is still unclear. We examined the associations of maternal caffeine intake, based on coffee and tea consumption, with fetal growth characteristics measured in each trimester of pregnancy and the risks of adverse birth outcomes. The associations were studied in 7346 pregnant women participating in a population-based prospective cohort study from early pregnancy onwards (the Netherlands, 2001-2005). First, second and third trimester caffeine intake was based on coffee and tea consumption and assessed by questionnaires. Fetal growth characteristics were repeatedly measured by ultrasound. Information about birth outcomes was obtained from hospital records. We observed no consistent associations of caffeine intake with fetal head circumference or estimated fetal weight in any trimester. Higher caffeine intake was associated with smaller first trimester crown-rump length, second and third trimester femur length and birth length (P_{trend} <0.05). Offspring of mothers who consumed 6 or more caffeine units per day tended to have increased risks of small-size-for-gestational-age at birth. Our results suggest that caffeine intake of 6 or more units per day during pregnancy is associated with impaired fetal length growth. Caffeine exposure might preferentially adversely affect fetal skeletal growth. Further studies are needed to assess these associations in non-European populations and to assess the postnatal consequences.

INTRODUCTION

Caffeine is a widely used and accepted pharmacologically active substance. The prevalence of the exposure is more than 80% in most Western countries[1]. Exposure to caffeine is mainly through coffee and tea consumption[2]. Maternal caffeine intake during pregnancy directly influences fetal caffeine exposure levels since caffeine freely passes through the placental barrier from the mother to the fetus and the principal caffeine metabolism enzyme, cytochrome CYP1A2, is absent in the placenta and fetus[3-5]. Fetal exposure to caffeine increases circulating catecholamine levels, which might subsequently lead to fetoplacental vasoconstriction and hypoxia, and eventually affect fetal growth and development[6].

The impact of caffeine intake during pregnancy on fetal growth and development is still unclear. Previous studies suggested that caffeine intake during pregnancy is associated with increased risks of miscarriage, and fetal death[7,8]. Similarly, some studies suggested associations of higher levels of maternal caffeine intake during pregnancy with lower birth weight[9-16]. However, results seem to be inconsistent, which might be due to differences in study designs, number of included subjects, methods for obtaining accurate caffeine intake and adjustment for potential confounders[10,17-22]. Also, studies focused on the associations of caffeine intake on only birth weight as measure of fetal growth and development are not able to identify specific fetal exposures and growth patterns. Studies on various fetal growth characteristics measured in different trimesters might give clues for specific critical periods and body proportions. The same birth weight might be the result of various fetal exposures and growth patterns.

Therefore, we examined in a population-based prospective cohort study among 7346 pregnant women the associations of maternal caffeine intake, based on coffee and tea consumption, with fetal growth characteristics measured in each trimester of pregnancy and the risks of adverse birth outcomes.

METHODS

Design

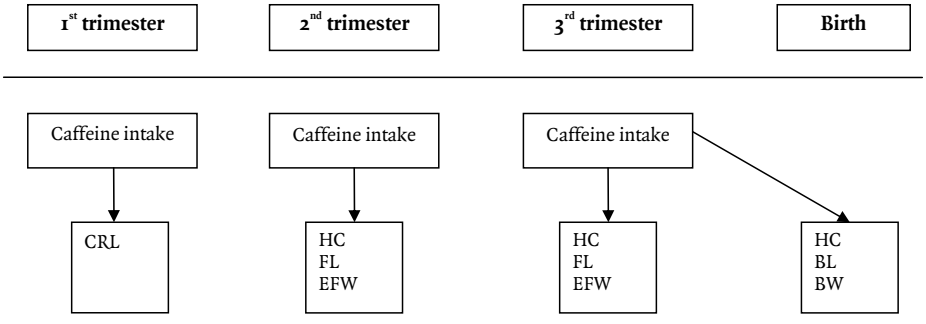
This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood[23,24]. The cohort includes 9778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands and has been described in detail previously[23,24]. All mothers were enrolled between 2001 and 2005, and all children were born between April 2002 and January 2006. Of all eligible children in the study area, 61% participated at birth in the study[24]. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study (MEC 198.782/2001/31). Written informed consent was obtained from all participants. Enrolment was aimed at early pregnancy (gestational age <18 weeks) at the routine fetal ultrasound examination in pregnancy but was allowed until birth of the child. Assessments in

pregnancy, including physical examinations, fetal ultrasound examinations, and questionnaires, were planned in early pregnancy (gestational age <18.0 weeks), mid-pregnancy (gestational age 18.0–24.9 weeks) and late pregnancy (gestational age ≥25.0 weeks). These measurements were considered as first, second and third trimester measurements, respectively. The individual timing of these assessments depended on the gestational age at enrolment[23,24].

Caffeine intake

Information about maternal caffeine intake was obtained by postal questionnaires in first, second, and third trimester of pregnancy. Response rates for these questionnaires were 91%, 80%, and 77%, respectively. Mothers who reported any coffee or tea drinking were asked to categorize their average number of cups of coffee or tea per day, and what type of coffee or tea they consumed (caffeinated; caffeinated and decaffeinated; decaffeinated). According to standard values for caffeine content, a regular coffee serving (125 ml) in the Netherlands contains about 90 mg of caffeine, decaffeinated coffee contains about 3 mg, and tea contains about 45 mg (25). To calculate the total caffeine intake in each trimester, we weighted the type of coffee or tea (caffeinated coffee=1; caffeinated and decaffeinated coffee=0.5; decaffeinated coffee=0; caffeinated tea=0.5; caffeinated and decaffeinated tea=0.25; decaffeinated tea=0; herbal tea=0; green tea=0.5). Thus, in our analyses each unit of caffeine intake reflects caffeine exposure based on one cup of caffeinated coffee (90 mg caffeine). Total caffeine intake was subsequently categorized (<2 units per day; 2 to 3.9 units per day; 4 to 5.9 units per day; ≥6 units per day). We used third trimester caffeine intake as exposure for both third trimester (≥25 weeks) and birth growth characteristics (Figure 1). Total caffeine intake in first, second, and third trimesters were correlated (Spearman's correlation coefficients ranged from 0.61 to 0.72 (P-value <0.01)). We used the mean caffeine intake during pregnancy to assess the associations with longitudinally measured fetal growth and the risks of adverse birth outcomes.

FIGURE 1. Design of the studied cross-sectional associations between caffeine intake during pregnancy and different fetal growth parameters



CRL=Crown-rump length; HC=Head circumference; FL=Femur length; EFW=Estimated fetal weight; BL=Birth length; BW=Birth weight

Fetal growth characteristics

Fetal ultrasound examinations were carried out in one of the two dedicated research centers in each trimester of pregnancy. These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics[24]. In the first trimester, we used crown-rump length to assess fetal growth only in mothers with a known and reliable first day of the last menstrual period and a regular menstrual cycle of 28 (range, 24-32) days, and who had crown-rump length measured between a gestational age of 10 and 15 weeks. The first day of the last menstrual period was obtained from the referring letter from the community midwife or hospital. This date was confirmed with the subjects at the ultrasound visit and additional information on the regularity and duration of cycle was obtained. Since using the last menstrual period has several limitations such as the large number of mothers who do not know the exact date of their last menstrual period or have irregular menstrual cycles[26], gestational age was established by fetal ultrasound examination for the second and third trimester growth measurements. In the second and third trimesters of pregnancy, we measured parameters of head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasound procedures[27]. Estimated fetal weight was subsequently calculated using the formula by Hadlock; $\log_{10}$ estimated fetal weight = 1.5662 – 0.0108 (head circumference) + 0.0468 (abdominal circumference) + 0.171 (femur length) + 0.00034 (head circumference)^2 – 0.003685 (abdominal circumference * femur length)[28].

Birth outcomes

Information about offspring sex, gestational age, weight, length, and head circumference at birth was obtained from medical records and hospital registries. Since head circumference and length at birth were not routinely measured at birth, missing birth measures were completed with data from the first month visit at the routine child health center. Of all measurements, 31% and 20% were based on the first month visit for head circumference and birth length, respectively. No differences in mean maternal caffeine intake between children with measurements at birth and those without measurements at birth were observed (T-tests p=0.73 for head circumference; p=0.92 for birth length). The regression models with neonatal head circumference and length as outcome were adjusted for postconceptional age (gestational age for measurements at birth or gestational age + postnatal age for measurement from the child health centers) and for the method of measurement (birth or child health center). Low birth weight was defined as birth weight below 2500 grams. Small-size-for-gestational-age at birth was defined as a gestational age adjusted birth weight below the 5th percentile in the study cohort (< -1.81 standard deviation score for boys and < -1.73 standard deviation score for girls), and preterm birth was defined as gestational age of less than 37 weeks at delivery.

Covariates

Information about educational level, ethnicity, parity and folic acid supplement use was obtained by a questionnaire at enrolment in the study. Maternal smoking and alcohol habits were assessed by questionnaires in each trimester. First trimester nutritional information (total energy intake, total carbohydrate intake, total fat intake, and total protein intake) was obtained by a food frequency questionnaire at enrolment. Mothers who were enrolled after first trimester of pregnancy did not receive this food frequency questionnaire. Maternal anthropometrics, including height (cm) and weight (kg), were measured without shoes and heavy clothing during visits at the research center, and subsequently body mass index (kg/m^2) was calculated for each trimester. Maternal weight measured at enrollment in the study (median gestational age: 14.4 weeks (95% range, 10.3-26.2)) was strongly correlated with pre-pregnancy weight ($r=0.95$, $p<0.01$). We used maternal weight and body mass index measured at enrollment in the analyses, because the numbers of missing values were smaller and data quality higher. These analyses were adjusted for gestational age at enrollment. Also, no differences in results were observed when we used maternal weight and body mass index based on the questionnaire data. Maternal age was registered at enrolment.

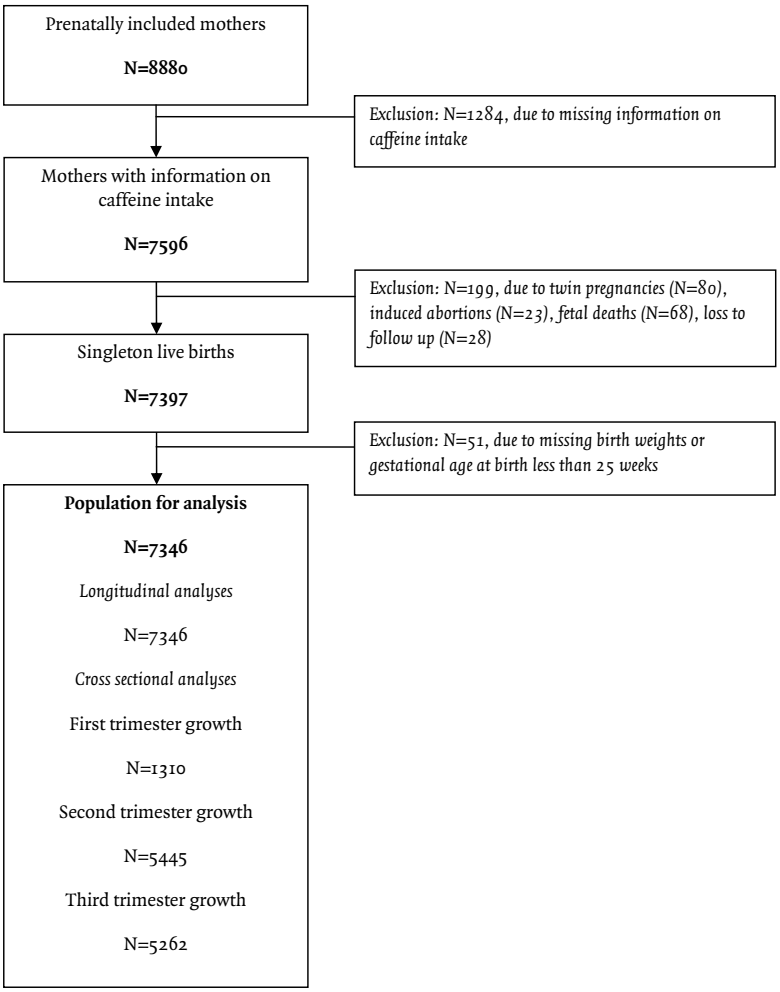
Population for analysis

In total 8880 mothers were enrolled during pregnancy[24]. Those without any information about coffee or tea consumption during pregnancy were excluded from the analyses (14.5%, n = 1284). Those pregnancies leading to twin births (n = 80), induced abortion (n = 23), fetal death (n = 68), or loss to follow up (n = 28) were excluded. Also, children with missing birth weight (n = 48) and gestational age at birth of less than 25 weeks (n = 3) were excluded. The associations of caffeine intake during pregnancy with fetal growth characteristics and risks of adverse birth outcomes were analyzed in the remaining 7346 mothers (Figure 2). Of these mothers, 5.4% were second (n = 394) or third (n = 3) pregnancies in the study. Since there were no differences in results after exclusion of these subjects, they were included in the analyses presented.

Statistical analyses

First, cross-sectional dose-response analyses in first, second, and third trimesters, and at birth were performed using multiple linear regression models with caffeine as independent and each growth characteristics as dependent variable (Figure 1). We used categories of units of caffeine intake and additional performed tests for trends using total caffeine intake per day as continuous variable in the models. Second, we assessed the associations between maternal caffeine intake and longitudinally measured standard deviation (SD) scores of head circumference, length (first trimester crown–rump length, second and third trimester femur length, and birth length) and weight (second and third trimester estimated fetal weight, and birth weight) using unbalanced repeated measurement analysis, which enables optimal use of available data, taking into account correlations within subjects and assessing both time dependent and independent associations. To calculate SD scores for each growth characteristic we used the following formula: SD score

FIGURE 2. Flow chart of participants included for analysis



= (observed value – median value of the reference population) / standard deviation value of the reference population. Models were constructed for gestational age adjusted SD scores. In total, we had 23695 measurements for SD scores of head circumference, 20657 measurements for SD scores of length, and 21216 for SD scores of weight. Third, we performed multiple logistic regression models to assess the associations of caffeine intake with the risks of low birth weight, small-size-for-gestational-age, and preterm birth. To increase the number of subjects, we combined the categories of caffeine intake into four, instead of five, categories (<2 units per day; 2 to 3.9 units per day; 4 to 5.9 units per day; ≥6 units per day). The regression models were adjusted for lifestyle-related and socioeconomic status-related confounders used in previous studies on maternal caffeine intake (maternal height, body mass index, educational level, smoking habits, alcohol consumption, folic acid supplement use, total energy intake, total carbohydrate intake,

total fat intake, and total protein intake) and known determinants of fetal growth (maternal age, maternal ethnicity, gestational diabetes, pregnancy-induced hypertension, preeclampsia, and parity and fetal sex)[29]. The percentages of missing values within the population for analysis were lower than 3%, except for folic acid supplement use data (14%) and nutritional data (24%). These higher percentages were due the large number of mothers who were not enrolled in first trimester and therefore did not receive the food frequency questionnaire. We imputed missing data of the covariates with the mean value for continuous variables, and a separate category for missing data for categorical variables. No differences in results were observed between analyses with imputed missing data or complete cases only. Also, no differences in results were observed with or without nutritional data. Therefore, only results including imputed missing data are presented.

All levels of association are presented with their 95% confidence intervals (CI). Cross-sectional analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA). The unbalanced repeated measurements analysis, including the Prox Mixed module, was performed with the Statistical Analysis System version 9.1 (SAS, Institute Inc. Gary NC, USA).

