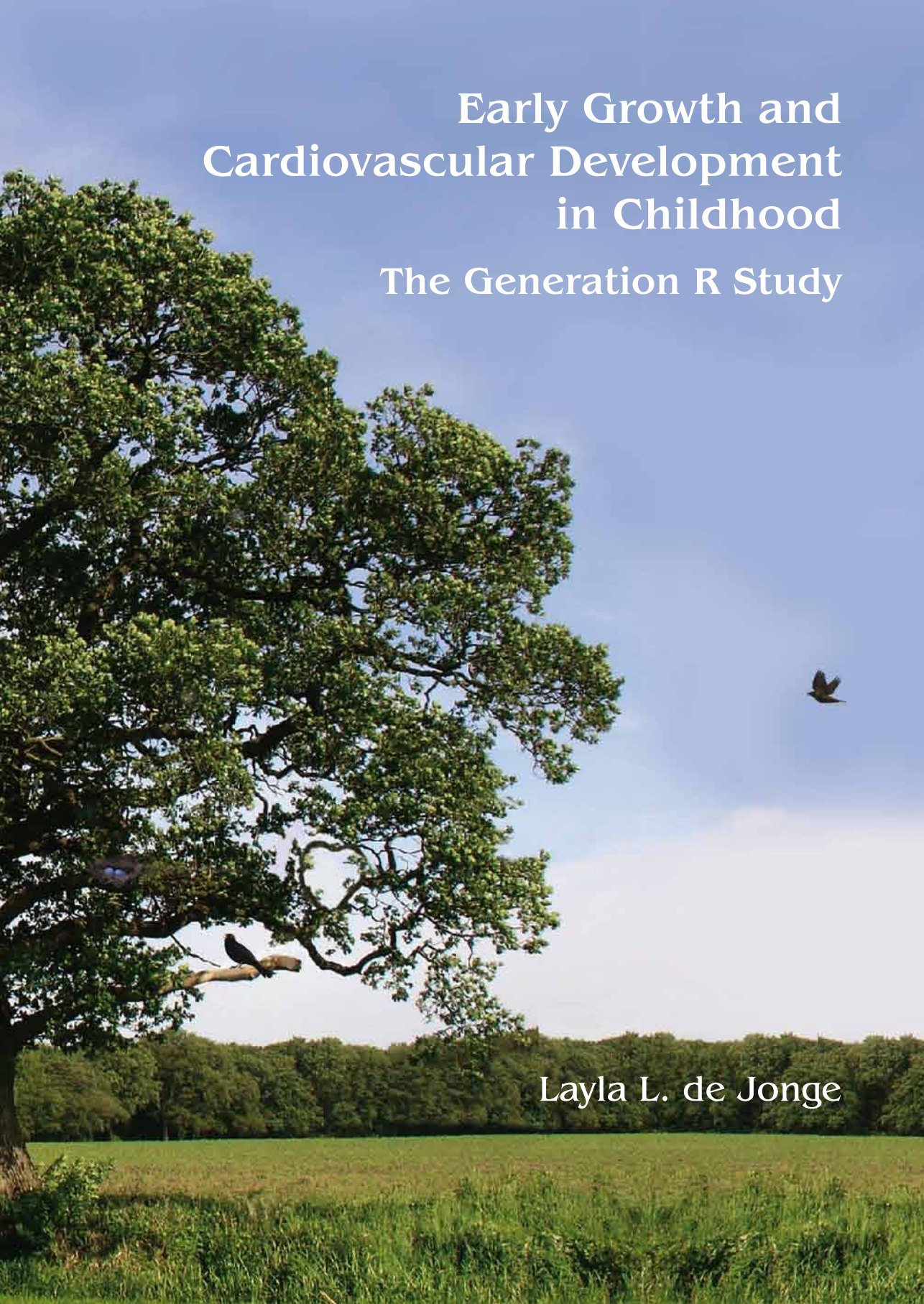
Early Growth and Cardiovascular Development in Childhood

The Generation R Study



Layla L. de Jonge

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Layla Leintje de Jonge

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Early Growth and Cardiovascular Development in Childhood

The Generation R Study

Vroege Groei en de Ontwikkeling van Hart en Bloedvaten in de Kindertijd

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.
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LIST OF ABBREVIATIONS

AC	Abdominal circumference
AGA	Appropriate for gestational age
BMI	Body mass index
BSA	Body surface area
CI	Confidence interval
CO	Cardiac output
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic blood pressure
EFW	Estimated fetal weight
FL	Femur length
GA	Gestational age
HC	Head circumference
Hs-CRP	High sensitivity C-reactive protein
LGA	Large for gestational age
NHS	Nurses' Health Study
OR	Odds ratio
PWV	Pulse wave velocity
RR	Rate ratio
SD	Standard deviation
SDS	Standard deviation score
SES	Socioeconomic status
SGA	Small for gestational age
SBP	Systolic blood pressure

Chapter 1

General introduction

INTRODUCTION

The developmental plasticity hypothesis proposes that environmental exposures, acting at different stages of fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems.[1] This early programming contributes to short-term survival, but increases the susceptibility of cardiovascular disease in adulthood.[1] This hypothesis is supported by observational studies demonstrating that low birth weight, as a measure of reduced growth in utero, is associated with an increased risk of the development of cardiovascular disease in later life.[2] Low birth weight has also been related to cardiovascular risk factors such as hypertension and cholesterol levels, although systematic reviews suggest small effects.[3-4] Birth weight is merely a marker of fetal growth and environment, and does not account for the influence of postnatal growth. Retrospective follow up studies have shown that individuals with a low birth weight and high rates of childhood weight gain have increased risks of cardiovascular disease[5-6], suggesting that fetal and childhood growth are both related to cardiovascular health and disease in later life. The exact growth patterns and underlying biological mechanisms linking fetal and childhood growth with disease in later life are not fully understood.

The increased risk of cardiovascular disease among children with a low birth weight may result from growth induced early developmental adaptations in cardiovascular structures and function. Detailed studies in animals and humans have demonstrated that fetal and childhood growth variation leads to cardiovascular structural and hemodynamic adaptations in early life.[7-14] Decreased fetal growth has been associated with adaptive fetal haemodynamic changes, consistent with an increase in cardiac afterload and compromised arterial compliance.[7] Also, it has been suggested that fetal growth restriction induces primary cardiac and vascular changes in childhood.[14] In addition, there is growing evidence for changes in cardiovascular properties due to factors that influence fetal growth. Maternal smoking during pregnancy has been associated with impaired endothelial function[15], thicker carotid artery intima media thickness and lower arterial distensibility in childhood.[16]

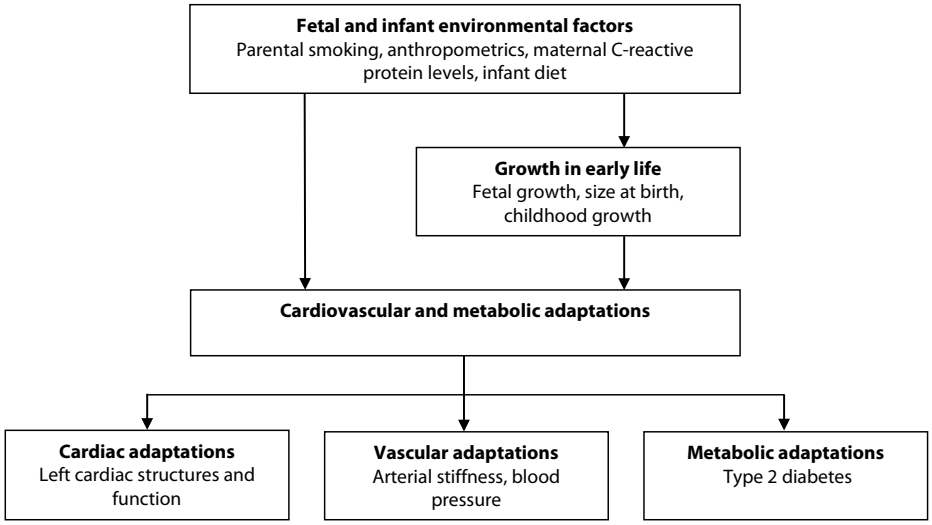
These cardiovascular adaptations due to early growth and its determinants, might partly explain the association between early growth and cardiovascular disease in adulthood. Studies focused on the effect of prospectively measured fetal and childhood growth patterns on cardiovascular development in childhood might therefore extend our knowledge on the origins of cardiovascular disease in the earliest phase of life.

OBJECTIVES

The specific aims of this thesis were to study (Figure 1):

1. Fetal, infant and childhood growth patterns and determinants related to the development of the cardiovascular system in childhood.
2. Early environmental determinants associated with cardiovascular and metabolic development.
3. The associations of inflammation in early pregnancy, as measured by first trimester C-reactive protein levels, with fetal growth and pregnancy complications.

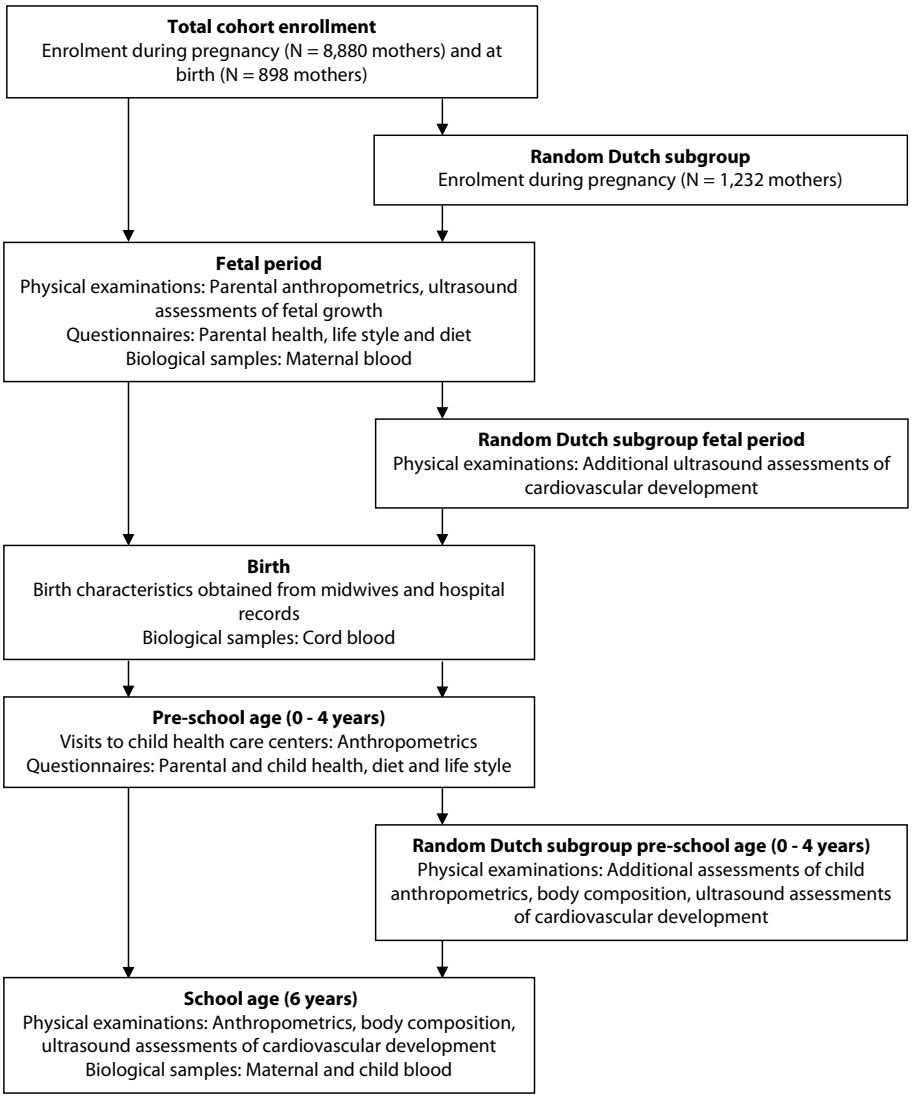
Figure 1. Proposed pathways through which early environment exposures and growth lead to cardiovascular and metabolic adaptations



GENERAL DESIGN

Most studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.[17-18] The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment was aimed at early pregnancy, but

Figure 2. Design and data collection in The Generation R Study



was possible until the birth of the child. In total, 9,778 mothers were enrolled in the study, of whom 8,880 (91%) were included during pregnancy (Figure 2). Assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18 - 25 weeks of gestation) and late pregnancy (≥25 weeks of gestation), and included physical examinations, blood and urine collection, fetal ultrasound examinations, and self-administered questionnaires. In a subgroup of 1,232 Dutch women and their children, more detailed assessments of fetal and postnatal

growth and development were conducted until the child age of 48 months. This subgroup is ethnically homogeneous, excluding confounding or effect modification by ethnicity. In the preschool period from birth to 48 months of age, data collection was performed in all children by questionnaires and visits to the routine child health care centers. At the age of 6 years, all participating children were invited to a dedicated research center in the Erasmus MC -Sophia Children's Hospital. Measurements during this visit included anthropometrics, body composition, cardiovascular development, and body fluid specimen collection.

In addition, two studies were performed in the Nurses' Health Study II.[19] The Nurses' Health Study II is an ongoing prospective cohort study of 116,430 female registered nurses from 15 US states, aged 25 to 42 years, that started in 1989. Participants are followed up via biennial self-administered questionnaires that inquire about health-related lifestyle, anthropometric variables and medical events. The average follow-up for the cohort exceeds 90% for each 2-year period. In 2001, the mothers of approximately 35,794 Nurses' Health Study II participants who were alive, still participating in the study, and had no history of cancer in 2001 were enrolled in the Nurses' Mothers' Cohort.[20] This cohort aimed to collect information on the prenatal, perinatal and early life exposures of the nurse daughters through questionnaires. The information provided by the participating nurses and their mothers has led to many new insights on health and disease.

OUTLINE OF THESIS

The objectives are addressed in several studies presented in this thesis. In **Chapter 2**, the relation between fetal, infant and childhood growth patterns and determinants and cardiovascular development is studied. In **Chapter 2.1**, cardiac reference growth charts until the age of 2 years are presented. **Chapters 2.2** and **2.3** describe the influence of fetal and infant growth, body composition and measures of obesity on cardiovascular development in childhood. **Chapter 2.4** presents the associations of third trimester placental and fetal haemodynamics and cardiovascular structures and function at the child age of 6 years.

In **Chapter 3**, specific early environmental determinants of cardiovascular development are discussed. **Chapter 3.1** addresses the association of parental smoking in pregnancy with cardiovascular development in childhood. **Chapters 3.2** and **3.3** focus on parental smoking in pregnancy and the risk of adult-onset hypertension and type 2 diabetes, respectively. In **Chapter 3.4** the associations of breastfeeding patterns in infancy with cardiovascular structures and function until the age of 24 months are evaluated. The associations of breastfeeding in infancy and the introduction of solid foods with cardiovascular development at the age of 6 years are assessed in **Chapter 3.5**.

Chapter 4 addresses the associations of inflammation in early pregnancy as measured by first trimester C-reactive protein levels, with maternal and neonatal complications. In

Chapter 4.1 the associations of C-reactive protein levels, maternal blood pressure and the risks of gestational hypertensive disorders are presented. **Chapter 4.2** describes the associations of C-reactive protein levels, fetal growth and the risks of neonatal complications.

Finally, **Chapter 5** provides a general discussion of our study and previous studies performed to identify the early determinants of cardiovascular development, health and disease. This discussion concludes with implications and directions for future research.

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Chapter 2

Early growth and cardiovascular development

Chapter 2.1

Reference charts for cardiac structures in children until the age of 24 months

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Chapter 2.2

Growth, obesity and cardiac adaptations in early childhood

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ABSTRACT

Cardiac structural adaptations in response to physical growth and obesity in older children have been identified and might have long term consequences. We examined the associations of growth and obesity with cardiac structures during the first two years of life. In a population-based prospective cohort study among 974 children, left atrial diameter, left ventricular diastolic diameter, left ventricular mass, aortic root diameter and fractional shortening were repeatedly measured by ultrasound at the ages of 1.5, 6 and 24 months. Height, weight and subcutaneous fat mass were measured at the same visits, and blood pressure was measured at the age of 24 months. Height, weight, body mass index and body surface area were positively associated with all cardiac structures during the first two years of life. At the age of 24 months, as compared to normal weight children, obese children had a greater left ventricular mass (1.04 SDS; 95% CI, 0.20 to 1.89), and a higher fractional shortening (0.91 SDS; 95% CI, 0.02 to 1.80). Nonsignificant tendencies were found for left atrial diameter, left ventricular diastolic diameter and aortic root diameter. Our results suggest that normal variation in growth affects cardiac structures in early life. Overweight and obese children show cardiac adaptations already at the age of two years. Further studies are needed to assess whether these structural adaptations influence the risk of cardiovascular disease in later life.

INTRODUCTION

In childhood and adolescence, cardiac dimensions are closely related to physical growth.[1-2] Previous studies have shown associations of height, weight, body mass index (BMI) and body surface area (BSA) with cardiac size in children and adolescents.[3-6] Body composition, especially lean body mass, has also been recognized as an important determinant of cardiac parameters in children.[4, 7-8] Because of the increasing prevalence of obesity in children, much attention has been focused on the influence of obesity on cardiovascular structure and function.[6, 9-11] In school age children and adolescents, both overweight and obesity are associated with structural cardiac changes, such as an increase in left atrial diameter, left ventricular dimensions and mass and an elevated blood pressure, as compared to normal weight individuals.[5-6, 12] Recent studies have also shown preclinical alterations in the aortic elastic properties in obese children.[13] Since cardiac structures track from childhood to adulthood, early cardiac structural adaptations in response to physical growth might have consequences in later life.

Therefore, we assessed in a population-based cohort study among 974 children the associations between growth characteristics, obesity and left cardiac structures during the first two years of life.

MATERIALS AND METHODS

Design and study population

The study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life onwards.[14] This cohort includes mothers, fathers and their children of different ethnicities living in Rotterdam, The Netherlands, and has been described in detail previously.[14] Mothers were enrolled between 2001 and 2005, and all children were born between April 2002 and January 2006. 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. More detailed assessments of fetal and postnatal growth and development were conducted in a random subgroup of 1,098 Dutch children (Supplementary material, Figure S1). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents of participants. The present analysis was limited to singleton live births (N = 1,071). None of these children had congenital heart disease. Detailed information on anthropometrics and body composition was available in 994 children. In 974 children, one or more echocardiographic measurements were performed during the first two years of life.

Child anthropometrics, body composition and blood pressure

Information about child anthropometrics was obtained by measurements at the research center at the ages of 1.5, 6 and 24 months. Length was measured in supine position to the nearest 0.1 cm by a neonatometer (Holtain Limited®) at the age of 1.5 and 6 months, and height was measured in upright position at the age of 24 months. Weight was measured in naked infants to the nearest grams by using an electronic scale (SECA®). BMI (kg/m^2) and BSA (m^2) were calculated.[15] For child BMI, we obtained age adjusted standard deviation scores (SDS) using Dutch reference growth curves (Growth Analyzer 3.0, Dutch Growth Research Foundation, Rotterdam, The Netherlands). Children were categorized as normal weight (SDS BMI <1.10), overweight (SDS BMI 1.10 – 2.29), or obese (SDS BMI ≥2.30) at the age of 24 months as defined by Cole *et al*.[16] Subcutaneous fat mass was measured as biceps, triceps, suprailiacal and subscapular skinfold thickness (SFT) using a standard skinfold caliper (SlimGuide, Creative Health Products®) as previously described.[17] Total subcutaneous fat mass was measured as the sum of biceps and triceps SFT (peripheral subcutaneous fat mass) and suprailiacal and subscapular SFT (central subcutaneous fat mass). At 24 months of age, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured to the nearest mmHg at the left upper arm by using an automatic sphygmomanometer (Vital Signs Monitor CAS 740; CAS Medical Systems, Inc., Branford, Connecticut, USA).[18] The mean of two consecutive measurements was used in the analyses. Blood pressure measurements were successfully performed in 70% of the children. In 215 children, blood pressure was measured only once due to crying and oppositional behavior.

Cardiac outcomes

Two-dimensional M-mode and Doppler echocardiographic measurements were performed using ATL-Philips Model HDI 5000 (Seattle, WA) equipment at the ages of 1.5, 6 and 24 months. In total, 86% of the measurements were performed by a single echocardiographer. The other measurements were performed by two other echocardiographers. The echocardiographers were supervised by a pediatric cardiologist. Echocardiographic measurements were successfully performed in 90%, 92% and 85% of the children examined at 1.5, 6 and 24 months. Missing echocardiograms were mainly due to crying or unavailability of equipment or echocardiographer. Left atrial diameter, aortic root diameter, interventricular end-diastolic septal thickness, left ventricular end-diastolic diameter as a measure of left ventricular end-diastolic volume, left ventricular end-diastolic posterior wall thickness, and shortening fraction were measured and left ventricular mass was calculated as described previously.[19]

Covariates

Gestational age was established by fetal ultrasounds.[20] Date of birth, infant sex and birth weight were obtained from midwife and hospital registries. Information on maternal age, pre-pregnancy weight, parity and smoking habits during pregnancy was obtained by questionnaires. Maternal height was measured without shoes and heavy clothing in the first trimester of

pregnancy. Socio-economic status was defined as highest completed education according to the classification of Statistics Netherlands.[21] Information about breastfeeding was collected by postnatal questionnaires and medical records.

Statistical methods

Associations of child anthropometrics with repeatedly measured cardiac structures and fractional shortening at 1.5, 6 and 24 months of age were first assessed using regression analysis for repeated measures using the Proc Mixed module of SAS. To compare effect estimates for the associations of child anthropometrics and body composition with cardiac structures at different ages, all measurements were converted to SDS. The best fitting models were constructed and the models can be written as:

Cardiac Outcome = $\beta_0 + \beta_1$ x Anthropometric Measurement + β_2 x Child Age + β_3 x Anthropometric Measurement x Child Age + Terms for additional variables

In these models, "$\beta_0 + \beta_1$ x Anthropometric Measurement" reflects the intercept, "β_2 x Child Age" reflects the slope of change in SDS of cardiac structures and fractional shortening per week. The term "β_3 x Anthropometric Measurement x Child Age" reflects the difference in change in SDS of cardiac structures and fractional shortening per week with increasing anthropometrics.

Subsequently, we performed multiple linear regression models to study the associations of blood pressure with cardiac structures at the age of 24 months, and the change in anthropometrics and body composition in the time period of 1.5 months to 24 months with cardiac structures at the age of 24 months, and with the change in cardiac structures in the time period of 1.5 to 24 months. We used the same models to assess the differences in cardiac structures and fractional shortening between normal weight, overweight and obese children. The category of normal weight children was taken as the referent. All analyses were adjusted for child gender, gestational age, birth weight, breastfeeding status, and maternal height, weight, parity, educational level and smoking habits during pregnancy. Multiple linear regression models were additionally adjusted for child age at measurement and analyses with growth rates as determinant were additionally adjusted for time between measurements. For all analyses, missing values were imputed with the mean for continuous variables or with an additional category for categorical variables.

Statistical analyses were performed using the SAS version 9.2 (SAS for Windows Version 9.2; SAS Institute, Cary, NC), including the Proc Mixed module for unbalanced repeated measurements and the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject characteristics

Maternal and child characteristics are shown in Table 1. The overall median gestational age was 40.3 weeks (95% range 35.8 – 42.4 weeks), with a mean birth weight of 3,510 grams (g) (SD 543 g). Descriptives of all cardiac measurements are given in the Supplementary material (Table S1). Overall, boys had a larger aortic root diameter and left ventricular mass at all ages. Of the 701 children who underwent successful echocardiographic examinations at the age of 24 months, 53 were overweight (8.0%) and 5 were obese (0.7%).

Table 1. Subject characteristics

Maternal characteristics	**Measure (N = 974)**
Height (cm)	170.0 (6.3)
Weight (kg)	71.3 (13.1)
Parity (%)	
0	602 (61.8)
≥1	370 (38.0)
Missing	2 (0.2)
Educational level (%)	
Primary school	19 (2.0)
Secondary school	320 (32.9)
Higher education	622 (63.9)
Missing	13 (1.3)
Smoking during pregnancy (%)	
Never	675 (69.3)
First trimester only	79 (8.1)
Continued	116 (11.9)
Missing	104 (10.7)
Child characteristics	
Birth	**(N = 974)**
Gestational age (weeks)	40.3 (35.8 – 42.4)
Boys (%)	499 (51%)
Weight (g)	3,510 (543)
Preterm birth (%)	42 (4.3)
Low birth weight (%)	37 (3.8)
Age 1.5 months	**(N = 861)**
Age (months)	1.5 (1.0 – 2.9)
Length (cm)	57.0 (2.6)
Weight (g)	4942 (710)

Table 1. Subject characteristics *(continued)*

Child characteristics	
Body mass index (kg/m^2)	15.2 (1.4)
Body surface area (m^2)	0.3 (0.0)
Subcutaneous fat mass (mm)	
Total	24.1 (6.7)
Central	12.1 (3.4)
Peripheral	12.1 (3.8)
Age 6 months	**(N = 867)**
Age (months)	6.3 (5.5 – 8.3)
Height (cm)	68.7 (2.6)
Weight (g)	7930 (883)
Body mass index (kg/m^2)	16.8 (1.3)
Body surface area (m^2)	0.4 (0.0)
Subcutaneous fat mass (mm)	
Total	27.2 (5.8)
Central	12.8 (3.2)
Peripheral	14.4 (3.5)
Age 24 months	**(N = 821)**
Age (months)	25.1 (23.7 – 28.2)
Height (cm)	89.0 (3.2)
Weight (g)	12,649 (1,377)
Body mass index (kg/m^2)	15.9 (1.3)
Body surface area (m^2)	0.5 (0.0)
Subcutaneous fat mass (mm)	
Total	27.3 (6.6)
Central	11.7 (3.1)
Peripheral	15.6 (4.4)
Systolic Blood Pressure (mmHg)	101.2 (10.6)
Diastolic Blood Pressure (mmHg)	62.3 (10.8)

Values represent means (SD), medians (95% range) or numbers (%).

Normal growth variation and cardiac structures

Table 2 presents regression coefficients of the associations of child anthropometrics and body composition with the repeatedly measured left cardiac structures and fractional shortening until the age of 24 months. Height, weight, BMI and BSA were positively associated with left ventricular diastolic diameter and left ventricular mass, (all *P* values <0.05) at all ages, and with aortic root diameter at the age of 6 and 24 months. For left atrial diameter, child anthropometrics also showed a positive tendency. At the age of 1.5 months, total subcutaneous fat mass was associated with left atrial diameter and both total and central fat mass were associated

Table 2. Associations of child growth characteristics with cardiac structures and fractional shortening until the age of 24 months

Child anthropometrics and body composition	**Left cardiac structures and fractional shortening**				
1.5 Months	**Left atrial diameter (SD = 1.89 mm)**	**Left ventricular diastolic diameter (SD = 1.89 mm)**	**Left ventricular mass (SD = 3.05 g)**	**Aortic root diameter (SD = 1.16 mm)**	**Fractional shortening (SD = 4.94%)**
Length (SD = 2.62 cm)	0.03 (-0.08, 0.15)	0.13 (0.02, 0.24)*	0.28 (0.17, 0.39)†	0.18 (0.08, 0.29)†	-0.10 (-0.22, 0.02)
Weight (SD = 710 g)	0.22 (0.10, 0.35)†	0.40 (0.29, 0.51)†	0.37 (0.25, 0.49)†	0.20 (0.08, 0.31)†	0.05 (-0.08, 0.18)
BMI (SD = 1.41 kg/m^2)	0.15 (0.06, 0.23)†	0.22 (0.14, 0.30)†	0.13 (0.05, 0.21)†	0.05 (-0.03, 0.12)	0.09 (0.00, 0.17)
BSA (SD = 0.02 m^2)	0.21 (0.07, 0.34)†	0.40 (0.27, 0.52)†	0.45 (0.32, 0.58)†	0.25 (0.13, 0.38)†	-0.02 (-0.16, 0.13)
Subcutaneous fat mass					
Total (SD = 6.66 mm)	0.09 (0.02, 0.17)*	0.00 (-0.07, 0.07)	0.04 (-0.03, 0.12)	-0.01 (-0.08, 0.06)	0.11 (0.03, 0.20)†
Central (SD = 3.40 mm)	0.06 (-0.02, 0.13)	0.02 (-0.05, 0.09)	0.04 (-0.03, 0.12)	0.01 (-0.06, 0.08)	0.13 (0.05, 0.22)†
Peripheral (SD = 3.80 mm)	0.10 (0.02, 0.17)†	0.00 (-0.07, 0.07)	0.03 (-0.04, 0.11)	-0.04 (-0.10, 0.03)	0.08 (0.00, 0.16)
6 Months	**Left atrial diameter (SD = 1.87 mm)**	**Left ventricular diastolic diameter (SD = 1.90 mm)**	**Left ventricular mass (SD = 4.02 g)**	**Aortic root diameter (SD = 1.22 mm)**	**Fractional shortening (SD = 4.69%)**
Length (SD = 2.63 cm)	0.00 (-0.10, 0.09)	0.19 (0.11, 0.28)†	0.26 (0.17, 0.25)†	0.14 (0.05, 0.22)†	-0.10 (-0.20, 0.00)
Weight (SD = 883 g)	0.10 (0.01, 0.19)*	0.20 (0.12, 0.28)†	0.29 (0.21, 0.38)†	0.18 (0.10, 0.27)†	-0.05 (-0.15, 0.04)
BMI (SD = 1.32 kg/m^2)	0.08 (0.01, 0.15)*	0.07 (0.00, 0.14)*	0.12 (0.04, 0.19)†	0.09 (0.03, 0.16)†	0.01 (-0.07, 0.09)
BSA (SD = 0.03 m^2)	0.07 (-0.02, 0.17)	0.25 (0.16, 0.34)†	0.34 (0.25, 0.44)†	0.21 (0.12, 0.29)†	-0.08 (-0.19, 0.02)
Subcutaneous fat mass					
Total (SD = 5.81 mm)	-0.03 (-0.10, 0.04)	-0.05 (-0.12, 0.01)	0.00 (-0.06, 0.07)	0.04 (-0.03, 0.10)	0.05 (-0.03, 0.12)
Central (SD = 3.24 mm)	0.01 (-0.06, 0.08)	0.01 (-0.06, 0.07)	0.01 (-0.06, 0.08)	0.02 (-0.04, 0.09)	0.04 (-0.03, 0.11)
Peripheral (SD = 3.55 mm)	-0.05 (-0.12, 0.02)	-0.07 (-0.13, 0.00)*	0.00 (-0.07, 0.07)	0.04 (-0.03, 0.10)	0.05 (-0.03, 0.12)
24 Months	**Left atrial diameter (SD = 2.44 mm)**	**Left ventricular diastolic diameter (SD = 2.37 mm)**	**Left ventricular mass (SD = 5.56 g)**	**Aortic root diameter (SD = 1.45 mm)**	**Fractional shortening (SD = 4.57%)**
Height (SD = 3.23 cm)	0.09 (0.00, 0.18)*	0.30 (0.22, 0.38)†	0.28 (0.20, 0.36)†	0.29 (0.22, 0.37)†	-0.07 (-0.16, 0.02)
Weight (SD = 1377 g)	0.16 (0.08, 0.25)†	0.31 (0.23, 0.39)†	0.33 (0.25, 0.41)†	0.26 (0.18, 0.34)†	-0.01 (-0.09, 0.08)

Table 2. Associations of child growth characteristics with cardiac structures and fractional shortening until the age of 24 months *(continued)*

Child anthropometrics and body composition	**Left cardiac structures and fractional shortening**				
BMI (SD = 1.27 kg/m^2)	0.11 (0.03, 0.18)†	0.11 (0.04, 0.18)†	0.17 (0.10, 0.25)†	0.07 (0.00, 0.14)*	0.04 (-0.04, 0.12)
BSA (SD = 0.04 m^2)	0.15 (0.06, 0.24)†	0.35 (0.27, 0.43)†	0.37 (0.28, 0.45)†	0.32 (0.24, 0.40)†	-0.04 (-0.13, 0.06)
Subcutaneous fat mass					
Total (SD = 6.55 mm)	0.00 (-0.08, 0.07)	-0.03 (-0.10, 0.04)	0.07 (0.00, 0.14)	-0.01 (-0.07, 0.06)	0.01 (-0.07, 0.09)
Central (SD = 3.13 mm)	0.00 (-0.08, 0.08)	-0.02 (-0.09, 0.05)	0.09 (0.02, 0.16)	0.03 (-0.04, 0.10)	-0.01 (-0.09, 0.07)
Peripheral (SD = 4.36 mm)	-0.01 (-0.08, 0.07)	-0.04 (-0.10, 0.03)	0.02 (-0.05, 0.09)	-0.05 (-0.12, 0.02)	0.02 (-0.05, 0.10)
SBP (SD = 10.63 mmHg)‡	0.08 (-0.01, 0.16)	0.05 (-0.04, 0.13)	0.02 (-0.07, 0.10)	0.04 (-0.04, 0.13)	0.03 (-0.05, 0.12)
DBP (SD = 10.83 mmHg)‡	0.07 (-0.01, 0.16)	0.05 (-0.04, 0.13)	-0.03 (-0.11, 0.06)	0.05 (-0.04, 0.13)	0.02 (-0.07, 0.11)

Values are regression coefficients (95% CI) and reflect the difference in SDS of left cardiac structure and fractional shortening per SDS of child anthropometrics and body composition, based on repeated measures regression models. Models are adjusted for child gender, gestational age, birth weight, breastfeeding status, age at visit, and maternal height and weight at intake, parity, educational level and smoking habits during pregnancy. *P <0.05 †P <0.01 ‡Regression coefficients of SBP and DBP are based on multiple linear regression models.

Table 3. Associations of child growth between the ages of 1.5 months to 24 months with left cardiac structures and fractional shortening at 24 months of age

Change in child anthropometrics and body composition	Left atrial diameter (SD = 2.44 mm)	Left ventricular diastolic diameter (SD = 2.37 mm)	Left ventricular mass (SD = 5.56 g)	Aortic root diameter (SD = 1.45 mm)	Fractional shortening (SD = 4.57%)
Height (SD = 3.07 cm)	0.13 (0.02, 0.23)*	0.30 (0.21, 0.40)†	0.22 (0.12, 0.32)†	0.20 (0.11, 0.30)†	-0.02 (-0.13, 0.09)
Weight (SD = 1290 g)	0.17 (0.07, 0.26)†	0.27 (0.19, 0.36)†	0.31 (0.23, 0.40)†	0.23 (0.14, 0.31)†	0.04 (-0.06, 0.13)
BMI (SD = 1.67 kg/m^2)	0.06 (-0.03, 0.15)	0.08 (-0.01, 0.17)	0.19 (0.10, 0.28)†	0.08 (0.00, 0.17)	0.04 (-0.06, 0.13)
BSA (SD = 0.03 m^2)	0.17 (0.07, 0.27)†	0.33 (0.24, 0.42)†	0.35 (0.25, 0.44)†	0.26 (0.16, 0.35)†	0.01 (-0.09, 0.12)
Subcutaneous fat mass					
Total (SD = 9.00 mm)	-0.07 (-0.16, 0.02)	-0.08 (-0.17, 0.00)	0.00 (-0.09, 0.09)	0.05 (-0.03, 0.13)	-0.03 (-0.12, 0.07)
Central (SD = 4.26 mm)	-0.06 (-0.15, 0.03)	-0.07 (-0.16, 0.01)	0.02 (-0.07, 0.11)	0.09 (0.01, 0.18)*	-0.02 (-0.11, 0.08)
Peripheral (SD = 5.80 mm)	-0.05 (-0.14, 0.04)	-0.09 (-0.17, -0.01)*	-0.02 (-0.11, 0.06)	-0.01 (-0.09, 0.08)	-0.03 (-0.12, 0.06)

Abbreviations: BMI: Body mass index, BSA: Body surface area. Values are regression coefficients (95% CI) and reflect the change in SDS of left cardiac structure and fractional shortening per change in SDS of increase in child anthropometrics and body composition. Models are adjusted for child gender, gestational age, birth weight, breastfeeding status, age at 24 months visit, time between moments of measurement and maternal height and weight at intake, parity, educational level and smoking habits during pregnancy. *P <0.05 †P <0.01

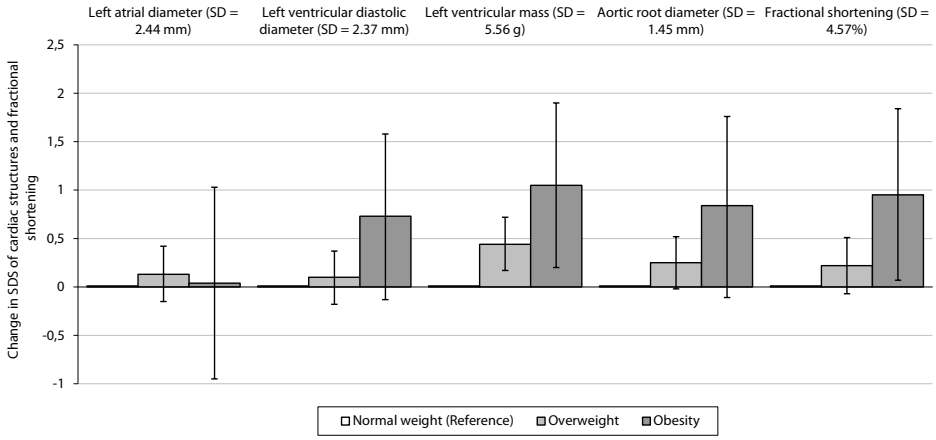
with fractional shortening. At older ages, no consistent associations were observed between subcutaneous fat mass and cardiac structures. No association with SBP or DBP measured at 24 months with any of the cardiac structures or fractional shortening was found.

Table 3 shows that gain in height, weight and BSA between the ages of 1.5 and 24 months were positively associated with left atrial diameter, aortic root diameter, left ventricular mass and left ventricular diastolic diameter at the age of 24 months. The increase in BSA was the strongest determinant, with 0.17 SDS (95% CI, 0.07 to 0.27), 0.33 SDS (95% CI, 0.24 to 0.42), 0.35 SDS (95% CI, 0.25 to 0.44) and 0.26 SDS (95% CI, 0.16 to 0.35) change in left atrial diameter, left ventricular diastolic diameter, left ventricular mass and aortic root diameter respectively per SDS change in BSA. A higher weight gain and increase in BSA tended to be associated with cardiac growth, particularly with left ventricular mass, whereas height gain between 1.5 and 24 months showed a strong association with aortic root diameter growth rate (Supplementary material, Table S2). The effect estimates for the associations between growth characteristics and cardiac structures at 24 months were not materially affected by adjustment for blood pressure (data not shown).

Overweight, obesity and cardiac structures

Figure 1 shows the differences of left cardiac structures and fractional shortening between normal weight, overweight and obese children. As compared to normal weight children, left ventricular mass was significantly greater in both overweight (0.44 SDS, 95% CI, 0.17 to 0.71)

Figure 1. Left cardiac structures and fractional shortening in normal, overweight and obese children at 24 months of age



Differences in cardiac structures and fractional shortening SDS between overweight and obese children, as compared to the reference group (normal weight). Adjustments were made for child gender, gestational age, birth weight, breastfeeding status, age at visit, and maternal height and weight at intake, parity, educational level and smoking habits during pregnancy.

and obese (1.04 SDS; 95% CI, 0.20 to 1.89) children. Nonsignificant tendencies were found for left atrial diameter, left ventricular diastolic diameter and aortic root diameter. The cardiac contractility indicated by fractional shortening was similar in normal and overweight children, and was higher in obese children (0.91 SDS; 95% CI, 0.02 to 1.80). SBP and DBP did not differ between the normal weight, overweight and obese children.

DISCUSSION

In this population-based prospective cohort study, we observed positive associations of weight, height, BMI and BSA with left atrial diameter, aortic root diameter, left ventricular diastolic diameter and left ventricular mass during the first two years of life. Furthermore, this study provides evidence that overweight and obesity already in early childhood exert an influence on the cardiac size.

Strengths and Limitations

A major strength of this study was its prospective design in a large cohort of children. Because of the repeated measurements, we were able to investigate the effect of early growth rates on the left cardiac structures. A limitation might be that information on left cardiac structures was missing in 10%, 8% and 15% of the children measured at the ages of 1.5, 6 and 24 months, respectively. Blood pressure measurements were successfully obtained in only 70% of the children. Missing echocardiographic or blood pressure measurements could lead to both selection bias and loss of power. Our results would be biased if the associations between anthropometrics, blood pressure and cardiac structures and function differ between those included and not included in the study. This seems unlikely, but cannot be excluded. Although we adjusted the regression models for several potential confounders, residual confounding might be an issue, as in any observational study. No information was available about dietary patterns, except for breastfeeding status. However, other components of child diet may influence the relationship of anthropometric measures and cardiac structures. More detailed information about child nutrition, including macronutrients and micronutrients intake, during the first two years would provide additional information.

Growth and Cardiac Structures

In children and adolescents, cardiac growth is influenced by physical growth, including body size and body composition. Results from the Bogalusa Heart study showed in 160 healthy children and young adults aged 9 to 22 years a strong association between height and left ventricular mass.[3] The same study demonstrated that there is a positive association between left ventricular mass in childhood and adolescence across BMI quartiles.[22] Various studies observed that body composition measured by SFT, bioelectric impedance or dual energy X-ray

absorptiometry, was also closely related to cardiac size in childhood and adolescence.[2-3, 7-8] Results from the Muscatine Study indicated fat free mass as a determinant of cardiac size and growth during adolescence[8], and another study among children supported the findings that lean tissue mass was a strong predictor in the youth.[7] In our study, we observed no consistent associations of subcutaneous fat mass measures with cardiac structures during the first two years of life. These results seem to be in contrast to the associations of BMI with left cardiac structures. BMI represents both fat free mass and fat mass though, and lean body mass might also be elevated in obese individuals. Our results are in line with the hypothesis that the demands of lean body mass are the primary determinants of left ventricular mass in children.[7]

In normal and hypertensive children, SBP and left ventricular mass are shown to be positively associated across a wide range of blood pressure values.[7, 11-12, 23] Sorof and colleagues observed left ventricular hypertrophy in 27% of adolescents with systolic hypertension, and that patients with left ventricular hypertrophy had a greater BMI than those without hypertrophy. These observations suggest that left ventricular hypertrophy can occur early in the course of hypertension in young individuals and that the combination of obesity and hypertension enhances the risk for adverse cardiovascular outcomes.[23] However, in our study, there was no difference in SBP or DBP between the normal weight, overweight and obese children, and the effect estimates for the associations between growth characteristics and cardiac structures were not materially affected by adjustment for blood pressure.

Obesity and Cardiac Structures

The recognition of obesity as an important determinant of cardiac remodeling, has led to increased interest in the influence of overweight and obesity on early cardiovascular development.[6, 9-12] In the Strong Heart Study among adolescents aged 14 to 20 years, both overweight and obese participants had a greater left ventricular diameter and mass than normal weight adolescents.[6] Di Salvo demonstrated that obesity, in absence of hypertension, was associated with significant reduction in systolic myocardial deformation properties in childhood, involving both the right and left ventricle.[10] In our cohort, we showed that overweight and obesity exert a significant influence on left cardiac structures already in early childhood. Severe obesity is also associated with arterial wall stiffness and endothelial dysfunction in children.[24] Obese children have a larger abdominal aortic diameter and increased aortic stiffness compared to normal weight children, suggesting that preclinical changes in the aortic elastic properties are already early detectable.[13] The present study showed a nonsignificant tendency towards a larger aortic root diameter in overweight and obese children.

Mechanisms

The associations of obesity with cardiac structures in childhood may be explained by various interrelated hemodynamic, metabolic, neurohormonal, and genetic mechanisms. In adulthood, obesity related hypertension creates a continuous pressure overload[25], inducing concentric

left ventricular hypertropy.[26] Elevated blood pressure and an increase in left ventricular mass have been previously described in obese children.[11-12] In our study, the associations between growth and cardiac structures were not explained by blood pressure. In normotensive patients, obesity is related to an elevated cardiac output and chronic volume overload[27-29], leading to an eccentric left ventricular hypertrophy. These changes are most likely due to the increased metabolic demand of the higher lean and fat mass in obesity[29], what might establish an effect already in early childhood. Another possible pathway that links obesity with cardiovascular remodeling, is through its association with insulin resistance and hyperinsulinemia. Studies in children found significant partial correlations of insulin with left ventricular mass and percentage of fat, suggesting that insulin may mediate the relation of fatness to cardiac structures.[30] In addition, obesity is related to a stimulated sympathetic outflow and inappropriately activated renin-angiotensin system.[29, 31-32] These neurohormonal mechanisms may induce hemodynamic changes such as peripheral vasoconstriction and volume retention. Finally, the influence of body size and obesity on left cardiac structures might be explained by a common genetic mechanism that modulates both somatic and cardiac growth.[33] The mechanisms that are associated with cardiac remodeling in childhood and adulthood remain subject to further investigation.

Perspectives

Cardiac structural adaptations in response to physical growth and obesity might have consequences in later life. This study demonstrated that physical growth is an important determinant of cardiac development in infancy and early childhood. Furthermore, the results of this study indicate that obesity is not only a risk factor for cardiovascular disease in later life, but also affects cardiac development as early as from the age of two years. Our results suggest that left cardiac size and function in adulthood have at least part of their origin in infancy and early childhood. Further studies are needed to investigate the possible influence of early cardiac growth and adaptations on the risk of cardiovascular disease in later life.

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SUPPLEMENTARY MATERIAL

Table S1. Cardiac structures and fractional shortening at the ages of 1.5, 6 and 24 months

Echocardiographic measurements	Boys	Girls	*P* value
1.5 Months	**(N = 402)**	**(N = 376)**	
Left atrial diameter (mm)	17.0 (1.9)	16.6 (1.9)	<0.01
Left ventricular diastolic diameter (mm)	22.5 (1.8)	21.5 (1.9)	<0.01
Left ventricular mass (g)	15.2 (3.1)	13.8 (2.8)	<0.01
Aortic root (mm)	12.0 (1.2)	11.5 (1.1)	<0.01
Fractional shortening (%)	35.4 (4.8)	35.4 (5.1)	0.92
6 Months	**(N = 420)**	**(N = 381)**	
Left atrial diameter (mm)	18.0 (1.9)	17.9 (1.8)	0.59
Left ventricular diastolic diameter (mm)	25.6 (1.8)	24.7 (1.9)	<0.01
Left ventricular mass (g)	20.3 (4.1)	18.4 (3.7)	<0.01
Aortic root (mm)	13.9 (1.2)	13.4 (1.2)	<0.01
Fractional shortening (%)	37.2 (4.6)	37.1 (4.8)	0.72
24 Months	**(N = 358)**	**(N = 343)**	
Left atrial diameter (mm)	20.7 (2.5)	20.5 (2.4)	0.23
Left ventricular diastolic diameter (mm)	31.9 (2.4)	30.9 (2.3)	<0.01
Left ventricular mass (g)	32.5 (5.7)	30.0 (5.1)	<0.01
Aortic root (mm)	16.7 (1.4)	16.0 (1.4)	<0.01
Fractional shortening (%)	35.4 (4.6)	35.4 (4.6)	0.99

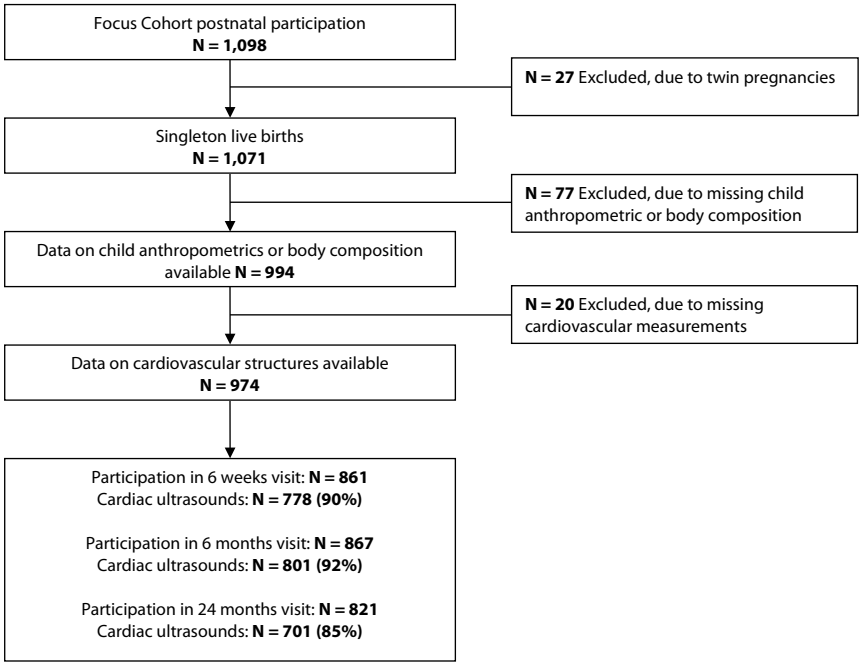
Values represent means (SD). Differences between boys and girls were compared using independent samples t-test.

Table S2. Associations of child growth characteristics with cardiac growth from 1.5 months to 24 months of age

Change in child anthropometrics and body composition	Increase in left atrial diameter (SD = 2.88 mm)	Increase in left ventricular diameter (SD = 2.45 mm)	Increase in left ventricular mass (SD = 5.35 g)	Increase in aortic root diameter (SD = 1.44 mm)
Increase in height (SD = 3.07 cm)	0.05 (-0.06, 0.16)	0.28 (0.17, 0.40)†	0.23 (0.10, 0.35)†	0.25 (0.14, 0.35)†
Increase in weight (SD = 1290 g)	0.10 (0.00, 0.20)	0.19 (0.09, 0.30)†	0.29 (0.19, 0.39)†	0.22 (0.12, 0.32)†
Increase in BMI (SD = 1.67 kg/m^2)	0.08 (-0.02, 0.18)	0.07 (-0.03, 0.18)	0.24 (0.13, 0.34)†	0.08 (-0.02, 0.18)
Increase in BSA (SD = 0.03 m^2)	0.09 (-0.01, 0.20)	0.24 (0.13, 0.35)†	0.33 (0.22, 0.44)†	0.26 (0.15, 0.36)†
Increase in subcutaneous fat mass				
Total (SD = 9.00 mm)	0.02 (-0.07, 0.12)	-0.13 (-0.23, -0.03)*	0.04 (-0.07, 0.14)	0.01 (-0.08, 0.11)
Central (SD = 4.26 mm)	-0.01 (-0.11, 0.09)	-0.14 (-0.24, -0.04)†	0.04 (-0.07, 0.15)	0.08 (-0.02, 0.18)
Peripheral (SD = 5.80 mm)	0.06 (-0.04, 0.15)	-0.09 (-0.19, 0.00)	0.04 (-0.06, 0.14)	-0.04 (-0.14, 0.05)

Values are regression coefficients (95% CI) and reflect the change in SDS of increase in left cardiac structure per change in SDS of increase in child anthropometrics and body composition. Models are adjusted for child gender, gestational age, birth weight, breastfeeding status, age at 24 months visit, time between moments of measurement, and maternal height and weight at intake, parity, educational level and smoking habits during pregnancy. *P <0.05 †P <0.01

Figure S1. Flow chart of the participants included in the analyses



Chapter 2.3

Fetal and infant growth patterns and cardiovascular development in children

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Submitted for publication

Chapter 2.4

Third trimester placental and fetal haemodynamics and cardiovascular outcomes in childhood

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Chapter 3

Environmental influences on cardiovascular development

Chapter 3.1

Parental smoking in pregnancy and cardiovascular structures and function in childhood

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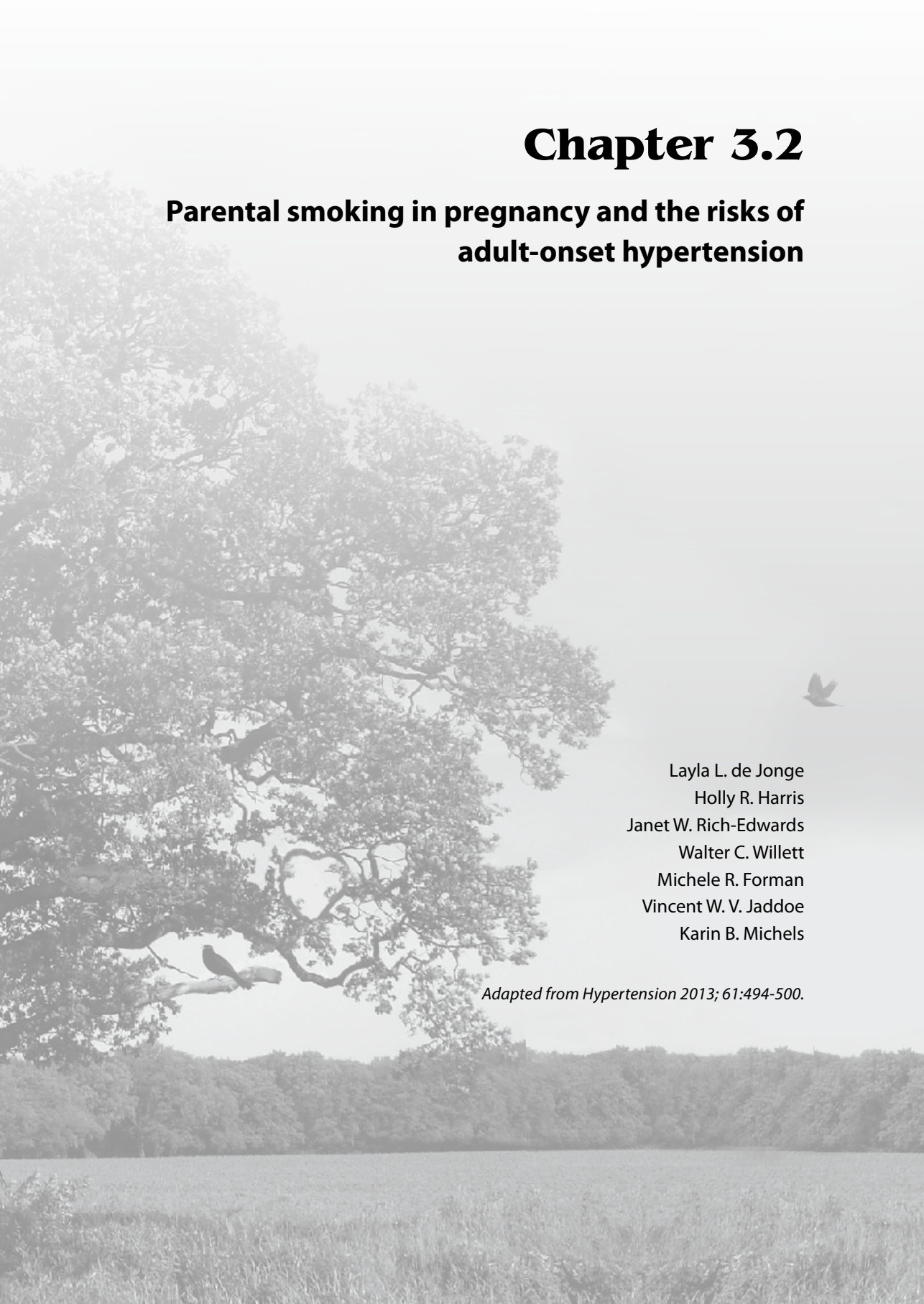
Chapter 3.2

Parental smoking in pregnancy and the risks of adult-onset hypertension



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ABSTRACT

Fetal exposure to parental smoking may lead to developmental adaptations and promote various diseases in later life. This study evaluated the associations of parental smoking during pregnancy with the risk of hypertension in the daughter in adulthood, and assessed whether these associations are explained by birth weight or body weight throughout life. We used data on 33,086 participants of the Nurses' Health Study II and the Nurses' Mothers' Cohort. Cox proportional hazards models were used to examine the associations of maternal and paternal smoking during pregnancy with the nurse daughter, with self-reported physician-diagnosed hypertension from 1989 until 2007. Overall, 8,575 (25.9%) mothers and 18,874 (57.0%) fathers smoked during pregnancy. During follow-up, 7,825 incident cases of adult-onset hypertension were reported. Both maternal and paternal smoking of ≥15 cigarettes/day during pregnancy were associated with increased risks of hypertension (RR 1.19, 95% CI 1.09 to 1.29, and RR 1.18, 95% CI 1.12 to 1.25, respectively) in the age-adjusted models. Further adjustment for birth weight did not affect the effect estimates appreciably, while additional adjustment for body shape and weight until age 18, or current body mass index, attenuated the associations with both maternal and paternal smoking (RR 1.07, 95% CI 0.98 to 1.16, and RR 1.06, 95% CI 1.01 to 1.12, respectively). The associations of parental smoking during pregnancy with the risk of hypertension in the offspring were largely explained by body weight throughout life, suggesting that these associations may not reflect direct intrauterine mechanisms.

INTRODUCTION

Fetal developmental adaptations to a suboptimal intrauterine environment may affect the structure, physiology and function of various organ systems. These adaptations lead to a better short-term survival through fetal growth retardation, but may increase the susceptibility of cardiovascular disease in adulthood.[1] This hypothesis is supported by various studies reporting consistent associations between lower birth weight and an elevated blood pressure in later life.[2-4] Indeed, birth weight is a product of genetic and environmental factors affecting fetal growth and gestation length, but the specific intrauterine exposures and mechanisms linking low birth weight to an elevated risk of hypertension are not well understood.

Parental smoking during pregnancy is an established risk factor of a compromised intrauterine environment and might lead to developmental adaptations influencing postnatal blood pressure levels. Epidemiologic studies of parental smoking during pregnancy and blood pressure levels in offspring have demonstrated inconsistent results[5-14], and the mechanisms through which prenatal smoke exposure might program blood pressure in later life are unclear. Prenatal smoke exposure is associated with reduced birth weight and increased risk of overweight in both childhood and adulthood. (Holly R. Harris, ScD, Walter C. Willett, MD, DrPH, Karin B. Michels, ScD, PhD, unpublished data, 2012)[14-16] Birth weight is inversely associated with blood pressure in later life[2-4], and overweight in adulthood is positively associated with the risk of hypertension, as demonstrated in Framingham Heart Study participants.[17] Therefore, birth weight and body weight throughout life might mediate the association between maternal smoking and blood pressure development. Thus far, the majority of studies has been conducted in child populations, and has mainly focused on the effect of maternal smoking only on blood pressure development. The impact of parental smoking during pregnancy on the risk of adult hypertension is unclear.

Therefore, the aims of this study were to assess the associations of maternal and paternal smoking during pregnancy with hypertension in adulthood, and to examine whether birth weight or body weight throughout life explain these associations, in a large cohort of US women.

METHODS

Study population

The Nurses' Health Study II (NHS II) is an ongoing prospective cohort study of 116,430 female registered nurses that started in 1989.[18] Participants are followed up via biennial self-administered questionnaires that inquire about health-related lifestyle, anthropometric variables and medical events. In 2001, the mothers of approximately 35,794 NHS II participants were enrolled in the Nurses' Mothers' Cohort.[19] The participating mothers completed a questionnaire on the

prenatal, perinatal and early-life exposures of the nurse daughters. The 113 Nurses who were adopted were excluded from the study, as were 1,013 nurses without information about maternal and paternal smoking during pregnancy with the nurse daughter. The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard School of Public Health, Boston, USA, and the National cancer Institute, Bethesda, Maryland, USA.