RESULTS

Subject characteristics

The mean age of the mothers in the whole cohort was 29.7 years (range, 15.3-46.3). Of all mothers 46.1% (n = 3388) were enrolled at a gestational age of less than 14 weeks, and 41.2% were higher educated. More than half of the mothers were of Dutch or other European ethnicity. The other largest ethnic groups were Surinamese, Turkish, and Moroccan (Table 1). Compared to mothers with no or less that 2 caffeine units per day, mothers consuming more caffeine tended to be older and taller. Also, they tended to have more previous births and be more frequently Dutch or European (Supplementary Table S1). They were less frequently smokers and more frequently alcohol consumers. Their total dietary energy intake was higher. Mean birth weight of the children was 3418 grams (SD, 559). Gestational age at birth ranged from 25.3 to 43.6 weeks with a median of 40.1 weeks. Of all singleton live births, 4.5% had a birth weight below 2500 grams, 4.6% were

TABLE 1. Characteristics of the mothers included[1]

	N=7346
Age, yrs	29.7 (5.3)
Height, cm	167.4 (7.3)
Weight at enrolment, kg	69.4 (13.3)
Body mass index at enrolment, kg/m^2	24.8 (4.5)
Parity, %	
0	57.0

TABLE 1. (continued)

≥1	42.9
Missing	0.1
Educational level, %	
Primary school	11.0
Secondary school	45.8
Higher education	41.2
Missing	2.0
Ethnicity, %	
Dutch and other-European	57.5
Surinamese	9.0
Turkish	8.9
Moroccan	6.6
Cape Verdian	4.0
Dutch Antilles	3.5
Other	10.0
Missing	0.5
Smoking habits, %	
No smoking in pregnancy	73.7
Smoking until pregnancy was known	8.1
Continued smoking in pregnancy	16.9
Missing	1.3
Alcohol consumption, %	
No alcohol in pregnancy	49.3
Alcohol until pregnancy was known	13.4
Continued alcohol in pregnancy	36.6
Missing	0.7
Total nutritional intake[2]	
Energy intake, kcal	2041 (567)
Carbohydrate intake, % of total energy intake	48.7
Fat intake, % of total energy intake	36.2
Protein intake, % of total energy intake	14.9
Folic acid supplementation use, %	
Preconception use	34.1
First 10 weeks of pregnancy	26.7
No use	24.9
Missing	14.3
Gestational age at enrollment, wks[3]	14.4 (10.3-26.2)

[1]Values are means (standard deviation) or percentages.
[2]Nutrional intake variables are only available in first trimester enrolled mothers.
[3]Median (95% range).

TABLE 2. Characteristics of the children included[1]

	N=7346
First trimester measurements (n=1310)	
Gestational age, wks[2]	13.4 (9.8-17.5)
Crown-rump length, mm	62.7 (12.8)
Second trimester measurements (n=5445)	
Gestational age, wks[2]	20.4 (18.5-23.5)
Head circumference, mm	179.5 (14.6)
Estimated fetal weight, g	382 (94)
Femur length, mm	33.5 (3.6)
Third trimester measurements (n=5262)	
Gestational age, wks[2]	30.2 (28.5-32.9)
Head circumference, mm	284.9 (12.6)
Estimated fetal weight, g	1617 (263)
Femur length, mm	57.5 (3.1)
Birth	
Gestational age, wks[2]	40.1 (18.3)
Head circumference, mm	350 (23)
Weight, g	3418 (559)
Length, mm	510 (28)
Sex, % boys	50.4
Low birth weight (<2500 g), %	4.5
Small-size-for-gestational-age (<-1.8 SD), %	4.6
Preterm (<37 wks of gestation), %	4.8

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

small-size-for-gestational-age, and 4.8% were born before a gestational age of 37 weeks (Table 2). Table 3 shows that the distribution total caffeine intake did differ between the trimesters (chi-square test; P<0.01). Most mothers consumed less than 2 units of caffeine per day.

Total caffeine intake and fetal growth characteristics

Maternal caffeine intake was not consistently associated with fetal head circumference in second and third trimester of pregnancy and at birth (Supplementary Table S2, S3 and S4). Also, no consistent associations were observed between maternal caffeine intake and estimated fetal weight in second and third trimester. We observed tendencies towards a lower weight at birth for using 6 or more caffeine units per day (difference: -100.27 grams (95% confidence interval (CI): -197.05, -3.49) compared to mothers who did not consume any caffeine during pregnancy). However, the overall tests for trend assessing the associations between the number of caffeine units and (estimated fetal) weight were not significant. Caffeine intake tended to be inversely associated with all length measures (all P-values for trend <0.05). Mothers who consumed 6 or more caffeine units

TABLE 3. Distribution of total caffeine intake in each trimester of pregnancy[1]

Daily total caffeine intake	First trimester		Second trimester[2]		Third trimester[2]	
	%	n	%	n	%	n
None	5.8	268	7.9	450	4.5	238
<2 units	52.6	2417	47.6	2704	48.8	2598
2 to 3.9 units	30.5	1404	32.9	1867	36.4	1936
4 to 5.9 units	8.2	377	9.2	523	8.3	441
≥6 units	2.9	133	2.4	136	2.1	111

[1]Values are percentages (absolute numbers).
[2]Significant differences in distributions of total caffeine intake between first trimester and second trimester, and between first trimester and third trimester were evaluated using chi-square tests (P<0.01).

per day had a smaller first trimester fetal crown-rump length (-4.54 mm (95% CI: -8.99, -0.09)) and a smaller third trimester femur length (-0.55 mm (95% CI: -1.09, -0.02)) compared to no caffeine intake. Analyses focused on the associations of caffeine consumption with head circumference at birth and length at birth, without imputing data from the first postnatal measurements at the child health centers, showed similar results (data not shown).

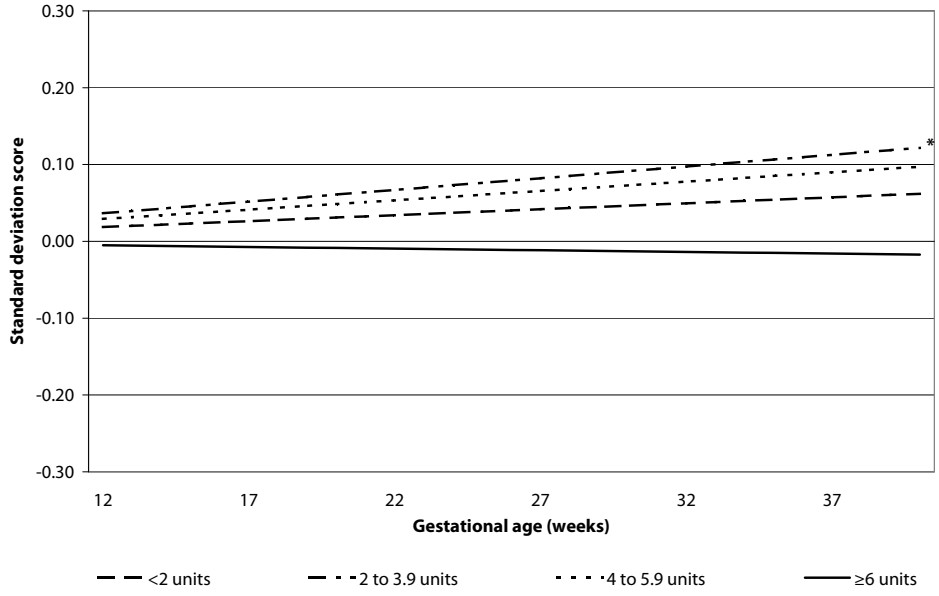
Results presented in Figures 3a, b, and c show the associations between the number of caffeine units and longitudinally measured fetal growth (head circumference, weight, length). These results were based on repeated regression models and to compare effect estimates, these results are presented as difference in gestational age adjusted standard deviation score. No consistent associations were found between the mean number of caffeine units per day and fetal head circumference growth. Compared to mothers who did not consume any caffeine containing beverages during pregnancy, those who consumed 6 or more units of caffeine per day showed impaired fetal weight and length growth (Supplementary Table S5).

Total caffeine intake and risks of adverse birth outcomes

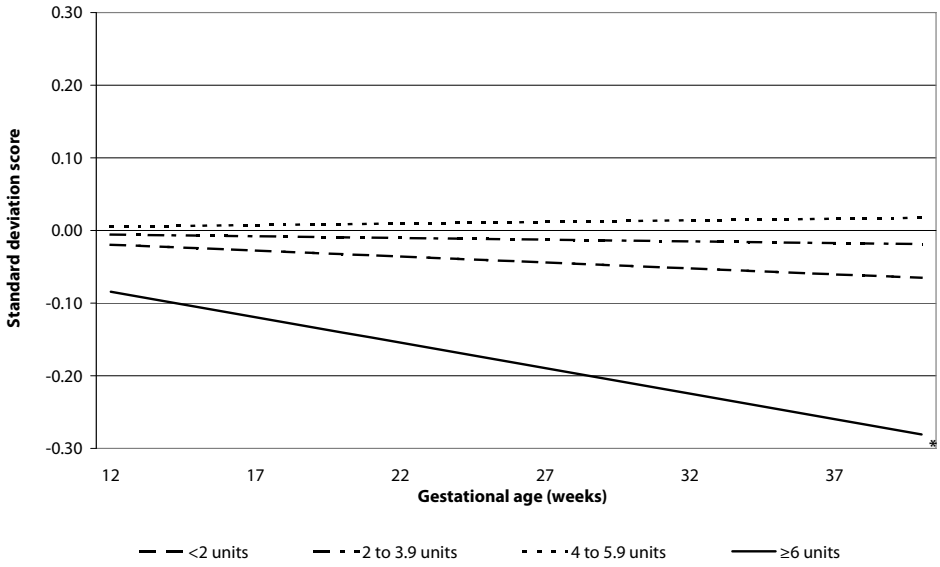
Caffeine intake of 6 or more units per day was associated with an increased risk of low birth weight (adjusted odds ratio (aOR), 2.58 (95% CI: 1.26, 5.30)). However, this effect estimate was based on small numbers (n = 9) and the overall tests for trend assessing the associations between the number of caffeine units and the risk of low birth weight were not significant. Caffeine intake was positively associated with the risks of delivering a small-size-for-gestational-age child (P-value <0.01). Compared to mothers who consumed less than 2 units of caffeine per day, the aOR were 1.38 (95% CI: 1.08, 1.76), 1.50 (95% CI: 0.96, 2.36) and 1.87 (95% CI: 0.84, 4.15) for mothers consuming 2 to 4 units, 4 to 6 units, and 6 or more units of caffeine per day, respectively (P_{trend} < 0.01). No associations were found between caffeine intake and the risk of preterm birth (Table 4).

FIGURE 3. Associations of caffeine intake during pregnancy and longitudinally measured growth characteristics

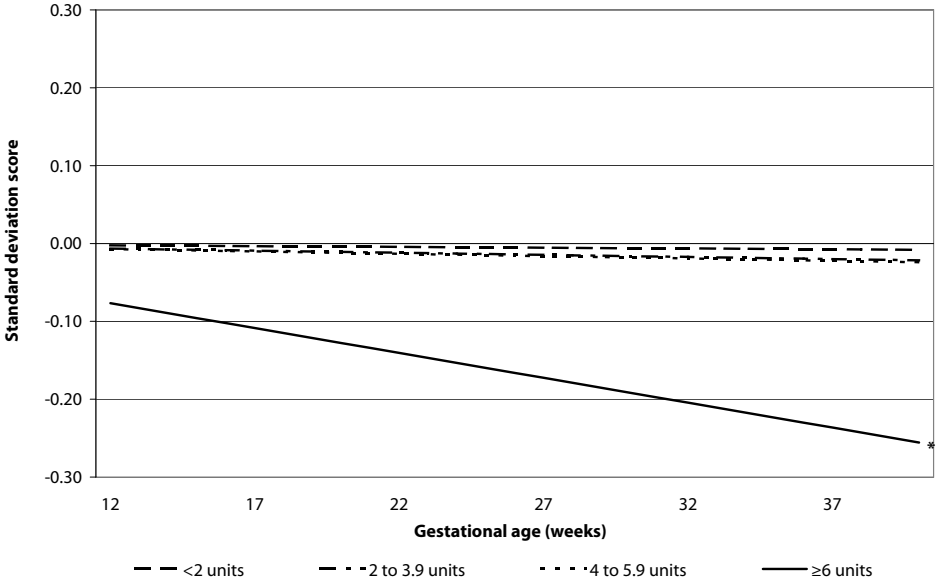
A. Head circumference growth



B. Weight growth



C. Length growth



Results are based on repeated linear regression models and reflect the differences in standard deviation score of head circumference (based on 23068 measurements), weight (based on 19519 measurements), and length (based on 20419 measurements) growth in offspring of mothers who consumed caffeine containing beverages compared to those from mothers who did not consume caffeine containing beverages (Reference is standard deviation score (SDS) =0). *P-value<0.05. The corresponding effect estimates and their confidence interval are given in the Supplementary Table S5.

DISCUSSION

Findings from this large population-based prospective cohort study suggest that caffeine intake of 6 or more units per day during pregnancy is associated with an impaired fetal length growth. Length or skeletal related fetal growth characteristics seemed to be consistently affected from first trimester onwards. No associations were observed between caffeine intake and fetal head circumference. For fetal weight no statistically significant associations were observed but there was a tendency toward lower fetal weight for higher caffeine intakes. In addition, we found a positive association between caffeine intake during pregnancy and the risks of offspring with a small-size-for-gestational-age at birth.

Methodological considerations

The strength of this study is the large number of subjects in a prospectively studied cohort. To our knowledge, this is the largest study focused on caffeine intake during pregnancy on fetal growth characteristics in different periods of pregnancy. Compared to many previous studies we were able to control for many possible confounders[14,16]. However, because of the observational

TABLE 4. Associations of maternal total caffeine intake during pregnancy with the risks of adverse birth outcomes[1]

Daily caffeine intake	**Total population**	**Low birth weight[2]**		
	n	n	aOR	95% CI
<2 units	4329	205	Reference	
2 to 3.9 units	2211	96	1.08	0.84, 1.40
4 to 5.9 units	439	19	1.19	0.73, 1.95
≥6 units	104	9	2.58*	1.26, 5.30
			P_{trend}[4]=0.14	
Daily caffeine intake	**Total population**	**Small-size-for-gestational-age[2]**		
	N	n	aOR	95% CI
<2 units	4329	204	Reference	
2 to 3.9 units	2211	119	1.38**	1.08, 1.76
4 to 5.9 units	439	24	1.50	0.96, 2.36
≥6 units	104	7	1.87	0.84, 4.15
			P_{trend}<0.01	
Daily caffeine intake	**Total population**	**Preterm birth[2]**		
	N	n	aOR	95% CI
<2 units	4329	193	Reference	
2 to 3.9 units	2211	116	0.92	0.72, 1.18
4 to 5.9 units	439	21	1.12	0.71, 1.73
≥6 units	104	7	1.35	0.58, 3.15
			P_{trend}=0.83	

Abbreviations: aOR, adjusted Odds Ratio; CI, confidence interval.
[1]Values are odds ratios (95% confidence interval) and reflect the risk of adverse birth outcomes among mothers who consumed 2 or more caffeine containing beverages compared to mothers who consumed less than 2 or no caffeine containing beverages.
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, smoking habits, alcohol consumption, height, body mass index at intake, nutritional intake (total energy, total carbohydrate, total fat, total protein), folic acid supplement use, maternal pregnancy complications (pregnancy-induced hypertension, preeclampsia, and gestational diabetes), and fetal sex.
[4]P-values for trend were based on multiple logistic regression models with caffeine intake as a continuous variable.
*P-value<0.05
**P-value<0.01

design, residual confounding due to socio-demographic and life style factors might still be an issue. Another potential limitation of our study is the missing data on coffee and tea consumption. Missing information about coffee and tea consumption may have led to loss of power. The associations may be underestimated if among mothers without caffeine data the percentage of consumers was higher than among mothers without missing data. On the contrary, if mothers without caffeine data were more likely to be no or light caffeine consumers and were at risk for adverse birth outcomes, the associations might be overestimated. This seems unlikely since no

other differences in characteristics between mothers with and without information about coffee and tea consumption were observed. Among mothers with information about coffee and tea consumption, we had a limited loss-to-follow-up. Therefore, we do not expect biased results due to loss-to-follow-up[30]. Information on coffee and tea consumption during pregnancy was collected by postal questionnaires. If any, misclassification would most likely be due to under-reporting and subsequently lead to underestimation of differences between dosages of caffeine intake[31]. Coffee is found to make up about 70% of all caffeine intake, tea 26%, and 4% other (cocoa, chocolate, soft drinks, and caffeine-containing medications)[32]. Although consumption of caffeinated soft drinks is increasing[1,30], analyzing only coffee and tea consumption in this study seems sufficient in assessing the effect of caffeine on the growth and development of the fetus. We categorized caffeine intake instead of calculating the exact milligrams of caffeine per day. However, the highest category of caffeine intake in our study (6 or more units) should be considered similar as caffeine intake of >540 mg per day. The amount of caffeine per coffee serving was estimated on 90 mg[25]. European coffee is typically stronger than coffee in the Unites States[1]. A standard coffee serving in the United States contains about 70 mg caffeine. This suggests that a caffeine intake of lower than 7 servings per day is not associated with any adverse birth outcome in the United States. However, our results should be interpreted very carefully for non-European populations. Further studies, specifically in other than European populations, are needed.