Assessment of parental smoking

Information on smoke exposure in utero was collected on the 2001 Nurses' Mothers' Cohort questionnaire.[20] Mothers were asked to report if they ever smoked cigarettes during pregnancy with the nurse daughter, the number of cigarettes they smoked daily during pregnancy, whether they stopped smoking during pregnancy, and if so, during which trimester. Maternal smoking was categorized as never smoked; stopped smoking in first trimester of pregnancy, continued smoking <15 cigarettes/day during pregnancy; and continued smoking ≥15 cigarettes/day during pregnancy. The questionnaire also inquired if the nurse's father ever smoked during pregnancy and the number of cigarettes he smoked. Paternal smoking was categorized as never smoked; smoked <15 cigarettes/day during pregnancy; and smoked ≥15 cigarettes/day during pregnancy. To investigate the association of both parents smoking independent and combined with the risk of hypertension, maternal and paternal smoking during pregnancy data were categorized into no parental smoking during pregnancy; only paternal smoking during pregnancy; and maternal smoking only and both parents smoking during pregnancy.

Ascertainment of hypertension

The baseline and biennial follow-up questionnaires of the NHS II asked participants about physician-diagnosed hypertension and the year of diagnosis. Self-reported hypertension was previously validated in the NHS II.[21] Review of the medical records of a randomly selected subset of participants, suggested a sensitivity of self-reported hypertension of 94% and a specificity of a nurse reporting no diagnosis of hypertension of 85%. For the current analysis, 1,582 women who reported physician-diagnosed hypertension at baseline (1989) were considered to have early onset, prevalent hypertension. Incident cases included participants who first reported hypertension on any of the subsequent questionnaires and whose date of diagnosis was after the return of the 1989 baseline NHS II questionnaire. For the analyses on incident cases, prevalent cases were excluded from the analysis, as the exact onset of their hypertension could not be dated.

Covariates

Information about covariates was obtained from the Nurses' Mothers' Cohort as well as the NHS II questionnaires. The 2001 Nurses' Mothers' Cohort questionnaire collected data on the nurse daughter's gestational age, birth weight and breastfeeding status, maternal and paternal age at

birth of the nurse daughter, educational level, occupation and home ownership at the time of the daughter's birth, maternal pre-pregnancy weight, paternal weight, parental height and the occurrence of pregnancy complications (preeclampsia and eclampsia) during the pregnancy of the nurse daughter. The 1989 NHS II questionnaire assessed age, height, weight at age 18, self-classified race of the nurse, and family history of hypertension at baseline. To assess body fatness in childhood, a nine-level figure drawing originally developed by Stunkard[22] was used to ascertain body shape at ages 5 and 10. Few participants reported their body fatness as greater than level 5 at ages 5 and 10, and therefore figures 5 - 9 were combined into a single category. Nurses' weight, smoking status and oral contraceptive use were additionally ascertained from the 1989 NHS II questionnaire and were updated with data from each biennial questionnaire cycle. Body mass index (BMI) at age 18 and current BMI were calculated by dividing the participants' reported weight in kilograms by height in meters squared as assessed in 1989. A Dietary Approaches to Stop Hypertension (DASH) score was calculated from the 1991 semi-quantitative food frequency questionnaire and updated every 4 years.[23] The DASH score was constructed based on foods and nutrients emphasized or minimized in the DASH diet[24], focusing on 8 components: high intake of fruits, vegetables, nuts and legumes, low-fat dairy products and whole grains, and low intake of sodium, sweetened beverages, and red and processed meats. Information on alcohol intake was also retrieved from the 1991 semi-quantitative food frequency questionnaire and updated every 4 years. Physical activity was expressed in metabolic equivalent task scores, and was ascertained in 1989, 1993, 1997, 2001 and 2005.

Statistical methods

Cox proportional hazards regression models were used to estimate the rate ratios (RR's) and 95% confidence intervals (CI's) of the risk of incident hypertension by maternal and paternal smoking. Participants contributed follow-up time from the return of the 1989 questionnaire to the report of physician diagnosed hypertension, death, or end of follow-up on June 1, 2007. To investigate the association of parental smoking on early onset hypertension, logistic regression models were used to estimate the odds ratio's (OR's) and 95% CI's of prevalent hypertension in 1989 by maternal and paternal smoking. We considered birth weight, childhood somatotype and BMI in adolescence and adulthood as potential explanatory variables for the associations of maternal and paternal smoking during pregnancy with the risk of hypertension.

Regression models were adjusted for age (Model 1), and in addition for perinatal variables (maternal and paternal age at delivery, maternal pre-pregnancy BMI, paternal BMI, maternal and paternal educational level, occupation, house ownership at the time of the daughter's birth, and the occurrence of pregnancy complications (preeclampsia and eclampsia) during the mothers' pregnancy, nurses' ethnicity, gestational age, breastfeeding status and family history of hypertension) (Model 2), for birth weight (Model 3), and for adult life variables (nurses' husbands' educational level, pretax household income, smoking, oral contraceptive use, height, DASH score, alcohol intake and physical activity) (Model 4). To examine whether body weight

throughout life explained the associations, we additionally adjusted Model 4for body shape of the nurses at age 5, body shape at age 10 and BMI at age 18 (Model 5) or current BMI (Model 6). The proportion of the associations potentially explained by current BMI was estimated using a SAS macro that calculates the point and interval estimates of the percent of exposure effect explained by the intermediate variable (Mediate SAS; Harvard School of Public Health; available at http://www.hsph.harvard.edu/faculty/donna-spiegelman/software/mediate/).[25]To investigate the association of both parents smoking independent and combined with the risk of hypertension, we compared the effect estimates of smoking by father only (N = 12,165), and mother only and both parents combined (N = 8,575), with no parental smoking during pregnancy (N = 12,346). Trend tests were performed across the level of the number of cigarettes smoked. Effect modification by current BMI was assessed with a likelihood ratio test comparing the model with the cross-product term between the exposure variable and the potential effect modifier, to the model with main effects only. Because time of observation was between the return of the NHS II questionnaire in 1989 and the NHS II questionnaire in 2007 and exposure was assessed in 2001, both prospective and retrospective analyses were combined in the analyses on incident hypertension. Therefore, sensitivity analyses were performed restricting

Table 1. Age-standardized characteristics of the study population of the mothers, fathers and nurse participants of the Nurses' Health Study II Cohort by maternal smoking during pregnancy

Subject characteristics	**Maternal smoking during pregnancy**			
	Nonsmoker (N = 24,511)	Quit smoking in first trimester (N = 1,092)	Continued smoking <15 cig/d (N = 4,232)	Continued smoking ≥15 cig/d (N = 2,626)
Maternal				
Age at daughter's birth (years)	26.5 (5.1)	25.4 (4.4)	26.2 (4.7)	25.8 (4.6)
BMI before pregnancy (kg/m^2)	21.4 (2.6)	20.9 (2.3)	20.9 (2.4)	21.0 (2.6)
Attended college (%)	36	42	41	38
Professional occupation (%)	3	4	3	3
Paternal				
Age at daughter's birth (years)	29.2 (5.7)	28.1 (5.3)	29.1 (5.4)	28.9 (5.3)
BMI (kg/m^2)	23.6 (2.8)	23.5 (2.8)	23.6 (2.7)	23.5 (2.8)
Attended college (%)	41	50	49	47
Professional occupation (%)	30	34	33	35
Smoked during pregnancy (%)	50	74	79	80
Family owned house at daughter's birth (%)	49	38	44	43
Pregnancy and childhood				
Preeclampsia or eclampsia during pregnancy (%)	4	4	3	3

Table 1. Age-standardized characteristics of the study population of the mothers, fathers and nurse participants of the Nurses' Health Study II Cohort by maternal smoking during pregnancy *(continued)*

Subject characteristics	**Maternal smoking during pregnancy**			
	Nonsmoker (N = 24,511)	Quit smoking in first trimester (N = 1,092)	Continued smoking <15 cig/d (N = 4,232)	Continued smoking ≥15 cig/d (N = 2,626)
Gestational age at birth (weeks)	39.4 (2.3)	39.5 (2.4)	39.3 (2.4)	39.2 (2.5)
Birth weight (g)	3,336 (502)	3,258 (510)	3,162 (512)	3,113 (512)
Ethnicity white (%)	96	96	97	98
Family history of hypertension (%)	50	54	49	50
Breastfed in infancy (%)	56	46	43	44
Body shape at age 5 <level 5 (%)	94	93	93	93
Body shape at age 10 <level 5 (%)	89	89	87	88
BMI at age 18 (kg/m^2)	20.9 (3.0)	21.1 (3.0)	21.3 (3.1)	21.5 (3.4)
Adulthood (baseline, 1989)				
Age in 1989 (years)*	34.3 (4.7)	33.8 (4.5)	34.0 (4.5)	33.2 (4.4)
Smoking				
Never (%)	71	62	61	62
Past (%)	20	25	25	23
Current (%)	9	13	14	15
Oral contraceptive use				
Never (%)	17	13	15	15
Past (%)	68	72	70	70
Current (%)	15	15	14	14
Pretax household income >75,000 dollar (%)	57	61	59	60
Husband attended college (%)	82	83	84	83
Height (cm)	165 (7.0)	165 (7)	165 (7)	164 (7)
BMI (kg/m^2)	23.3 (4.4)	23.5 (4.5)	23.6 (4.5)	24.0 (4.7)
Physical activity (METs/wk)†	24.1 (34.1)	24.4 (31.9)	25.0 (34.6)	26.1 (39.1)
Alcohol intake (g/day)	2.9 (5.7)	3.6 (5.4)	3.8 (6.5)	3.7 (6.9)
DASH score	24.0 (5.1)	24.2 (5.1)	24.1 (5.1)	23.8 (5.2)

Abbreviations: BMI, body mass index; METs, metabolic equivalents; and DASH, Dietary Approaches to Stop Hypertension

Values are means (SD) unless otherwise indicated

*Value is not age adjusted

†Metabolic equivalents from recreational and leisure-time activities

follow-up to the prospective component, between the return of the NHSII Questionnaire 2001 (exposure assessment) and the NHSII Questionnaire 2007 (outcome assessment).

All statistical analyses were performed using the Statistical Analysis System (SAS) statistical software version 9.2 (SAS Institute Inc, Cary, North Carolina).

RESULTS

1,582 Women reported physician-diagnosed hypertension at baseline (1989). During 533,902 person-years of follow up, 7,825 incident cases of physician-diagnosed hypertension were reported. Participant characteristics by maternal smoking during pregnancy are presented in Table 1. As compared to mothers who never smoked during pregnancy, mothers who continued smoking 15 cigarettes/day or more, were more likely to have attended college, and did less often own a house at the time of the nurses' birth. Daughters of mothers who continued smoking ≥15 cigarettes/day during pregnancy, were born with lower birth weight, were less frequently breastfed, were more often smokers in adulthood, and had a higher alcohol intake than daughters of mothers who did not smoke during pregnancy. In total 12,346 (37.3%) mothers of the study participants reported that neither parent smoked during pregnancy, 1,866 (5.6%) reported maternal smoking only, 12,165 (36.8%) reported paternal smoking only, and 6,709 (20.3%) reported that both parents smoked during pregnancy.

In the age-adjusted Cox proportional hazards model, maternal smoking of ≥15 cigarettes/day during pregnancy was associated with an increased incidence of hypertension in the offspring as compared to no maternal smoking during pregnancy (RR 1.19, 95% CI 1.09, 1.29) (Table 2). Adjustment for perinatal variables, birth weight and adult life variables did not appreciably change the observed associations. The RR's were decreased by additional adjustment for body shape at age 5, body shape at age 10 and BMI at age 18 (RR 1.13, 95% CI 1.03, 1.23), and inclusion of current BMI completely eliminated the associations (RR 1.07, 95% CI 0.98, 1.16). In the age-adjusted models we did not observe an increased risk when mothers reported first trimester smoking only, or continued smoking of <15 cigarettes/day during pregnancy, although our results suggested a dose-response trend (*P* for trend <0.01). The trend test was no longer significant after additional adjustment for current BMI of the nurse. Body mass index was estimated to mediate 60% (95% CI 10%, 111%) of the association between maternal smoking of 15 cigarettes/day or more and the risks of adult-onset hypertension accounting for possible confounding variables. Using similar models focused on the associations of the number of cigarettes smoked during pregnancy by the father with the risk of hypertension in the offspring, we observed in the age-adjusted models that as compared to no paternal smoking during pregnancy, paternal smoking of 15 cigarettes/day or more was associated with an increased risk of hypertension in the adult offspring (RR 1.18, 95% CI 1.12, 1.25) (Table 2). The RR's only slightly changed after adjustment for perinatal variables, birth weight and adult

Table 2. Maternal and paternal smoking during pregnancy and the risk of incident hypertension in the participants of the Nurses' Health Study II Cohort

	Maternal smoking					Paternal smoking			
Level of adjustment	Nonsmoker	Quit smoking in 1st trimester	Continued smoking <15 cig/d	Continued smoking ≥15 cig/d	*P* for trend*	Nonsmoker	<15 cig/d	≥15 cig/d	*P* for trend*
Person years	395,471	17,795	68,490	42,138		231,171	113,948	179,442	
No. of cases	5,777	247	988	650		3,092	1,740	2,848	
RR (95% CI)									
Model 1 Adjusted for age	*Reference*	1.02 (0.89, 1.16)	1.01 (0.94, 1.08)	1.19 (1.09, 1.29)	<0.01	*Reference*	1.07 (1.00, 1.13)	1.18 (1.12, 1.25)	<0.01
Model 2 Additionally adjusted for perinatal variables†	*Reference*	1.04 (0.91, 1.19)	1.02 (0.95, 1.10)	1.20 (1.10, 1.31)	<0.01	*Reference*	1.05 (0.99, 1.12)	1.15 (1.09, 1.21)	<0.01
Model 3 Additionally adjusted for birth weight	*Reference*	1.03 (0.90, 1.18)	1.01 (0.94, 1.08)	1.18 (1.08, 1.28)	<0.01	*Reference*	1.04 (0.98, 1.11)	1.14 (1.08, 1.20)	<0.01
Model 4 Additionally adjusted for adult life variables‡	*Reference*	1.05 (0.92, 1.19)	1.00 (0.93, 1.08)	1.18 (1.08, 1.28)	<0.01	*Reference*	1.04 (0.98, 1.11)	1.13 (1.07, 1.19)	<0.01
Model 5 Model 4 additionally adjusted for body shape and weight until age 18§	*Reference*	1.03 (0.90, 1.17)	0.97 (0.90, 1.04)	1.13 (1.03, 1.23)	0.03	*Reference*	1.03 (0.97, 1.10)	1.11 (1.05, 1.17)	<0.01
Model 6 Model 4 additionally adjusted for current BMI	*Reference*	0.99 (0.87, 1.13)	0.95 (0.88, 1.01)	1.07 (0.98, 1.16)	0.31	*Reference*	1.01 (0.95, 1.08)	1.06 (1.01, 1.12)	0.02

Abbreviations: CI, confidence interval; BMI, body mass index; RR, Rate Ratio; and DASH, Dietary Approaches to Stop Hypertension

**P* for trend across nonsmokers, smoking 1-14 cigarettes/d and smoking ≥15 cigarettes/d

†Maternal and paternal age at time of daughter's birth, maternal pre-pregnancy BMI, paternal BMI, maternal and paternal educational level, maternal and paternal occupation, house ownership of parents at the time of the daughter's birth, the occurrence of pregnancy complications during pregnancy, and nurse's ethnicity, gestational age, breastfeeding status, and family history of hypertension

‡Nurse's husbands' educational level, pretax household income, nurse's smoking behavior, oral contraceptive use, height, DASH score, alcohol intake and physical activity

§Nurses' body shape at age 5, body shape at age 10, and BMI at age 18

Table 3. Parental smoking during pregnancy and the risk of incident hypertension in the participants of the Nurses' Health Study II Cohort

	Parental smoking during pregnancy			
Level of adjustment	No parental smoking	Paternal smoking only	Maternal or both parents smoking	*P* for trend*
Person-years	200,551	194,920	138,431	
No. of cases	2,699	3,078	2,048	
RR (95% CI)				
Model 1 Adjusted for age	*Reference*	1.12 (1.06, 1.18)	1.13 (1.07, 1.20)	<0.01
Model 2 Additionally adjusted for perinatal variables†	*Reference*	1.09 (1.03, 1.15)	1.13 (1.06, 1.20)	<0.01
Model 3 Additionally adjusted for birth weight	*Reference*	1.08 (1.03, 1.14)	1.11 (1.04, 1.18)	<0.01
Model 4 Additionally adjusted for adult life variables‡	*Reference*	1.08 (1.02, 1.14)	1.11 (1.04, 1.18)	<0.01
Model 5 Model 4 additionally adjusted for body shape and weight until age 18§	*Reference*	1.07 (1.01, 1.13)	1.06 (1.00, 1.13)	0.04
Model 6 Model 4 additionally adjusted for current BMI	*Reference*	1.04 (0.99, 1.10)	1.01 (0.95, 1.08)	0.59

Abbreviations: CI, confidence interval; BMI, body mass index; RR, Rate Ratio; and DASH, Dietary Approaches to Stop Hypertension

**P* for trend across no parental smoking, paternal smoking only and maternal or both parents smoking

†Maternal and paternal age at time of daughter's birth, maternal pre-pregnancy BMI, paternal BMI, maternal and paternal educational level, maternal and paternal occupation, house ownership of parents at the time of the daughter's birth, the occurrence of pregnancy complications during pregnancy, and nurse's ethnicity, gestational age, breastfeeding status, and family history of hypertension

‡Nurse's husbands' educational level, pretax household income, nurse's smoking behavior, oral contraceptive use, height, DASH score, alcohol intake and physical activity

§Nurses' body shape at age 5, body shape at age 10, and BMI at age 18

life variables. Similarly, the effect of the quantity of paternal smoking attenuated after adjustment for body weight throughout life, although the associations of paternal smoking of 15 cigarettes/day or more remained significant. The estimated mediation proportion indicated that current BMI accounted for 49% (95% CI 22%, 76%) of the association between paternal smoking of 15 cigarettes/day or more and the risks of adult-onset hypertension adjusting for possible confounders.

In the age-adjusted models, paternal smoking only, as well as maternal smoking only and both parents smoking during pregnancy combined, were associated with an increased risk of adulthood hypertension (Table 3); the strongest association was found for both parents smoking during pregnancy (RR 1.13, 95% CI 1.07, 1.20). However, the associations were reduced after adjusting for childhood body shape and BMI at age 18, and were eliminated when current BMI was added to the models (RR 1.01, 95% CI 0.95, 1.08).

No significant interactions were observed for the risk of hypertension, by current BMI. When restricting follow-up to incident events counted after exposure assessed in 2001, the RR's of the fully adjusted models were slightly higher for both maternal smoking in first trimester only and continued smoking of <15 cigarettes/day, and marginally lower for maternal smoking ≥15 cigarettes/day. The RR's of the fully adjusted models of paternal smoking remained stable, with larger confidence intervals.

Comparably, in the logistic regression models on prevalent hypertension cases in 1989, the associations of the quantity of both maternal and paternal smoking during pregnancy decreased after adding body weight throughout life to the models, and were completely eliminated after adjustment for current BMI (OR 0.99, 95% CI 0.81, 1.21, and OR 1.05, 95% CI 0.92, 1.18, respectively) (Supplementary material). Adding body weight throughout life to the logistic regression models similarly decreased the association of both parents smoking independent and combined, compared to no parental smoking during pregnancy with the risk of early onset hypertension (Supplementary material).

DISCUSSION

In this prospective cohort study of 33,086 nurses, we observed weak positive associations between both maternal and paternal smoking during pregnancy with the risk of hypertension in their adult daughters. However, observed associations were largely eliminated after adjustment for body weight throughout life, with BMI closest to the diagnosis of hypertension having the greatest impact. Most of the effect of parental smoking during pregnancy on the daughter's hypertension risk was mediated through current BMI, but some independent effect remained.

Blood pressure shows moderate tracking between childhood and adulthood[26], and factors that influence blood pressure in early life might have consequences for the risk of hypertension in later life. Studies of parental smoking during pregnancy in relation to the development of

blood pressure in the offspring have mainly focused on childhood blood pressure and had inconsistent results. Several studies suggested a positive association of fetal smoke exposure with blood pressure during childhood[5-8], while other studies found no evidence of an effect of maternal smoking on blood pressure.[9-10] A systematic review of nine observational studies suggested that maternal smoking during pregnancy was associated with a slightly elevated blood pressure in children.[12]The inconsistent results between studies might be explained by a diminishing influence of parental smoking behavior during pregnancy on offspring blood pressure with increasing age, when influences from behavior and environmental factors such as BMI become increasingly important. In adult participants of the 1958 British Birth Cohort, adult offspring of mothers who smoked during pregnancy had a higher blood pressure on average than offspring of non-smokers, but the associations were attenuated after adjustment for postnatal influences across life.[14] Second, previous studies varied in the level of adjustment for possible confounders, most notably postnatal weight or BMI.

In the present analysis, adjustment for body weight throughout life attenuated considerably the association of parental smoking during pregnancy and adult hypertension. Previous studies suggested that intrauterine smoke exposure leads to adaptations in weight and predispose to an increased risk of overweight and obesity in the offspring in childhood, adolescence and adulthood.(Holly R. Harris, Walter C. Willett, Karin B. Michels, unpublished data, 2012)[14-16] Obesity and weight gain are major risk factors for developing hypertension.[17, 27] Thus the association of parental smoking during pregnancy with the risk of hypertension in adulthood might be mediated through the programming of body weight throughout life. These findings are supported by a study in children, which suggested that childhood BMI and weight trajectory largely mediated the associations of maternal smoking during pregnancy with the offspring's systolic blood pressure at the age of seven.[11]However, parental smoking in pregnancy may also be a proxy of behavioral lifestyle factors clustering in smoking families that not only affect the risk of adult-onset hypertension, but also fosters the development of overweight and obesity throughout life.

Because of the inverse relation between birth weight and blood pressure in later life, and the widely acknowledged association of maternal smoking during pregnancy with birth weight, we also investigated the influence of birth weight on the association of parental smoking during pregnancy and adult hypertension. The influence of birth weight in the pathway appeared to be limited. In line with our findings, in a recent meta-analysis, the associations of fetal smoke exposure with child overweight were independent of birth weight.[15]

In the present study cohort, we observed similar effect estimates for the associations of paternal smoking during pregnancy with the risk of hypertension in the offspring, compared to maternal smoking. Although passive smoke exposure is known to cause cardiovascular disease in adults[28], and maternal exposure to second hand smoke in pregnancy is associated with a reduction in birth weight[29], the effect of maternal active smoking is expected to be larger than the effect of maternal second hand smoke exposure in pregnancy. If the associations between

maternal smoking during pregnancy with blood pressure in the offspring would be due to direct intrauterine mechanisms, we would expect stronger effects for maternal than paternal smoking on health outcomes in the offspring. Moreover, the associations of numbers of cigarettes smoked by the parents and the specific period during pregnancy of fetal smoke exposure with risk of hypertension also attenuated after adjustment for life course body weight. Brion and colleagues reported in children aged seven years similar associations for maternal and paternal smoking during pregnancy with offspring systolic blood pressure.[13] The authors subsequently concluded that differences in child blood pressure found in minimally adjusted models are not because of a biological influence of maternal smoking on the intrauterine environment, but rather are a marker for other environmental factors. In addition, the effects of postnatal passive smoke exposure might be more important than the effects of intrauterine smoke exposure, especially in childhood when children live in close proximity to their parents. In healthy preschool children, parental smoking during childhood was an independent risk factor for higher blood pressure in the offspring.[30]

A limitation of the study was that data on parental smoking and associated covariates were collected retrospectively after several decades. We expect that any resulting misclassification is likely to be non-differential with respect to adult disease status and would therefore bias the associations towards the null. However, in a subgroup of our study population a high agreement of the daughters' reports of maternal smoking during pregnancy as compared to the mothers' reports was reported (sensitivity ranged from 74% to 85% and specificity ranged from 90% to 95%, kappa = 0.72 - 0.81).[20] Second, both prospective and retrospective analyses were combined. However, actual exposure took place a considerable time before the outcome of interest, and it is assumed that reporting of exposure is independent of the outcome of interest. In addition, in the sensitivity analysis performed with time of observation between 2001 and 2007, the results were not appreciably changed. Third, the diagnosis of hypertension was obtained through self-reports. Although hypertension reporting has been validated previously in the NHS II[21], and self-reported hypertension appeared strongly predictive of coronary heart disease in the Nurses' Health Study I[31], it cannot be excluded that misclassification has occurred. Finally, although comprehensive information about covariates was available, the possibility of residual confounding due to insufficient measured or unmeasured factors should be considered. The major strengths of the study are the large sample size and the availability of information on paternal smoking during pregnancy. The study relied primarily on maternal reports of parental smoking during pregnancy, which are likely to be superior to the reports of the daughters.

Perspectives

Parental smoking is a modifiable risk factor of compromised intrauterine environment and there is broad evidence of its deleterious effects on fetal health. Understanding the influence of adverse fetal environment on cardiovascular disease and its risk factors may have important

implications on the knowledge of the pathogenesis and prevention of cardiovascular disease. This study provides limited evidence of a direct intrauterine effect of prenatal smoke exposure on vascular function. The weak associations of maternal and paternal smoking during pregnancy with the risk of hypertension in adulthood were largely attenuated after adjustment for body weight throughout life. Our observations may be explained by a mediating role of BMI of the effect of parental smoking on hypertension through the programming of body weight throughout life, or a marker of unhealthy lifestyle behaviors clustering in smoking families, that influence the development of BMI. The exact mechanisms linking parental smoking, body weight and the risks of incident hypertension in adulthood remain subject for further investigation.

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SUPPLEMENTARY MATERIAL

Table S1. Maternal and paternal smoking during pregnancy and the risk of prevalent hypertension in 1989 in the participants of the Nurses' Health Study II Cohort

	Maternal smoking					Paternal smoking			
Level of adjustment	Nonsmoker	Quit smoking in first trimester	Continued smoking <15 cig/d	Continued smoking ≥15 cig/d	*P* for trend*	Nonsmoker	<15 cig/d	≥15 cig/d	*P* for trend*
Number of participants	25,679	1,138	4,436	2,755		14,829	7,428	11,799	
No. of cases	1,168	46	204	129		617	352	586	
OR (95% CI)									
Model 1 Adjusted for age	*1.00 (Reference)*	0.93 (0.69, 1.26)	1.04 (0.89, 1.21)	1.14 (0.94, 1.37)	0.17	*1.00 (Reference)*	1.07 (0.93, 1.22)	1.17 (1.04, 1.31)	<0.01
Model 2 Additionally adjusted for perinatal variables†	*1.00 (Reference)*	0.98 (0.72, 1.33)	1.10 (0.94, 1.28)	1.18 (0.98, 1.42)	0.06	*1.00 (Reference)*	1.07 (0.93, 1.22)	1.17 (1.04, 1.32)	<0.01
Model 3 Additionally adjusted for birth weight	*1.00 (Reference)*	0.99 (0.73, 1.34)	1.08 (0.92, 1.26)	1.16 (0.95, 1.40)	0.10	*1.00 (Reference)*	1.07 (0.93, 1.23)	1.14 (1.01, 1.29)	0.03
Model 4 Additionally adjusted for adult life variables‡	*1.00 (Reference)*	1.01 (0.74, 1.37)	1.09 (0.93, 1.28)	1.16 (0.95, 1.40)	0.09	*1.00 (Reference)*	1.10 (0.95, 1.26)	1.16 (1.03, 1.31)	0.02
Model 5 Model 4 additionally adjusted for body shape and weight until age 18§	*1.00 (Reference)*	0.97 (0.71, 1.33)	1.00 (0.86, 1.18)	1.03 (0.85, 1.26)	0.74	*1.00 (Reference)*	1.07 (0.93, 1.23)	1.11 (0.98, 1.25)	0.11
Model 6 Model 4 additionally adjusted for current BMI	*1.00 (Reference)*	0.93 (0.68, 1.27)	0.99 (0.85, 1.17)	0.99 (0.81, 1.21)	0.93	*1.00 (Reference)*	1.03 (0.90, 1.19)	1.05 (0.92, 1.18)	0.51

Abbreviations: BMI, body mass index; OR, Odds Ratio; and DASH, Dietary Approaches to Stop Hypertension

**P* for trend across non-smokers, smoking 1-14 cigarettes/d and smoking ≥15 cigarettes/d

†Maternal and paternal age at time of daughter's birth, maternal pre-pregnancy BMI, paternal BMI, maternal and paternal educational level, maternal and paternal occupation, house ownership of parents at the time of the daughter's birth, the occurrence of pregnancy complications during pregnancy, and nurse's ethnicity, gestational age, breastfeeding status, and family history of hypertension

‡Nurse's husbands' educational level, pretax household income, nurse's smoking behavior, oral contraceptive use, height, DASH score, alcohol intake, and physical activity

§Nurses' body shape at age 5, body shape at age 10, and BMI at age 18

Table S2. Parental smoking during pregnancy and the risk of prevalent hypertension in 1989 in the participants of the Nurses' Health Study II Cohort

	Parental smoking during pregnancy			
Level of adjustment	No parental smoking	Paternal smoking only	Maternal or both parents smoking	*P* for trend*
Number of participants	12,887	12,792	8,989	
No. of cases	541	627	414	
OR (95% CI)				
Model 1 Adjusted for age	*1.00 (Reference)*	1.10 (0.98, 1.24)	1.12 (0.98, 1.27)	0.08
Model 2 Additionally adjusted for perinatal variables†	*1.00 (Reference)*	1.09 (0.97, 1.23)	1.17 (1.02, 1.34)	0.02
Model 3 Additionally adjusted for birth weight	*1.00 (Reference)*	1.08 (0.96, 1.22)	1.15 (1.00, 1.31)	0.05
Model 4 Additionally adjusted for adult life variables‡	*1.00 (Reference)*	1.10 (0.98, 1.25)	1.17 (1.02, 1.34)	0.03
Model 5 Model 4 additionally adjusted for body shape and weight until age 18§	*1.00 (Reference)*	1.09 (0.96, 1.23)	1.06 (0.92, 1.22)	0.36
Model 6 Model 4 additionally adjusted for current BMI	*1.00 (Reference)*	1.05 (0.92, 1.19)	1.01 (0.88, 1.16)	0.84

Abbreviations: BMI, body mass index; OR, Odds Ratio; and DASH, Dietary Approaches to Stop Hypertension

**P* for trend across no parental smoking, paternal smoking only and maternal or both parents smoking

†Maternal and paternal age at time of daughter's birth, maternal pre-pregnancy BMI, paternal BMI, maternal and paternal educational level, maternal and paternal occupation, house ownership of parents at the time of the daughter's birth, the occurrence of pregnancy complications during pregnancy, and nurse's ethnicity, gestational age, breastfeeding status, and family history of hypertension

‡Nurse's husbands' educational level, pretax household income, nurse's smoking behavior, oral contraceptive use, height, DASH score, alcohol intake, and physical activity

§Nurses' body shape at age 5, body shape at age 10, and BMI at age 18

Chapter 3.3

Fetal exposure to parental smoking and the risk of type 2 diabetes in adulthood

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Chapter 3.4

Breastfeeding and left cardiac structures and function in early childhood

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ABSTRACT

Background Shorter duration of breastfeeding in infancy has been suggested to be associated with an increased risk of cardiovascular disease in adulthood. Early cardiovascular adaptations due to breastfeeding may explain these associations.

Aim To investigate whether breastfeeding affects left cardiac structures and blood pressure development in early childhood.

Study Design Prospective cohort study from fetal life until the age of two years.

Subjects Information about the duration and exclusivity of breastfeeding was collected by questionnaires at the ages of 2, 6 and 12 months in 933 children.

Outcome Measures Left cardiac structures (left atrial diameter, aortic root diameter and left ventricular mass), fractional shortening and blood pressure at the ages of 1.5, 6 and 24 months.

Results No differences in cardiac structures, fractional shortening and blood pressure were observed between breastfed and non-breastfed children. Duration and exclusivity of breastfeeding were not consistently associated with any cardiac structure, fractional shortening, or blood pressure until the age of 24 months. Also, there was no association of breastfeeding with cardiac growth between 6 months and 24 months. All analyses were adjusted for child age and sex. Additional adjustment for child anthropometrics, maternal age, anthropometrics, family history, maternal cardiovascular risk factors, pregnancy or delivery complications, parity, socio-economic status, smoking status and alcohol consumption during pregnancy did not materially change the effect estimates.

Conclusions Our results do not support the hypothesis that early postnatal cardiovascular adaptations underlie the previously shown associations between breastfeeding and cardiovascular disease in adulthood. Further studies are needed to investigate whether and at what age the associations appear.

INTRODUCTION

Prolonged breastfeeding in infancy may have a favorable effect on cardiovascular disease and its risk factors in later life.[1] Prior research demonstrated inverse associations of breastfeeding with body mass index[2], total cholesterol and low density lipoprotein (LDL) cholesterol levels[3] and a positive association with high density lipoprotein (HDL) cholesterol levels[4] in adulthood. Two meta-analyses suggested that adults who had been breastfed had a slightly lower systolic blood pressure than those who were not breastfed.[5-6] Human breast milk differs from infant formula in several ways that affect the growth of human infants.[7] Human breast milk has a distinct lipid profile and contains hormones and growth factors that may influence the development of the cardiovascular system.[8-9]Associations of breastfeeding with cardiovascular disease might be explained by developmental plasticity, which refers to the ability of an organism to develop in various ways, depending on the particular environment or setting.[10] The increased risks of cardiovascular disease among subjects who were not breastfed may reflect the long term consequences of adaptive responses in cardiovascular structure and function to nutrition in infancy. However, results from studies relating breastfeeding with cardiovascular morbidity and mortality seem to be inconsistent. A recent meta-analysis of four studies suggested no association between breastfeeding and the risk of death from cardiovascular disease.[11] Data from previous studies are confined by the retrospective gathered information on breastfeeding, providing suboptimal data and potential for biased results.

Left ventricular mass, left atrial size, aortic root size and blood pressure are related to cardiovascular morbidity and mortality. Studies in children demonstrated that left ventricular mass and blood pressure track from childhood to adulthood.[12-14] We have recently shown tracking of left cardiac structures from birth until the age of 2 years[15], suggesting that left cardiac structures and function are at least partly established in early life. Early exposures, such as breastfeeding, may affect cardiovascular and blood pressure development, and this association might already be present in early life. Prospectively collected data on breastfeeding and early cardiovascular development enables identification of potential developmental adaptations and critical time periods, which might have consequences for later life.

Therefore the aim of this study was to investigate whether breastfeeding duration affects left cardiac structures and blood pressure development in early childhood.

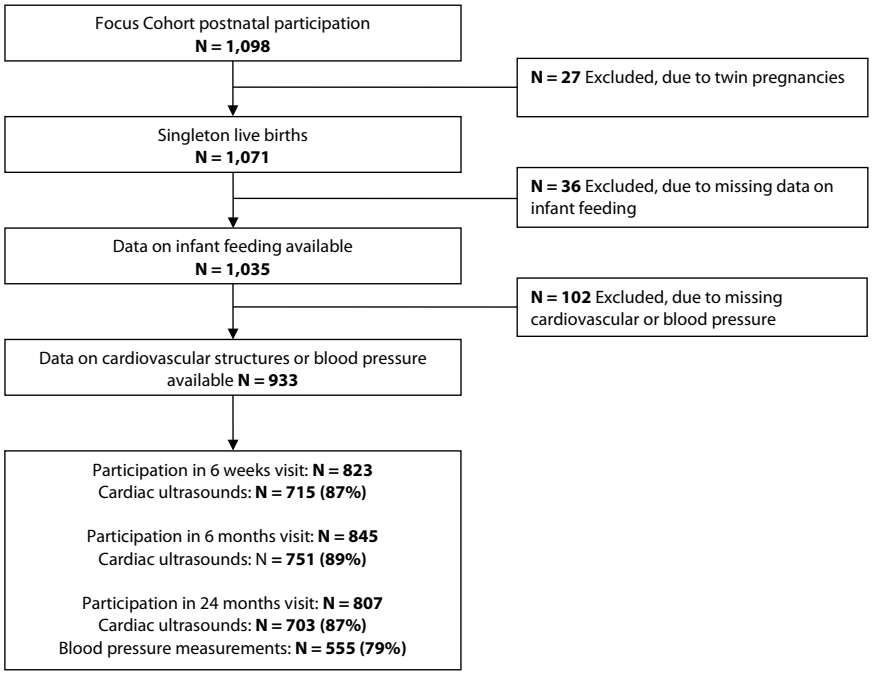
MATERIALS AND METHODS

Design and study population

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life until young adulthood.[16-17] The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health. The

total cohort consists of 9,778 mothers and their children living in Rotterdam, The Netherlands. Mothers were aimed to enroll during early pregnancy, but enrollment was allowed until birth. Additional and more detailed assessments of growth and development are conducted in a random subgroup of Dutch children.[17] In total 1,106 children participated in this subgroup during the postnatal phase and 1,098 children participated in the postnatal assessments at the research center (Figure 1). Multiple births (N = 27) were excluded from the analysis. The present analysis was limited to singleton live births (N = 1,071). None of the remaining children had congenital heart disease. Detailed information on infant feeding was available in 1,035 children. In 933 children, one or more echocardiographic measurements or blood pressure measurements were performed during the first two years of life. A total of 823 (74%) singleton live births participated in the assessments at the age of 1.5 months, 845 (76%) at 6 months, and 807 (73%) at 24 months of age (Figure 1).

Figure 1. Flowchart of the participants included in the analyses



Breastfeeding

Information about breastfeeding was collected by postnatal questionnaires at the infant ages of 2, 6 and 12 months. Medical records were used to establish breastfeeding initiation in case of missing information on the starting of breastfeeding in the questionnaires. Breastfeeding was coded as ever breastfed or never breastfed. Breastfeeding duration was categorized as <2 months, 2 - 4 months, 4 - 6 months and ≥6 months. The duration of exclusive breastfeeding was derived from information about duration of breastfeeding and at what age formula feeding, other types of milk and solid food were introduced in the first 6 months of life. This resulted in three categories: never breastfed, partially breastfed (indicating breastfeeding, other milk and/or solid food) and exclusively breastfed for at least 4 months (only breastfeeding, no other milk, solid food, or fluids other than water).

Cardiovascular outcomes

Two-dimensional M-mode and Doppler echocardiographic measurements were performed using ATL-Philips Model HDI 5000 (Seattle, WA) equipment at the child age of 1.5, 6 and 24 months. In total, 86% of the measurements were performed by a single echocardiographer. The other measurements were performed by 2 other echocardiographers. The echocardiographers were supervised by a pediatric cardiologist. Echocardiographic measurements were successfully performed in 87%, 89% and 87% of the children examined at 1.5, 6 and 24 months. Missing echocardiograms were mainly due to crying or unavailability of equipment or echocardiographer. Left atrial diameter, aortic root diameter, interventricular end-diastolic septal thickness (IVSTD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-diastolic posterior wall thickness (LVPWTD), and shortening fraction as a quantification of cardiac function, were measured using methods recommended by the American Society of Echocardiography.[18] Left ventricular mass was computed using the formula derived by Devereux *et al.*[19]:

$$\text{Left ventricular mass} = 0.80 \times 1.04((\text{IVSTD} + \text{LVEDD} + \text{LVPWTD})^3 - (\text{LVEDD})^3) + 0.6$$

Previously, a good intra and inter observer reproducibility of the ultrasound measurements was shown in this study. The intraclass correlation coefficient between and among observers was calculated in 30 subjects and varied between 0.85 and 0.99.[15] The procedure of the blood pressure measurements has been described in detail elsewhere.[20] Briefly, systolic blood pressure and diastolic blood pressure were measured to the nearest mmHg at the left upper arm by using an automatic sphygmomanometer (Vital Signs Monitor CAS 740; CAS Medical Systems, Inc., Branford, Connecticut, USA[21]). The child was seated quietly during the measurements, and a cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference.[22] Diastolic blood pressure was taken at the fifth Korotkoff phase. The mean of the two readings was used for the data analysis. Blood pressure measurements were successfully performed in 69% of the children examined at 24 months. In 218 children, it was not possible to measure blood pressure twice due to crying and opposi-

tional behavior. Thus, their blood pressure was based on one measurement. No differences in blood pressure values between those with two or one measurement were observed.

Covariates

Date of birth, infant sex, Apgar score at 5 minutes, and birth weight were obtained from midwife and hospital registries. Gestational age adjusted SD scores were constructed for birth weight. Weight and height were measured at the visits at the age of 1.5, 6 and 24 months. Body mass index (kg/m^2) was calculated. Information on maternal age, pre-pregnancy weight, parity, maternal hypertension, high cholesterol and heart abnormalities, family history of hypertension, stroke and myocardial infarction at an age <65 years, highest education finished, family household income, smoking status and alcohol consumption during pregnancy was obtained by the questionnaires. Socio-economic status was indicated by maternal education and family household income in this population. Maternal education was defined as highest followed education according to the classification of Statistics Netherlands and categorized in primary, secondary and higher.[23] Family income, defined by the total net month income of the household, was categorized as '<1200 €' (below social security level), '1200 - 2000 €' and '>2000 €' (more than modal income). Information about doctor-diagnosed pregnancy complications, i.e. pregnancy induced hypertension, preeclampsia and gestational diabetes, was retrieved from medical records and verified according to current guidelines.[24-25] Maternal height and weight were measured without shoes and heavy clothing and body mass index was calculated (kg/m^2) at the research center in the first trimester of pregnancy.

Statistical methods

Differences in characteristics between children who were ever breastfed versus never breastfed were tested using independent samples t-tests and Chi-square tests. Differences in baseline characteristics among the different categories of breastfeeding duration were assessed using the ANOVA tests and Chi-square tests. Associations of duration and exclusivity of breastfeeding with left cardiac structures and blood pressure were assessed with linear regression models. At all ages, the category with highest breastfeeding duration was taken as the referent for the analyses. For the analyses on cardiac structures at the age of 1.5 months, this was the combined group of breastfeeding categories with a duration of >1.5 months. For the analyses on cardiac structures at the age of 6 months, this was the combined group of breastfeeding categories with a duration of >4 months and for the analysis at 24 months, this was the category with a duration of breastfeeding ≥6 months. Tests for trends were performed using breastfeeding duration as a continuous variable in the linear regression analyses. First, these models were adjusted for infant sex and age. Subsequently, to take account for maternal and child anthropometrics analyses were adjusted for gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, height and weight at measurement, child weight gain from birth to moment of measurement, maternal age, height, weight, parity, maternal hypertension or heart

abnormalities, family history of hypertension, stroke and myocardial infarction at an age <65 years, pregnancy complications, educational level, family household income, smoking status and alcohol consumption during pregnancy. The selection of covariates was based on results from previous studies and correlations with breastfeeding status and left cardiac structures. All statistical analyses were performed using the Statistical Package for the Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 presents the characteristics of the mothers and infants by breastfeeding status. The total percentage of boys was 51.0%. The overall median ages (95% range) of the children at the visits were 1.5 months (1.0 - 2.8), 6.3 months (5.5 - 8.3), and 25.1 months (23.7 - 28.2). The majority of children (N = 842, 90%) were breastfed during infancy, of which in 83.8% (N = 706) the exact duration of breastfeeding was known. The median (95% range) duration of breastfeeding was 5 (0.5 - 12.0) months. In total, 24.6% (N = 207) of all children were exclusively breastfed during at least 4 months. The prevalence of breastfeeding initiation did not differ by sex and birth weight. Higher educated mothers tended to initiate breastfeeding more frequently.

Table 1. Subject characteristics (N = 933)

	Breastfeeding (N = 842)	**No breastfeeding (N = 91)**	***P* value**
Maternal characteristics			
Age (years)	32.2 (22.8, 39.5)	32.3 (19.5, 38.4)	0.18
Height (cm)	171.1 (6.3)	169.9 (6.2)	0.09
Weight (kg)	71.3 (12.6)	73.5 (17.0)	0.24
Body mass index (kg/m^2)	24.4 (4.1)	25.4 (5.4)	0.08
Parity ≥1 (%)	311 (37.5)	39 (42.9)	0.31
Highest completed educational level mother			<0.01
Primary (%)	14 (1.7)	4 (4.4)	
Secondary (%)	250 (29.7)	52 (57.1)	
Higher (%)	569 (67.6)	34 (37.4)	
Missing (%)	9 (1.1)	1 (1.1)	
Family household income, no. (%)			0.12
<1,200	6 (0.7)	2 (2.2)	
1,200 – 2,000	12 (1.4)	3 (3.3)	
>2,000	774 (91.9)	78 (85.7)	
Missing	50 (5.9)	8 (8.8)	
Smoking during pregnancy, no. (%)			0.17
Not	596 (70.8)	61 (67.0)	
First trimester only	69 (8.2)	5 (5.5)	
Continued	85 (10.1)	16 (17.6)	
Missing	91 (10.8)	9 (9.9)	

Table 1. Subject characteristics (N = 933) *(continued)*

Maternal characteristics	Breastfeeding (N = 842)	No breastfeeding (N = 91)	*P* value
Alcohol consumption during pregnancy, no. (%)			<0.01
Not	205 (24.3)	41 (45.1)	
First trimester only	125 (14.8)	9 (9.9)	
Continued	425 (50.5)	32 (35.2)	
Missing	87 (10.3)	9 (9.9)	
Family history of cardiovascular diseases, no. (%)[a]	340 (40.4)	40 (44.0)	0.64
Risk factors for cardiovascular diseases, no. (%)[b]	21 (2.5)	5 (5.5)	0.25
Pregnancy or delivery complications, no. (%)[c]	69 (8.2)	11 (12.1)	0.24
Childhood characteristics			
Gestational age (weeks)	40.3 (36.0, 42.4)	40.1 (34.1, 42.5)	0.02
Male, no. (%)	425 (50.5)	51 (56.0)	0.31
Apgar score at 5 minutes <7, no. (%)	8 (1.0)	1 (1.1)	0.61
Birth weight (g)	3,518.9 (534.2)	3,477.5 (586.6)	0.49
Low birth weight (<2,500 grams), no. (%)	29 (3.4)	5 (5.5)	0.32
Preterm birth (<37 weeks), no. (%)	32 (3.8)	6 (6.6)	0.20
Duration of breastfeeding, no. (%)			
<2 months	190 (22.6)		
2 - 4 months	158 (18.8)		
4 - 6 months	93 (11.0)		
≥6 months	264 (31.4)		
Missing	137 (16.3)		
Visit 1.5 months, no. (%)	740 (87.9)	83 (91.2)	0.35
Visit 6 months, no. (%)	759 (90.1)	86 (94.5)	0.18
Visit 24 months, no. (%)	733 (87.1)	74 (81.3)	0.13

Values are means (SD), medians (95% range), or no.'s (%). Differences between ever and never breastfed were compared using independent samples t-tests or Chi-square tests.

[a]Includes family history of hypertension, stroke and myocardial infarction <65

[b]Includes maternal cardiovascular risk factors, consisting of maternal hypertension, high cholesterol and heart abnormalities

[c]Includes pregnancy or delivery complications, consisting of maternal preeclampsia, pregnancy induced hypertension and diabetes gravidarum

Table 2 presents the associations of breastfeeding status, duration and exclusivity with the repeatedly measured left cardiac structures and fractional shortening, adjusted for child sex and age at visit. No significant associations of breastfeeding with any of the cardiac outcomes were observed. Additionally adjusting for gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, height and weight at measurement, child weight gain from birth to moment of measurement, and for maternal age, height, weight, parity, maternal hypertension, high cholesterol or heart abnormalities, family history of hypertension, stroke or myocardial infarction at an age <65 years, pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy did not materially change these associations. Supplementary Table S1 shows the effect of adjusting for child anthropometrics and maternal factors. Similar results were observed for the association of breastfeeding with systolic and diastolic blood

Table 2. Associations of duration and exclusivity of breastfeeding with left cardiac structures at the ages of 1.5, 6 and 24 months

	Left atrial diameter (mm)			Aortic root diameter (mm)			Left ventricular mass (g)			Fractional shortening (%)		
Breastfeeding	1.5 months (N = 491)	6 months (N = 725)	24 months (N = 685)	1.5 months (N = 493)	6 months (N = 723)	24 months (N = 684)	1.5 months (N = 447)	6 months (N = 680)	24 months (N = 656)	1.5 months (N = 666)	6 months (N = 683)	24 months (N = 650)
Ever												
Never (N = 91)	-0.09 (-0.68, 0.51)	-0.37 (-0.80, 0.06)	-0.61 (-1.27, 0.05)	0.22 (-0.14, 0.58)	-0.09 (-0.37, 0.19)	0.26 (-0.12, 0.64)	0.67 (-0.26, 1.59)	-0.09 (-1.05, 0.86)	0.74 (-0.78, 2.26)	-0.44 (-1.73, 0.86)	0.73 (-0.41, 1.88)	0.65 (-0.61, 1.91)
Ever (N = 842)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Duration												
Never (N = 91)	0.10 (-0.50, 0.69)	-0.39 (-0.85, 0.07)	-0.57 (-1.28, 0.15)	0.12 (-0.24, 0.48)	-0.09 (-0.40, 0.21)	0.15 (-0.27, 0.56)	0.73 (-0.22, 1.67)	-0.18 (-1.20, 0.83)	0.46 (-1.18, 2.11)	-0.19 (-1.52, 1.14)	1.05 (-0.18, 2.27)	0.53 (-0.83, 1.89)
0 - 2 months (N = 190)	0.07 (-0.37, 0.50)	-0.15 (-0.51, 0.21)	-0.01 (-0.54, 0.52)	-0.22 (-0.48, 0.05)	0.00 (-0.24, 0.24)	-0.15 (-0.45, 0.16)	0.51 (-0.18, 1.20)	-0.59 (-1.39, 0.21)	-1.06 (-2.28, 0.17)	0.29 (-0.72, 1.30)	0.93 (-0.03, 1.90)	-0.13 (-1.13, 0.88)
2 - 4 months (N = 158)		-0.51 (-0.89,-0.12)*	-0.06 (-0.62, 0.50)		0.03 (-0.23, 0.28)	-0.18 (-0.50, 0.14)		-0.48 (-1.34, 0.37)	-0.10 (-1.39, 1.19)		-0.07 (-1.11, 0.96)	-0.80 (-1.86, 0.27)
4 - 6 months (N = 93)			-0.21 (-0.88, 0.46)			-0.19 (-0.57, 0.20)			0.09 (-1.49, 1.67)			0.64 (-0.64, 1.91)
≥6 months (N = 264)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
P for trend			0.77			0.11			0.40			0.89
Exclusivity												
Never (N = 61)			-0.23 (-0.96, 0.51)			0.11 (-0.31, 0.54)			0.34 (-1.36, 2.05)			1.01 (-0.40, 2.43)
Partial ≥4 months (N = 340)			0.30 (-0.16, 0.76)			-0.18 (-0.45, 0.09)			-0.74 (-1.82, 0.34)			0.49 (-0.40, 1.37)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for child's sex and age at visit. *P <0.05

Table 3. Association of duration and exclusivity of breastfeeding with systolic and diastolic blood pressure at the age of 24 months

	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Breastfeeding	24 months (N = 555)	24 months (N = 555)
Ever		
Never (N = 91)	0.45 (-2.34, 3.23)	0.85 (-2.04, 3.74)
Ever (N = 842)	*Reference*	*Reference*
Duration		
Never (N = 91)	0.58 (-2.42, 3.57)	0.49 (-2.63, 3.60)
0 - 2 months (N = 190)	1.28 (-0.97, 3.54)	0.72 (-1.62, 3.06)
2 - 4 months (N = 158)	-2.30 (-4.68, 0.07)	-2.44 (-4.91, 0.03)
4 - 6 months (N = 93)	-0.34 (-3.09, 2.42)	-2.25 (-5.11, 0.61)
≥6 months (N = 264)	*Reference*	*Reference*
P for trend	0.84	0.56
Exclusivity		
Never (N = 61)	1.22 (-1.91, 4.34)	1.78 (-1.47, 5.02)
Partial ≥4 months (N = 340)	0.97 (-1.02, 2.96)	1.13 (-0.93, 3.19)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for child's sex and age at visit.

pressure, as presented in Table 3 and Supplementary Table S2. Table 4 shows the associations of breastfeeding status, duration and exclusivity with the growth of left cardiac structures in the time period 6 months to 24 months. Breastfeeding was not significantly associated with the growth of any of the cardiac structures. The effect estimates were not substantially affected by adjustment of the models for gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, height and weight at measurement, child weight gain from birth to moment of measurement, and maternal age, height, weight, parity, maternal cardiovascular risk factors, family history for cardiovascular diseases, pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy as shown in Supplementary Table S3.

Table 4. Association of breastfeeding with growth of left cardiac structures between 6 and 24 months

	Change in left atrial diameter (mm)	Change in aortic root diameter (mm)	Change in left ventricular mass (g)
Breastfeeding	(N = 532)	(N = 531)	(N = 487)
Ever			
Never (N = 91)	-0.20 (-0.99, 0.58)	0.19 (-0.23, 0.61)	1.34 (-0.44, 3.12)
Ever (N = 842)	*Reference*	*Reference*	*Reference*
Duration			
Never (N = 91)	-0.20 (-1.07, 0.66)	0.06 (-0.40, 0.52)	0.86 (-1.07, 2.80)
0 - 2 months (N = 190)	0.11 (-0.55, 0.76)	-0.16 (-0.51, 0.19)	-0.39 (-1.88, 1.11)
2 - 4 months (N = 158)	0.10 (-0.60, 0.81)	-0.27 (-0.64, 0.11)	-0.48 (-2.05, 1.09)
4 - 6 months (N = 93)	-0.25 (-1.09, 0.59)	-0.26 (-0.70, 0.19)	-0.55 (-2.44, 1.35)
≥6 months (N = 264)	*Reference*	*Reference*	*Reference*
P for trend	0.41	0.22	0.93
Exclusivity			
Never (N = 61)	0.07 (-0.83, 0.96)	0.10 (-0.38, 0.58)	0.74 (-1.28, 2.77)
Partial ≥4 months (N = 340)	0.34 (-0.24, 0.93)	-0.13 (-0.44, 0.18)	-0.68 (-2.00, 0.65)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for child's sex and age at visit.

DISCUSSION

In this prospective cohort study among children, we found no evidence of an association of type of infant feeding with left cardiac morphology, fractional shortening and blood pressure at the age of 1.5, 6 and 24 months. Also no associations of duration or exclusivity of breastfeeding with any of the cardiovascular outcomes were observed. Adjusting for infant sex, gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, age and anthropometrics and for maternal age, anthropometrics, parity, maternal cardiovascular risk factors, family history for cardiovascular diseases, pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy did only slightly affect the effect estimates.

A major strength of this study is its prospective design from fetal life onwards, in a large cohort of children. Most of the studies conducted so far, were based on recall of infant feeding after several decades, making recall bias an important concern. Another strength is the availability of detailed information about the duration and exclusivity of breastfeeding. This enabled us to investigate the influence of duration of breastfeeding and whether results differ among children who were exclusively or partially breastfed. Furthermore, during the first 2 years of life, the effect of other postnatal exposures related to cardiovascular disease in adulthood, such as obesity and smoking, are confined. A potential limitation of the study is that the associations between breastfeeding and left cardiac structures and blood pressure may

depend on additional dietary patterns. In the analysis, we did not adjust for other components of infant diet. More detailed information about infant nutrition, including macronutrients and micronutrients intake, during the first two years would provide additional information. Another limitation was that because of the young age at examination and the limited time available at the three visits, we were not able to get a higher percentage of successful cardiovascular and blood pressure measurements. Missing outcome measurements could have led to selection bias. In our study, mothers of children who did not have any measurements were younger at enrolment than the mothers of children who had at least one cardiovascular or blood pressure measurement. Our results would be biased if the associations between breastfeeding and the cardiovascular or blood pressure measurements differ between those included and not included in the study. This seems unlikely, but cannot be excluded. Also, the Generation R Focus cohort comprises a relatively healthy and high educated group of Dutch women and children. Although the starting rates of breastfeeding in our study were fairly high relative to those in earlier reports[26-27], they were consistent with the upward trend toward breastfeeding that has been taking place in Western countries since the 1990.[28] The analyses were based on internal comparison within this selective study population, and the effect estimates are valid for this cohort. The selection towards a more healthy and higher educated population might result in a limited generalizability of the results.

Over the past decades there has been increased interest in the influence of infant nutrition, especially breastfeeding, on development of cardiovascular morbidity and mortality in later life. A possible association between breastfeeding and cardiovascular disease might be explained by developmental plasticity.[10] The association of breastfeeding with cardiovascular diseases and its risk factors in later life may reflect the long term consequences of adaptive responses in cardiovascular morphology to nutrition in infancy. Singhal and Lucas proposed the more specified growth acceleration hypothesis, suggesting that a high-nutrient diet in infancy adversely programs the principal components of the metabolic syndrome by promoting growth acceleration.[29] This suggests that relative slower or normal infant growth would be protective for later cardiovascular health. Breastfed infants show slower growth than those who are formula fed.[30] Thus reduced early growth due to a lower nutrient intake is the potential link between breastfeeding and reduced cardiovascular disease late in life.[29] Our study provided no support for the hypothesis that breastfeeding leads to cardiovascular developmental adaptations in early life. No association between partial or exclusive breastfeeding duration with left cardiac structures and their growth, fractional shortening or blood pressure was found. It is still possible that more detailed functional cardiovascular measurements, such as endothelial dysfunction, arterial stiffness or left ventricular diastolic filling patterns might be influenced by breastfeeding.

Various other studies have been conducted to investigate the association of breastfeeding in infancy with the development of cardiovascular disease and its risk factors. Systematic reviews and meta-analyses showed inconsistent results and publication bias and socioeconomic

confounding appeared of important concern.[6] Studies focused on cardiovascular risk factors in younger subjects, which make recall bias less likely, presented conflicting results as well. Two meta-analyses on the association of breastfeeding with blood pressure in later life both found that adults who were breastfed had a slightly lower systolic blood pressure.[5-6] The evidence for publication bias was strong though, with smaller effects of marginal statistical significance in the larger studies. A meta-analysis on blood cholesterol levels in infancy, childhood and adult life showed that breastfeeding is associated with elevated total cholesterol and LDL-cholesterol levels in infancy but lower levels in adult life.[3] In a randomized trial, adolescents born preterm who received breastfeeding , had a lower ratio of LDL-cholesterol to HDL-cholesterol and also a lower serum C-reactive protein compared to those who were formula fed, indicative of a lower risk for cardiovascular disease.[31] On the contrary, a large cohort study among 2818 adults, provided no evidence of a protective influence of breast feeding on the total cholesterol and LDL-cholesterol levels.[2] Several studies have shown associations between breastfeeding and in obesity in children and in adults consistently[32-34], although a study from Belarus reported that prolonged breastfeeding had no effect on the rate of obesity.[35]

Thus both the beneficial effects of breastfeeding on cardiovascular diseases outcomes and the potential underlying mechanisms are still not clear.

CONCLUSION

Our results did not show any cardiovascular adaptations, measured as left cardiac structures and function and blood pressure in early childhood, in response to breastfeeding duration. Further studies are needed to assess whether and at what age the associations of infant nutrition with cardiac morphology and function appear. Also, studies on more detailed dietary patterns and cardiovascular function in early childhood are needed.