We assessed the effects of caffeine intake on crown-rump length only in mothers with a certain first day of last menstrual period and regular menstrual cycle. For second and third trimester growth measurements, gestational age was established by fetal ultrasound examination. This method appears superior to the use of the last menstrual period[24]. The major disadvantage of establishing gestational age by ultrasound is that the growth variation of the fetal characteristics used for pregnancy dating is assumed to be zero. Since pregnancy dating characteristics and growth characteristics are correlated throughout pregnancy, growth variation in head circumference, and femur length may be reduced by dating the pregnancy on crown-rump length and biparietal diameter. This may have led to underestimation of our effect estimates. However, we expect this effect to be small in our results. The longitudinal analyses were focused on fetal growth during pregnancy within individuals. This relative change in size is unlikely to have been materially affected by our pregnancy dating method. Since gestational age and fetal growth were not established concurrently, we believe that we minimized the effect of pregnancy dating on growth variation. We measured fetal growth repeatedly in each trimester of pregnancy by ultrasound. These measurements were performed in dedicated research centers according to standardized protocols. Intra-class correlation coefficients for intra- and inter-observer reproducibility of fetal growth measurements were above 0.95[26,33]. Since body length cannot be measured by fetal ultrasound, we used SD scores of crown-rump length, femur length and birth length as measures of fetal length to assess relative changes in fetal skeletal growth. Results from these combined repeated regression models should be interpreted carefully since these measurements might

reflect different body parts, as we used crown-rump length, femur length and body length as length measurements throughout pregnancy.

Caffeine intake and fetal growth

Caffeine crosses the placental barrier easily, where it can directly affect the fetus in several different aspects. The half-life of caffeine is greatly increased in pregnancy, as it cannot be metabolized by the fetus or the placenta[34]. In the first trimester the half life is about ten hours, while it increases up to 18 hours during the third trimester, as the enzymes in the human liver do not exist until the eight month of life[35,36]. Previous studies focused primarily on the associations between caffeine intake during pregnancy and birth weight and showed inconsistent associations[10,17-22,37,38]. Moreover, most studies used low birth weight as outcome measurement instead of studying the actual fetal growth. Examining fetal growth characteristics instead of birth weight is a more appropriate approach to assess the effects of caffeine intake during pregnancy. This enables identification of specific critical periods during pregnancy for the exposure on various patterns of fetal growth and development. We found negative associations between caffeine intake levels and weight and length growth. Previous results from the CARE Study Group suggested adverse effects of caffeine consumption on fetal growth at lower levels[16]. This difference might be due differences in study design and populations. Also, calculations of caffeine intake should be interpreted carefully and might be country specific. We found consistent effects on fetal growth measures which reflect skeletal growth. These include first trimester crown-rump length, second and third trimester femur length and birth length. Although, these measures reflect different body proportions, they seem to be consistent. Caffeine intake might selectively affect bone and skeletal development. Further follow up studies are needed focused on the effects of fetal caffeine exposure on postnatal skeletal and bone measurements.

Conclusion

We showed that caffeine intake of 6 or more units per day during pregnancy is associated with an impaired fetal weight and length growth. Length or skeletal related fetal growth characteristics seemed to be most consistently affected from first trimester onwards. Further structural and functional studies are needed to assess organ specific effects. Our results suggest that pregnant women should be advised to not use 6 or more caffeine units (>540 mg) per day during pregnancy.

REFERENCES

1. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol. 1996;34:119-29.
2. Knight CA, Knight I, Mitchell DC, et al. Beverage caffeine intake in US consumers and subpopulations of interest: estimates from the Share of Intake Panel survey. Food Chem Toxicol. 2004;42:1923-30.
3. Weathersbee PS, Lodge JR. Caffeine: its direct and indirect influence on reproduction. J Reprod Med. 1977;19: 55-63.
4. Arnaud MJ, Bracco I, Sauvageat JL, et al. Placental transfer of the major caffeine metabolite in the rat using 6-amino-5[N-formylmethylamino]1,3[Me-14C]-dimethyluracil administered orally or intravenously to the pregnant rat. Toxicol Lett. 1983;16:271-9.
5. Aldridge A, Aranda JV, Neims AH. Caffeine metabolism in the newborn. Clin Pharmacol Ther. 1979;25:447-53.
6. Kirkinen P, Jouppila P, Koivula A, et al. The effect of caffeine on placental and fetal blood flow in human pregnancy. Am J Obstet Gynecol. 1983;147:939-42.
7. Cnattingius S, Signorello LB, Anneren G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med. 2000;343:1839-45.
8. Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol. 2008;198:279 e1-8.
9. Beaulac-Baillargeon L, Desrosiers C. Caffeine-cigarette interaction on fetal growth. Am J Obstet Gynecol. 1987; 157:1236-40.
10. Martin TR, Bracken MB. The association between low birth weight and caffeine consumption during pregnancy. Am J Epidemiol. 1987;126:813-21.
11. Peacock JL, Bland JM, Anderson HR. Effects on birthweight of alcohol and caffeine consumption in smoking women. J Epidemiol Comm Health. 1991;45:159-63.
12. Hinds TS, West WL, Knight EM, et al. The effect of caffeine on pregnancy outcome variables. Nutr Rev. 1996; 54:203-7.
13. Vlajinac HD, Petrovic RR, Marinkovic JM, et al. Effect of caffeine intake during pregnancy on birth weight. Am J Epidemiol. 1997;145:335-8.
14. Fernandes O, Sabharwal M, Smiley T, et al. Moderate to heavy caffeine consumption during pregnancy and relationship to spontaneous abortion and abnormal fetal growth: a meta-analysis. Reprod Toxicol. 1998;12: 435-44.
15. Vik T, Bakketeig LS, Trygg KU, et al. High caffeine consumption in the third trimester of pregnancy: gender-specific effects on fetal growth. Paediat Perinat Epidemiol. 2003;17:324-31.
16. The CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ. 2008;337:a2332.
17. Linn S, Schoenbaum SC, Monson RR, et al. No association between coffee consumption and adverse outcomes of pregnancy. N Engl J Med. 1982;306:141-5.
18. Fenster L, Eskenazi B, Windham GC, et al. Caffeine consumption during pregnancy and fetal growth. Am J Public Health. 1991;81:458-61.
19. Fortier I, Marcoux S, Beaulac-Baillargeon L. Relation of caffeine intake during pregnancy to intrauterine growth retardation and preterm birth. Am J Epidemiol. 1993;137:931-40.
20. Cook DG, Peacock JL, Feyerabend C, et al. Relation of caffeine intake and blood caffeine concentrations during pregnancy to fetal growth: prospective population based study. BMJ. 1996;313:1358-62.
21. Eskenazi B, Stapleton AL, Kharrazi M, et al. Associations between maternal decaffeinated and caffeinated coffee consumption and fetal growth and gestational duration. Epidemiology. 1999;10:242-9.
22. Bech BH, Obel C, Henriksen TB, et al. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. BMJ. 2007;334:409.
23. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
24. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25: 823–841.

25. NEVO tabel 2006. Nederlands Voedingsstoffenbestand 2006/Stichting Nederlands Voedingsstoffenbestand. (2006) Den Haag: Voedingscentrum.
26. Verburg BO, Steegers EA, De Ridder M, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31: 388-96.
27. Routine ultrasound screening in pregnancy: protocol. London, UK: RCOG Press. 2000.
28. Hadlock FP, Harrist RB, Sharman RS, et al. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gyneacol. 1985;151:333-7.
29. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bull World Health Organ. 1987;65:663-737.
30. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
31. Bracken MB, Triche E, Grosso L, et al. Heterogeneity in assessing self-reports of caffeine exposure: implications for studies of health effects. Epidemiology. 2002;13:165-71.
32. Clausson B, Granath F, Ekbom A, et al. Effect of caffeine exposure during pregnancy on birth weight and gestational age. Am J Epidemiol. 2002;155:429-36.
33. Verburg BO, Mulder PG, Hofman A, et al. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenat Diagn. 2008;28:323-31.
34. Aldridge A, Bailey J, Neims AH. The disposition of caffeine during and after pregnancy. Sem Perinat. 1981;5: 310-4.
35. Grosso LM, Bracken MB. Caffeine metabolism, genetics, and perinatal outcomes: a review of exposure assessment considerations during pregnancy. Ann Epidemiol. 2005;15:460-6.
36. Springhouse, ed. Physician's Drug Handbook. 11th edition ed: Lippincott Williams & Wilkins January 1, 2005.
37. Larroque B, Kaminski M, Lelong N, et al. Effects of birth weight of alcohol and caffeine consumption during pregnancy. Am J Epidemiol. 1993;137:941-50.
38. Shu XO, Hatch MC, Mills J, et al. Maternal smoking, alcohol drinking, caffeine consumption, and fetal growth: results from a prospective study. Epidemiology. 1995;6:115-20.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLE S1. Characteristics of the mothers according to their caffeine intake[1]

	Overall n=7346	<2 units/ day[2] n=4329	2-3.9units/ day[2] n=2211	4-5.9 units/ day[2] n=439	≥6 units/ day[2] n=104	Missing n=263	Trend test p-value[3]
Age, yrs	29.7 (5.3)	28.9 (5.3)	30.9 (4.9)	31.8 (4.7)	32.1 (4.6)	28.3 (5.5)	<0.001
Height, cm	167.4 (7.3)	166.7 (7.3)	168.5 (7.2)	169.0 (7.2)	168.0 (7.1)	164.9 (7.4)	<0.001
Weight at enrolment, kg	69.4 (13.3)	69.0 (13.8)	70.1 (12.5)	70.5 (12.3)	71.1 (11.8)	69.0 (14.5)	<0.01
BMI at enrolment, kg/m^2	24.8 (4.5)	24.8 (4.7)	24.7 (4.3)	24.7 (4.2)	25.2 (3.8)	25.3 (4.8)	0.53
Parity, %							<0.001
0	57.0	60.2	53.2	49.7	40.4	54.4	
≥1	42.9	39.7	46.6	50.1	59.6	45.6	
Missing	0.1	0.1	0.2	0.2	0.0	0.0	
Educational level, %							<0.001
Primary school	11.0	11.9	8.8	8.4	10.6	22.1	
Secondary school	45.8	49.5	38.9	39.6	53.8	48.7	
Higher education	41.2	36.4	50.6	51.0	34.6	26.2	
Missing	2.0	2.2	1.8	0.9	1.0	3.0	
Ethnicity, %							<0.001
Dutch and other-European	57.5	51.2	68.0	76.5	76.0	33.1	
Surinamese	9.0	11.2	5.1	3.2	5.8	16.0	
Turkish	8.9	8.1	9.8	9.1	11.5	15.1	
Moroccan	6.6	7.1	6.0	3.9	1.9	10.0	
Cape Verdian	4.0	5.2	2.2	0.7	0.0	6.1	
Dutch Antilles	3.5	5.1	1.1	0.7	0.0	4.2	
Other	10.0	11.5	7.6	5.4	3.9	14.0	
Missing	0.5	0.6	0.2	0.5	0.0	1.5	
Smoking habits, %							<0.001
No smoking	73.7	77.8	69.3	60.6	41.3	77.9	
Until pregnancy was known	8.1	7.6	9.1	9.3	7.7	6.8	
Continued smoking	16.9	13.2	20.4	29.4	51.0	12.9	
Missing	1.3	1.4	1.3	0.7	0.0	2.3	
Alcohol consumption, %							<0.001
No alcohol	49.3	54.8	39.3	36.4	36.5	68.1	
Until pregnancy was known	13.4	14.0	12.1	14.4	8.7	14.1	
Continued alcohol	36.6	30.5	47.8	49.0	53.8	16.7	
Missing	0.7	0.7	0.8	0.2	1.0	1.1	
Total nutritional intake							
Energy intake, kcal	2041 (567)	1996 (573)	2093 (539)	2198 (547)	2193 (588)	1969 (650)	<0.001
Carbohydrate intake, % of total energy intake	48.7	49.0	48.4	47.4	47.9	48.6	<0.001

SUPPLEMENTARY TABLE S1. (continued)

Fat intake, % of total energy intake	36.2	36.0	36.4	37.2	36.7	36.4	<0.001
Protein intake, % of total energy intake	14.9	14.8	14.9	15.0	15.0	14.9	0.56
Folic acid supplementation use, %							<0.001
Preconception use	34.1	32.1	38.4	39.9	25.0	25.5	
First 10 weeks of pregnancy	26.7	26.7	27.7	28.5	22.1	16.3	
No use	24.9	26.1	21.6	22.1	29.8	36.5	
Missing	14.3	15.2	12.3	9.6	23.1	21.7	
Gestational diabetes, %	1.0	1.1	0.9	1.1	0.0	0.8	<0.001
Missing	3.6	3.3	3.6	5.0	4.8	5.3	
PIH or preeclampsia, %	5.8	6.1	5.2	6.6	4.8	4.9	<0.001
Missing	2.5	2.7	2.2	1.4	2.9	3.0	

Abbreviations: BMI, body mass index; PIH, pregnancy-induced hypertension.
[1]Values are means (standard deviation) or percentages.
[2]The mean caffeine intake during pregnancy was calculated based on coffee and tea consumption in each trimester of pregnancy. Mean caffeine intake was categorized in the following categories; no or <2 units per day, 2 to 3.9 units per day, 4 to 5.9 units per day, 6 or more units per day. In total, 263 women did not report the dosage of caffeine intake.
[3]Differences in maternal characteristics (compared with the lowest caffeine intake category) were evaluated using ANOVA tests for continuous variables and chi-square tests for proportions. The missing category was excluded from these analyses.

SUPPLEMENTARY TABLE S2. Associations of maternal total caffeine intake during pregnancy with fetal and birth head circumference[1,2]

Daily caffeine intake	**Second trimester**		**Third trimester**		**Birth[3,4]**	
	HC (mm) n=5432	95% CI	HC (mm) n=5218	95% CI	HC (mm) n=4296	95% CI
None	Reference		Reference		Reference	
<2 units	-0.27	-1.12, 0.58	-0.21	-1.50, 1.09	2.26	-0.02, 4.53
2 to 3.9 units	0.30	-0.58, 1.18	-0.26	-1.60, 1.08	2.34*	0.01, 4.67
4 to 5.9 units	-0.17	-1.25, 0.92	-0.66	-2.24, 0.91	1.79	-0.91, 4.48
≥6 units	-0.06	-1.70, 1.58	-0.64	-2.85, 1.58	0.58	-3.12, 4.27
	P_{trend}[5]= 0.57		P_{trend}= 0.90		P_{trend}= 0.35	

Abbreviations: HC, head circumference; CI, confidence interval.
[1]Values are regression coefficients (95% confidence interval) and reflect the difference in head circumference in offspring of mothers who consumed caffeine containing beverages compared to mothers who did not consume caffeine containing beverages.
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, smoking habits, alcohol consumption, height, body mass index at visit, nutritional intake (total energy, total carbohydrate, total fat, total protein), folic acid supplement use, and fetal sex.
[3]Models are also adjusted for maternal pregnancy complications (pregnancy-induced hypertension, preeclampsia, and gestational diabetes).
[4]Combined measurements at birth of head circumference are additionally adjusted for the postconceptional age and the origin of data (measured at birth or at the first child health center visit).