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SUPPLEMENTARY MATERIAL

Table S1. Association of duration and exclusivity of breastfeeding with left cardiac structures at the ages of 1.5, 6 and 24 months

	Left atrial diameter (mm)			Aortic root diameter (mm)			Left ventricular mass (g)			Fractional shortening (%)		
Breastfeeding	1.5 months (N = 491)	6 months (N = 725)	24 months (N = 685)	1.5 months (N = 493)	6 months (N = 723)	24 months (N = 684)	1.5 months (N = 447)	6 months (N = 680)	24 months (N = 656)	1.5 months (N = 666)	6 months (N = 683)	24 months (N = 650)
Ever												
Never (N = 91)	0.26 (-0.43, 0.94)	-0.06 (-0.56, 0.45)	-0.87 (-1.62, -0.12)*	0.21 (-0.18, 0.60)	-0.04 (-0.36, 0.27)	0.30 (-0.10, 0.70)	0.94 (-0.04, 1.93)	0.03 (-1.02, 1.08)	0.13 (-1.52, 1.78)	0.13 (-1.23, 1.49)	0.21 (-1.14, 1.56)	0.57 (-0.86, 1.99)
Ever (N = 842)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Duration												
Never (N = 91)	0.51 (-0.19, 1.20)	-0.08 (-0.61, 0.46)	-0.82 (-1.63, 0.00)	0.11 (-0.29, 0.50)	-0.05 (-0.39, 0.29)	0.14 (-0.29, 0.57)	1.07 (0.06, 2.08)*	0.03 (-1.10, 1.16)	-0.26 (-2.05, 1.54)	-0.03 (-1.62, 1.57)	0.52 (-0.95, 1.98)	0.23 (-1.33, 1.78)
0 - 2 months (N = 190)	0.32 (-0.17, 0.81)	-0.10 (-0.52, 0.31)	0.12 (-0.49, 0.72)	-0.18 (-0.46, 0.10)	-0.01 (-0.28, 0.25)	-0.23 (-0.56, 0.09)	0.68 (-0.04, 1.39)	-0.25 (-1.12, 0.62)	-1.01 (-2.37, 0.34)	0.44 (-0.72, 1.60)	0.83 (-0.29, 1.95)	-0.79 (-1.96, 0.37)
2 - 4 months (N = 158)		-0.58 (-1.03, -0.13)*	-0.17 (-0.81, 0.47)		0.03 (-0.26, 0.32)	-0.27 (-0.61, 0.06)		-0.14 (-1.09, 0.81)	-0.33 (-1.76, 1.10)		0.09 (-1.15, 1.32)	-0.72 (-1.96, 0.52)
4 - 6 months (N = 93)			0.00 (-0.76, 0.77)			-0.23 (-0.64, 0.17)			-0.10 (-1.82, 1.61)			0.09 (-1.37, 1.56)
≥6 months (N = 264)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
P for trend			0.96			0.06			0.45			0.38
Exclusive breastfeeding												
Never (N = 61)			-0.56 (-1.39, 0.28)			0.11 (-0.33, 0.56)			-0.36 (-2.21, 1.49)			0.80 (-0.80, 2.40)
Partial ≥4 months (N = 340)			0.26 (-0.27, 0.78)			-0.26 (-0.54, 0.02)			-0.86 (-2.04, 0.31)			0.30 (-0.72, 1.32)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for infant sex, gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, age, height and weight at measurement, weight gain from birth to moment of measurement, and for maternal age, anthropometrics, parity, maternal family history, cardiovascular risk factors, delivery or pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy. *P <0.05

Table S2. Association of duration and exclusivity of breastfeeding with systolic and diastolic blood pressure at the age of 24 months

	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Breastfeeding	24 months (N = 555)	24 months (N = 555)
Ever		
Never (N = 91)	1.50 (-1.85, 4.85)	-0.33 (-3.02, 3.68)
Ever (N = 842)	*Reference*	*Reference*
Duration		
Never (N = 91)	2.00 (-1.59, 5.60)	-0.17 (-3.79, 3.44)
0 - 2 months (N = 190)	2.65 (-0.09, 5.39)	0.26 (-2.49, 3.01)
2 - 4 months (N = 158)	-2.51 (-5.31, 0.30)	-2.83 (-5.64, -0.01)*
4 - 6 months (N = 93)	-1.18 (-4.50, 2.15)	-3.35 (-6.69, -0.01)*
≥6 months (N = 264)	*Reference*	*Reference*
P for trend	0.58	0.21
Exclusive breastfeeding		
Never (N = 61)	2.64 (-1.11, 6.39)	0.86 (-2.90, 4.62)
Partial ≥4 months (N = 340)	1.42 (-0.97, 3.80)	0.36 (-2.03, 2.75)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for infant sex, gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, age, height and weight at measurement, weight gain from birth to moment of measurement, and for maternal age, anthropometrics, parity, maternal family history, cardiovascular risk factors, delivery or pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy. *P <0.05

Table S3. Association of breastfeeding with growth of left cardiac structures between 6 and 24 months

	Change in left atrial diameter (mm)	Change in aortic root diameter (mm)	Change in left ventricular mass (g)
Breastfeeding	(N = 532)	(N = 531)	(N = 487)
Ever			
Never (N = 91)	-0.60 (-1.49, 0.30)	0.27 (-0.21, 0.74)	0.66 (-1.43, 2.75)
Ever (N = 842)	*Reference*	*Reference*	*Reference*
Duration			
Never (N = 91)	-0.59 (-1.56, 0.39)	0.15 (-0.37, 0.68)	-0.01 (-2.27, 2.25)
0 - 2 months (N = 190)	-0.11 (-0.63, 0.85)	-0.19 (-0.59, 0.21)	-0.96 (-2.67, 0.75)
2 - 4 months (N = 158)	-0.07 (-0.74, 0.87)	-0.27 (-0.69, 0.17)	-0.67 (-2.52, 1.18)
4 - 6 months (N = 93)	0.03 (-0.91, 0.98)	-0.08 (-0.59, 0.42)	-0.30 (-2.47, 1.86)
≥6 months (N = 264)	*Reference*	*Reference*	*Reference*
P for trend	0.68	0.21	0.58

Table S3. Association of breastfeeding with growth of left cardiac structures between 6 and 24 months *(continued)*

Exclusive breastfeeding	Change in left atrial diameter (mm)	Change in aortic root diameter (mm)	Change in left ventricular mass (g)
Never (N = 61)	-0.37 (-1.38, 0.65)	0.18 (-0.37, 0.72)	-0.14 (-2.50, 2.21)
Partial ≥4 months (N = 340)	0.37 (-0.29, 1.02)	-0.14 (-0.50, 0.21)	-1.05 (-2.56, 0.46)
Exclusive ≥4 months (N = 169)	*Reference*	*Reference*	*Reference*

Values are regression coefficients (95% confidence interval) based on multiple linear regression models. Models are adjusted for infant sex, gestational age adjusted SD scores for birth weight, Apgar score at 5 minutes, age, height and weight at measurements, weight gain from birth to moment of measurement, and for maternal age, anthropometrics, parity, maternal family history, cardiovascular risk factors, delivery or pregnancy complications, socio-economic status, smoking status and alcohol consumption during pregnancy.

Chapter 3.5

Breastfeeding, introduction of solid foods and cardiovascular development in children

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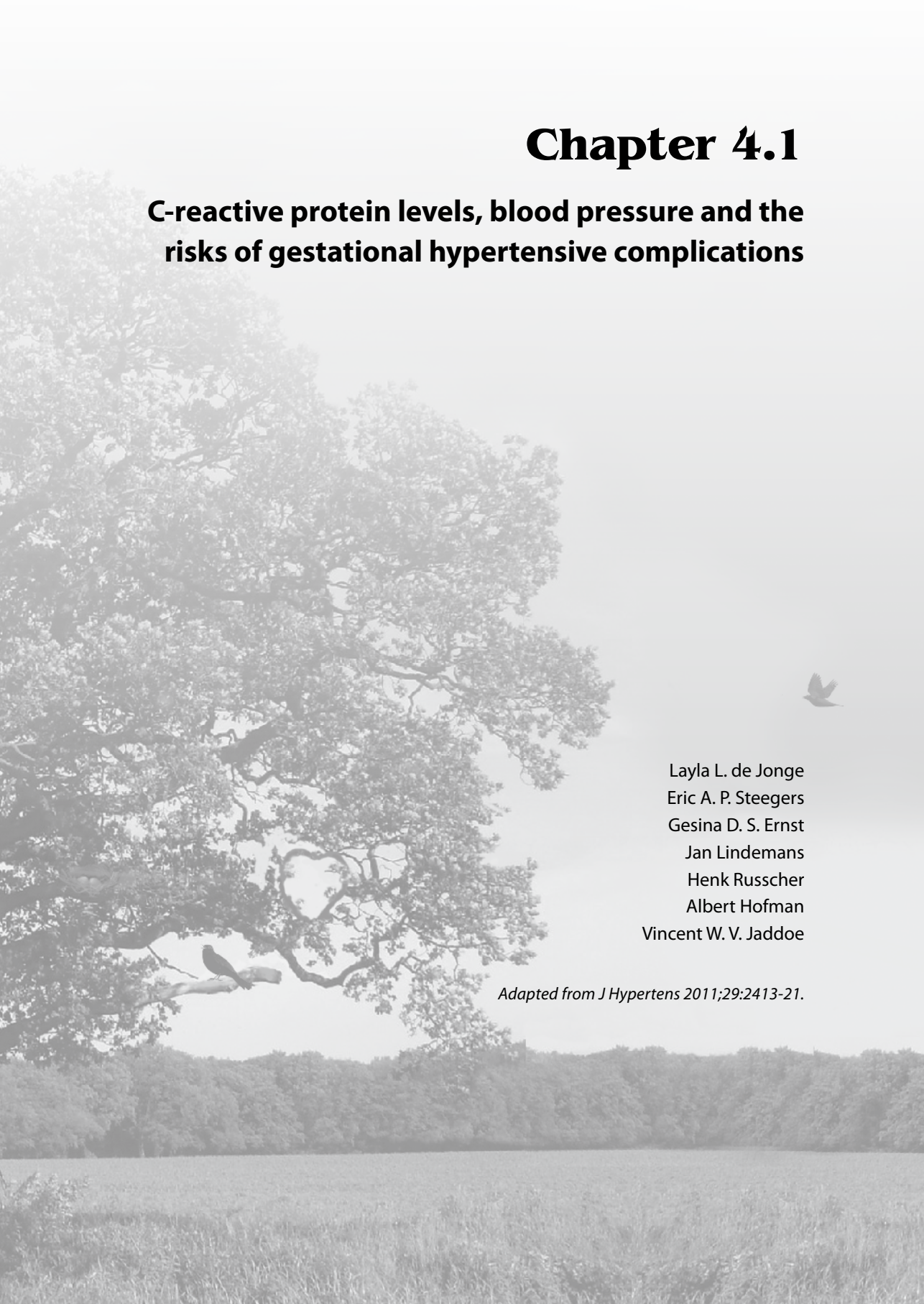
Submitted for publication

Chapter 4

Inflammation and pregnancy outcomes

Chapter 4.1

C-reactive protein levels, blood pressure and the risks of gestational hypertensive complications



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ABSTRACT

Background Aim of this study was to investigate the associations of C-reactive protein levels, as marker of low grade inflammation, with blood pressure development during pregnancy and the risks gestational hypertensive complications. We also explored the role of maternal body mass index in these associations.

Methods High sensitivity C-reactive protein levels were measured in early pregnancy (median 13.2 weeks, 95% range 9.6 to 17.6) in 5,816 mothers participating in a population-based prospective cohort study in the Netherlands. Blood pressure measurements were performed in each trimester. Information about pregnancy induced hypertension and preeclampsia was retrieved from hospital charts of the women.

Results Longitudinal analyses showed that C-reactive protein levels were not associated with systolic and diastolic blood pressure patterns throughout pregnancy. Trimester specific multivariate linear regression models showed that as compared to low C-reactive protein levels (<5.0 mg/L), elevated levels (≥20.0 mg/L) were associated with maternal systolic and diastolic blood pressure. Elevated C-reactive protein levels in early pregnancy were associated with the risks of pregnancy induced hypertension (Odds ratio 2.78, 95% CI 1.66 to 4.66). After adjustment for maternal body mass index, all associations attenuated.

Conclusion Our results suggest that first trimester C-reactive protein levels are associated with systolic and diastolic blood pressure levels throughout pregnancy and with gestational hypertensive complications, but these associations are largely explained by maternal body mass index.

INTRODUCTION

Pregnancy induced hypertension and preeclampsia are leading causes of maternal and fetal morbidity and mortality worldwide.[1-2] The clinical features of preeclampsia can partly be explained as clinical responses to generalized endothelial dysfunction.[3] An exaggerated maternal systemic inflammatory response to pregnancy is considered to have a crucial role in the pathophysiologic mechanisms leading to endothelial dysfunction.[4-5]

C-reactive protein is an acute phase protein[6-7], and is used as a diagnostic indicator of tissue damage in both acute and chronic inflammation. Circulating C-reactive protein levels are elevated in normal pregnancy[8], and this elevation is suggested to be more profound in preeclamptic women.[9-14] However, results from studies focused on C-reactive protein levels in pregnant women, and its potential use as a risk factor for preeclampsia have been conflicting.[15] Various studies showed higher C-reactive protein levels in preeclamptic pregnancies[9, 11-12, 14], and C-reactive protein levels seem to be associated with the severity of preeclampsia.[16] Other studies suggested that the effect of C-reactive protein levels on the risk of preeclampsia might be explained by maternal obesity.[10, 13] A more profound activation of inflammatory markers and alterations C-reactive protein levels has been shown in overweight and obese pregnant women.[17-18] Inflammation in early pregnancy might also be associated with blood pressure changes within the normal range during pregnancy. These subclinical vascular adaptations to inflammation might be involved in the pathways that predispose to maternal gestational hypertensive disorders.

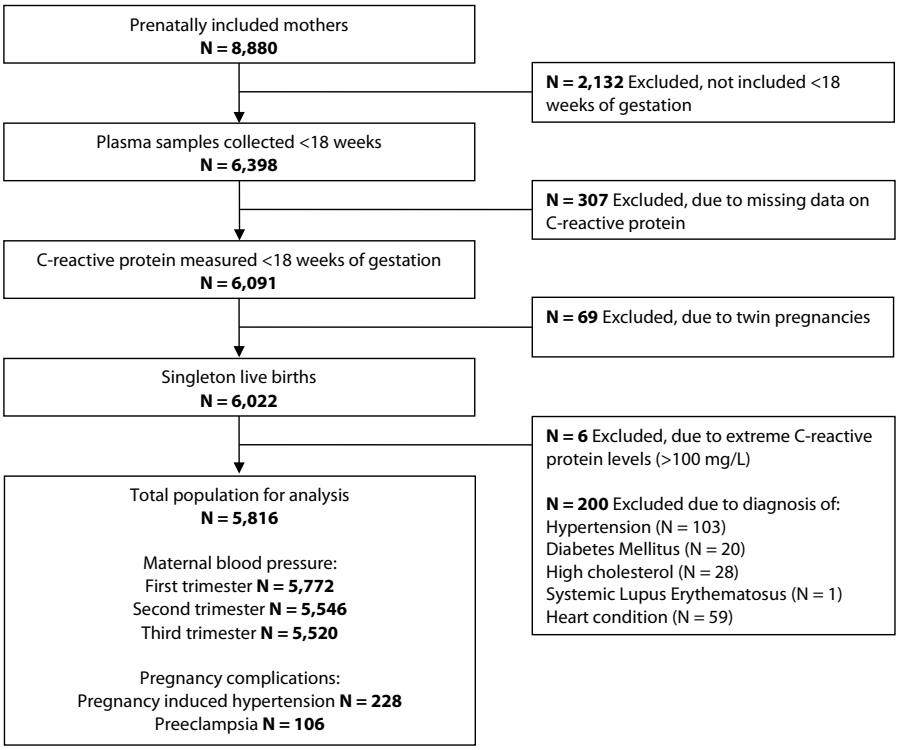
Therefore, we investigated in a large population-based prospective cohort study the associations of high sensitivity C-reactive protein levels in early pregnancy with blood pressure in different periods of pregnancy and the risk of gestational hypertensive disorders. Next, we explored how maternal body mass index could be a confounding factor in these associations.

METHODS

Study design and population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in the city of Rotterdam, The Netherlands.[19-20] All children were born between April 2002 and January 2006. The overall response rate was 61% and was based on the number of children born to eligible mothers during the inclusion period of the study.[20] The study has been approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants. Of the total of 8,880 mothers who were enrolled during pregnancy, 76% (N = 6,748) were enrolled before a gestational age of 18 weeks. Of these mothers, blood plasma samples were collected in 95% (N = 6,398) and C-reactive protein was successfully measured in 90% (N = 6,091). To exclude women

Figure 1. Flowchart of participants included in the analyses (N = 5,816)



with high-risk pregnancies and women predisposed for gestational hypertensive disorders, we excluded twin pregnancies (N = 69), mothers with extremely high C-reactive protein levels (>100 mg/L) (N = 6), pre-existing hypertension (N = 103), pre-existing diabetes (N = 20), high cholesterol (N = 28), Systemic Lupus Erythematosus (N = 1) and a chronic heart condition (N = 59). The total cohort comprised 5,816 mothers with singleton live births for analysis (65% of 8,880) (Figure 1). Women excluded from the study because of extremely high C-reactive protein levels and an increased risk of developing hypertensive disorders, were of higher age at intake, were heavier, were less inclined to drink alcohol during pregnancy, had higher C-reactive protein levels and had higher systolic and diastolic blood pressures during the three trimesters of pregnancy as shown in Supplementary material (Table S1).

High-sensitivity C-reactive protein levels

Maternal venous blood samples were collected in early pregnancy and transported to the regional laboratory (Star-MDC, Rotterdam, The Netherlands) for processing and storage.[19] Processing was aimed to be finished within a maximum of 3 hours after venous puncture. Blood samples were immediately stored at -80 ºC after processing. C-reactive protein concentrations

were measured in EDTA plasma samples at the Department of Clinical Chemistry of the Erasmus MC in 2009. We measured high-sensitivity C-reactive protein since traditional clinically used C-reactive protein methods lack the sensitivity in low ranges needed for predicting future risk of events in apparently healthy individuals.[21] C-reactive protein levels were analyzed using an immunoturbidimetric assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, The Netherlands). The within run precision for C-reactive protein was 1.3% at 12.9 mg/L and 1.2% at 39.9 mg/L. The lowest level of detection was 0.2 mg/L. Assessments of C-reactive protein were blinded to outcome. We created five categories of C-reactive protein levels (<5.0, 5.0 - 9.9, 10.0 - 15.0, 15.0 - 19.9, and ≥20 mg/L). Levels <5.0 mg/L and ≥20.0 mg/L were considered as low (reference) and elevated levels, respectively.[22]

Blood pressure measurements

Systolic and diastolic blood pressure were measured in first, second and third trimester with the Omron 907 automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, The Netherlands), which was validated in non-pregnant adults.[23] Participants were seated in a chair with back support and blood pressure measurement started after 5 to 10 minutes of rest. A cuff was placed around the nondominant upper arm, which was supported at the level of the heart, with the bladder midline over the brachial artery pulsation. If the circumference of the upper arm exceeded 33 centimeter (cm), a larger cuff (32-42 cm) was used. For each participant, the mean value of two blood pressure readings over a 60-second interval was documented. In total, N = 5,772, N = 5,546, and N = 5,520 blood pressure measurements were available in first, second and third trimester, 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.[24]

Doctor-diagnosed pregnancy induced hypertension was defined according to the criteria described by the International Society for the Study of Hypertension in Pregnancy (ISSHP) as the development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg without proteinuria after 20 weeks of gestation in previously normotensive women.[25]Doctor-diagnosed preeclampsia was defined as the development of systolic blood pressure at least 140 mmHg and/or diastolic blood pressure at least 90 mmHg after 20 weeks of gestation in previously normotensive women and 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).

Covariates

Information about maternal age, educational level, ethnicity and parity was obtained by a questionnaire at enrolment in the study. Maternal smoking and alcohol consumption habits were assessed by questionnaires in each trimester. Maternal height and weight were measured without shoes and heavy clothing at enrolment and body mass index was calculated as weight/height^2 (kg/m^2). Information about maternal weight just before pregnancy was obtained by questionnaires. In our population for analysis, 63% and 100% of all women were enrolled and assessed before a gestational age of 14 and 18 weeks, respectively. As enrolment in our study was in pregnancy, we were not able to measure maternal weight before pregnancy. However, correlation of pre-pregnancy weight obtained by questionnaire and weight measured at enrolment was 0.95 (P <0.001).

Statistical analysis

First, the associations of C-reactive protein levels with repeatedly measured maternal systolic and diastolic blood pressure were analyzed using the unbalanced repeated measurement regression analysis. This regression technique takes the correlation of multiple measurements within one subject into account and allows for incomplete outcome data.[26] The best fitting models were constructed using fractional polynomials of gestational age.[27] C-reactive protein was included in the models as intercept and as interaction term with gestational age. The models can be written as:

Systolic Blood Pressure = $\beta_0 + \beta_1$ x C-reactive protein + β_2 x gestational age + β_3 x gestational age^{-2} + β_4 x C-reactive protein x gestational age

Diastolic Blood Pressure = $\beta_0 + \beta_1$ x C-reactive protein + β_2 x gestational age + β_3 x gestational age$^{0.5}$ + β_4 x C-reactive protein x gestational age

In these models, "$\beta_0 + \beta_1$ x C-reactive protein" reflects the intercept, "β_2 x gestational age + β_3 x gestational age^{-2}" reflects the slope of change in systolic blood pressure per week, and "β_2 x gestational age + β_3 x gestational age$^{0.5}$" reflects the slope of change in diastolic blood pressure per week. The term "β_4 x C-reactive protein x gestational age" reflects the difference in change in blood pressure per week between the different C-reactive protein categories for systolic and diastolic blood pressure. Second, the trimester specific associations of first trimester C-reactive protein levels with first, second and third trimester maternal blood pressure were assessed using multiple linear regression models. To explore the role of maternal obesity, we repeated these analyses in strata of body mass index (<20.0 kg/m^2, 20.0-24.9 kg/m^2, 25.0-29.9 kg/m^2 and ≥30.0 kg/m^2, representing respectively lean, normal weight, overweight and obese women). Finally we assessed the associations of first trimester C-reactive protein level with the risks of pregnancy complications (pregnancy induced hypertension and preeclampsia) with multiple

logistic regression models. Tests for trends were performed using logarithmically transformed C-reactive protein as continuous variable in the linear and logistic regression analyses. All models were adjusted for gestational age at venous puncture, gestational age at blood pressure measurement, maternal age, education, ethnicity, parity, smoking, alcohol consumption and body mass index. The percentages of missing values within the population for analysis were lower than 1% for continuous data and lower than 13% for the categorical data. We applied multiple imputation for covariates.[28] Since there were no major differences in the observed results between analyses with imputed missing data or complete cases only, only results including imputed missing data are presented. Statistical analyses were performed using the Statistical Analysis System version 9.2 (SAS for Windows Version 9.2; SAS Institute, Cary, NC, USA), including the PROC MIXED module for unbalanced repeated measurements, as well as the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject Characteristics

Subject characteristics and data on maternal and fetal complications are presented in Table 1. Median maternal age was 30.3 years, with a 95% range of 19.5 to 38.8 years. Of all women included in the study, 56.7% was primipara. Median C-reactive protein level was 4.4 mg/L (95% range 0.60 to 25.22). In total, 228 (3.9%) and 106 (1.8%) mothers developed pregnancy induced hypertension and preeclampsia, respectively.

Table 1. Characteristics of study participants included in the analyses

Maternal characteristics at enrolment	**N = 5,816**
Age (years)	30.3 (19.5 – 38.8)
Height (cm)	167.6 ± 7.4
Weight (kg)[a]	68.5 ± 12.8
Body mass index (kg/m^2)	24.4 ± 4.3
Parity	
0	3,298 (56.7)
≥1	2,468 (42.8)
Missing	50 (0.9)
Highest completed educational level	
Primary school	524 (9.0)
Secondary school	2,438 (41.9)
Higher education	2,433 (41.8)
Missing	421 (7.2)
Ethnic background	

Table 1. Characteristics of study participants included in the analyses *(continued)*

Maternal characteristics at enrolment	N = 5,816
European	3,385 (58.2)
Non-European	2,094 (36.0)
Missing	337 (5.8)
Smoking during pregnancy	
No smoking	3,723 (64.0)
First trimester only	462 (7.9)
Continued smoking	879 (15.1)
Missing	752 (12.9)
Alcohol consumption during pregnancy	
No alcohol consumption	2,320 (39.9)
First trimester only	767 (13.2)
Continued alcohol consumption	2,004 (34.5)
Missing	725 (12.5)
Gestational age at enrolment (weeks)	13.2 (9.6-17.6)
Maternal pregnancy complications	
Pregnancy induced hypertension	228 (3.9)
Preeclampsia	92 (1.6)
HELLP	11 (0.2)
Preeclampsia and HELLP	14 (0.2)
Eclampsia	0 (0.0)
Fetal pregnancy complications	
Small for gestational age[b]	287 (4.9)
Preterm birth[c]	279 (4.8)
Stillbirth	0 (0.0)
Neonatal death[d]	11 (<0.1)
C-reactive protein level (mg/L)	4.4 (0.60-25.22)

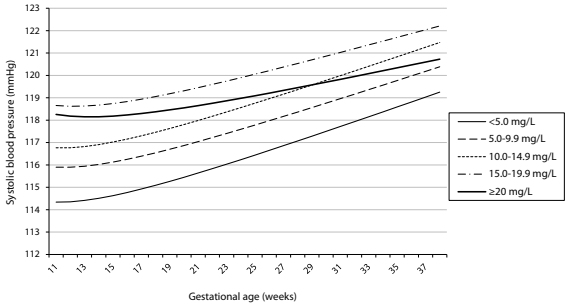
Abbreviations: HELLP, Hemolysis Elevated Liver enzymes, Low Platelets
Values are means (± SD), medians (95% range), or no.'s (%).
[a]Weight was measured at enrolment in the study
[b]Defined as sex specific gestational age adjusted birth weight <5th percentile in study cohort
[c]Defined as birth <37 weeks of gestation
[d]Defined as infant death during the first 28 competed days of life

C-reactive protein levels and blood pressure in different trimesters

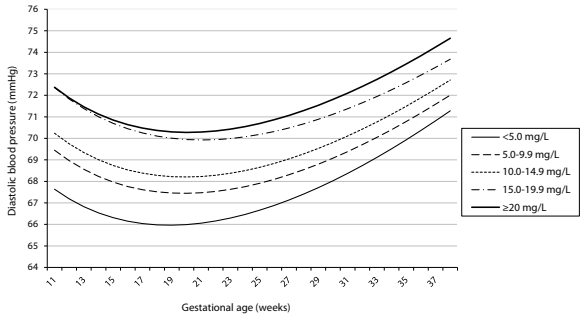
Figure 2 shows the longitudinally measured maternal systolic and diastolic blood pressure patterns in the different C-reactive protein level categories. Systolic blood pressure increased continuously with increasing gestational age. For diastolic blood pressure, a mid-pregnancy dip in was found in all C-reactive protein categories. As compared to the lowest C-reactive protein category of <5.0 mg/L, higher C-reactive protein levels were not consistently associated

Figure 2. Systolic and diastolic blood pressure patterns in different C-reactive protein categories.

A. Systolic blood pressure



B. Diastolic blood pressure



The change in blood pressure in mmHg per C-reactive protein category, compared to the reference group of women with a C-reactive protein level of <5.0 mg/L, is shown based on repeated measurement analysis.

with the systolic or diastolic blood pressure pattern. The specific effect estimates for gestational age independent (intercept) and gestational age dependent differences (interaction C-reactive protein and gestational age) are given in Supplementary material (Table S2).

Table 2 shows the associations between C-reactive protein levels in early pregnancy and maternal blood pressure in different trimesters of pregnancy. As compared to low C-reactive protein levels (<5.0 mg/L), elevated levels (≥20.0 mg/L) were not consistently associated with systolic blood pressure, but were positively associated with higher diastolic blood pressure in each trimester (differences 1.69 mmHg (95% CI: 0.54 to 2.85) in the first trimester, 1.00 mmHg (95% CI: -0.16 to 2.16) in the second trimester and 1.39 mmHg (95% CI: 0.21 to 2.57) in the third trimester (all *P* values for trend <0.05). Body mass index was the only covariate which attenuated the associations of C-reactive protein with maternal blood pressure. The results for the analyses without adjustment for body mass index are given in the Supplementary material (Table S3) and showed consistent positive associations between C-reactive protein levels and both systolic and diastolic blood pressure.

Table 2. Associations of maternal C-reactive protein levels in early pregnancy with maternal blood pressure

Systolic blood pressure						
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
C-reactive protein level	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<5.0 mg/L	N = 3,178	*Reference*	N = 3,063	*Reference*	N = 3,056	*Reference*
5.0 – 9.9 mg/L	N = 1,578	0.36 (-0.33, 1.05)	N = 1,506	0.34 (-0.35, 1.03)	N = 1,503	0.39 (-0.31, 1.10)
10.0 – 14.9 mg/L	N = 557	0.58 (-0.47, 1.61)	N = 539	0.36 (-0.68, 1.39)	N = 531	0.74 (-0.32, 1.80)
15.0 – 19.9 mg/L	N = 207	0.09 (-1.53, 1.70)	N = 197	0.35 (-1.27, 1.97)	N = 191	0.30 (-1.37, 1.97)
≥20.0 mg/L	N = 252	0.17 (-1.31, 1.65)	N = 241	-0.62 (-2.10, 0.86)	N = 239	-0.62 (-2.14, 0.90)
Trend test	*P* for trend 0.051	0.77 (0.00, 1.54)	*P* for trend 0.196	0.51 (-0.26, 1.29)	*P* for trend 0.182	0.54 (-0.25, 1.33)
Diastolic blood pressure						
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
C-reactive protein level	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<5.0 mg/L	N = 3,178	*Reference*	N = 3,063	*Reference*	N = 3,056	*Reference*
5.0 – 9.9 mg/L	N = 1,578	0.55 (0.01, 1.09)*	N = 1,506	0.67 (0.13, 1.22)*	N = 1,503	0.20 (-0.35, 0.75)
10.0 – 14.9 mg/L	N = 557	0.72 (-0.10, 1.53)	N = 539	0.20 (-0.62, 1.01)	N = 531	0.46 (-0.38, 1.29)
15.0 – 19.9 mg/L	N = 207	1.43 (0.17, 2.69)*	N = 197	0.69 (-0.58, 1.96)	N = 191	0.45 (-0.85, 1.76)
≥20.0 mg/L	N = 252	1.69 (0.54, 2.85)**	N = 241	1.00 (-0.16, 2.16)	N = 239	1.39 (0.21, 2.58)*
Trend test	*P* for trend <0.001	1.54 (0.93, 2.15)	*P* for trend 0.002	0.94 (0.33, 1.55)	*P* for trend 0.010	0.82 (0.20, 1.44)

Values were based on multivariate linear regression models and reflect the difference (95% CI) in maternal systolic and diastolic blood pressure for each C-reactive protein level group compared to the reference group (<5.0 mg/L). Models were adjusted for gestational age at blood puncture, gestational age at blood pressure measurement, maternal age, education, ethnicity, parity, smoking and alcohol consumption, and maternal body mass index. Tests for trend were based on multiple linear regression models with the logarithmically transformed C-reactive protein as a continuous variable. *P <0.05 **P <0.01

Table 3 shows the results for the analyses focused on the associations of C-reactive protein levels with systolic and diastolic blood pressure in different trimesters of pregnancy within strata of body mass index. We observed tendencies towards positive associations between C-reactive protein and systolic blood pressure. C-reactive protein was positively associated with diastolic blood pressure in normal weight, overweight and obese women. For both systolic and diastolic blood pressure, the effect estimates seem to be largest in obese mothers.

Table 3. Associations of maternal C-reactive protein levels in early pregnancy with maternal blood pressure, stratified by maternal body mass index

Systolic blood pressure						
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
Body mass index	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<20.0 kg/m^2	N = 577	0.27 (-0.59, 1.12)	N = 555	0.15 (-0.69, 0.98)	N = 551	0.39 (-0.42, 1.19)
20.0 - 24.9 kg/m^2	N = 3,206	0.46 (0.05, 0.87)*	N = 3,104	0.39 (-0.02, 0.79)	N = 3,078	0.46 (0.04, 0.87)*
25.0 - 29.9 kg/m^2	N = 1,394	0.33 (-0.36, 1.03)	N = 1,324	0.44 (-0.29, 1.16)	N = 1,337	-0.22 (-0.96, 0.51)
≥30.0 kg/m^2	N = 595	1.92 (0.63, 3.21)**	N = 563	1.02 (-0.24, 2.28)	N = 554	0.98 (-0.33, 2.28)
Diastolic blood pressure						
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
Body mass index	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<20.0 kg/m^2	N = 577	0.32 (-0.38, 1.03)	N = 555	0.46 (-0.24, 1.15)	N = 551	0.33 (-0.38, 1.03)
20.0 - 24.9 kg/m^2	N = 3,206	0.72 (0.41, 1.04)**	N = 3,104	0.36 (0.04, 0.68)*	N = 3,078	0.38 (0.06, 0.71)*
25.0 - 29.9 kg/m^2	N = 1,394	0.75 (0.21, 1.30)**	N = 1,324	0.64 (0.08, 1.21)*	N = 1,337	0.24 (-0.32, 0.80)
≥30.0 kg/m^2	N = 595	1.93 (0.97, 2.88)**	N = 563	1.76 (0.86, 2.66)**	N = 554	1.70 (0.77, 2.63)**

Estimates reflect the change (95% CI) in systolic- and diastolic blood pressure per change in SD C-reactive protein in four different categories of maternal body mass index. Models were adjusted for gestational age at blood puncture, gestational age at blood pressure measurement, maternal age, education, ethnicity, parity, smoking and alcohol consumption. *P <0.05 **P <0.01

C-reactive protein levels and risks of hypertensive disorders

Table 4 presents the associations of C-reactive protein levels with the risks of pregnancy complications, in different adjustment models. The multivariate models unadjusted for maternal body mass index show that compared to the reference category (C-reactive protein levels <5.0 mg/L) mothers with elevated C-reactive protein levels (≥20.0 mg/L) had a higher risk of pregnancy induced hypertension (Odds ratio 2.78, 95% CI 1.66 to 4.66). No significant association was observed for the risk of preeclampsia in mothers with elevated C-reactive protein levels, but the trend test showed an association of higher C-reactive protein levels with an increased risk of preeclampsia (P for trend <0.05). However, all effect estimates attenuated towards the null after adjustment for maternal body mass index.

Table 4. Associations of maternal C-reactive protein levels in early pregnancy with the risks of maternal pregnancy complications

Pregnancy induced hypertension			**Model A**	**Model B**	**Model C**
C-reactive protein level	No. of mothers	No. of cases	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
<5.0 mg/L	3,060	112	*Reference*	*Reference*	*Reference*
5.0 – 9.9 mg/L	1,520	62	1.12 (0.82, 1.54)	1.28 (0.93, 1.76)	0.91 (0.62, 1.33)
10.0 – 14.9 mg/L	533	21	1.08 (0.67, 1.74)	1.39 (0.86, 2.26)	0.50 (0.25, 0.99)
15.0 – 19.9 mg/L	195	14	2.04 (1.15, 3.62)*	2.56 (1.41, 4.62)**	1.20 (0.58, 2.48)
≥20.0 mg/L	243	19	2.23 (1.35, 3.70)**	2.78 (1.66, 4.66)**	1.23 (0.64, 2.36)
Trend test			1.43 (1.03, 2.00) *P* for trend 0.035	1.84 (1.30, 2.59) *P* for trend 0.001	0.87 (0.59, 1.27) *P* for trend 0.472
Preeclampsia			**Model A**	**Model B**	**Model C**
C-reactive protein level	No. of mothers	No. of cases	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
<5.0 mg/L	3,001	53	*Reference*	*Reference*	*Reference*
5.0 – 9.9 mg/L	1,485	27	1.03 (0.65, 1.64)	1.09 (0.68, 1.75)	0.81 (0.46, 1.42)
10.0 – 14.9 mg/L	526	14	1.52 (0.84, 2.76)	1.71 (0.93, 3.13)	1.41 (0.71, 2.80)
15.0 – 19.9 mg/L	188	7	2.15 (0.96, 4.80)	2.41 (1.07, 5.45)*	1.49 (0.54, 4.16)
≥20.0 mg/L	229	5	1.24 (0.49, 3.14)	1.38 (0.54, 3.52)	0.61 (0.17, 2.16)
Trend test			1.66 (1.02, 2.71) *P* for trend 0.041	1.87 (1.13, 3.09) *P* for trend 0.015	1.29 (0.75, 2.22) *P* for trend 0.358

Values were bases on multivariate logistic regression models and reflect the odds ratio (95% CI) for pregnancy complications for each C-reactive protein level group compared to the reference group (<5.0 mg/L). Model A was unadjusted. Model B was adjusted for gestational age at blood puncture, maternal age, education, ethnicity, parity, smoking and alcohol consumption. Model C was adjusted for gestational age at blood puncture, maternal age, education, ethnicity, parity, smoking, alcohol consumption and maternal body mass index. Tests for trend were based on multiple linear regression models with the logarithmically transformed C-reactive protein as a continuous variable. *P <0.05 **P <0.01

DISCUSSION

In this population-based, prospective cohort study we observed associations between first trimester maternal C-reactive protein levels and maternal systolic and diastolic blood pressure measured in the three trimesters of pregnancy. However, these associations were largely explained by maternal body mass index. Similarly, we demonstrated that the associations between first trimester maternal C-reactive protein levels and the risks of pregnancy induced hypertension and preeclampsia were fully explained by body mass index. In addition, we did not observe consistent associations of C-reactive protein levels with the systolic or diastolic blood pressure pattern during pregnancy.

A strength of this study was the large population-based cohort from early pregnancy onwards and information about a large number of potential confounders being available. The

design of the study allowed the retrospective assessment of maternal systemic inflammation status determined in early pregnancy and the subsequent development of maternal blood pressure and hypertensive complications. However, potential limitations of the study warrant discussion. The response rate of the study was 61%. Pregnant women who participated in the study were higher educated, healthier and more frequently of European origin than those who did not participate.[20]The mothers in this study do therefore not fully represent the source population and this selective participation might have led to bias. Although selection bias in a prospective follow up studies arises primary from loss to follow up instead of non-response at baseline, it is difficult to ascertain whether the association between C-reactive protein and blood pressure development would be different in non-responders. Our results should therefore be carefully interpreted to the general population of pregnant women. The small number of pregnancy induced hypertension cases (N = 228, 3.9%) and preeclampsia cases (N = 106, 1.8%) might be a reflection of this selection towards a more healthy and homogeneous population, and might have led to lower statistical power to detect the associations of C-reactive protein levels with gestational hypertensive complications. Second, as in all observational studies, residual confounding due to unmeasured covariates might be a potential source of bias. Third, C-reactive protein levels were assayed only once in the first trimester of pregnancy. Therefore, the change in maternal inflammation status during the course of pregnancy and its influence on blood pressure development could not be determined. Finally, the OMRON 907 device has been validated only in non-pregnant adults.[23] Further validation studies are needed to make definite statements about the accuracy of this device in pregnant women.

Normal pregnancy evokes a mild systemic inflammatory response, with evidence for an acute-phase reaction and activation of multiple components of the inflammatory network.[29] C-reactive protein provides an objective and sensitive index of overall inflammatory activity in the body[7], and C-reactive protein levels have been shown to be elevated in healthy pregnancy as compared to the non-pregnant state.[8] C-reactive protein levels are raised already at the fourth week of gestation, suggesting that the maternal inflammatory response is established during the earliest phases of implantation.[30]

It has been postulated that the pathogenesis of preeclampsia may involve an excessive generalized maternal inflammatory response to pregnancy. Redman and colleagues introduced the concept that the processes that generate preeclampsia are an intrinsic part of normal pregnancy and that the systemic inflammatory response of preeclampsia is not a different condition but a more extreme part of the spectrum common to all pregnancies.[29] Studies on inflammation in pregnancies complicated by hypertensive disorders have shown conflicting results. C-reactive protein levels have been shown to be elevated in preeclamptic women, and concentrations were significantly higher in severe preeclampsia as compared to mild preeclampsia.[16] C-reactive protein levels have been demonstrated to be elevated already in the first trimester of pregnancy in women who subsequently developed preeclampsia.[9] However, in a prospective study by Wolf *et al*., the association between elevated first-trimester C-reactive protein levels

and subsequent development of preeclampsia was mitigated after adjusting for maternal body mass index.[13] In our study, C-reactive protein levels in early pregnancy were positively associated with maternal systolic and diastolic blood pressure in all trimesters of pregnancy and with the development of gestational hypertensive disorders, providing evidence of involvement of systemic inflammation. After the adjustment for maternal body mass index however, the associations attenuated substantially. Second, the results of the longitudinal analyses do not show a consistent association of C-reactive protein levels with the systolic or diastolic blood pressure pattern during pregnancy, indicating that C-reactive protein levels in early pregnancy might not influence the longitudinal blood pressure development during pregnancy. However, blood pressure, most notably diastolic blood pressure, typically falls early in gestation and increases from mid-pregnancy onwards.[31] These changes in blood pressure during pregnancy might mask the associations between C-reactive protein levels and blood pressure before pregnancy. The role of C-reactive protein levels before pregnancy in the pathways leading to gestational hypertensive disorders should be studied in further detail.

Body mass index is an important determinant of C-reactive protein in pregnant women[17], and obesity is a well-established risk factor for gestational hypertensive disorders.[32] Inflammation has therefore been proposed to have a mediating role in the association between body mass index and preeclampsia. Previous studies suggested that approximately 31% of the total effect of body mass index on the risk of preeclampsia, was mediated through inflammation.[33] To study the effects of C-reactive protein on maternal blood pressure and gestational hypertensive disorders independent of maternal body mass index, we performed stratified analyses. In normal weight, overweight and obese women, C-reactive protein concentrations were positively associated with maternal diastolic blood pressure throughout pregnancy. This suggests not only a combined contribution to maternal blood pressure development, but also an effect of C-reactive protein on diastolic blood pressure independent of maternal body mass index.

The mechanisms through which C-reactive protein levels are related to maternal blood pressure and the development of gestational hypertensive disorders are unclear though. In the non-pregnant state, C-reactive protein levels are associated with changes indicative of progressive atherosclerosis and endothelial dysfunction.[34-35] Structural and functional changes in the endothelium influence blood pressure development and ultimately lead to hypertension.[34, 36] These changes could also be involved in the pathway of inflammation, blood pressure development and the pathogenesis of hypertensive disorders in pregnancy.

Conclusion

In conclusion, this study showed that the associations of first trimester high sensitivity C-reactive protein levels with maternal blood pressure and the risks of pregnancy induced hypertension and preeclampsia were largely explained by maternal body mass index. Whether C-reactive protein is an intermediate in the pathway between maternal body mass index and gestational hypertensive disorders remains subject to further research.

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SUPPLEMENTARY MATERIAL

Table S1. Characteristics of study participants included and excluded from the analyses

Characteristics of women	Excluded[1] N = 206	Included N = 5,816	*P* value
Age (years)	31.2 (19.2 – 40.1)	30.3 (19.5 – 38.8)	<0.01
Height (cm)	167.6 ± 7.2	167.6 ± 7.4	0.999
Weight (kg)	76.8 ± 18.9	68.5 ± 12.8	<0.01
Body mass index (kg/m^2)	27.4 ± 6.3	24.4 ± 4.3	<0.01
Parity			0.88
0	115 (55.8)	3,298 (56.7)	
≥1	90 (43.7)	2,468 (42.8)	
Missing	1 (0.5)	50 (0.9)	
Highest completed educational level			0.05
Primary school	25 (12.1)	524 (9.0)	
Secondary school	101 (49.0)	2,438 (41.9)	
Higher education	73 (35.4)	2,433 (41.8)	
Missing	7 (3.4)	421 (7.2)	
Ethnic background			0.94
European	123 (59.7)	3,385 (58.2)	
Non-European	77 (37.4)	2,094 (36.0)	
Missing	6 (2.9)	337 (5.8)	
Smoking during pregnancy			0.77
Not	142 (68.9)	3,723 (64.0)	
First trimester only	18 (8.7)	462 (7.9)	
Continued	29 (14.1)	879 (15.1)	
Missing	17 (8.3)	752 (12.9)	
Alcohol consumption during pregnancy			0.04
Not	104 (50.5)	2,320 (39.9)	
First trimester only	25 (12.1)	767 (13.2)	
Continued	61 (29.6)	2,004 (34.5)	
Missing	16 (7.8)	725 (12.5)	
Gestational age at enrollment (weeks)	13.6 (9.4-17.3)	13.2 (9.6-17.6)	0.73
C-reactive protein level (mg/L)	6.2 (0.51-113.19)	4.4 (0.60-25.22)	<0.01
First trimester systolic blood pressure (mmHg)	125.6 ± 17.1	115.2 ± 12.0	<0.01
First trimester diastolic blood pressure (mmHg)	77.2 ± 13.2	67.9 ± 9.3	<0.01
Second trimester systolic blood pressure (mmHg)	125.5 ± 14.7	116.9 ± 11.8	<0.01
Second trimester diastolic blood pressure (mmHg)	75.3 ± 11.5	67.0 ± 9.2	<0.01
Third trimester systolic blood pressure (mmHg)	125.0 ± 15.0	118.3 ± 11.9	<0.01
Third trimester diastolic blood pressure (mmHg)	76.3 ± 12.1	68.9 ± 9.2	<0.01

Values are means (± SD), medians (95% range), or no.'s (%). Differences between included and excluded women were compared using independent samples t-tests or Chi-square tests.

[1]Mothers with extremely high C-reactive protein levels (>100 mg/L) (N = 6), pre-existing hypertension (N = 103), pre-existing diabetes (N = 20), high cholesterol (N = 28), Systemic Lupus Erythematosus (N = 1) and a chronic heart condition (N = 59).

Table S2. Longitudinal associations between C-reactive protein levels and systolic and diastolic blood pressure

	Difference in systolic blood pressure			
C-reactive protein level	**Intercept** mmHg (95% CI)	***P* value**	**Slope** mmHg (95% CI)	***P* value**
<5.0 mg/L	110.46 (109.22, 111.71)	<0.01	*Reference*	
5.0 – 9.9 mg/L	112.20 (109.88, 114.53)	<0.01	-0.02 (-0.06, 0.03)	0.47
10.0 – 14.9 mg/L	112.97 (110.11, 115.84)	<0.01	-0.01 (-0.07, 0.06)	0.82
15.0 – 19.9 mg/L	115.33 (111.55, 119.13)	<0.01	-0.05 (-0.15, 0.05)	0.33
≥20.0 mg/L	115.30 (111.74, 118.87)	<0.01	-0.09 (-0.18, 0.00)	0.06
	Difference in diastolic blood pressure			
C-reactive protein level	**Intercept** mmHg (95% CI)	***P* value**	**Slope** mmHg (95% CI)	***P* value**
<5.0 mg/L	96.03 (91.98, 100.07)	<0.01	*Reference*	
5.0 – 9.9 mg/L	98.29 (93.41, 103.17)	<0.01	-0.04 (-0.07, -0.01)	0.02
10.0 – 14.9 mg/L	99.01 (93.70, 104.31)	<0.01	-0.04 (-0.09, 0.01)	0.12
15.0 – 19.9 mg/L	101.70 (95.68, 107.72)	<0.01	-0.09 (-0.17, -0.01)	0.03
≥20.0 mg/L	101.18 (95.33, 107.02)	<0.01	-0.05 (-0.12, 0.03)	0.22

Values are based on repeated measurement analysis and reflect the change in blood pressure in mmHg per C-reactive protein category, compared to the reference group of women with a C-reactive protein level of <5.0 mg/L.

Table S3. Associations of maternal C-reactive protein levels in early pregnancy with maternal blood pressure

	Systolic blood pressure					
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
C-reactive protein level	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<5.0 mg/L	N = 3,178	*Reference*	N = 3,063	*Reference*	N = 3,056	*Reference*
5.0 – 9.9 mg/L	N = 1,578	1.97 (1.26, 2.68)‡	N = 1,506	2.11 (1.40, 2.83)‡	N = 1,503	1.78 (1.06, 2.50)‡
10.0 – 14.9 mg/L	N = 557	3.32 (2.26, 4.38)‡	N = 539	3.24 (2.17, 4.30)‡	N = 531	3.22 (2.15, 4.29)‡
15.0 – 19.9 mg/L	N = 207	4.69 (3.05, 6.33)‡	N = 197	5.10 (3.45, 6.76)‡	N = 191	4.09 (2.40, 5.77)‡
≥20.0 mg/L	N = 252	4.52 (3.02, 6.01)‡	N = 241	3.78 (2.27, 5.29)‡	N = 239	3.24 (1.72, 4.76)‡
Trend test	*P* for trend <0.001	4.08 (3.31, 4.84)	*P* for trend <0.001	3.95 (3.18, 4.71)	*P* for trend <0.001	3.40 (2.63, 4.17)
	Diastolic blood pressure					
	First trimester (N = 5,772)		**Second trimester** (N = 5,546)		**Third trimester** (N = 5,520)	
C-reactive protein level	No.	Difference (95% CI)	No.	Difference (95% CI)	No.	Difference (95% CI)
<5.0 mg/L	N = 3,178	*Reference*	N = 3,063	*Reference*	N = 3,056	*Reference*
5.0 – 9.9 mg/L	N = 1,578	1.74 (1.19, 2.29)‡	N = 1,506	2.02 (1.46, 2.57)‡	N = 1,503	1.33 (0.77, 1.89)‡
10.0 – 14.9 mg/L	N = 557	2.78 (1.95, 3.60)‡	N = 539	2.42 (1.59, 3.26)‡	N = 531	2.44 (1.60, 3.28)‡
15.0 – 19.9 mg/L	N = 207	4.78 (3.50, 6.06)‡	N = 197	4.29 (2.99, 5.59)‡	N = 191	3.45 (2.13, 4.77)‡
≥20.0 mg/L	N = 252	4.92 (3.76, 6.09)‡	N = 241	4.31 (3.13, 5.49)‡	N = 239	4.47 (3.28, 5.66)‡
Trend test	*P* for trend <0.001	3.98 (3.39, 4.58)	*P* for trend <0.001	3.56 (2.96, 4.16)	*P* for trend <0.001	3.10 (2.50, 3.71)

Values were based on multivariate linear regression models and reflect the difference (95% CI) in maternal systolic and diastolic blood pressure for each C-reactive protein level group compared to the reference group (<5.0 mg/L). Models were adjusted for gestational age at blood puncture, gestational age at blood pressure measurement, maternal age, education, ethnicity, parity, smoking and alcohol consumption. Tests for trend were based on multiple linear regression models with the logarithmically transformed C-reactive protein as a continuous variable. ‡*P* <0.01

Chapter 4.2

C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications

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ABSTRACT

Objective To examine the associations of maternal C-reactive protein levels with fetal growth and the risks of neonatal complications.

Study design C-reactive protein levels were measured in early pregnancy in 6,016 women. Main outcome measures were fetal growth in each trimester and neonatal complications.

Results As compared to the reference group (C-reactive protein levels (<5 mg/L), elevated maternal C-reactive protein levels (≥25 mg/L) were associated with lower estimated fetal weight in third trimester and lower weight at birth (differences: -29 grams, 95% CI -58, 0 and -128 grams, 95% CI -195, -60, respectively). Elevated maternal C-reactive protein levels were also associated with an increased risk of a small size for gestational age in the offspring (adjusted odds ratios 2.94, 95% CI 1.61, 5.36).

Conclusion Maternal C-reactive protein levels in early pregnancy are associated with fetal growth restriction and increased risks of neonatal complications.

INTRODUCTION

C-reactive protein is an acute-phase reactant and a frequently used marker of low grade systemic inflammation, which levels increase in response to both infectious and non-infectious exposures.[1] Elevated C-reactive protein levels are associated with increased risks of common diseases such as cardiovascular disease and type 2 diabetes.[2-3] However, it is still not clear whether these associations reflect causal pathways.[4-5] Elevated C-reactive protein levels during pregnancy, as a marker of low grade inflammation, have also been suggested to be associated with increased risks of fetal growth restriction and neonatal complications, like preterm birth, low birth weight and small size for gestational age.[6-8] Low grade inflammation is associated with endothelial dysfunction, leading to vascular dysfunction and suboptimal placental development. Maternal systemic inflammation might also be a response to ischemia of the placenta, due to suboptimal placentation.[9-10] Subsequently, suboptimal placental development might predispose mothers to increased risks for various pregnancy complications.[11-12] Although the association of elevated C-reactive protein levels with preterm birth has been shown in several studies, results from studies relating C-reactive protein levels with fetal growth measures or neonatal complications are not consistent [7-8,13,14]. Differences in results might be due to differences in study designs and populations. It is not known whether and in which trimester C-reactive protein levels affect fetal growth measures.

In a population-based prospective cohort study among 6,016 pregnant women, we examined the associations of maternal C-reactive protein levels, as marker of low grade inflammation in early pregnancy, with fetal growth characteristics in different trimesters of pregnancy and the risks of neonatal complications.

MATERIALS AND METHODS

Design and population

This study was embedded in the Generation R Study, a population-based prospective cohort study from early fetal life onwards in the city of Rotterdam, the Netherlands.[15] Enrollment was aimed in early pregnancy but was allowed until birth of the child. All mothers were enrolled between 2001 and 2005. Response rate was 61%.[15] The study has been approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants.

Of the total of 8,880 mothers who were enrolled during pregnancy, 76% (N = 6,748) were enrolled before a gestational age of 18 weeks.[15] Of these mothers, blood plasma samples were collected in 95% (N = 6,398) and C-reactive protein was successfully measured in 90% (N = 6,091). Mothers with extremely high C-reactive protein levels (>100 mg/L) (N = 6), and mothers

with twin pregnancies were excluded (N = 69), leaving 6,016 mothers with singleton live births for analysis (Figure S1 of Supplementary material shows the study participants flow chart).

High sensitivity C-reactive protein levels

Maternal venous blood samples were collected in early pregnancy (median 13.2, 95% range 9.6 to 17.6 weeks) and transported to the regional laboratory (Star-MDC, Rotterdam, The Netherlands) for processing and storage.[16] Blood samples were stored at -80 ºC. C-reactive protein concentrations were measured in EDTA plasma samples at the Department of Clinical Chemistry of the Erasmus MC in 2009. We measured high-sensitivity C-reactive protein since traditional clinically used C-reactive protein methods lack the sensitivity in low ranges needed for predicting future risk of events in apparently healthy individuals.[17] C-reactive protein levels were analyzed using an immunoturbidimetric assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, The Netherlands). The within run precision for C-reactive protein was 1.3% at 12.9 mg/L and 1.2% at 39.9 mg/L. The lowest level of detection was 0.2 mg/L. We created 6 categories of C-reactive protein levels (<5.0, 5.0 - 9.9, 10.0 - 14.9, 15.0 - 19.9, 20.0 - 24.9 and ≥25 mg/L). Levels <5.0 mg/L and ≥25 mg/L were considered as low (reference) and elevated levels, respectively.

Fetal growth characteristics

Fetal ultrasound examinations were performed in one of the two dedicated research centers in each trimester of pregnancy. Median gestational age for first, second and third trimester visits were 12.4 weeks (95% range 10.7 to 14.5), 20.5 weeks (95% range 18.7 to 23.1), and 30.4 weeks (95% range 28.6 to 32.8), respectively. In the second and third trimester of pregnancy, we measured fetal head circumference, abdominal circumference and femur length to the nearest millimeter using standardized ultrasoundprocedures.[18] Estimated fetal weight was calculated using the formula by Hadlock.[19] Longitudinal growth curves and gestational age-adjusted standard deviation scores (SDS) were constructed for all fetal growth measurements.[20]

Information about offspring sex, gestational age, weight, length, and head circumference at birth was obtained from medical records and 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. Preterm birth was defined as a gestational age of <37 weeks at delivery, low birth weight was defined as birth weight <2,500 grams, and small size for gestational age at birth was defined as a sex specific gestational age adjusted birth weight below the fifth percentile in the study cohort (standard deviation score ≤1.81 for boys, and ≤1.78 for girls).

Covariates

Information about maternal educational level, ethnicity and parity was obtained by a questionnaire at enrollment in the study. Maternal smoking and alcohol consumption habits were

assessed by questionnaires in each trimester. Maternal anthropometrics, including height and weight, were measured without shoes and heavy clothing and body mass index (BMI) was calculated (weight/height^2 (kg/m^2)) at enrollment.[21] Maternal systolic and diastolic blood pressure were measured at intake, using standardized methods. For each participant, the mean value of two blood pressure readings over a 60-second interval was documented.[22] Folate levels were analyzed from venous samples drawn in the first trimester of pregnancy. Maternal age was registered at enrolment.

Statistical analysis

We assessed the associations of maternal characteristics with C-reactive protein levels as outcome levels using multivariate linear regression models. Since C-reactive protein levels were not normally distributed, we applied a logarithmic transformation for these analyses. Results are presented as geometric means (95% range) per determinant category and an overall *P* for trend based on these regression models. Associations of C-reactive protein levels with fetal growth characteristics were assessed using linear regression models. These models were adjusted for gestational age at the measurement, fetal sex, and maternal age, BMI, education, ethnicity, parity, smoking, alcohol consumption and folate level at intake. BMI is known to be highly correlated with levels of C-reactive protein[23], and with birth weight[21,24], and therefore expected to be our main confounder. Further variables were included in these models based on their association with both the C-reactive protein levels and pregnancy outcomes, or a 10% change in the effect estimate. Next, we assessed the associations of C-reactive protein level with the risks of neonatal complications (preterm birth, low birth weight and small size for gestational age). These models were adjusted for maternal age, BMI, education, ethnicity, parity, smoking, alcohol consumption and folate level at intake. Tests for trends were performed using C-reactive protein as continuous variable in multivariate linear and logistic regression analyses. The percentages of missing values within the population for analysis were <1% for continuous data and <13% for the categorical data. We applied multiple imputations for covariates.[25] Since there were no major differences in the observed results between analyses with imputed missing data or complete cases only, only results including imputed missing data are presented. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Maternal age ranged from 15.3 years to 46.3 years, with a mean of 29.8 years (Table 1). Median C-reactive protein level was 4.5 mg/L (95% range 0.60 - 25.46). Mean offspring birth weight was 3,420 grams (standard deviation (SD) 564 grams), and median gestational age at birth

Table 1. Characteristics of study participants

Maternal characteristics at enrolment	**(N = 6,016)**
Age (years)	29.8 (5.1)
Height (cm)	167.6 (7.4)
Weight (kg)	68.8 (13.1)
Body-mass index, (kg/m^2)	24.5 (4.4)
Parity	
0	3,412 (56.7)
1	1,797 (29.9)
≥2	756 (12.6)
Missing	51 (0.8)
Highest completed educational level	
Primary school	549 (9.1)
Secondary school	2,534 (42.1)
Higher education	2,505 (41.6)
Missing	428 (7.1)
Ethnic background	
European	3,502 (58.2)
Non-European	2,171 (36.1)
Missing	343 (5.7)
Smoking during pregnancy	
Not	3,865 (64.2)
First trimester only	480 (8.0)
Continued	908 (15.1)
Missing	763 (12.7)
Alcohol consumption during pregnancy	
Not	2,424 (40.3)
First trimester only	792 (13.2)
Continued	2,065 (34.3)
Missing	735 (12.2)
Gestational age at enrolment	13.2 (9.6-17.6)
C-reactive protein level (mg/L)	4.5 (0.60-25.46)
Folate level (nmol/L)	15.70 (5.50-37.50)
Birth characteristics	
Birth weight (grams)	3,420 (564)
Gestational age at birth (weeks)	40.1 (35.6-42.3)
Males	3,032 (50.4)
Preterm birth (<37 weeks)	302 (5.0)
Low birth weight (<2,500 grams)	293 (4.9)
Small size for gestational age (≤5th percentile)	299 (5.0)

Values are presented as means (SD), medians (95% range), or number (%).

was 40.1 weeks (95% range 35.6, 42.3). Maternal education, BMI, parity and gestational age were associated with C-reactive protein levels. Mothers with a higher parity had a higher mean C-reactive protein level (means for C-reactive protein levels from 3.84 mg/L for a parity of 0 to 6.15 mg/L with a parity of ≥2) and lower maternal education was associated with higher C-reactive protein levels (means for C-reactive protein levels from 5.40 mg/L for mothers with primary school only to 3.62 mg/L for mothers with higher education). A strong effect was seen for maternal BMI, with monotonous increasing means for C-reactive protein from 2.78 mg/L for the group with BMI<20 to 10.87 mg/L for the group with BMI ≥35 (data shown in Table S1 and Figure S2 of Supplementary material).