[5]P-values for trend were based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value<0.05

SUPPLEMENTARY TABLE S3. Associations of maternal total caffeine intake during pregnancy with fetal and birth weight[1,2]

	Second trimester		Third trimester		Birth[3]	
Daily caffeine intake	EFW (g) n=5422	95% CI	EFW (g) n=5250	95% CI	BW (g) n=5324	95% CI
None	Reference		Reference		Reference	
<2 units	-1.07	-6.64, 4.49	-1.41	-27.92, 25.10	-27.18	-83.90, 29.54
2 to 3.9 units	-0.06	-5.83, 5.71	-3.67	-30.96, 23.62	-19.26	-77.63, 39.12
4 to 5.9 units	-3.25	-10.34, 3.83	-5.49	-37.61, 26.64	-43.63	-112.25, 25.00
≥6 units	-6.27	-16.97, 4.43	-24.75	-69.87, 20.38	-100.27*	-197.05, -3.49
	P_{trend}[4]= 0.18		P_{trend}= 0.68		P_{trend}= 0.19	

Abbreviations: EFW, estimated fetal weight; BW, birth weight; CI, confidence interval.
[1]Values are regression coefficients (95% confidence interval) and reflect the difference for every weight measure in offspring of mothers who consumed caffeine containing beverages compared to mothers who did not consume caffeine containing beverages.
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, smoking habits, alcohol consumption, height, body mass index at visit, nutritional intake (total energy, total carbohydrate, total fat, total protein), folic acid supplement use, and fetal sex.
[3]Models are also adjusted for maternal pregnancy complications (pregnancy-induced hypertension, preeclampsia, and gestational diabetes).
[4]P-values for trend were based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value<0.05

SUPPLEMENTARY TABLE S4. Associations of maternal total caffeine intake during pregnancy with fetal and birth length measures[1,2]

Daily caffeine intake	**First trimester**		**Second trimester**		**Third trimester**		**Birth[3,4]**	
	CRL (mm) n=1310	95% CI	FL (mm) n=5445	95% CI	FL (mm) n=5262	95% CI	BL (mm)[5] n=4378	95% CI
None	Reference		Reference		Reference		Reference	
<2 units	1.18	-1.41, 3.77	-0.10	-0.32, 0.13	-0.12	-0.43, 0.20	-0.53	-3.69, 2.62
2 to 3.9 units	1.20	-1.47, 3.86	-0.18	-0.41, 0.05	-0.27	-0.60, 0.05	-1.14	-4.38, 2.09
4 to 5.9 units	0.59	-2.49, 3.68	-0.28	-0.57, 0.00	-0.32	-0.70, 0.06	-2.16	-5.92, 1.61
≥6 units	-4.54*	-8.99, -0.09	-0.37	-0.80, 0.06	-0.55*	-1.09, -0.02	-3.79	-8.97, 1.39
	P_{trend}[5]<0.05		P_{trend}<0.01		P_{trend}< 0.01		P_{trend}=0.01	

Abbreviations: CRL, crown-rump length; FL, femur length; BL, birth length; CI, confidence interval.
[1]Values are regression coefficients (95% confidence interval) and reflect the difference for every length measure in offspring of mothers who consumed caffeine containing beverages compared to mothers who did not consume caffeine containing beverages.

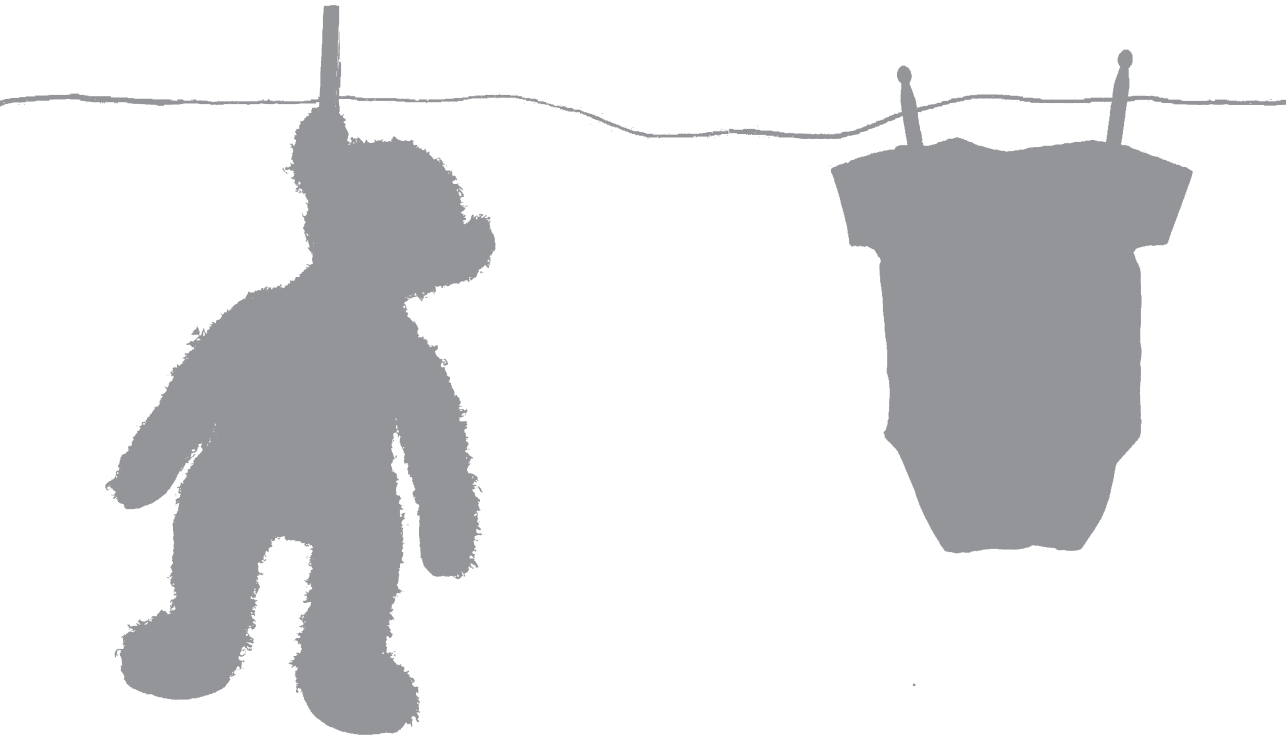
[2]Models are adjusted for gestational age at visit, maternal age, educational level, ethnicity, parity, smoking habits, alcohol consumption, height, body mass index at visit, nutritional intake (total energy, total carbohydrate, total fat, total protein), folic acid supplement use, and fetal sex.
[3]Models are also adjusted for maternal pregnancy complications (pregnancy-induced hypertension, preeclampsia, and gestational diabetes).
[4]Combined measurements at birth of length growth are additionally adjusted for the postconceptional age and the origin of data (measured at birth or at the first child health center visit).
[5]P-values for trend were based on multiple linear regression models with caffeine intake as a continuous variable.
*P-value<0.05

SUPPLEMENTARY TABLE S5. Associations of caffeine intake during pregnancy and longitudinally measured growth measures[1]

	Difference in growth rate (SDS/ week)		
	Effect estimate	95% CI	p-value
Daily caffeine intake	Head circumference		
None	Reference		
<2 units	0.0015	-0.0013, 0.0043	0.28
2 to 3.9 units	0.0030	0.0001, 0.0060	<0.05
4 to 5.9 units	0.0024	-0.0011, 0.0060	0.18
≥6 units	-0.0004	-0.0058, 0.0049	0.88
Daily caffeine intake	Weight growth		
None	Reference		
<2 units	0.0016	-0.0045, 0.0013	0.27
2 to 3.9 units	-0.0004	-0.0034, 0.0025	0.75
4 to 5.9 units	0.0004	-0.0032, 0.0041	0.81
≥6 units	-0.0070	-0.0126, -0.0015	<0.05
Daily caffeine intake	Length growth		
None	Reference		
<2 units	0.0002	-0.0030, 0.0026	0.89
2 to 3.9 units	-0.0005	0.0034, 0.0024	0.72
4 to 5.9 units	-0.0006	-0.0041, 0.0029	0.74
≥6 units	-0.0064	-0.0118, -0.0009	<0.05

[1]Values are based on repeated linear regression models and reflect the differences in growth in standard deviation score (SDS) of head circumference (based on 23068 measurements), weight (based on 19519 measurements), and length (based on 20419 measurements) in offspring of mothers who consumed caffeine containing beverages compared to those from mothers who did not consume caffeine containing beverages (Reference is standard deviation score (SDS)=0).

Part 5 | **General discussion**



INTRODUCTION

In Western countries, the most common adverse maternal lifestyle habits during pregnancy include smoking, alcohol consumption, and caffeine consumption. These adverse maternal lifestyle habits may influence maternal cardiovascular adaptations during pregnancy, and increase the risks of maternal hypertensive disorders during pregnancy. Although not directly lifestyle related, maternal age is also considered as a modifiable risk factor for adverse pregnancy outcomes.

Maternal cardiovascular adaptations are suggested to play an important role in the pathway between an adverse fetal environment and subsequent neonatal complications. Because of hypertensive complications, impaired placental perfusion may occur, and subsequently the oxygen and nutrient supply to the fetus might be reduced. Therefore, these adverse lifestyle habits are also considered as modifiable risk factors for fetal and neonatal complications. Consistent findings have been shown in previous studies for the associations of high levels of exposure of maternal smoking, alcohol consumption, and caffeine intake with the risks of perinatal mortality and morbidity[1-8]. Less is known about the effects of low-to-moderate exposure levels on these outcomes. Furthermore, maternal age has been associated with birth weight in an inverse U-shaped manner[9]. Whether maternal lifestyle habits explain this association is not known.

Most studies have focussed on birth weight as main outcome measure, instead of fetal growth. Different fetal growth patterns may result in the same weight at birth. Exposure of maternal lifestyle habits in different trimesters of pregnancy might have differential effects on fetal growth patterns. Therefore, studies on exposure effects in different trimesters might identify specific critical periods.

The main objectives of the studies described in this thesis were to examine the associations of maternal lifestyle habits with hypertensive complications during pregnancy, and with fetal growth and neonatal complications in the offspring. This part of the thesis provides a general discussion of the main findings (Figure 1), discusses general methodological issues and provides future research perspectives.

MAIN FINDINGS

Smoking assessment

Single assessment of maternal smoking during pregnancy by questionnaires is an often-used method in population-based cohorts. The use of questionnaires seems to be a valid method; however, misclassification may occur, due to underreporting or failure of smoking cessation among pregnant women[10]. We found a Cohen's Kappa coefficient of 0.86 between smoking categories based on single and repeated questionnaires. Pregnant women who more often underreported their smoking status of failed to quit smoking were younger, shorter of stature, lower educated, more often non-European, experienced more stress, consumed more alcohol and less frequently

FIGURE 1. Schematic overview of the described findings in the studies on maternal lifestyle, fetal and hypertensive complications

Exposure	Blood pressure	Hypertensive complications	Fetal growth	Neonatal complications
Smoking	↑SBP, ↑DBP		↓FG*[11]	↑ LBW*, ↑PTB*[12]
Alcohol			↑ΔEFW	↑LBW
Caffeine		↓PE	↓FL	↑SGA
Younger age			↓BW	
Older age	↓SBP, ↑DBP		↓BW	
Blood pressure	n.a.		↓HC, ↓FL, ↓EFW, ↓BW	
Hypertensive complications		n.a.		↑LBW, ↑PTB, ↑SGA

SBP, systolic blood pressure; DBP, diastolic blood pressure; PIH, pregnancy-induced hypertension; PE, preeclampsia; FG, fetal growth, HC, head circumference; FL, femur length; EFW, estimated fetal weight; BW, birth weight; LWB, low birth weight; PTB, preterm birth, SGA, small-size-for-gestational-age; LGA, large-size-for-gestational-age. *not in this thesis.

used folic acid supplements during pregnancy. After comparing the effect estimates of the associations of maternal smoking with neonatal outcomes, including birth weight, preterm birth, low birth weight, and small-size-for-gestational-age, based on single or repeated assessment, only marginal differences were found. Our results suggest that single assessment of smoking during pregnancy, does lead to underestimation of the continued smoking prevalence, especially among women who reported quitting smoking in first trimester. However, this underestimation does not materially change the effect estimates for the associations between maternal smoking and neonatal outcomes. Women who quit smoking after first trimester of pregnancy may need more assistance to continue this smoking cessation (**Chapter 2.2**).

Maternal lifestyle and hypertensive complications during pregnancy

Blood pressure tracking can be used as a concept to examine the predictability of future values by early measurements. We found correlations coefficients between first and third trimester for systolic and diastolic blood pressure of 0.47 and 0.46, respectively. Maternal age, maternal height, gestational weight gain and ethnic background influenced these correlation coefficients. Furthermore, systolic and diastolic blood pressure change from second to third trimester, but not from first to second trimester, were positively associated with the risks of pregnancy-induced hypertension and preeclampsia. In conclusion, our results suggest that blood pressure tracking moderately during pregnancy, and is influenced by maternal characteristics. Second to third trimester increases in systolic and diastolic blood pressure are associated with an increased risk of gestational hypertensive disorders (**Chapter 3.1**).

Increased age is associated with increased risks of hypertension and cardiovascular disease[13,14]. Less is known about maternal age and the development of hypertensive disorders during pregnancy. We have shown that higher maternal age is associated with lower second and third

trimester systolic blood pressure, but higher third trimester diastolic blood pressure. These small differences in blood pressure levels between younger and older women were within the physiological range of blood pressure variability. Maternal age was not consistently associated with the risk of gestational hypertensive disorders (**Chapter 3.2**).

Epidemiological studies have shown that smoking during pregnancy lowers the risk of preeclampsia[15,16]. We examined the associations of smoking during pregnancy and repeatedly measured blood pressure. We found that as compared to non-smoking, both first trimester only and continued smoking were associated with a steeper increase for systolic blood pressure and a lowest mid-pregnancy level and steeper increase thereafter for diastolic blood pressure throughout pregnancy. We did not find any significant associations in risk of preeclampsia for first trimester only smoking and continued smoking. Our results suggest that both first trimester only and continued smoking are associated with persistent maternal cardiovascular adaptations during pregnancy. The effects of smoking in early and late pregnancy on the risk of preeclampsia should be further explored (**Chapter 3.3**).

Habitual caffeine intake has been suggested to be associated with the risk of hypertension[17-19]. Our longitudinal analyses suggested no significant differences in both systolic and diastolic blood pressure between maternal caffeine intake groups. The cross-sectional analyses showed that higher caffeine intake tended to be associated with higher systolic blood pressure in first and third trimester, but not in second trimester. Caffeine intake was not consistently associated with diastolic blood pressure levels, or the risk of pregnancy-induced hypertension. As compared to women with caffeine intake of less than 2 units per day, those using 2 to 3.9 units per day had a lower risk of preeclampsia. The unexpected finding of a possible protective association with moderate caffeine intake and the risk of preeclampsia needs further investigation (**Chapter 3.4**).

Previous studies have shown that maternal hypertensive disorders during pregnancy may result in offspring with lower birth weight[20,21]. Less is known about blood pressure levels and fetal growth in different trimesters of pregnancy. Our results suggested that higher blood pressure was not associated with fetal growth characteristics in second trimester, but with impaired fetal growth from third trimester onwards. We found that offspring of women with higher blood pressure had smaller fetal head circumference and femur length, and lower fetal weight. Largest effects were observed for diastolic blood pressure, and at older gestational age. Not first to second trimester changes of systolic and diastolic blood pressure, but second to third trimester changes of systolic blood pressure was associated with an increased risk of low birth weight, and of diastolic blood pressure with increased risks of preterm birth, low birth weight, and small-size-for-gestational-age. As compared to non-hypertensive pregnancies, women with preeclampsia had increased risks of delivering preterm, after restriction to spontaneous deliveries only, low birth weight, and small-size-for-gestational-age children (**Chapter 3.5**).

Maternal lifestyle and fetal and neonatal complications

Previous studies showed lower birth weight among offspring of younger women, but suggested an inverse U-shaped relationship between maternal age and birth weight[22,23]. Several mechanisms may explain this association between maternal age and birth weight. Our analyses showed that women younger than 20 years had the highest risk of delivering small-size-for-gestational-age children; however, this increased risk disappeared after adjustment for socio-demographic and lifestyle related determinants. Women older than 40 years had the highest risk of delivering large-size-for-gestational-age children. The association of maternal age with the risks of delivering large-size-for-gestational-age children could not be fully explained by socio-demographic and lifestyle related determinants (**Chapter 4.1**).

Excessive alcohol consumption during pregnancy has adverse effects on fetal growth and development, and neonatal outcomes[24]. Less consistent associations have been shown for the associations of light-to-moderate maternal alcohol consumption during pregnancy with health outcomes in the offspring[25]. We found that 37% of all pregnant women continued alcohol consumption during pregnancy, of whom the majority used less than three drinks per week. We observed no differences in growth rates of fetal head circumference, abdominal circumference or femur length between women with and without continued alcohol consumption during pregnancy. As compared to women without alcohol consumption, women with continued alcohol consumption during pregnancy had a small increased fetal weight gain. This effect might be explained by an increased calory intake through alcoholic bevarages. Cross-sectional analyses in mid- and late pregnancy showed no consistent associations between the number of alcoholic consumptions and fetal growth characteristics. In summary, our results suggest that low-to-moderate maternal alcohol consumption during pregnancy does not adversely affect fetal growth characteristics; however, developmental effects cannot be excluded (**Chapter 4.2**). We also showed that maternal alcohol consumption during pregnancy was not associated with the risks of adverse birth outcomes, including low birth weight, preterm birth, and small-size-for-gestational-age. However, dose-response analyses showed non-significant tendencies towards adverse effects of low daily alcohol consumption in early pregnancy on birth weight and neonatal outcomes. Similar effects were found in late pregnancy (**Chapter 4.3**).

Maternal smoking during pregnancy is known to be associated with increased risks of neonatal complications[26]. Use of folic acid supplements might reduce the adverse effects of maternal smoking on DNA-methylation, since folate provides methyl groups for the syntheses of methionine[27]. The derivate of methionine is an important methyl donor in the hyuman body for DNA-methylation[28,29]. We showed that continued maternal smoking was associated with higher first trimester homocysteine levels, lower third trimester fetal weight and birth weight. Periconceptional folic acid supplement use decreased these differences. Among women who continued smoking during pregnancy, those who did not use folic acid supplements, tended to have the highest risks of low birth weight and small-size-for-gestational-age at birth children as compared to those who did use periconceptional folic acid supplements. Our finding suggest that the adverse effects of maternal

smoking on first trimester homocysteine levels, fetal growth and risks of neonatal complications might be reduced by the use of folic acid supplements (**Chapter 4.4**). However, because of the observational design of this study, these results should be considered as hypothesis generating.

Caffeine is a widely used and accepted pharmacologically active substance[30]. The impact of caffeine intake during pregnancy on fetal growth and development is still unclear. We observed no consistent associations of caffeine intake, based on coffee and tea consumption, with fetal head circumference or estimated fetal weight in any trimester. Higher caffeine intake was associated with smaller first trimester crown-rump length, second and third trimester femur length and birth length. Offspring of women who consumed six or more caffeine units per day tended to have increased risks of low birth weight children. Our results suggest that caffeine intake of 6 or more units per day during pregnancy is associated with impaired fetal length growth. Caffeine exposure might preferentially adversely affect fetal skeletal growth (**Chapter 4.5**).

METHODOLOGICAL CONSIDERATIONS

The studies described in this thesis all have been conducted within the Generation R Study, a population-based prospective cohort study[31]. Specific methodological considerations of the studies have been discussed in the separate chapters. In this paragraph, general methodological issued regarding selection bias, information bias and confounding are discussed.

Selection bias

In general, selection bias may occur either if the association between the determinant and outcome measurement is different in those who participate and those who were eligible, but do not participate or due to selective loss to follow-up[32]. First, of all eligible children at birth, 61% participated in the Generation R Study. Information on maternal smoking, alcohol consumption and caffeine intake during pregnancy at enrolment was missing in 14.4%, 13.9%, and 8.5%, respectively, of the participating women. None of the women had missing data on their age at enrolment. Non-response due to non-participation at baseline among the eligible participants is not likely to be random. The percentages of women from ethnic minorities and lower socio-economic status, and of women or children with medical complications are lower among the participants than expected from the population figures in Rotterdam, the Netherlands[33]. The selection towards a more healthy study population may probably affect the prevalence rates and, consequently, the statistical power in our studies and generalizibility of our findings. Second, selection bias due to selective loss to follow-up may have occurred if the associations of maternal lifestyle habits during pregnancy with fetal growth or the risks of hypertensive disorders differed between those lost and those not loss to follow-up. Due to the prospective nature of the study, selection on the outcome at baseline is not a issue. A previous study confirms that biased estimates, due to selection bias, in

prospective cohort studies primarily arise from loss to follow-up rather than from non-response at baseline[34].

Information bias

The main determinants studied in this thesis, including maternal smoking, alcohol consumption, and caffeine intake during pregnancy, were collected prospectively in the Generation R Study by self-reported questionnaires. This information was obtained without reference to fetal growth characteristics or information on hypertensive complications. The women were not aware of the specific research questions addressed in this thesis. Although assessing lifestyle habits in pregnancy by questionnaires seems to be a valid method, misclassification may have occurred[35]. Due to socially acceptable behavior, assessment of adverse lifestyle habits by questionnaires, mainly smoking habits and alcohol consumption, may have led to underreporting. We do not expect similar reporting issues on caffeine intake, since caffeine intake was calculated from coffee and tea consumption during pregnancy. Furthermore, less is known among pregnant women of the possible harmful effects of this exposure. Random misclassification of maternal lifestyle habits during pregnancy would have led to bias towards the null. However, when misclassification of the determinant is related to the outcome, information bias may occur. Exposure information in our studies was mainly collected before assessment of the outcome, which makes non-random misclassification of the exposure unlikely. In addition, the examiners who collected information on fetal growth characteristics by ultrasound, and information on hypertensive disorders during pregnancy were blinded to the exposure status of the participant, which also makes non-random misclassification even more unlikely.

If underreporting of maternal smoking and alcohol consumption is selectively more present among women with higher exposures that did report low-to-moderate exposure, the effect estimates found on for these latter groups would be overestimated. To overcome these limitations, previous studies have used biomarkers of smoking exposure, including cotinine, in maternal urine samples[36,37]. However, low correlations between cotinine levels and self-reported smoking habits have been demonstrated[38]. Possible explanations for these low correlations include inaccurate maternal reporting of smoking during pregnancy, use of categorical rather than continuous variables for assessing the number of cigarettes smoked per day, but also individual differences in inhalation, absorption, and metabolism. It has been demonstrated that use of cotinine levels is not superior to the use of self-reporting questionnaires in studying the effect of maternal smoking in pregnancy on pregnancy outcomes[38]. For maternal alcohol consumption, current available biomarkers, including carbohydrate-deficient transferrin and gamma-glutamyltransferase, seem to be inappropriate for assessment of low-to-moderate alcohol exposure[39]. For high maternal alcohol consumption, fatty acid ethyl esters (FAEEs) extracted from meconium is found to be a reliable biomarker[40,41]. However, these data were not available in our study.

Confounding

Within the Generation R Study information on many variables related to lifestyle habits of the pregnant women were collected. Therefore, a wide range of potential confounding factors was available for the analyses in the described studies. Confounding may be considered as biased effects, in which the apparent effect of the exposure of interest is distorted because the effect of an extraneous factor is mistaken for or mixed with the actual exposure effect. A confounding factor should be associated with both the exposure and the outcome, and cannot be an intermediate in the causal chain from exposure to outcome. Adjustment for an intermediate in the causal pathway from exposure to outcome, or adjustment for a variable that is causally related to the exposure but only correlated to the outcome, is inappropriate. Although, we had information on many variables of interest, we may have missed potential confounders. Residual confounding due to unmeasured variables such as maternal nutrition, medication use, and physical activity during pregnancy might still be possible. Thus, missing information on other adverse exposures in fetal life may have introduced residual confounding in the studies presented in this thesis.

FUTURE PERSPECTIVES

The described studies in this thesis only considered the prenatal phase. Follow-up of these women and their children may provide more insight in the underlying mechanisms, but also provide information on long-term consequences of adverse maternal lifestyle. Also, more detailed information on patterns of exposure may benefit the knowledge on this area of research. Especially, alcohol consumption is thought of being more harmful if consumed at once, e.g. binge drinking, instead of frequent low intakes. This might also be the case in caffeine consumption. Some specific areas of future research should be mentioned.

Fetal nutrition and epigenetics

Maternal nutrition and subsequent fetal nutrition is an important factor of healthy fetal circumstances. Maternal nutrition is closely related to maternal lifestyle. Previous studies have shown that critical periods for exposure to famine seem to be mainly in early fetal life but also in early childhood[42,43]. However, inconsistent associations of macronutrient intake in pregnant women and adverse health outcomes in the offspring were shown[44,45]. Not only energy and macronutrient intake, but also variation of dietary patterns and micronutrient intake are of interest. Also, the mechanisms by which micronutrients may influence cardiovascular adaptations are still largely unknown. In response to, fetal nutrition variation, epigenetic modifications may occur, including DNA methylation[46]. Currently, it is unclear whether these epigenetic modifications may underlie the associations between adverse fetal nutritional exposures and adverse fetal outcomes[47]. Since the largest variation of methylation is expected periconceptionally, it is of great importance that future population-based cohort studies start as early in preconceptional or fetal life as possible[48].

Paternal factors

Previous studies have shown that not only maternal determinants influence development and growth of the fetus, but also paternal determinants may play an important role. For example, paternal smoke exposure might affect health of the fetus by passive maternal smoke exposure or direct effects on the sperm. It is known that passive smoke exposure is associated with a lower birth weight. Paternal smoking may damage paternal DNA and therefore may lead to fetal mutations[49]. Also, advanced paternal age is shown to be associated with increased risk of fetal death[50]. Furthermore, previous studies in small populations and rats suggested that paternal smoking and alcohol consumption are associated with fetal morbidity and mortality[51-53]. Most studies were not able to assess the separate effects of paternal smoking in fetal and neonatal complications because of the correlations with maternal smoking or small numbers. Furthermore, Zusterzeel *et al.* found certain polymorphisms in not only the mothers, but also in the fathers, are associated with increased risk of preeclampsia[54]. Also, distortions in genomic imprinting in placental tissue, resulting from impaired paternal versus maternal gene expression, is suggested to be associated with an increased risk of preeclampsia[47,55]. Therefore, population-based cohort studies also including fathers might be of great interest.

CONCLUSION

The associations of maternal lifestyle habits with fetal and hypertensive complications seem to be within the normal and physiological ranges. They also suggest that specific exposures in different periods of fetal life have differential consequences for fetal development. The mechanisms underlying these associations are not known, but may include epigenetic modifications. Although our findings might be of important public health relevance, they should be interpreted carefully because of the observational design. Future studies should be focused on identification of these mechanisms. Furthermore, studies should focus on detailed follow-up of the studied women and their children. In the end, results from these proposed studies may lead to improved health in childhood and adulthood by creating a better fetal environment.

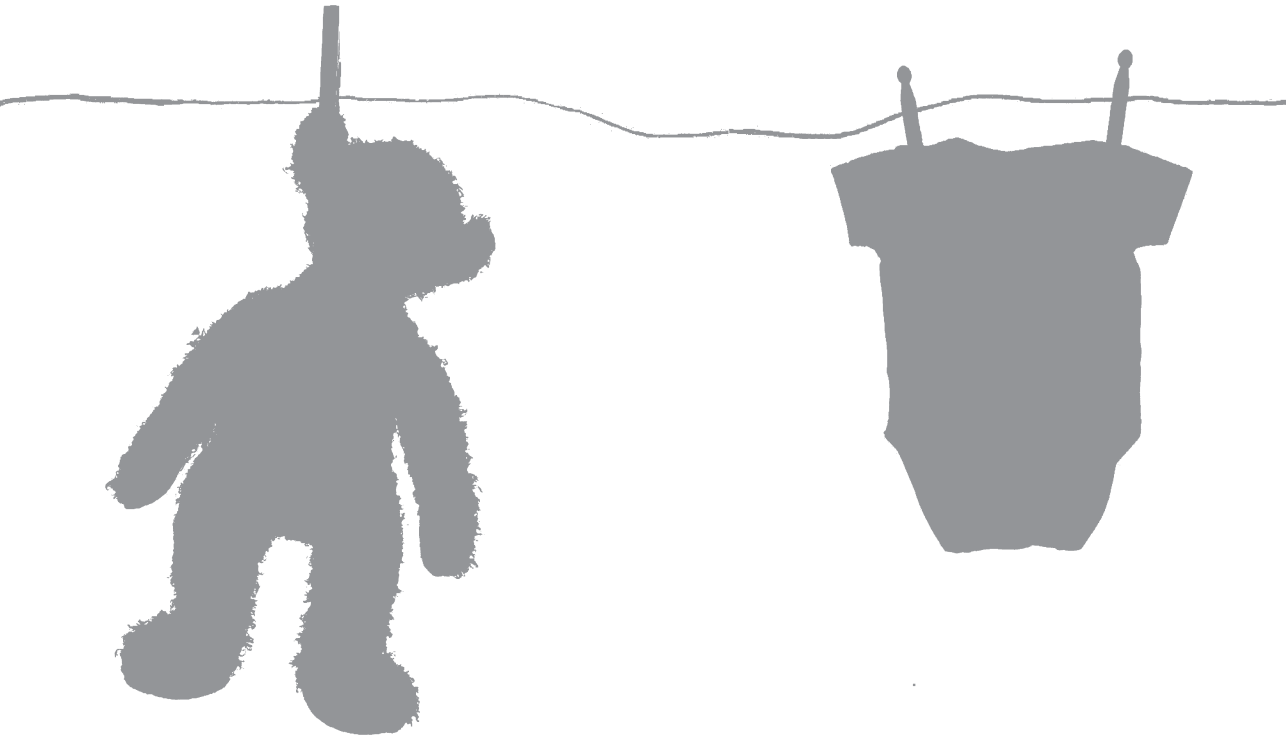
REFERENCES

1. Kline J, Stein ZA, Susser M, et al. Smoking: a risk factor for spontaneous abortion. N Engl J Med. 1977;297: 793-6.
2. Cnattingius S, Haglund B, Meirik O. Cigarette smoking as risk factor for late fetal and early neonatal death. BMJ. 1988;297:258-61.
3. Kline J, Shrout P, Stein Z, et al. Drinking during pregnancy and spontaneous abortion. Lancet. 1980;2:176-80.
4. Ouellette EM, Rosett HL, Rosman NP, et al. Adverse effects on offspring of maternal alcohol abuse during pregnancy. N Engl J Med. 1977;297:528-30.
5. O'Leary CM, Nassar N, Kurinczuk JJ, et al. The effect of maternal alcohol consumption on fetal growth and preterm birth. BJOG. 2009;116:390–400.
6. Cnattingius S, Signorello LB, Annerén G, et al. Caffeine intake and the risk of first-trimester spontaneous abortion. N Engl J Med. 2000;343:1839-45.
7. Wisborg K, Kesmodel U, Bech BH, et al. Maternal consumption of coffee during pregnancy and stillbirth and infant death in first year of life: prospective study. BMJ. 2003;326:420.
8. CARE Study Group. Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. BMJ. 2008;337:a2332.
9. MacLeod S, Kiely JL. The effects of maternal age and parity on birthweight: a population-based study in New York City. Int J Gynaecol Obstet. 1988;26:11-9.
10. Pickett KE, Kasza K, Biesecker G, et al. Women who remember, women who do not: a methodological study of maternal recall of smoking in pregnancy. Nicotine Tob Res. 2009;11:1166-74.
11. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R Study. Am J Epidemiol. 2007;165:1207-15.
12. Jaddoe VW, Troe EJ, Hofman A, 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;22:162-71.
13. Klebanoff MA, Levine RJ, Morris CD, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediat Perinat Epidemiol. 2001;15:140-3.
14. Steegers EAP, von Dadelszen P, Duvekot JJ, et al. Pre-eclampsia. Lancet. 2010;376:631-44.
15. Zhang J, Klebanoff MA, Levine RJ, et al. The puzzling association between smoking and hypertension during pregnancy. Am J Obstet Gyneacol. 1999;181:1407-13.
16. England L, Zhang J. Smoking and risk of preeclampsia: a systematic review. Front Biosci. 2007;12:2471-83.
17. Klag MJ, Wang NY, Meoni LA, et al. Coffee intake and risk of hypertension: the Johns Hopkins precursors study. Arch Int Med. 2002;162:657-62.
18. Winkelmayer WC, Stampfer MJ, Willett WC, et al. Habitual caffeine intake and the risk of hypertension in women. JAMA. 2005;294:2330-5.
19. Noordzij M, Uiterwaal CS, Arends LR, et al. Blood pressure response to chronic intake of coffee and caffeine: a meta-analysis of randomized controlled trials. J Hypertens. 2005;23:921-8.
20. Waugh J, Perry IJ, Halligan AW, et al. Birth weight and 24-hour ambulatory blood pressure in nonproteinuric hypertensive pregnancy. Am J Obstet Gynecol. 2000;183:633-7.
21. Zhang J, Klebanoff MA, Roberts JM. Prediction of adverse outcomes by common definitions of hypertension in pregnancy. Obstet Gyneacol. 2001;97:261-7.
22. MacLeod S, Kiely JL. The effects of maternal age and parity on birthweight: a population-based study in New York City. Int J Gynaecol Obstet. 1988;26:11-9.
23. Jahromi BN, Husseini Z. Pregnancy outcome at maternal age 40 and older. Taiwan J Obstet Gynecol. 2008;47: 318-21.
24. Henderson J, Kesmodel U, Gray R. Systematic review of the fetal effects of prenatal binge-drinking. J Epidemiol Community Health. 2007;61:1069–73.
25. Henderson J, Gray R, Brocklehurst P. Systematic review of effects of low-moderate prenatal alcohol exposure on pregnancy outcome. BJOG. 2007;114:243–52.