As compared to maternal C-reactive protein levels of <5.0 mg/L, maternal C-reactive protein levels of ≥25 mg/L were inversely associated with estimated fetal weight in third trimester and with birth weight (difference -29 grams, 95% CI -58, 0 and -128 grams, 95% CI -195, -60 respectively). No consistent associations were observed between maternal C-reactive protein levels in early pregnancy and fetal head circumference in second or third trimester or at birth (Table 2). We used femur length in second and third trimester and body length at birth as fetal length measures. Maternal C-reactive protein levels were not consistently associated with fetal length.

Table 2. C-reactive protein levels and fetal growth

	Second trimester		**Third trimester**		**Birth**	
Head circumference						
	Head circumference (N = 5,774)		Head circumference (N = 5,748)		Head circumference (N = 4,743)	
C-reactive protein level	No. of children	Difference (mm) (95% CI)	No. of children	Difference (mm) (95% CI)	No. of children	Difference (mm) (95% CI)
<5.0 mg/L	N = 3,162	*Reference*	N = 3,153	*Reference*	N = 2,638	*Reference*
5.0 – 9.9 mg/L	N = 1,573	-0.07 (-0.44, 0.31)	N = 1,566	0.10 (-0.45, 0.66)	N = 1,300	-0.08 (-1.05, 0.88)
10.0 – 14.9 mg/L	N = 570	-0.21 (-0.77, 0.35)	N = 563	-0.34 (-1.17, 0.48)	N = 437	-1.85 (-3.33, -0.36)
15.0 – 19.9 mg/L	N = 210	-0.37 (-1.24, 0.50)	N = 206	0.07 (-1.22, 1.36)	N = 162	-0.50 (-2.80, 1.81)
20.0 – 24.9 mg/L	N = 109	0.44 (-0.73, 1.61)	N = 107	-1.21 (-2.95, 0.54)	N = 82	-1.02 (-4.18, -2.14)
≥25.0 mg/L	N = 150	-0.75 (-1.76, 0.25)	N = 153	-0.51 (-1.99, 0.96)	N = 124	-1.49 (-4.08, 1.10)
P for trend		0.240		0.300		0.109

Table 2. C-reactive protein levels and fetal growth *(continued)*

Length						
	Femur length (N = 5,787)		Femur length (N = 5,789)		Birth length (N = 4,804)	
C-reactive protein level	No. of children	Difference (mm) (95% CI)	No. of children	Difference (mm) (95% CI)	No. of children	Difference (mm) (95% CI)
<5.0 mg/L	N = 3,174	*Reference*	N = 3,176	*Reference*	N = 2,659	*Reference*
5.0 – 9.9 mg/L	N = 1,569	0.10 (-0.01, 0.21)	N = 1,578	0.03 (-0.10, 0.17)	N = 1,313	-0.01 (-1.38, 1.37)
10.0 – 14.9 mg/L	N = 575	0.08 (-0.08, 0.24)	N = 565	0.04 (-0.16, 0.24)	N = 458	-1.00 (-3.08, 1.08)
15.0 – 19.9 mg/L	N = 210	0.03 (-0.22, 0.28)	N = 208	0.06 (-0.25, 0.37)	N = 168	-0.23 (-3.46, 3.01)
20.0 – 24.9 mg/L	N = 109	0.22 (-0.12, 0.56)	N = 108	0.18 (-0.25, 0.37)	N = 80	-3.08 (-7.63, 1.46)
≥25.0 mg/L	N = 150	-0.18 (-0.47, 0.11)	N = 154	-0.27 (-0.63, 0.09)	N = 126	-2.74 (-6.40, 0.92)
P for trend		0.972		0.395		0.275
Weight						
	Estimated fetal weight (N = 5,763)		Estimated fetal weight (N = 5,768)		Birth weight (N = 5,979)	
C-reactive protein level	No. of children	Difference (g) (95% CI)	No. of children	Difference (g) (95% CI)	No. of children	Difference (g) (95% CI)
<5.0 mg/L	N = 3,161	*Reference*	N = 3,167	*Reference*	N = 3,271	*Reference*
5.0 – 9.9 mg/L	N = 1,559	-1 (-3, 2)	N = 1,570	7 (-4, 18)	N = 1,630	10 (-15, 36)
10.0 – 14.9 mg/L	N = 575	1 (-3, 4)	N = 562	-3 (-20, 13)	N = 586	-47 (-85, -9)
15.0 – 19.9 mg/L	N = 209	0 (-6, 6)	N = 207	14 (-12, 46)	N = 221	-28 (-87, 30)
20.0 – 24.9 mg/L	N = 109	3 (-5, 10)	N = 108	12 (-23, 46)	N = 112	-86 (-165, -6)
≥25.0 mg/L	N = 150	-6 (-12, 1)	N = 154	-29 (-58, 0)	N = 159	-128 (-195, -60)
P for trend		0.274		0.115		<0.001

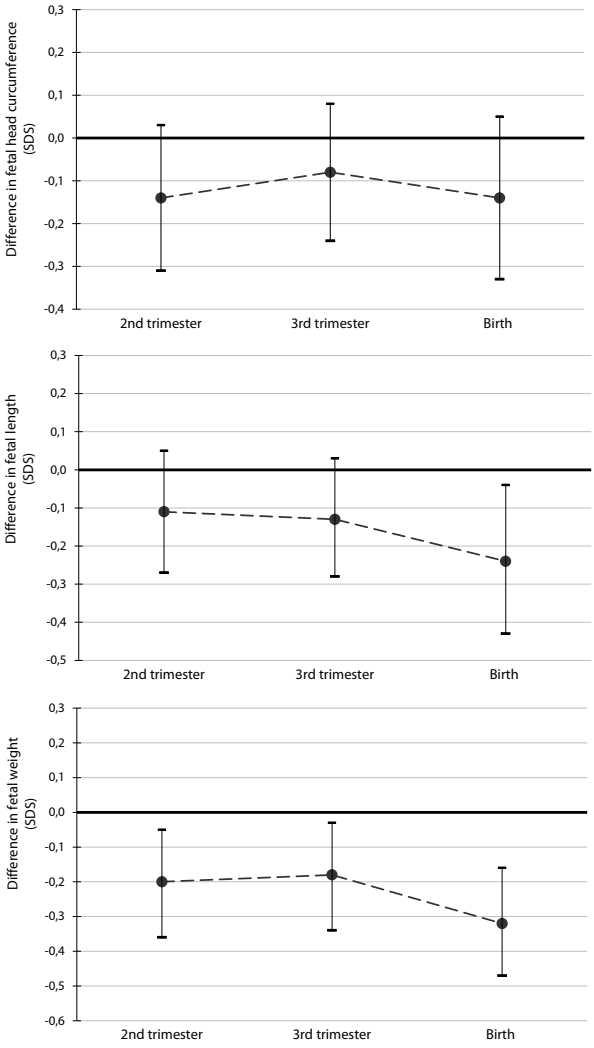
All models were adjusted for maternal age, body mass index, height, systolic and diastolic blood pressure at intake, education, ethnicity, parity, smoking, alcohol, folate level at intake, gestational age at measurement, gestational age at blood collection and fetal sex.

Figure 1 shows that, as compared to levels of <5.0 mg/L, the relative effect of C-reactive protein levels of ≥25.0 mg/L on fetal head circumference, length, and weight, presented as difference in standard deviation score, tended to be greater at birth.

Table 3 presents both the unadjusted and adjusted associations of C-reactive protein levels with the risks of neonatal complications. The differences between the unadjusted and adjusted

models were mainly explained by including maternal BMI in the models. The adjusted models show that as compared to the reference category (C-reactive protein levels <5.0 mg/L) mothers with elevated C-reactive protein levels (≥25.0 mg/L) were more likely to deliver children with a small size for gestational age at birth (adjusted odds ratio 2.94, 95% CI 1.61, 5.36).

Figure 1. Elevated maternal C-reactive protein levels and fetal growth characteristics



Values are regression coefficients (95% CI) and represent the difference in growth standard deviation score (SDS) between elevated levels of maternal C-reactive protein levels in early pregnancy (≥25 mg/L) as compared to the reference group (<5.0 mg/L). All models were adjusted for maternal age, body mass index, height, systolic and diastolic blood pressure at intake, education, ethnicity, parity, smoking, alcohol, use of folate level at intake, gestational age at blood collection and fetal sex.

Table 3. C-reactive protein levels and neonatal complications

			Preterm birth, <37 weeks of gestation			
C-reactive protein level	No. of children	No. of cases	Odds Ratio (95% CI)	*P*value	Adjusted Odds Ratio (95% CI)	*P* value
<5.0 mg/L	3,284	141	1.00		1.00	
5.0 – 9.9 mg/L	1,646	88	1.26 (0.96, 1.65)	0.10	1.25 (0.94, 1.66)	0.12
10.0 – 14.9 mg/L	590	40	1.62 (1.27, 2.32)	0.01	1.69 (1.15, 2.47)	0.01
15.0 – 19.9 mg/L	222	17	1.85 (1.09, 3.11)	0.02	1.86 (1.07, 3.23)	0.03
20.0 – 24.9 mg/L	112	7	1.49 (0.68, 3.24)	0.32	1.69 (0.64, 3.23)	0.38
≥25.0 mg/L	159	9	1.34 (0.67, 2.66)	0.42	1.42 (0.69, 2.89)	0.34
P for trend			0.075		0.109	
			Low birth weight, <2,500 grams			
C-reactive protein level	No. of children	No. of cases	Odds Ratio (95% CI)	*P*value	Adjusted Odds Ratio (95% CI)	*P* value
<5.0 mg/L	3,271	154	1.00		1.00	
5.0 – 9.9 mg/L	1,630	84	1.09 (0.83, 1.43)	0.54	1.18 (0.89, 1.57)	0.25
10.0 – 14.9 mg/L	586	31	1.12 (0.75, 1.66)	0.59	1.40 (0.92, 2.12)	0.12
15.0 – 19.9 mg/L	221	11	1.05 (0.56, 1.95)	0.89	1.35 (0.70, 2.59)	0.38
20.0 – 24.9 mg/L	112	5	0.94 (0.38, 2.32)	0.89	1.11 (0.43, 2.86)	0.82
≥25.0 mg/L	159	8	1.05 (0.51, 2.17)	0.90	1.43 (0.67, 3.03)	0.36
P for trend			0.990		0.280	
			Small for gestational age, ≤5%			
C-reactive protein level	No. of children	No. of cases	Odds Ratio (95% CI)	*P*value	Adjusted Odds Ratio (95% CI)	*P* value
<5.0 mg/L	3,263	168	1.00		1.00	
5.0 – 9.9 mg/L	1,629	73	0.86 (0.65, 1.14)	0.30	0.98 (0.73, 1.31)	0.88
10.0 – 14.9 mg/L	585	29	0.96 (0.64, 1.43)	0.82	1.32 (0.86, 2.01)	0.21
15.0 – 19.9 mg/L	221	10	0.87 (0.45, 1.66)	0.67	1.31 (0.66, 2.59)	0.44
20.0 – 24.9 mg/L	112	5	0.86 (0.74, 2.12)	0.74	1.23 (0.48, 3.16)	0.66
≥25.0 mg/L	159	14	1.76 (1.00, 3.11)	0.05	2.94 (1.61, 5.36)	<0.01
P for trend			0.379		0.003	

Adjusted odds ratio's were adjusted for maternal age, body mass index, height, systolic and diastolic blood pressure at intake, education, ethnicity, parity, smoking, alcohol, folate level at intake and gestational age at blood collection.

Mothers with elevated C-reactive protein levels (≥25.0 mg/L) tended to be more likely to deliver preterm (adjusted odds ratio 1.42, 95% CI 0.69, 2.89) or a child with a low birth weight (adjusted odds ratio 1.43, 95% CI 0.67, 3.03), as compared to the reference category, although not significantly.

DISCUSSION

In this population-based prospective cohort study, we observed associations of maternal socio-demographic and anthropometric measures and life style habits with C-reactive protein levels in early pregnancy. After adjustment for these variables, elevated maternal C-reactive protein levels (≥25.0 mg/L)in early pregnancy were associated with fetal growth restriction, and increased risks of preterm birth and small size for gestational age at birth.

C-reactive protein is known to be slightly elevated during pregnancy, due to the maternal inflammatory reaction to the pregnancy.[26] Previous studies showed that measures of unhealthy life style habits such as cigarette smoking are also associated with elevated C-reactive protein levels, whereas moderate alcohol consumption and increased physical activity are associated with lower C-reactive protein levels.[27-29] Increased BMI and adiposity are strongly associated with elevated C-reactive protein levels.[30-31] In our population-based cohort among pregnant women, maternal BMI, parity and smoking during pregnancy were all positively associated with C-reactive protein levels. Higher maternal education, European background, and continued maternal alcohol consumption were associated with lower C-reactive protein levels. Our results suggest that markers of unhealthy life style habits lead to elevated C-reactive protein levels.

Our results indicate that elevated C-reactive protein levels, as a marker of maternal low grade inflammation, are associated with fetal growth restriction. The effect estimates are relatively small. Our findings may be important from an etiological perspective on population level. Future studies should explore the role of C-reactive protein levels in clinical practice.

We did observe an association between elevated maternal C-reactive protein levels in early pregnancy and the risk of small size for gestational age, which remained significant after adjustment. The differences between the unadjusted and adjusted models were mainly explained by including maternal BMI in the model. Maternal BMI is positively associated with C-reactive protein levels[32] and fetal growth.[21] After adjusting our models for maternal BMI, elevated C-reactive protein levels were still associated with fetal growth restriction and increased risks of small size for gestational age, suggesting that these associations are independent of maternal BMI. Our results are in line with previous smaller studies, which suggested an increased risk of preterm delivery among mothers with elevated C-reactive protein in early or mid-pregnancy. However, these studies did not demonstrate associations with fetal growth retardation.[7,14]

The mechanisms underlying these associations should further be explored, but might include that maternal low grade systemic inflammation, indicated by elevated C-reactive

protein levels, results in suboptimal placental development[9-10], and subsequently increase the risks of pregnancy complications. However, higher levels of C-reactive protein may also be associated with vascular dysfunction that leads in turn to suboptimal placental development.[9-10] Both fetal growth and pregnancy induced hypertensive disorders might have at least part of their origin in suboptimal early placenta development.[33] Also, the associations might not reflect causality, since elevated C-reactive protein levels can be markers of other risk factors or processes leading fetal growth restriction. Previous studies focused on the associations of C-reactive protein genotypes and levels with cardiovascular disease, which used a Mendelian randomization approach, suggested that C-reactive protein is not causally related to cardiovascular disease but rather a marker of other risk factors.[4-5, 34] Such studies have not yet been performed in pregnancy.

Some methodological issues need to be considered. To our knowledge, this is the largest cohort study that has examined the associations of maternal C-reactive protein levels in early pregnancy and placenta related complications in mother and child. Of all mothers enrolled in pregnancy, 76% was enrolled in early pregnancy. Non-response at baseline would lead to selection bias if the associations would differ between those with and without complete data. This seems unlikely. Biased estimates in large cohort studies primarily arise from loss to follow-up rather than from non-response at baseline.[35] Since follow-up information at birth was available in 93%, we do not expect biased results due to loss to follow-up. We were able to adjust or models for multiple potential confounders, related to maternal socio-demographic status, anthropometrics and life style habits. However, as in all observational studies, residual confounding due to unmeasured covariates might still be the case.

In conclusion, maternal low grade inflammation in early pregnancy, as measured by C-reactive protein levels, was associated with fetal growth restriction and increased risks of neonatal complications. Further studies are needed to explore the underlying mechanisms and causality for these associations.

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SUPPLEMENTARY MATERIAL

Table S1. Associations of maternal characteristics with C-reactive protein levels in early pregnancy

	N = 6,016	C-reactive protein
Maternal age		
<20 years	N = 204	3.42 (0.51-21.00)
20 - 24.9 years	N = 965	4.61 (0.70-27.62)
25 - 29.9 years	N = 1,673	4.68 (0.60-27.01)
30 - 34.9 years	N = 2,337	4.12 (0.60-25.21)
35 - 39.90 years	N = 759	4.18 (0.60-23.50)
≥40 years	N = 78	4.73 (1.13-21.48)
P for trend		0.127
Adjusted *P* for trend		0.475
Maternal body mass index at intake		
<19.9 kg/m^2	N = 542	2.78 (0.35-17.92)
20 – 24.9 kg/m^2	N = 3,260	3.54 (0.60-20.75)
25 – 29.9 kg/m^2	N = 1,453	5.58 (1.00-25.22)
30 – 34.9 kg/m^2	N = 470	8.45 (1.98-29.92)
≥35 kg/m^2	N = 229	10.87 (1.50-43.02)
Missing	N =62	4.92 (0.51-21.53)
P for trend		<0.001
Adjusted *P* for trend		<0.001
Parity		
0	N = 3,412	3.84 (0.60-23.07)
1	N = 1,797	4.67 (0.60-28.21)
≥2	N = 756	6.15 (0.99-27.10)
Missing	N = 51	5.66 (0.40-36.83)
P for trend		<0.001
Adjusted *P* for trend		<0.001
Highest completed educational level		
Primary School	N = 549	5.40 (0.50-30.22)
Secondary School	N = 2,534	4.75 (0.70-26.46)
Higher Education	N = 2,505	3.62 (0.60-22.34)
Missing	N = 428	5.40 (0.67-32.60)
P for trend		<0.001
Adjusted *P* for trend		<0.001
Ethnic background		
European	N = 3,502	3.95 (0.60-24.50)
Non-European	N = 2,171	4.85 (0.60-27.07)
Missing	N = 343	5.41 (0.70-33.43)

Table S1. Associations of maternal characteristics with C-reactive protein levels in early pregnancy *(continued)*

	N = 6,016	**C-reactive protein**
P for trend		<0.001
Adjusted *P* for trend		0.103
Smoking		
Not	N = 3,865	4.23 (0.60-26.04)
First trimester only	N = 480	3.95 (0.60-22.09)
Continued	N = 908	4.60 (0.60-22.13)
Missing	N = 763	4.84 (0.70-28.93)
P for trend		0.048
Adjusted *P* for trend		0.956
Alcohol consumption		
Not	N = 2,424	4.79 (0.60-26.18)
First trimester only	N = 792	4.04 (0.70-22.25)
Continued	N = 2,065	3.80 (0.74-24.10)
Missing	N = 735	4.86 (0.70-30.30)
P for trend		<0.001
Adjusted *P* for trend		0.095

Values are presented as geometric means (95% range). Values are based on multivariate linear regression models with adjustment for all maternal characteristics from this table.

Figure S1. Flow chart of participants included in the study

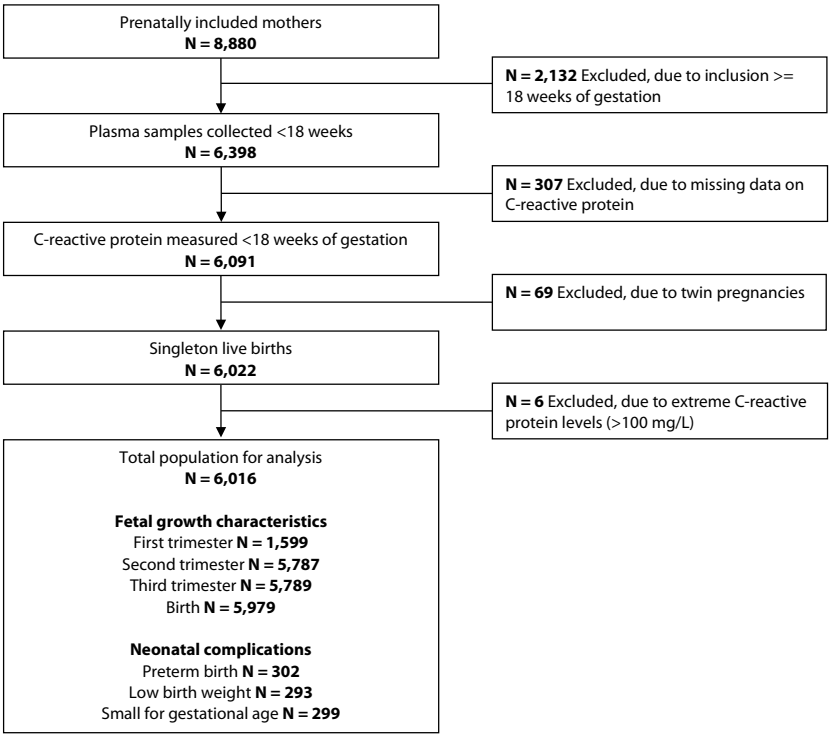
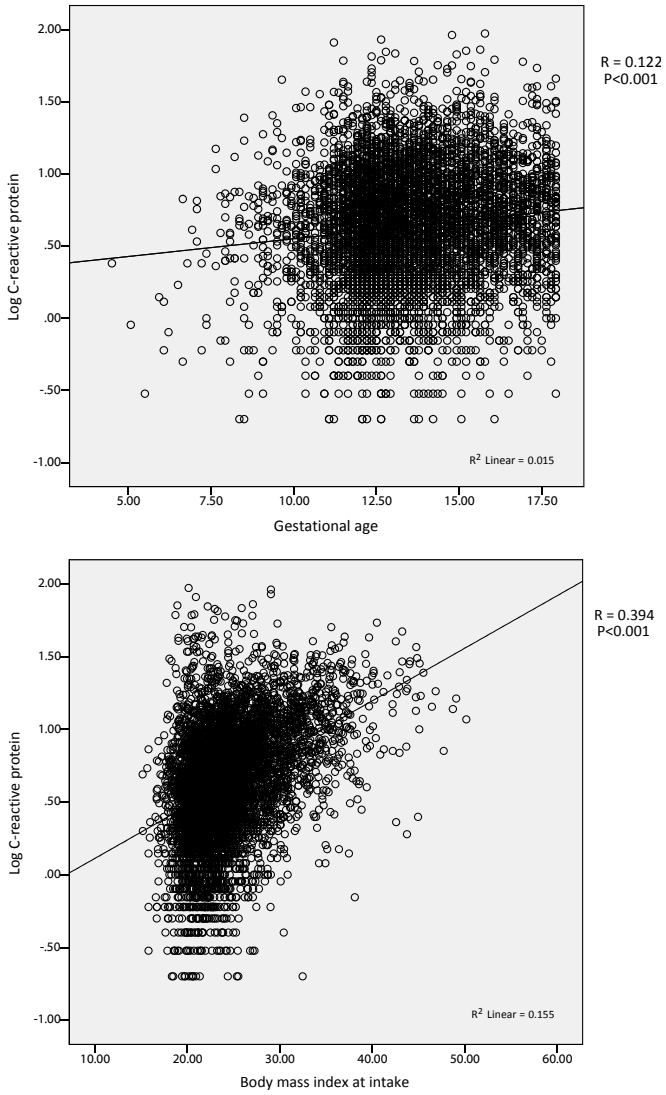


Figure S2. Correlation of gestational age and maternal body mass index with C-reactive protein levels



Plots represent the correlation of gestational age at blood collection and body mass index at intake with C-reactive protein levels, respectively. C-reactive protein was not normally distributed and therefore log-transformation was performed.

Chapter 5

General discussion

INTRODUCTION

Over the past decades, epidemiological studies have provided evidence that early environmental exposures influence the risk of various health outcomes in adulthood. Low birth weight, as an indicator of impaired fetal growth, has been associated with the development of cardiovascular disease[1] and its risk factors.[2] Later studies suggested that high growth rates in childhood following low birth weight may have important influences on the increased risk of cardiovascular disease in later life.[3-4] These findings have led to the "Developmental Plasticity" hypothesis, which describes the ability of an organism to develop in various ways, depending on the particular environment.[5] Developmental adaptations in response to environmental exposures might contribute to better short-term survival, but may increase the susceptibility of cardiovascular disease in adulthood. Many of the published studies examining the associations between fetal factors and the risk of cardiovascular disease in later life have been performed retrospectively in cohorts of adults. The interpretation of the influence of early growth and its determinants is complicated by the possibility of confounding by factors related to cardiovascular disease over the course of adult life. In early life, the effect of these confounding factors is expected to be limited. Therefore, research on the early origins of cardiovascular disease has been extended from studies in adults on birth weight, into studies on the influence of fetal and early postnatal life measures in childhood on cardiovascular risk development.

The exact underlying biological mechanisms linking early growth with cardiovascular disease in later life are largely unknown. It has been hypothesized that the association between early growth and cardiovascular disease in later life might be explained by cardiovascular adaptations due to early suboptimal growth and environment. Therefore, this thesis focused on the effect of prospectively measured fetal and childhood growth patterns and its determinants on cardiovascular and metabolic development in childhood and adulthood. The majority of the studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.[6-7] This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. In addition, two studies were performed in the Nurses' Health Study II, an ongoing prospective cohort study of 116,430 female registered nurses that started in 1989.[8] In this chapter, the most important studies designed to explore the associations between early growth and cardiovascular diseases in adulthood are discussed, and the results of studies performed as part of the current thesis are considered in the context of this previous research. In addition, general methodological issues are described and perspectives for future research are provided.

CARDIOVASCULAR DEVELOPMENT FROM FETAL LIFE TO ADULTHOOD

The heart is the first functional organ of a human embryo, and starts beating approximately 21 days after conception. The fetal heart grows mainly through proliferation of the small and mononucleated cardiomyocytes, referred to as cardiomyocyte hyperplasia.[9] The plastic nature of the developing fetal heart provides the opportunity to adjust to the increased workload imposed to it by the enlarging vascular system.[10] At the end of gestation the cardiomyocytes tend to differentiate, and their capacity to proliferate decreases. Postnatal cardiac growth is mainly established by hypertrophia of cardiomyocytes and non-muscle cell hyperplasia.[9] The growth and maturation of conduit arteries and peripheral vascular beds are determined during intrauterine life and in the early postnatal months[11], and are suggested to be influenced by blood flow.[12] The rates of elastin synthesis in blood vessels increase to a maximum in the perinatal period, followed by a rapid fall in the synthesis rates thereafter.[13-14] Factors that interfere in these early processes might permanently influence cardiovascular development.

Previous studies demonstrated that various measures of the cardiovascular system track in childhood and adulthood, describing that individuals within a particular structural or functional range relative to other individuals tend to remain in this range.[15-20] A large number of studies have been conducted to investigate the development of blood pressure over time.[15] A review and meta-analyses based on cohort studies in diverse pediatric populations showed moderate blood pressure tracking between childhood and adulthood.[15] On the cardiac structural level, moderate tracking of left cardiac structures in pre-school children during the first 2 years of life has been demonstrated within the Generation R Study.[16] Data from older children and young adults indicated more profound tracking of left ventricular mass.[17-18] In adults, longitudinal observations have shown that left ventricular mass and left atrial diameter progressively increase over the course of life, particularly in the presence of risk factors such as elevated blood pressure and BMI.[19-20]

In addition to the tracking of cardiovascular structural and functional measures, studies have shown that factors that influence cardiovascular remodeling measured in childhood, remain in their rank order into adulthood. Children who have measures of body size in a high percentile rank tend to remain in this high rank as they mature, while children who are in a low percentile, stay low.[21]

In adulthood, various cardiovascular structural and functional properties are independently associated with cardiovascular health outcomes. High blood pressure is a well-established risk factor of the premature development of cardiovascular disease, forming a major health issue in all world regions.[22] The clinical value of arterial elastic properties in assessing cardiovascular risk is now recognized and arterial stiffness is increasingly used for the prediction of cardiovascular outcomes.[23-24] Structurally, an increase in left atrial diameter is a sign of atrial remodeling and has a prognostic significance with regard to the risk of cardiovascular morbidity.[25] A reduced cross-sectional area of the aorta and large conduit arteries will decrease the volume of blood

flow and might lead to tissue ischemia[26], while aortic root and ascending aortic dilatation might form a thoracic aneurysm.[27] Increased left ventricular mass, as detected by echocardiography, is a predictor of a higher incidence of cardiovascular disease related clinical events.[28]

These data suggest that factors that contribute to cardiovascular remodeling in early life might have implications for cardiovascular structures and function in later life. Early markers of cardiovascular adaptations might therefore provide insight in the mechanisms linking early growth and cardiovascular disease in later life.

EARLY GROWTH AND CARDIOVASCULAR DEVELOPMENT

Fetal growth and cardiovascular adaptations

Although the underlying mechanisms linking low birth weight with cardiovascular disease in later life are largely unknown, recent evidence from diverse animal and human populations suggests a potentially crucial role of both fetal and early childhood cardiovascular adaptations linked to early growth. Fetal growth restriction due to placental insufficiency is associated with fetal circulatory adaptations, contributing to the preferential perfusion of vital fetal organs, such as the heart and the brain, at the expense of less critical organs. This "brain sparing" is caused by a reduction of the vascular resistance in the fetal brain, and an increase in the peripheral vascular resistance.[29] Growth restricted fetuses showed coronary vasodilatation and it is suggested that this occurs in order to maintain myocardial oxygenation in the presence of hypoxemia and deteriorating venous flow velocity waveforms.[30] Within the Generation R Study, decreased fetal growth was associated with cardiac remodeling and cardiac output changes consistent with a gradual increase in afterload and previously compromised arterial compliance.[31] These changes were already noticeable before the onset of clinical apparent fetal growth restriction.