26. Jaddoe VW, Verburg BO, de Ridder MA, et al. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the generation R study. Am J Epidemiol. 2007;165:1207-15.
27. Timmermans S, Jaddoe VW, Hofman A, et al. Periconception folic acid supplementation, fetal growth and the risks of low birth weight and preterm birth: the Generation R Study. Br J Nutr. 2009;102:777-85.
28. McDonald SD, Perkins SL, Jodouin CA, et al. Folate levels in pregnant women who smoke: an important gene/environment interaction. Am J Obstet Gyneacol. 2002;187:620-5.
29. Tamura R, Picciano MF. Folate and human reproduction. Am J Clin Nutr. 2006;83:993-1016.
30. Barone JJ, Roberts HR. Caffeine consumption. Food Chem Toxicol. 1996;34:119-29.
31. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol. 2010;25:823–841.
32. Greenland S. Response and follow-up bias in cohort studies. Am J Epidemiol. 1977;106:184-187.
33. Center for Research and Statistics, Rotterdam (COS); http:// www.cos.rotterdam.nl. In; 2005.
34. Nohr EA, Frydenberg M, Henriksen TB, et al. Does low participation in cohort studies induce bias? Epidemiology. 2006;17:413-8.
35. Klebanoff MA, Levine RJ, Morris CD, et al. Accuracy of self-reported cigarette smoking among pregnant women in the 1990s. Paediatr Perinat Epidemiol. 2001;15:140-3.
36. Wang X, Tager IB, Van Vunakis H, et al. Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. Int J Epidemiol. 1997;26:978-88.
37. English PB, Eskenazi B, Christianson RE. Black-white differences in serum cotinine levels among pregnant women and subsequent effects on infant birthweight. Am J Public Health. 1994;84:1439-43.
38. Haddow JE, Knight GJ, Palomaki GE, et al. Cigarette consumption and serum cotinine in relation to birthweight. Br J Obstet Gyneacol. 1987;94:678-81.
39. van Pelt J, Lueisink GL, Nierop PW, et al. Test characteristics of carbohydrate-deficient transferrin and gamma-glutalmyltransferase in alcohol-using perimenopausal women. Alcohol Clin Exp Res. 2000;24:176-179.
40. Bearer CF, Jacobson JL, Jacobson SW, et al. Validation of a new biomarker of fetal exposure to alcohol. J Pediatr. 2003;143:463-469.
41. Bearer CF, Bailey SM, Hoek JB. Advancing alcohol biomarkers research. Alcohol Clin Exp Res. 2010;34:941-945.
42. Roseboom T, de Rooij S, Painter R. The Dutch famine and its long-term consequences for adult health. Early Hum Dev. 2006;82:485-91.
43. Li Y, Jaddoe VW, Qi L, et al. Exposure to the Chinese Famine in Early Life and the Risk of Metabolic Syndrome in Adulthood. Diabetes Care. 2011; doi: 10.2337/dc10-2039
44. Campbell DM, Hall MH, Barker DJ, et al. Diet in pregnancy and the offspring's blood pressure 40 years later. Br J Obstet Gyneacol. 1996;103:273-80.
45. Huh SY, Rifas-Shiman SL, Kleinman KP, et al. Maternal protein intake is not associated with infant blood pressure. Int J Epidemiol. 2005;34:378-84.
46. Waterland RA, Michels KB. Epigenetic epidemiology of the developmental origins hypothesis. Annu Rev Nutr. 2007;27:363-88.
47. Nafee TM, Farrell WE, Carroll WD, et al. Epigenetic control of fetal gene expression. BJOG. 2008;115:158-68.
48. Inskip HM, Godfrey KM, Robinson SM, et al. Cohort profile: the Southampton women's survey. Int J Epidemiol. 2006;35:42-8.
49. Aitken RJ, Koopman P, Lewis SEM. Seeds of concern. Nature. 2004;432:48-52.
50. Andersen AN, Hansen KD, Andersen PK, et al. Advanced paternal age and risk of fetal death: A cohort study. Am Epidemiol. 2004;160:1214-22.
51. Uncu Y, Ozcakir A, Ercan I, et al. Pregnanct women quit smoking; What about fathers? Survey study in Bursa Region, Turkey. Croat Med J. 2005;46:832-837.
52. Cicero TJ, Nock B, O'Connor LH, et al. Acute paternal alcohol exposure impairs fertility and fetal outcome. Life Sci. 1994;55:PL33-6.
53. Little J, Vianio H. Mutagenic lifestyles? A review of evidence of associations between germ-cell mutations in humans and smoking, alcohol consumption and use of 'recreational' drugs. Mutat Res. 1994;313:131-51.

54. Zusterzeel PLM, te Morsche R, Raijmakers MTM. Paternal contribution to the risk of preeclampsia. J Med Genet. 2002;39:44-45.
55. Oudejans CB, van Dijk M. Placental gene expression and preeclampsia. Placenta. 2008;29:78-82.

Part 6 | **Summary**



In Western countries, the most common adverse maternal lifestyle habits during pregnancy include smoking, alcohol consumption, and caffeine intake. Although not directly lifestyle related, maternal age is also considered as a modifiable risk factor for pregnancy complications and adverse outcomes. These adverse maternal lifestyle habits may influence maternal cardiovascular adaptations during pregnancy, and subsequently increase the risks of maternal hypertensive disorders during pregnancy, including pregnancy-induced hypertension and preeclampsia. Maternal cardiovascular adaptations might also be involved in the pathway leading to an adverse fetal environment and subsequently neonatal complications because of impaired placental perfusion that may lead to limited oxygen and nutrient supply to the fetus. Therefore, these adverse lifestyle habits are also considered as modifiable risk factors for fetal and neonatal complications.

Previous studies showed consistent robust evidence for the associations of high levels of exposure to adverse maternal lifestyle habits during pregnancy with the risks of perinatal mortality and morbidity; however, less is known about the effects of lower levels of exposure. In addition, most previous studies focussed on birth weight as main outcome, but birth weight is just a proxy of fetal growth. Different fetal growth characteristics and body proportions might result in the same birth weight. Exposure to adverse maternal lifestyle habits in different trimesters of pregnancy might also have differential effects on fetal growth. Therefore, studies on exposure effects in different trimesters might identify specific critical periods. Finally, examination of factors that may explain the established relationship between maternal age and adverse pregnancy outcomes may help understand the underlying mechanisms.

The main objectives of the studies presented in this thesis are to examine the associations of maternal lifestyle habits with hypertensive complications during pregnancy, and with fetal growth and the risks of neonatal complications.

In **Part 2** we present the overall design of the Generation R study, a population-based prospective cohort study from fetal life until young adulthood. Pregnant women with a delivery date between April 2002 and January 2006 were eligible for enrolment. In total, 9778 pregnant women were enrolled in the study, of which 8880 women were enrolled during pregnancy. Assessments during pregnancy were planned in first, second and third trimester, and mainly included physical examinations, questionnaires, and fetal ultrasound examinations. Information on pregnancy complications and outcomes were collected from medical records (**Chapter 2.1**).

Data collection of adverse lifestyle habits is often debated. Single assessment of maternal smoking during pregnancy by questionnaires is a common used method in population-based cohorts. The use of questionnaires seems to be valid; however, misclassification may occur, mainly due to underreporting or failure of smoking cessation. We found a Cohen's Kappa coefficient, a measure of intra- and inter-agreement of observations, of 0.86 between maternal smoking status based on single and repeated questionnaires. Younger, smaller, lower educated, non-European, pregnant women who experienced more stress, consumed more alcohol and less frequently used folic acid supplements were more often to be misclassified based on single assessment. Single assessment

of smoking status did lead to underestimation of the continued smoking prevalence, especially among women who reported quitting smoking in first trimester. However, this underestimation did not materially change the effect estimates for the associations between maternal smoking and neonatal outcomes (**Chapter 2.2**).

Part 3 presents studies of adverse maternal lifestyle habits and blood pressure, and hypertensive complications during pregnancy. In **Chapter 3.1** we found correlations coefficients of blood pressure tracking between first and third trimester for systolic and diastolic blood pressure of 0.47 and 0.46, respectively. Maternal age, maternal height, gestational weight gain and ethnic background influenced these correlation coefficients. Systolic and diastolic blood pressure change from second to third trimester was positively associated with the risks of pregnancy-induced hypertension and preeclampsia. Our results suggest that blood pressure tracking is present during pregnancy, influenced by maternal characteristics. Not first to second but second to third trimester increase in blood pressure levels is associated with increased risks of gestational hypertensive disorders.

In **Chapter 3.2** we showed that higher maternal age is associated with lower second and third trimester systolic blood pressure, but higher third trimester diastolic blood pressure. In addition, maternal age was not consistently associated with the risk of gestational hypertensive disorders.

Furthermore, we found that as compared to non-smoking women, both first trimester only and continued smoking were associated with a steeper increase for systolic blood pressure and a lowest mid-pregnancy level and steeper increase thereafter for diastolic blood pressure throughout pregnancy. We did not find any significant associations in risk of preeclampsia for first trimester only smoking and continued smoking. These results suggest that both first trimester only and continued smoking are associated with persistent maternal cardiovascular adaptations during pregnancy (**Chapter 3.3**).

Longitudinal analyses showed no significant differences in both systolic and diastolic blood pressure between different maternal caffeine intake levels. Cross-sectional analyses showed that higher caffeine intake tended to be associated with higher systolic blood pressure in first and third trimester, but not in second trimester. Caffeine intake was not consistently associated with diastolic blood pressure levels, or the risk of pregnancy-induced hypertension. As compared to women with caffeine intake of less than 2 units per day, those using 2 to 3.9 units per day had a lower risk of preeclampsia (**Chapter 3.4**).

In **Chapter 3.5** we showed that higher blood pressure was not associated with fetal growth characteristics in second trimester, but with impaired fetal growth from third trimester onwards. We found that offspring of women with higher blood pressure had smaller fetal head circumference and femur length, and lower fetal weight. We observed the largest effects for diastolic blood pressure, and at older gestational age. Not first to second trimester changes of systolic and diastolic blood pressure, but second to third trimester changes of systolic blood pressure were associated with an increased risk of low birth weight, and second to third trimester changes of diastolic blood pressure were associated with increased risks of preterm birth, low birth weight,

and small-size-for-gestational-age. As compared to non-hypertensive pregnancies, women with preeclampsia had increased risks of delivering preterm, low birth weight, and small-size-for-gestational-age children.

In **Part 4** we present studies focused on the associations of maternal lifestyle factors with fetal growth patterns and the risks of neonatal complications. In **Chapter 4.1** we showed that women younger than 20 years had the highest risk of delivering small-size-for-gestational-age children; however, this increased risk disappeared after adjustment for socio-demographic and lifestyle related determinants. Women older than 40 years had the highest risk of delivering large-size-for-gestational-age children. The association of maternal age with the risks of delivering large-size-for-gestational-age children could not be fully explained by socio-demographic and lifestyle related determinants.

Chapter 4.2 and **Chapter 4.3** describe the associations of alcohol consumption during pregnancy with fetal growth and adverse pregnancy outcomes. We found that 37% of all pregnant women continued alcohol consumption during pregnancy, of whom the majority used less than three drinks per week. We observed no differences in growth rates of fetal head circumference, abdominal circumference or femur length between women with and without continued alcohol consumption during pregnancy. As compared to women without alcohol consumption, women with continued alcohol consumption during pregnancy had a small increased fetal weight gain. Cross-sectional analyses in mid- and late pregnancy showed no consistent associations between the number of alcoholic consumptions and fetal growth characteristics (**Chapter 4.2**). We also showed that maternal alcohol consumption during pregnancy was not associated with the risks of adverse birth outcomes, including low birth weight, preterm birth, and small-size-for-gestational-age. However, dose-response analyses showed non-significant tendencies towards adverse effects of low daily alcohol consumption in early pregnancy on birth weight and neonatal outcomes. We found similar effects in late pregnancy (**Chapter 4.3**).

In **Chapter 4.4** we showed that continued maternal smoking was associated with higher first trimester homocysteine levels, lower third trimester fetal weight and birth weight. Periconceptional folic acid supplement use decreased these differences. Among women who continued smoking during pregnancy, those who did not use folic acid supplements, tended to have the highest risks of low birth weight and small-size-for-gestational-age at birth children as compared to those who did use periconceptional folic acid supplements. Our findings suggest that the adverse effects of maternal smoking on first trimester homocysteine levels, fetal growth and risks of neonatal complications might be reduced by the use of folic acid supplements.

Finally, we observed no consistent associations of caffeine intake, based on coffee and tea consumption, with fetal head circumference or estimated fetal weight in any trimester. Higher caffeine intake was associated with smaller first trimester crown-rump length, second and third trimester femur length and birth length. Offspring of women who consumed 6 or more caffeine units per day tended to have increased risks of low birth weight children. Our results suggest

that caffeine intake of 6 or more units per day during pregnancy is associated with impaired fetal length growth. Caffeine exposure might preferentially adversely affect fetal skeletal growth (**Chapter 4.5**).

Part 5 describes the main findings of the studies in this thesis. In addition, general methodological issues, including selection bias, information bias, and confounding are discussed. Finally, future research perspectives are provided.

In conclusion, the associations of maternal lifestyle habits with fetal and hypertensive complications seem to be within the normal and physiological ranges. The findings described in this thesis also suggest that specific exposures in different periods of fetal life have differential consequences for fetal development. The mechanisms underlying these associations still unknown, but may include epigenetic modifications. Although our findings might be of important public health relevance, they should be interpreted carefully because of the observational design. Future studies should be focused on identification of the underlying mechanisms. Furthermore, studies should focus on detailed follow-up of the studied women and their children. In the end, results from these proposed studies may lead to improved health in childhood and adulthood by providing a better fetal environment.

Samenvatting

Roken, alcoholgebruik en cafeïne inname zijn in de Westerse wereld de meest voorkomende potentieel ongunstige leefgewoontes van de moeder tijdens de zwangerschap. Ook wordt de leeftijd van de moeder beschouwd als een risicofactor voor zwangerschapscomplicaties en nadelige geboorte-uitkomsten. Deze ongunstige maternale leefgewoonten kunnen invloed hebben op de maternale cardiovasculaire aanpassingen tijdens de zwangerschap, en vervolgens mogelijk het risico op hypertensieve aandoeningen tijdens de zwangerschap verhogen. Met hypertensieve aandoeningen worden zwangerschapsgeïnduceerde hoge bloeddruk en pre-eclampsie, ofwel zwangerschapsvergiftiging, bedoeld. Maternale cardiovasculaire aanpassingen kunnen leiden tot een ongunstige foetale omgeving en leiden tot neonatale complicaties als gevolg van een verminderde doorbloeding van de placenta dat kan leiden tot beperkte zuurstof en voedingsstof overdracht naar de foetus. Hierdoor worden de benoemde ongunstige leefgewoontes van de moeder ook beschouwd als beïnvloedbare risicofactoren voor foetale en neonatale complicaties.

Eerdere studies toonden consistente associaties aan tussen hoge niveaus van blootstelling aan deze nadelige maternale leefgewoonten tijdens de zwangerschap en het risico op perinatale sterfte en morbiditeit. Er is echter minder bekend over de effecten van lagere niveaus van blootstelling. Bovendien zijn de meeste eerdere studies gericht op het geboortegewicht als belangrijkste uitkomst. Geboortegewicht is echter slechts een grove maat voor de groei van de foetus. Verschillende foetale groeipatronen kunnen resulteren in hetzelfde geboortegewicht. Blootstelling aan ongunstige maternale leefgewoontes in de verschillende trimesters van de zwangerschap hebben wellicht ook verschillende effecten op de groei kenmerken en patronen van de foetus. Daarom kunnen studies naar het effect van blootstelling in verschillende trimesters mogelijk specifieke kritieke perioden vaststellen. Ten slotte kan door middel van onderzoek naar factoren die de eerder vastgestelde associatie tussen leeftijd van de moeder en ongunstige zwangerschapsuitkomsten verklaren, inzicht verkregen worden in onderliggende mechanismen.

De belangrijkste doelstellingen van de studies beschreven in dit proefschrift zijn het bestuderen van de associaties van maternale levensstijl met hypertensieve complicaties tijdens de zwangerschap, en met groei van de foetus en het risico op neonatale complicaties.

In **Deel 2** beschrijven we de algemene opzet van de Generation R studie, een populatiegebaseerde prospectieve cohortstudie vanaf het foetale leven tot aan jong volwassenheid. Zwangere vrouwen met een bevallingsdatum tussen april 2002 en januari 2006 kwamen in aanmerking voor deelname. In totaal werden er 9778 zwangere vrouwen in de studie geïncludeerd, waarvan 8880 vrouwen al tijdens de zwangerschap. Metingen tijdens de zwangerschap waren gepland in het eerste, tweede en derde trimester, en bestonden voornamelijk uit lichamelijk onderzoek, vragenlijsten en foetaal echo-onderzoek. Informatie over de zwangerschapscomplicaties en de geboorte-uitkomsten werden verzameld met behulp van medische dossiers (**Hoofdstuk 2.1**).

Het verzamelen van informatie over schadelijke leefgewoonten is vaak onderwerp van discussie. Eenmalige rapportage van roken tijdens de zwangerschap met behulp van vragenlijsten is een veelgebruikte methode in populatiegebaseerde onderzoeken. Het gebruik van vragenlijsten lijkt valide te zijn, maar misclassificatie kan optreden, voornamelijk als gevolg van onderrapportage van werkelijk rookgedrag of door het falen van stoppen met roken. Wij vonden een Cohen's Kappa coëfficiënt, een maat voor de intra- en inter-overeenstemming van waarnemingen, van 0.86 voor rookstatus van de moeder op basis van eenmalige en herhaalde vragenlijsten. Jongere, kleinere, lager opgeleide, niet-Europese zwangere vrouwen die meer stress hadden, meer alcohol en minder vaak foliumzuur supplementen gebruikten werden vaker gemisclassificeerd op basis van eenmalige rapportage van rookgedrag. Eenmalige rapportage van de rookstatus leidt tot een onderschatting van de moeders die doorroken tijdens de gehele zwangerschap, vooral onder vrouwen die aangaven te zijn gestopt met roken in het eerste trimester. Echter deze onderschatting veranderde niet wezenlijk de associaties tussen roken van de moeder en neonatale uitkomsten (**Hoofdstuk 2.2**).

Deel 3 bevat studies naar het effect van ongunstige maternale levensstijl op de bloeddruk, en op het risico op hypertensieve complicaties tijdens de zwangerschap. In **Hoofdstuk 3.1** vonden we correlatiecoëfficiënten van bloeddruk tussen het eerste en derde trimester in systolische en diastolische bloeddruk van 0.47 en 0.46. Leeftijd, lengte, gewichtstoename tijdens de zwangerschap en etnische achtergrond van de vrouw waren van invloed op deze correlatiecoëfficiënten. Systolische en diastolische bloeddruk stijging van de tweede naar derde trimester was positief geassocieerd met het risico op zwangerschapsgeïnduceerde hoge bloeddruk en pre-eclampsie. Onze resultaten suggereren dat derde trimester stijging in bloeddruk geassocieerd met een verhoogd risico op hypertensieve aandoeningen tijdens de zwangerschap.

In **Hoofdstuk 3.2** toonden we aan dat hogere leeftijd van de zwangere vrouw is geassocieerd met een lagere tweede en derde trimester systolische bloeddruk, maar hoger derde trimester diastolische bloeddruk. We vonden geen consistente associatie tussen de leeftijd van de vrouw en het risico op hypertensieve aandoeningen tijdens de zwangerschap.

Verder vonden wij dat in vergelijking met niet-rokende vrouwen, zowel vrouwen die alleen in het eerste trimester rookten, als vrouwen die blijven roken tijdens de gehele zwangerschap, een hogere stijging in de systolische bloeddruk hadden. Zij toonden ook een laagste diastolische bloeddruk niveau in het midden van de zwangerschap en daarna een hogere stijging voor de diastolische bloeddruk. Wij vonden geen significant verhoogd of verlaagd risico op pre-eclampsie onder vrouwen die in het eerste trimester rookten of gedurende de gehele zwangerschap rookten. Deze resultaten suggereren dat zowel roken in enkel het eerste trimester als roken tijdens de gehele zwangerschap gerelateerd is aan maternale cardiovasculaire aanpassingen tijdens de zwangerschap (**Hoofdstuk 3.3**).

Longitudinale analyses toonden geen significante verschillen in zowel de systolische als diastolische bloeddruk bij vrouwen met verschillende hoeveelheden cafeïne inname tijdens de zwangerschap. Cross-sectionele analyses toonden aan dat een hogere inname van cafeïne geassocieerd

lijkt te zijn met een hogere systolische bloeddruk in de eerste en derde trimester, maar niet in het tweede trimester. Inname van cafeïne was niet consequent geassocieerd met diastolische bloeddruk, of het risico op zwangerschapsgeïnduceerde hoge bloeddruk. In vergelijking met vrouwen met een cafeïne inname van minder dan 2 eenheden per dag, hadden vrouwen die gebruik maakten van 2 tot 3.9 eenheden per dag een lager risico op pre-eclampsie (**Hoofdstuk 3.4**).

In **Hoofdstuk 3.5** hebben we laten zien dat een hogere bloeddruk niet geassocieerd is met foetale groeipatronen in het tweede trimester, maar met een verminderde groei van de foetus vanaf het derde trimester van de zwangerschap. We vonden dat kinderen van vrouwen met een hogere bloeddruk een kleinere foetale hoofdomtrek en beenlengte hadden en een lager foetaal gewicht. De grootste effecten werden waargenomen voor diastolische bloeddruk, en in het derde trimester van de zwangerschap. Niet eerste tot tweede trimester verandering in bloeddruk, maar tweede tot derde trimester stijging in systolische bloeddruk was geassocieerd met een verhoogd risico op laag geboortegewicht. Tweede tot derde trimester stijging van diastolische bloeddruk was ook gerelateerd met een verhoogd risico op vroeggeboorte, een laag geboortegewicht, en te klein geboren kinderen ten opzichte van hun zwangerschapsduur. In vergelijking met niet-hypertensieve zwangerschappen, hadden vrouwen met pre-eclampsie een verhoogd risico van het krijgen van premature kinderen, kinderen met een laag geboortegewicht, en te klein geboren kinderen ten opzichte van hun zwangerschapsduur.

Deel 4 bevat studies gericht op de associaties van maternale leefstijlfactoren met foetale groeipatronen en het risico op neonatale complicaties. In **Hoofdstuk 4.1** hebben we laten zien dat moeders jonger dan 20 jaar het hoogste risico hebben op te klein geboren kinderen ten opzichte van hun zwangerschapsduur. Dit verhoogde risico verdween na correctie voor sociaal demografische en levensstijl gerelateerde factoren. Moeders ouder dan 40 jaar hadden het hoogste risico van het leveren van een te groot geboren kind ten opzichte van hun zwangerschapsduur. De associatie van leeftijd van de moeder en het risico van het krijgen van een te groot geboren kinder ten opzichte van hun zwangerschapsduur kon niet volledig worden verklaard door sociaal demografische en levensstijl gerelateerde factoren.

Hoofdstuk 4.2 en **Hoofdstuk 4.3** beschrijven de associaties tussen alcoholgebruik tijdens de zwangerschap en de foetale groei en ongunstige zwangerschapsuitkomsten. Wij vonden dat 37% van alle zwangere vrouwen alcoholgebruik voortzet tijdens de zwangerschap, van wie de meerderheid minder dan drie drankjes per week gebruikt. We zagen geen verschillen in groei van de hoofdomtrek, buikomtrek of beenlengte van de foetus tussen moeders met en zonder alcoholgebruik tijdens de zwangerschap. In vergelijking met moeders zonder alcoholgebruik, hadden foetussen van moeders met een aanhoudend alcoholgebruik tijdens de zwangerschap een grotere toename in gewicht. Cross-sectionele analyses in midden en late zwangerschap toonden geen consistente associaties tussen het aantal alcoholische consumpties en groei van de foetus (**Hoofdstuk 4.2**). We toonden tevens aan dat alcoholgebruik van de moeder tijdens de zwangerschap niet geassocieerd is met het risico op nadelige geboorteuitkomsten, zoals een laag geboortegewicht,

vroeggeboorte, en te klein geboren kind ten opzichte van hun zwangerschapsduur. Echter, dosis response analyses toonden een niet-significante tendens aan voor negatieve effecten van lage dagelijkse alcoholconsumptie in het begin van de zwangerschap en het geboortegewicht en neonatale uitkomsten. Wij vonden soortgelijke effecten in de late zwangerschap (**Hoofdstuk 4.3**).

In **Hoofdstuk 4.4** hebben wij laten zien dat het blijven roken van de moeder tijdens de gehele zwangerschap geassocieerd is met hogere eerste trimester homocysteïne niveaus en een lager derde trimester foetale gewicht en geboortegewicht. Onder rokende moeders die periconceptioneel foliumzuur gebruikten daalde deze verschillen. Bij moeders die bleven roken tijdens de zwangerschap en geen gebruik van foliumzuur maakten hadden het hoogste risico op kinderen met een laag geboortegewicht en te klein geboren ten opzichte van hun zwangerschapsduur in vergelijking met moeders die wel periconceptioneel foliumzuur gebruikten. Onze bevindingen suggereren dat de negatieve gevolgen van roken van de moeder op het eerste trimester homocysteïne gehalte, groei van de foetus en het risico op neonatale complicaties kan worden verminderd door het gebruik van foliumzuur.

Tot slot zagen we geen consistente associaties van cafeïne inname, op basis van koffie en thee consumptie, met de foetale hoofdomtrek of het foetaal gewicht. Hogere inname van cafeïne was geassocieerd met een kleiner eerste trimester kruin-romp lengte, tweede en derde trimester beenlengte en geboortelengte. Kinderen van moeders die 6 of meer cafeïne eenheden per dag consumeerden hadden een verhoogd risico op laag geboortegewicht. Onze resultaten suggereren dat cafeïne inname van 6 of meer eenheden per dag tijdens de zwangerschap geassocieerd is met een verminderde foetale lengtegroei. Cafeïne blootstelling lijkt vooral een nadelig invloed te hebben op de foetale groei van het skelet (**Hoofdstuk 4.5**).

Deel 5 beschrijft de belangrijkste bevindingen van de studies in dit proefschrift. Tevens worden algemene methodologische kwesties, te weten selectie bias, informatie bias en confounding, besproken. Ten slotte worden toekomstige onderzoeksperspectieven beschreven.

Samengevat lijken de associaties van maternale levensstijl factoren met foetale en hypertensieve complicaties ook bij lagere blootstellingniveaus zichtbaar. De bevindingen in dit proefschrift suggereren dat specifieke blootstelling in verschillende perioden van het foetale leven verschillende gevolgen heeft voor de ontwikkeling van de foetus. De onderliggende mechanismen van deze gevonden associaties zijn nog onbekend. Wellicht is er sprake van epigenetische modificaties. Hoewel onze bevindingen relevant zijn voor de volksgezondheid, moeten ze zorgvuldig worden geïnterpreteerd. Dit met name als gevolg van de observationele opzet van dit onderzoek. Toekomstige studies zouden moeten worden gericht op de identificatie van de onderliggende mechanismen. Bovendien zouden deze studies zich moeten richten zich op een gedetailleerde follow-up van de reeds onderzochte vrouwen en hun kinderen. Uiteindelijk kunnen deze resultaten van de voorgestelde studies mogelijk leiden tot betere gezondheid tijdens de kindertijd en volwassenheid als gevolg van een verbeterde foetale omgeving.

Authors' affiliations

In order of appearance in this thesis

The Generation R Study, Erasmus MC, Rotterdam, the Netherlands
Rachel Bakker, Claudia J Kruithof, Vincent VW Jaddoe, Romy Gaillard, Angelique A Biharie, Liane E Pluimgraaff, Aleksandra Obradov

The Department of Epidemiology, Erasmus MC, Rotterdam, the Netherlands
Rachel Bakker, Claudia J Kruithof, Henning Tiemeier, Albert Hofman, Vincent VW Jaddoe, Romy Gaillard, Angelique A Biharie, Liane E Pluimgraaff, Jacqueline C Witteman, Aleksandra Obradov

The Department of Biostatistics, Erasmus MC, Rotterdam, the Netherlands
Sten Willemsen

The Department of Obstetrics & Gyneacology, Erasmus MC, Rotterdam, the Netherlands
Eric AP Steegers, Sarah Timmermans

The Department of Child and Adolescent Psychiatrie, Erasmus MC, Rotterdam, the Netherlands
Henning Tiemeier

The Department of Public Health, Erasmus MC, Rotterdam, the Netherlands
Johan P Mackenbach, Hein Raat

The Department of Paediatrics, Erasmus MC, Rotterdam, the Netherlands
Vincent VW Jaddoe, Romy Gaillard, Henriette A Moll

Publication list

1. Ledebt A, Becher J, Kapper J, Rozendaal RM, **Bakker R**, Leenders IC, Savelsbergh GJ. Balance training with visual feedback in children with hemiplegic cerebral palsy: effect on stance and gait. Motor Control. 2005;9:459-68.
2. Jaddoe VWV, **Bakker R**, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JCM. Moderate alcohol consumption during pregnancy and the risk of low birth weight and preterm birth. The Generation R Study. Ann Epidemiol. 2007;17:834-40.
3. Jaddoe VWV, **Bakker R**, van Duijn CM, van der Heijden AJ, Lindemans J, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Uitterlinden AG, Verhulst FC, Hofman A. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol. 2007;22:917-23.
4. **Bakker R**, Rifas-Shiman SL, Kleinman KP, Lipshultz SE, Gillman MW. Maternal calcium intake during pregnancy and blood pressure in the offspring at age 3 years: a follow-up analysis of the Project Viva cohort. Am J Epidemiol. 2008;168:1374-80.
5. Ay L, Kruithof CJ, **Bakker R**, Steegers EA, Witteman JC, Moll HA, Hofman A, Mackenbach, JP, Hokken-Koelega AC, Jaddoe VW. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. BJOG. 2009;116:953-63.
6. **Bakker R**, Pluimgraaff LE, Steegers EAP, Raat H, Tiemeier H, Hofman A, Jaddoe VWV. Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy. The Generation R Study. Int J Epidemiol. 2010;39:777-89.
7. **Bakker R**, Steegers EAP, Obradov A, Raat H, Hofman A, Jaddoe VWV. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: The Generation R Study. Am J Clin Nutr. 2010;91:1691-8.
8. **Bakker R**, Steegers EAP, Mackenbach JP, Hofman A, Jaddoe VWV. Maternal smoking and blood pressure in different trimesters of pregnancy. The Generation R Study. J Hypertens. 2010;28:2210-8.
9. **Bakker R**, Steegers EAP, Bihari AA, Mackenbach JP, Hofman A, Jaddoe VWV. Explaining differences in birth outcomes in relation to maternal age. The Generation R Study. BJOG. 2011;118:500-9.
10. Patra J*, **Bakker R***, Irving H, Jaddoe VWV, Rehm J. Dose-response relationship between alcohol consumption before and during pregnancy and the risks of low birth weight and preterm birth – A systematic review and meta-analyses (*authors contributed equally). *Submitted*
11. **Bakker R**, Steegers EAP, Raat H, Hofman A, Jaddoe VWV. Maternal caffeine intake, blood pressure and the risk of hypertensive complications during pregnancy. The Generation R Study. Am J Hypertens. 2010;24:421-8.
12. Gaillard R, **Bakker R**, Steegers EAP, Hofman A, Jaddoe VWV. Maternal age during pregnancy is associated with third trimester blood pressure level. The Generation R Study. *Submitted*

13. Timmermans S, Steegers-Theunissen RPM, Vujkovic M, **Bakker R**, den Breeijen H, Russcher H, Lindemans J, Hofman A, Jaddoe VWV, Steegers EAP. Major dietary patterns and blood pressure patterns during pregnancy. The Generation R Study. Am J Obstet Gyneacol. *In press*
14. **Bakker R**, Timmermans S, Steegers EAP, Hofman A, Jaddoe VWV. Maternal smoking, folic acid supplement use and the risks of fetal complications. The Generation R Study. *Submitted*
15. **Bakker R**, Kruithof CJ, Steegers EAP, Tiemeier H, Mackenbach JP, Hofman A, Jaddoe VWV. Assessment of maternal smoking status during pregnancy and the associations with neonatal outcomes. *Submitted*
16. **Bakker R**, Steegers EAP, Hofman A, Jaddoe VWV. Blood pressure in different trimesters, fetal growth and the risks of adverse birth outcomes. The Generation R Study. Am J Epidemiol. *In press*
17. Gaillard R, **Bakker R**, Willemsen S, Hofman A, Steegers EAP, Jaddoe VWV. Blood pressure tracking during pregnancy and the risk of gestational hypertensive complications. The Generation R Study. *Submitted*

About the author

Rachel Bakker was born on September 21th 1978 in Amersfoort, the Netherlands. She studied Human Movement Sciences at the Vrije Universiteit in Amsterdam, the Netherlands and obtained her Master of Science degree in December 2003. From 2004 to 2006, she worked as data manager at the Generation R Study, Erasmus Medical Center, Rotterdam. In 2006, she worked for 6 months at the Harvard School of Public Health, Boston, MA, United States. Her research was focused on the effects of maternal calcium intake and blood pressure in the offspring at age 3 in the Project Viva (head Prof. M.W. Gillman). During this period, she participated in several courses on Biostatistics and Epidemiology. In 2007, she started working as PhD student and study coordinator in the Generation R Study. Her research was focused on maternal lifestyle habits and pregnancy complications (Supervisors Prof. A. Hofman, Prof. E.A.P. Steegers and Dr. V.W.V. Jaddoe). In 2009, she obtained her Master of Science degree in Epidemiology at the Netherlands Institute for Health Sciences. From December 2010 until March 2011, she worked as research fellow at the Department of Epidemiology at the University of Aarhus, Denmark (Head Prof. J. Olsen). In Aarhus, she worked on a research project of the effect of paternal smoking habits on the risk of fetal death. This project was performed within the Danish National Birth Cohort.

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student:	R. Bakker
Research School:	NIHES
Erasmus MC Department:	Epidemiology
PhD period:	January 2008 – March 2011
Promotor(s):	Prof.dr. A. Hofman, Prof.dr. E.A.P. Steegers
Supervisor:	Dr. V.W.V. Jaddoe

PhD training

	Year	Workload (ECTS)
Research skills		
✓ Paediatric Clinical Epidemiology Course, KEK-NIHES:		
Principles and Methods of Clinical Scientific Research in Paediatrics	2005	0.7
✓ Harvard School of Public Health, Boston, USA:		
Principles of Biostatistics I	2006	5.7
Principles of Biostatistics II	2006	4.3
Principles of Epidemiology	2006	0.7
Research with Large Databases	2006	0.7
✓ MSc Epidemiology, NIHES:		
Principles of Research in Medicine	2005	0.7
Cohort studies	2005	0.7
Methods of Public Health Research	2007	0.7
Health Economics	2007	0.7
Case-control studies	2007	0.7
Introduction to Public Health	2007	0.7
Prevention Research	2007	0.7
Study Design	2007	4.3
Methodological Topics in Epidemiological Research	2007	1.4
Genome Wide Association Analysis	2008	1.4
Conceptual Foundation of Epidemiologic Study Design	2008	0.7
Introduction to Decision-making in Medicine	2008	0.7
History of Epidemiological Ideas	2008	0.7
Clinical Epidemiology	2008	5.7
In-depth courses		
✓ SNP's and Human Diseases	2007	1.4
✓ Missing Values in Clinical Research	2009	0.7

✓ Analysis of Time-varying Exposures	2009	0.7
✓ Maternal and Child Health	2009	0.7
National and international conferences, seminars, and workshops		
✓ The Generation R Study Group research meetings	2007 - 2009	0.7
✓ Symposium Generation R 2007: Fetal growth and development, Rotterdam, the Netherlands	2007	0.7
✓ RIVM – Centrum voor Voeding en Gezondheid – Bilthoven	2007	0.7
✓ Kennispoort conference – Utrecht	2008	0.7
✓ Symposium Onderzoek over de grens, Research Unit KG, Erasmus MC, Rotterdam, the Netherlands	2009	0.7
✓ DOHaD - Santiago de Chili, Chili Poster: Maternal smoking, blood pressure and placental haemodynamics and risk of preeclampsia. Poster: Does folic acid supplementation in early pregnancy modify the associations of maternal smoking during pregnancy with fetal growth and birth weight?	2009	1.4
✓ Generation R Retraite: Growth and Development research group, Rotterdam, the Netherlands	2010	0.3
✓ WEON – Nijmegen, the Netherlands Poster: Maternal caffeine intake, fetal growth and the risks of adverse birth outcomes. Poster: Maternal smoking and blood pressure in different trimesters of pregnancy.	2010	1.4
▪ EUCCONET meeting London Presentation: Tracking sample members in longitudinal studies	2010	0.7

Teaching activities

	Year	Workload (ECTS)
Supervising Master's theses		
▪ **Liane Pluimgraaff**, Biomedical Sciences student, University of Leiden. Project title: Associations of light and moderate maternal alcohol consumption with fetal growth characteristics in different periods of pregnancy.	2008	2.0
▪ **Aleksandra Obradov**, Biochemistry and Molecular Biophysics student , University of Arizona, USA. Project title: Maternal caffeine intake, fetal growth and the risks of adverse birth outcomes.	2009	2.0
▪ **Angelique Bihari**, Medical student, Erasmus University, Rotterdam. Project title: Explaining differences in birth outcomes in relation to maternal age.	2009	2.0
▪ **Romy Gaillard**, Medical student, Erasmus University, Rotterdam. Project title: Maternal age, blood pressure in different trimesters, and the risks of hypertensive disorders during pregnancy & Blood pressure tracking during pregnancy.	2010	4.0
▪ **Guest lecture**, Zorgacademie, Erasmus MC: Wetenschappelijk Onderzoek	2011	0.7

Other skills

	Year
Reviewed for European Journal of Epidemiology	2008 - 2010
Reviewed for BMC Research Notes	2008
Reviewed for Archives of General Psychiatry	2009
Reviewed for Paediatric and Perinatal Epidemiology	2010
Reviewed for Archives of Disease in Childhood	2010
Reviewed for Arteriosclerosis, Thrombosis and Vascular Biology	2010
Reviewed for British Medical Journal	2010
Reviewed for American Journal of Epidemiology	2010
Reviewed for Journal of the American Medical Association	2011
Reviewed for International Journal of Epidemiology	2011
Reviewed for Human Reproduction Update	2011
Reviewed for European Journal of Clinical Nutrition	2011
Reviewed for Epidemiology	2011
Reviewed for Early Human Development	2011

Dankwoord

Als eerste wil ik alle deelnemers van Generation R bedanken. Alle dappere 5-6 jarigen die met hun moeders en/of vaders helemaal naar ons onderzoekscentrum komen, en al jaren zoveel gegevens aan ons doorgeven waardoor dit soort onderzoek mogelijk gemaakt wordt. Bedankt voor jullie inzet, tijd en moeite!

Graag bedank ik mijn promotoren Professor Bert Hofman and Professor Eric Steegers. Beste Bert, bedankt voor de begeleiding en bedankt voor de epidemiologie lessen in Rotterdam en ook in Boston! Ik heb de afgelopen jaren veel geleerd. Beste Eric, bedankt voor de begeleiding, alle (klinisch relevante) onderzoekstips, en mooie onderzoeksideeën. Er ligt nog zo enorm veel data met vele mooie plannen om dit te gaan analyseren; daar gaan we na mijn promotie mee aan de slag. Ik kijk er naar uit.

Mijn kleine commissie, Professor Bert van der Heijden, Professor Johan Mackenbach en Professor Jørn Olsen, wil ik bedanken. Beste Bert en Johan, bedankt voor het lezen van het manuscript en voor jullie aanwezigheid tijdens mijn verdediging. Dear Jørn, thank you very much for your hospitality in Aarhus, the inspiring meetings, and great research ideas! Aarhus was a wonderful experience for me. 'Tag for alt!'

Mijn grote commissie, Professor Simone Buitendijk, Professor Ewout Steyerberg en Professor Gouke Bonsel, wil ik alvast bedanken. Hartelijk dank voor de voorbereiding en aanwezigheid tijdens mijn verdediging.

Tevens wil ik alle co-auteurs bedanken voor alle suggesties tijdens het schrijven van de artikelen.

Alle lieve collega's bij Generation R in het Ae; wat heb ik veel collega's ontmoet, gedag gezegd, en weer opnieuw ontmoet; iets te veel om iedereen hier apart op te noemen. Ik heb een fantastische tijd gehad als zowel datamanager, studiecoördinator, en promovendus. Iedereen bedankt hiervoor! Inclusief mijn kamergenoten op de 21e tijdens mijn eerste tijd bij Generation R. In het bijzonder wil ik mijn nieuwe collega's Natalia en Marian bedanken; ik wist wel dat ik mijn kamer (en taken!) met een gerust hart kon overlaten aan dit sfeervolle duo! De etentjes om bij te kletsen blijven we doen hoor! Ook het datamanagement-team Ronald en Claudia, wat moeten we toch zonder jullie. Bedankt voor alle inzet! Natuurlijk ook mijn kamergenootjes tijdens de laatste maanden; Edith en Denise, bedankt voor de gezelligheid, en sorry voor alle spullen en onrust die ik in het begin heb meegebracht. Verder uiteraard mijn 'G&O borrel' maatjes; Dennis, Nienke, Elise, Miranda en Sarah. Iedere keer iets lekkers eten, wijntje of cocktail erbij, en bijkletsen... dat was een leuke tijd, laten we een poging doen het in stand te houden! Een speciale dank voor Patricia; ik ken niemand behulpzamer en handiger! Bedankt voor de fijne samenwerking. Gaan we van de week mijn lijstje ophangen in de vergaderzaal?! Tot slot, een speciaal bedankje voor de studentes Liane, Aleksandra [Thanks Aleks; I really enjoyed working with you during your time in Holland. Good luck with everything; no doubt all of your plans will be successful!], Angelique en Romy, met wie ik mooie artikelen heb mogen schrijven! In het bijzonder wil ik Romy bedanken; altijd zo hard

werkend en attent, bedankt voor de fijne samenwerking. Ben erg benieuwd naar al je volgende mooie papers!

Lieve Elise, Miran en Saar, ook jullie wil ik bedanken. Van collega's hele fijne vriendinnen geworden. Door dik en dun, altijd in voor een wijntje. En dat moet zo blijven. Dankjewel dat jullie er voor mij zijn.

Dan een zeer grote dank aan alle medewerkers op het onderzoekscentrum; onderzoeksmedewerkers, promovendi en studenten. Na het eerste bezoek van ruim 5 uur [sorry, Micky en Margot...] redden we het nu al zoveel bezoeken (5000+!!) in veel minder tijd. Bedankt voor jullie inzet en enthousiasme met de kinderen; dag in, dag uit. Het is en blijft een teamresultaat om verschrikkelijk trots op te zijn!

Mijn lieve vrienden door heel Nederland, iedereen bedankt. In het speciaal Leonie, vriendschap vanaf onze eerste studiedag in Utrecht, en altijd heb je er vertrouwen in gehad dat het wel goed zou komen met mij en studeren. Dankjewel voor je steun. En kleine Susan, het voorbeeld waarom dit soort onderzoek zo belangrijk is. Wat is ze toch mooi en lief! Ook Brita en Jitske, bedankt voor de warme vriendschap, wat is dat toch fijn.

Speciale dank aan Vincent; bovenal een bijzonder mooie vriendschap en daarnaast een top samenwerking. Ik ben je enorm dankbaar voor alles wat ik van je heb geleerd! Ontelbare track changes... ['tja, dat is mijn enthousiasme' en 'het is een kwestie van smaak...'], maar met zoveel mooi resultaten. Echt, zonder jou had dit boekje er nooit zo uitgezien en was ik nu nog roosterproblemen aan het oplossen. Dankjewel!

Mijn paranimfen; lieve Clau, dankjewel dat je zo'n goede vriendin bent geworden. Ik kan op je bouwen, en ik weet ook dat ik dat voor en tijdens mijn verdediging kan, en uiteraard ook daarna! En na deze inspanning; hoogste tijd voor een wijntje. Lieve zus, dankjewel dat je naast me staat. Ben er trots op! Als beste vriendin is het zo lekker dat je altijd alles kunt bespreken met elkaar. En als ik het op 27 mei echt niet meer weet neem je het over hè, valt vast niet op!

Last but certainly not least, mijn familie; mijn lieve broer ['(Para)nimfen zijn toch meisjes?' Ben het geheel met je eens dat naamgeving wat vreemd is... dus sorry, voor meisjes gekozen inderdaad]. Dankjewel voor je enorm nuchtere kijk op alles en je lieve steun als grote kleine broer. Mijn lieve kleine sista; dankjewel voor je knusheid, geklets, (strenge maar lieve) adviezen en luisterend oor. Lieve Carla en Pascal, mijn extra zus en broer, heb met jullie te doen als het weer zo een chaos kan zijn met z'n allen bij elkaar, maar ben zo blij dat jullie erbij horen. In het bijzonder wil ik mijn lieve ouders enorm bedanken. Met recht bij alle hoogte en diepte punten; altijd staan jullie voor me klaar! Waar ik ook weer mee kom of mee zit, er is altijd begrip en geduld, en zeer waardevol advies. Jullie hebben mij zoveel meegegeven van klein meisje af aan, en nog steeds met grote regelmaat. Ik ben jullie daar erg dankbaar voor. En natuurlijk is er altijd weer interesse voor het feit dat ik weer een 'zo enorm spannend' artikel heb geschreven... Lieve pap, mam, Marcel, Carla, Marinka en Pascal, het blijft heerlijk om met z'n allen te lachen en te dollen, enorm fijn om uit zo'n gezin te komen. Dank jullie wel!

Hoe ver je gaat
Heeft met afstand niets te maken
Hoogstens met de tijd

Hoe diep je gaat
Heeft met denken niets te maken
Hoogstens met een wil

Hoe recht je staat
Heeft met zwaarte niets te maken
Hoogstens met de wind

Bløf