Fetal growth and cardiovascular adaptations in childhood and adulthood

To investigate the hypothesis that impaired fetal growth has lasting effects on future cardiovascular health, various studies have been performed on the relation of fetal growth characteristics and postnatal cardiovascular adaptations. Martyn and Greenwald suggested that fetal growth restriction was associated with impaired elastin synthesis in the walls of the aorta and other large arteries, and that this deficiency could initiate permanent loss of arterial elasticity.[14] Indeed, adaptations in arterial properties are detectable in both children[32-33] and adults[34-35] born with low birth weight, although results are inconsistent.[36-37] In the current study, we observed an inverse association of birth weight and birth weight for gestational age with systolic and diastolic blood pressure in 6,415 children aged 6 years. We did not find consistent evidence of associations of second or third trimester fetal growth with carotid-femoral pulse wave velocity. Montgomery *et al*. also observed no association of birth measures and arterial stiffness in adults aged 25 years, and the authors explained the difference with their study

and previous studies in adults by a possible increasing influence of atherosclerosis on arterial compliance in older individuals.[37]

Studies on the relation between birth measures and arterial diameter showed positive associations in both preterm and term individuals[16, 38-39], although the results do not seem consistent.[40] In a study designed to define echocardiographic normative data, aortic root dimensions tended to increase with increasing birth weight in healthy neonates, although increments were reduced at higher birth weights.[41] In our study, we observed that length and weight growth rates from 2nd to 3rd trimester of pregnancy were consistently associated with aortic root diameter at the age of 6 years, suggesting the presence of a critical period of developmental programming of the vascular system. In addition, we observed positive associations of birth weight with aortic root diameter at the child age of 6 years, consistent with results from previous studies in children aged 24 months[16] and 9 years.[39]

Studies on the influence of early growth on cardiac dimensions show inconsistent results.[16, 39, 42-43] In a study conducted in 200 young children, fetal growth restriction was suggested to induce primary changes in cardiac morphology, with a linear increase with the severity of growth restriction.[44] In a recent study among 418 adolescents, a direct positive association between birth weight and left ventricular mass was described, independent of current weight.[45] In contrast, in a study of 9 year old children, no significant associations of birth weight with left ventricular mass, left ventricular diameter or atrial diameter were found.[39] In adult males, birth weight was not related to left ventricular mass either.[42] We observed a smaller left ventricular mass in 6 year old children with restricted fetal growth from 2nd to 3rd trimester and born with a small size for their gestational age. It has been hypothesized that cardiac hypertrophy may have a developmental origin in the reduction in cardiomyocyte number through either reduced cellular proliferation or increased apoptosis.[46] Our results may be explained by the inability of the left ventricle to hypertrophy according to the increased workload that is imposed at birth, due to a lower number of cardiomyocytes.

We also hypothesized that fetal haemodynamic adaptations related to fetal growth restriction may have a persistent influence on cardiovascular development. Twin studies showed that in uncomplicated monochorionic twins, no differences were seen in fetal haemodynamic structures between the bigger fetus and the smaller co-twin. However, in the twin-twin transfusion syndrome, the recipient fetuses had increased aortic and pulmonary velocities compared with their donor co-twin.[47] In human infants, pulse wave velocity in the brachioradial arterial segment was higher in the donor twin of the twin-twin transfusion syndrome, implying that cardiovascular adaptations of the donor fetus to haemodynamic disturbances may influence arterial properties.[48] We found no evidence that third trimester placental or fetal haemodynamics were associated with blood pressure or arterial stiffness, but we observed weak associations with cardiac structural outcomes in childhood.

Growth in childhood and cardiovascular development

For an adequate evaluation of echocardiographically measured cardiovascular structures, an evidence-based determination of reference values is essential.[49] To correctly differentiate normal from pathological cardiac development, normalization of cardiac structures to body size is necessary. Based on previous studies on cardiovascular allometry, BSA as calculated by the Haycock formula[50] seems a more important determinant of the size of cardiovascular structures than age, height, or weight.[51] The longitudinal collected data on child BSA and cardiovascular development in the Generation R Study provided the opportunity to establish reference charts for left cardiac structures until the age of 24 months. Moreover, to increase the usefulness of the reference charts in clinical practice, Z-scores were created to facilitate the assessment of the deviation of a particular measurement from the mean for a given BSA.[52]

In childhood and adolescence, cardiovascular structures and function are closely related to physical growth.[53-54] In a retrospective review based on clinical data from pediatric primary care practices, blood pressure already increased with rising BMI in children aged 2 to 5 years.[55] Studies on cardiac development have shown associations of height, weight, BMI and BSA with various cardiac dimensions.[18, 56-58] In a subgroup consisting of Dutch mothers and their children, we observed positive associations of child anthropometrics and left cardiac structures in the first 24 months of life. In addition, infant growth in the first 24 months of life was associated with blood pressure and left ventricular mass at the age of 6 years. Our results suggest that not only cross-sectionally measured child anthropometrics are closely related to cardiovascular structures and function, but that growth in the first months of life influences cardiovascular development in later childhood.

Obesity and cardiometabolic health in childhood

The worldwide prevalence of obesity, known for the adverse effect on the cardiovascular system in later life, is increasing rapidly in the pediatric population.[59] Childhood obesity is already associated with several metabolic and hemodynamic abnormalities in childhood[60], and is a major risk factor for the development of cardiovascular disease in later life.[61] The recognition of obesity as an important determinant of cardio-metabolic health has led to increased interest in the influence of overweight measurements and obesity on early cardiovascular structural and functional development. The increase in the prevalence of obesity in children results in an overall elevation of childhood blood pressure.[62] Preclinical alterations in the aortic elastic properties of obese children have been found[63] and childhood obesity is related to endothelial dysfunction[64], a marker of the early stage of atherosclerotic disease.[65] In children and adolescents, overweight measurements and obesity are associated with cardiac structural changes, such as an increase in left atrial diameter and left ventricular dimensions.[57-58, 66] We observed that obesity, defined by BMI cutoffs, was related to cardiovascular structures and function already at the age of 2 years.

A major disadvantage of the use of BMI as an indicator of obesity is that it does not account for the distribution of different body tissues. Studies in both adults and children provided evidence that next to BMI, body fat mass and an android fat pattern are major predictors of the cardiometabolic profile in children[67], and cardiovascular disease in later life.[68] The findings from Project HeartBeat! indicated that body size, especially when measured as fat free mass, was the strongest determinant of left ventricular mass in children and adolescents.[56] In a cross-sectional study by Daniels *et al*. on body fat distribution determined by DXA and cardiovascular risk factors in children and adolescents, the authors observed that an android fat pattern was associated with a higher systolic blood pressure and left ventricular mass.[69] Studies focused on the effect of fat mass and fat distribution may therefore be of great value in understanding the association of body size and cardiovascular risk development.

Longitudinal approach of fetal and childhood growth

It has been proposed that the pattern of childhood growth mediates the association between restricted fetal growth and the risk of cardiovascular disease. Barker and colleagues described that reduced birth weight followed by accelerated child growth was related to a higher risk of coronary heart disease in later life.[3] In 1,258 men participating in the Caerphilly study, the risk of coronary heart disease associated with low birth weight was present only in individuals with a high BMI in adulthood.[70] In the current thesis, we investigated whether infant growth patterns influenced the association of size at birth on childhood cardiovascular development. We observed that children born small for gestational age or appropriate for gestational age that subsequently experienced catch-up growth in the first 24 months of life, had a higher systolic blood pressure than children born appropriate for gestational age followed by normal growth. In addition, children born small for gestational age with overweight or obese measurements had the highest blood pressure, whereas children born large for gestational age with catch-up growth in infancy or recorded as overweight or obese in childhood had the highest left ventricular mass. These results support the hypothesis that variations in both fetal and childhood growth lead to adaptations in the cardiovascular system.

FETAL AND INFANT EXPOSURES AND CARDIOVASCULAR DEVELOPMENT

The hypothesis that early adverse exposures may lead to postnatal cardiovascular disease by affecting cardiovascular properties is supported by data from a broad range of epidemiological studies. Research has been focused on parental and childhood life style habits, behavior, health and disease.

Parental smoking in pregnancy

Maternal smoking in pregnancy is one of the most important, potentially modifiable adverse exposures in Western countries[71-72], and the effect of fetal smoke exposure on cardiovascular disease and its risk factors have been studied extensively. Fetal smoke exposure is associated with numerous complications, such as fetal growth restriction and preterm birth.[73-74] There is a growing body of evidence for changes in cardiovascular structures and function in children born to mothers who smoked during pregnancy, although results are inconsistent.[75-80] It is not clear whether the association of maternal smoking with cardiovascular outcomes is explained by direct intrauterine mechanisms, or by unmeasured confounders.[76] The results of the Generation R Study suggest that continued maternal smoking during pregnancy is associated with higher diastolic blood pressure and fractional shortening, but not with left cardiac structures and carotid-femoral pulse wave velocity in children at the age of 6 years. To assess the role of confounding, we also investigated whether paternal smoking during pregnancy was associated with cardiovascular structures and function. Among mothers who did not smoke during pregnancy, paternal smoking was associated with aortic root diameter, but not with other cardiovascular outcomes. The stronger effect estimates for maternal smoking, compared to paternal smoking, might suggest that direct intrauterine mechanisms are involved. However, we must be careful to conclude intrauterine effects, since effect estimates were small and paternal smoking was associated with childhood aortic root diameter.

Thus far, the majority of studies on the effect of fetal smoke exposure has been conducted in childhood populations, and has mainly been focused on the effect of maternal smoking only on blood pressure development. To investigate the impact of smoking of both parents in pregnancy on the risk of adult hypertension, we assessed the associations of maternal and paternal smoking in pregnancy with hypertension in adulthood, in a large cohort of US women participating in the Nurses' Health Study II. We observed weak positive associations between both maternal and paternal smoking during pregnancy with the risk of hypertension in their adult daughters. However, observed associations were largely eliminated after adjustment for body weight throughout life, with BMI closest to the diagnosis of hypertension having the greatest impact. Previous studies suggested that intrauterine smoke exposure leads to adaptations in weight and predisposition to an increased risk of being overweight and obesity in the offspring in childhood, adolescence and adulthood.[81-83] Obesity and weight gain are major risk factors for developing hypertension.[84-85] Thus the association of parental smoking during pregnancy with the risk of hypertension in adulthood might be mediated through the programming of body weight throughout life. These findings are supported by a large study in children, which suggested that childhood BMI and weight trajectory largely mediated the associations of maternal smoking during pregnancy with the offspring's systolic blood pressure at the age of seven.[86] However, BMI should also be considered as a potential confounder of the associations. The influence of birth weight in the pathway appeared to be limited. In line

with our findings, in a recent meta-analysis the associations of fetal smoke exposure with child overweight were independent of birth weight.[82]

In the same ongoing longitudinal cohort study, we investigated the associations of maternal and paternal smoking in pregnancy with the risks of type 2 diabetes. The first report of an inverse association between low birth weight and impaired glucose tolerance in adulthood dated from 1991.[87] A recent meta-analysis among 30 studies including more than 150,000 individuals suggested a decrease in risk of type 2 diabetes with increasing birth weight. Maternal smoking leads to restricted blood flow in the vascular beds of the fetus and placenta, leading to suboptimal nutrition supply to various organs, such as the fetal pancreas.[88] Studies in rats demonstrated that high maternal nicotine levels during pregnancy influenced the development of pancreas islets, control of fat storage and homeostasis of energy expenditure and blood vessel structure and reactivity.[88-89] We observed positive associations between maternal smoking during pregnancy and the risk of type 2 diabetes in adulthood, independent of birth weight and adult risk factors for type 2 diabetes. However, observed associations attenuated after adjustment for body weight throughout life, with BMI closest to the diagnosis of type 2 diabetes having the greatest impact.

Early nutrition in childhood

Early nutritional exposures may chronically affect cardiovascular development and lead to cardiovascular disease in later life.[90] Previous studies suggested a protective effect of breastfeeding on cardiovascular disease in later life[91-92], although a large meta-analysis did not confirm these results.[93] In studies focused on cardiovascular risk factors, breastfeeding duration was inversely associated with blood pressure[94-96], obesity[97], cholesterol levels[98], and insulin resistance[99]. In addition, the timing of weaning has been inversely related to cholesterol levels and percentage of body fat and weight in childhood[92, 100], although results were inconsistent.[101-102] The effect of breastfeeding on the risk of cardiovascular disease in later life might be explained by early cardiovascular structural and functional adaptations due to early nutritional exposures.[103-104] School age children exclusively breastfed for 3 to 6 months had a greater carotid intima-media thickness compared to exclusively formula-fed children[103], and young adults breastfed for 4 months or more had a lower arterial distensibility.[104] We observed that never breastfed children tended to have a smaller left atrial diameter during the first 24 months of life compared to breastfed children, although results were nonsignificant. At the age of 6 years, children who had never been breastfed had a higher carotid-femoral pulse wave velocity, smaller left atrial diameter and lower left ventricular mass compared to breastfed children. Children with a younger age at introduction of solid foods had a higher blood pressure. Results from this study suggest support the hypothesis that early nutrition may influence cardiovascular structural and functional development in childhood.

INFLAMMATION IN PREGNANCY, MATERNAL AND NEONATAL COMPLICATIONS

Worldwide, pregnancy-induced hypertension and preeclampsia are leading causes of maternal and fetal morbidity and mortality.[105-106] The clinical features of preeclampsia can partly be explained as responses to endothelial dysfunction[107], with a potential crucial role for an exaggerated maternal systemic inflammatory response to pregnancy.[108-109] C-reactive protein is an acute-phase protein[110-111], and is used as a diagnostic indicator of tissue damage in both acute and chronic inflammation. Although circulating C-reactive protein levels show a more profound elevation in preeclamptic women than in normal pregnancy[112-115], its potential use as a predictive factor for gestational hypertensive disorders is not clear.[116] Previous studies showed higher C-reactive protein levels in preeclamptic pregnancies, while other studies postulated that this might be explained by maternal obesity.[113, 117] BMI is an important determinant of C-reactive protein levels in pregnancy[118-119], and obesity is a well-established risk factor for gestational hypertensive disorders.[119] In this study, we demonstrated that the associations between first trimester maternal C-reactive protein levels and maternal systolic and diastolic blood pressure measured throughout pregnancy, were largely explained by maternal BMI. Similarly, we observed that the associations between first-trimester maternal C-reactive protein levels and the risks of pregnancy-induced hypertension and preeclampsia disappeared after adjustment for maternal BMI. This study provides further ground for the hypothesis of a mediating role of BMI in the association between inflammation and preeclampsia.

Elevated C-reactive protein levels during pregnancy have also been suggested to be associated with increased risks of fetal growth restriction and neonatal complications.[114, 120-121] We observed associations of elevated first trimester maternal C-reactive protein levels (≥25.0 mg/L) with fetal growth restriction, and increased risks of preterm birth and SGA. After adjusting for maternal BMI, as this is associated with both C-reactive protein levels and fetal growth, these associations remained present. The mechanisms underlying these associations are largely unknown. A possible explanation might be that maternal low-grade systemic inflammation results in suboptimal placental development.[122-123] In addition, C-reactive protein levels might be associated with vascular dysfunction, leading to suboptimal placental development.[122-123] However, C-reactive protein levels could also be markers of other risk factors or processes leading to fetal growth restriction. Further studies are needed to explore whether maternal or fetal systemic inflammatory markers also affect cardiovascular development of the fetus and child.

METHODOLOGICAL CONSIDERATIONS

Most of the studies presented in this thesis have been performed within the Generation R Study, a prospective, population-based cohort study from fetal life onwards. Furthermore, two studies

have been embedded in the Nurses' Health Study II, an ongoing prospective cohort study of female registered nurses.[8] Specific methodological considerations of the presented studies have been discussed in the separate chapters of this thesis. In the following paragraphs, some general methodological issues regarding three types of bias that may occur in epidemiological studies are discussed: Selection bias, information bias and confounding.

Selection bias

Selection bias occurs if the relation between determinant and outcome is different in those who participate and those who were eligible, but do not participate. Of all eligible children at birth, is estimated that 61% participated in the Generation R Study. This non response at baseline is not likely to be random. Participants in the Generation R Study generally had a higher socio-economic status and were more likely to be native Dutch compared to non-participants. The percentages of children born preterm or with low birth weight are smaller than expected on population figures in Rotterdam, reflecting a selection toward a relative healthy study population. This selection towards a more affluent and healthy population is expected to lead to bias in etiological association studies if the selection mechanisms are related to both the determinant and the outcome measures, and the associations differ between the study population and the total eligible population. The selection towards a more homogeneous population might affect the generalizability of the results and the frequency rates of our determinant and outcome measures, reducing the statistical power of our studies. In addition, selection bias may also arise from selective loss to follow up if the associations of early growth and cardiovascular development differ between participants lost to follow up and those that continued participating in the study. Of all children included in the Generation R Study, 83.9% (N = 8,305) participated in the follow up studies at age 6 years, and 67.0% participated in one of the cardiovascular follow up measurements. Selection bias due to selective loss to follow up is of concern if the associations of the various determinants with cardiovascular structures and function differ between those included and those not included in the analyses. Although this seems unlikely, these associations cannot be investigated.

Information bias

Information bias is an error that arises in a study because of misclassification of the determinants or outcome measurements. Misclassification of either determinant or outcome can be non-differential and differential.[124] Non-differential misclassification refers to independent misclassification, where the determinant status is not related to the outcome status, and vice versa. In the case of differential misclassification, determinant and outcome status are related. Whereas non-differential misclassification in general will attenuate results, differential misclassification can either exaggerate or underestimate an effect.[124] For the current thesis, data on exposures and outcomes were mainly obtained by physical examinations, questionnaires and biological samples. The main determinants of the studies performed within the Generation

R Study were obtained prospectively without reference to the cardiovascular outcome measures. This makes differential misclassification of the exposure unlikely. A major limitation of the Nurses' Health Study II was the retrospective collection of data on parental smoking and associated perinatal covariates. It is assumed that any resulting misclassification is likely to be non-differential with respect to hypertension status. This would bias the associations towards the null. However, in a subgroup of the Nurses' Health Study II population, a good agreement of the daughters' reports of maternal smoking during pregnancy as compared to the mothers' accounts was reported.[125] Second, part of the data on exposure was obtained through maternal self-report by questionnaires in both the Generation R Study and the Nurses' Health Study II. It has proven to be difficult to acquire reliable and valid measures of life style habits, and assessment by questionnaire might lead to differential misclassification. For example, mothers might underreport the number of cigarettes they smoked during pregnancy[126], causing an underestimation of the differences in cardiovascular structures and function between children of non-smoking and smoking mothers. A possibility to overcome these limitations is the use of biomarkers for the exposure or outcome measures, such as cotinine in maternal urine samples as a biomarker for measuring tobacco exposure.[127-128] However, the use of cotinine levels was not superior to the use of self-reporting by questionnaires in studying the effect of maternal smoking in pregnancy on birth weight[129], and the use of biomarkers is usually not possible in the case of retrospective data collection.

Confounding

A confounder is an extraneous variable that correlates with both the determinant and the outcome, and is not an intermediate on the causal pathway of the variables of interest. A confounder might lead to biased effect estimates. For example, in the study on the association of C-reactive protein with the risk of gestational hypertensive disorders, the associations were largely explained by maternal BMI. Inflammation has therefore been proposed as a mediator in the association between BMI and preeclampsia. The studies on parental smoking in pregnancy and cardiovascular development in later life found comparable effect estimates for maternal and paternal smoking. Similar effect sizes suggest that the association of maternal smoking is explained by other environmental factors, rather than a biological influence of maternal smoking on the intrauterine environment. Although comprehensive information about various covariates was collected in both the Generation R Study and the Nurses' Health Study, the possibility of residual confounding due to insufficient measured or unmeasured factors should always be considered in observational studies. The problem of residual confounding might be addressed by randomization, ensuring that confounders are randomly distributed between groups.

IMPLICATIONS FOR FUTURE RESEARCH

Cardiovascular disease is a leading cause of death in the adult general population. The observation that early life events have an important role for the susceptibility to cardiovascular diseases in adulthood, has led to increased insight in initial development of the disease and its risk factors. This thesis provided new evidence for an influence of early growth and its determinants on cardiovascular development in childhood. However, the significance of our observations with regard to the risk of cardiovascular disease in later life remains unclear. Although cardiovascular structures and function seem to track in childhood and adulthood, the implications of the cardiovascular changes due to early growth and development should be topic of future research. Our findings need to be followed over time to learn if the altered cardiovascular structures and function persist and are a precursor to cardiovascular disease in later life. To gain more insight in the longitudinal associations between early influences and cardiovascular development, regular cardiovascular assessments during childhood beyond the age of 6 years and adolescence should be performed.

In the Generation R Study, blood pressure and carotid-femoral pulse wave velocity measurements have been obtained to investigate cardiovascular function, and echocardiographic measurements of left cardiac structures have been performed to gain insight into cardiac development. Other markers of cardiovascular adaptations might provide complementary views on the underlying mechanism of the association between low birth weight and cardiovascular disease in later life. Autopsy studies in children have shown that the atherosclerotic process, associated with cardiovascular disease in later life, begins to develop many years before cardiovascular complications. Endothelial dysfunction and impaired vascular reactivity induced by dyslipidemia is an initial step in atherosclerosis[130], and carotid intima media thickness is considered as a marker of preclinical atherosclerosis in adults.[131] Ultrasound techniques make it possible to visualize endothelial function and the intima media thickness non-invasively, and this might provide adequate markers of preclinical atherosclerosis already in childhood.[132] The retina is the only site of the body where the human vasculature can be directly and non-invasively visualized.[133] Retinal vascular imaging provides a unique opportunity to study the influence of environmental factors on the microvasculature in children. Biomarkers such as cholesterol and C-reactive protein are well-established determinants of cardiovascular disease in adulthood, and future studies should investigate how subtle changes in biomarker levels relate to cardiovascular development at younger ages. In addition, imaging techniques such as magnetic resonance imaging and three dimensional ultrasounds of the cardiovascular system can visualize cardiovascular development in more detail. These imaging techniques do not use harmful ionizing radiation to acquire images, which improves their application to the pediatric population.

The epigenetics is an emerging field of research and comprises the study of heritable changes in gene expression that are not caused by modification of the DNA sequence.[134]

Epigenetic dysregulation causes human disease and it has been proposed that epigenetic modifications might be involved in the biological mechanisms underlying the developmental origins hypothesis.[135] The epigenetic epidemiology of this hypothesis focuses on the concept that early environmental influences induce epigenetic variation and thereby permanently affect metabolism and the risk of chronic diseases.[135] A study testing for the effects of maternal smoking in pregnancy demonstrated alterations in methylation of placental CYP1A1, and showed that these changes are correlated with CYP1A1 gene expression and fetal growth restriction.[136] In adults exposed to undernutrition during the Dutch Famine, lower methylation of the IGF2 gene, a main factor in human growth and development, was found.[137] This suggests that epigenetic modifications induced by early exposures, such as maternal smoking and fetal nutrition, may have phenotypic consequences throughout life. Therefore, future studies on the role of epigenetics may contribute to the understanding of the biological mechanisms linking early exposures and disease in adulthood.

Knowledge of the early origins of cardiovascular disease may provide a window of opportunity for new prevention initiatives in pregnancy and childhood. The results of the studies presented in this thesis suggest that risk control for long-term prevention of cardiovascular disease should begin from pregnancy onwards. The development of non-invasive measurements and tests could facilitate the detection of high risk children. Lowering risk factors in these children may prevent or postpone the processes that ultimately lead to cardiovascular disease in later life. Evidence for the pathogenic importance of parental and childhood modifiable behavioral and life style factors should be translated into public health policies on the population level.

CONCLUSION

To conclude, both fetal and childhood growth and determinants seem to influence cardiovascular development from early childhood onwards. Although the long-term clinical importance of cardiovascular changes is largely unknown, structural and functional adaptations may possibly have a lasting influence on cardiovascular risk in later life. Therefore, risk control for prevention of future cardiovascular disease should begin from early life onwards.

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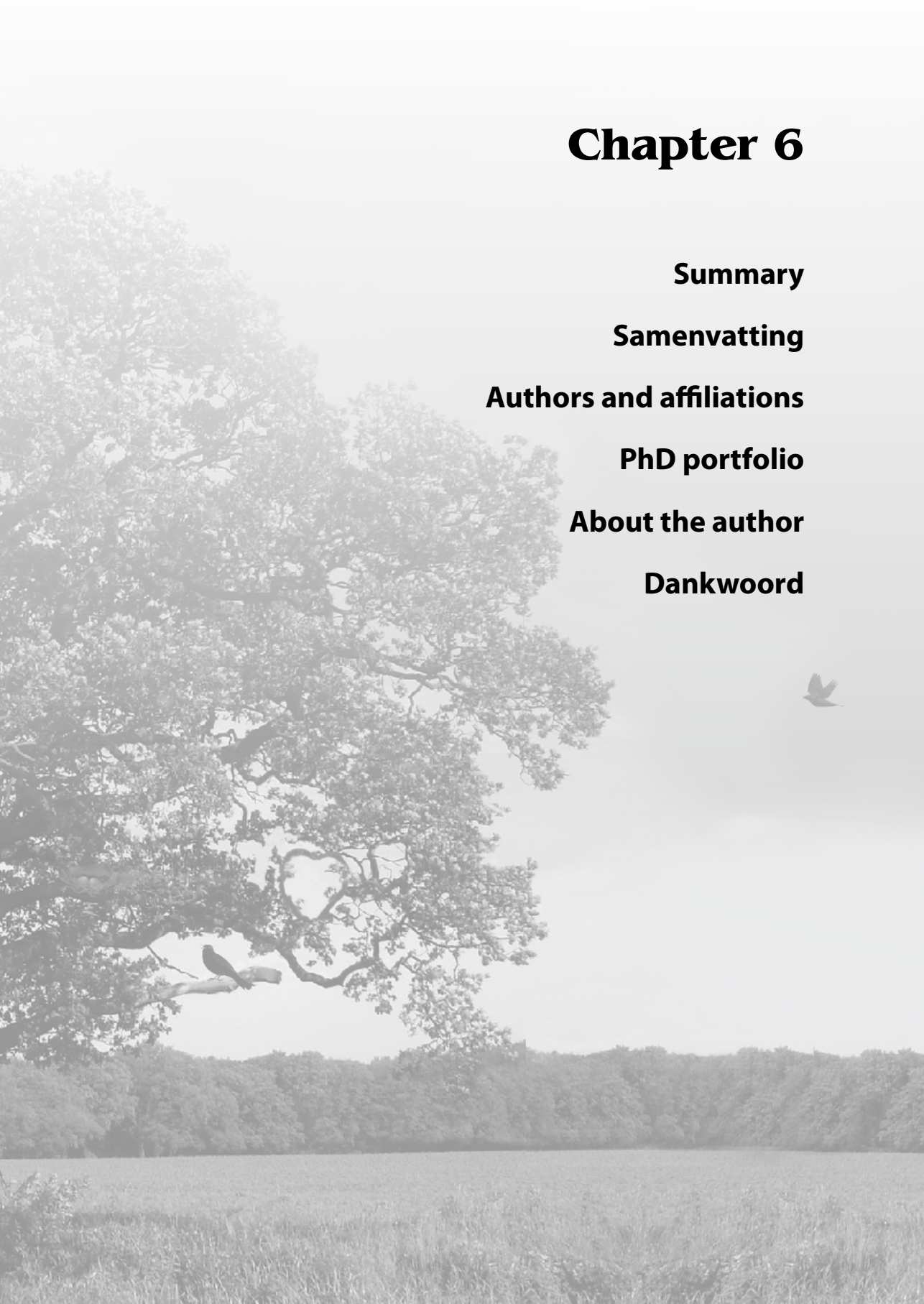
Chapter 6



SUMMARY

Worldwide, cardiovascular diseases are leading causes of morbidity and mortality in adulthood. Epidemiological studies have provided evidence that early environmental exposures influence the susceptibility to disease in later life. Low birth weight has been associated with an increased risk for the development of cardiovascular disease and its risk factors, particularly in individuals who show postnatal catch-up growth. These findings have led to the "Developmental plasticity" hypothesis, describing the ability of an organism to develop in various ways, depending on the particular environment. This developmental plasticity contributes to short-term survival, but may increase the susceptibility of cardiovascular disease in adulthood.

The exact underlying biological mechanisms linking early growth with cardiovascular disease in later life are largely unknown. It has been hypothesized that the association between early growth and the susceptibility to cardiovascular disease in later life might partly be explained by suboptimal cardiovascular adaptations in response to impaired growth and an adverse environment. Therefore, this thesis focused on the effects of prospectively measured fetal and childhood growth patterns and their determinants on cardiovascular outcomes in childhood and adulthood.

Most studies presented in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from the fetal stage until young adulthood in Rotterdam, The Netherlands. The Generation R Study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. In addition, two studies were performed within the Nurses' Health Study II, an ongoing prospective cohort study of female registered nurses from 15 US states that commenced in 1989.

In **Chapter 2** we described the influence of early growth patterns on cardiovascular development in childhood. In **Chapter 2.1**, we established sex-specific longitudinal reference charts and corresponding Z-scores for left cardiac structures and function according to BSA in healthy children until the age of 24 months. These reference charts may contribute to the quantitative assessment of left cardiac structures by ultrasound in early childhood in clinical settings and epidemiological studies. In **Chapter 2.2**, we examined the associations of child anthropometrics with cardiovascular structures and function during the first 24 months of life in a subgroup of Dutch children participating in the Generation R Study. We observed positive associations of length, weight, BMI and BSA with left cardiac structures until the age of 24 months. Furthermore, this study provided evidence that overweight factors and obesity already exert an influence on cardiac size in early childhood. To investigate the hypothesis that impaired growth in early life has lasting effects on future cardiovascular health, we studied the associations of fetal and infant growth patterns with cardiovascular structures and function in childhood (**Chapter 2.3**). Preterm birth and small size at birth were associated with higher systolic blood pressure, and smaller left atrial diameter, aortic root diameter, and left ventricular mass in

children aged 6 years. We observed that fetal and infant growth characteristics were associated with cardiovascular structural and functional outcomes in childhood, independent of current BMI. Children with fetal growth restriction and accelerated growth in the first 24 months of life have higher blood pressure, whereas children with both high fetal and infant growth rates have larger left ventricular mass. The study described in **Chapter 2.4** explored the associations of third trimester placental and fetal haemodynamics with the cardiovascular system in childhood. The results did not provide evidence for an association of placental, fetal cerebral or fetal cardiac haemodynamics with blood pressure or carotid-femoral pulse wave velocity, but we observed associations of third trimester cardiac structural and functional measures with cardiac structural outcomes in childhood.

The aims of the studies described in **Chapter 3** were to identify fetal and infant exposures that affect cardiovascular development in children. In **Chapter 3.1**, we examined the associations of fetal smoke exposure with cardiovascular structures and function in childhood in the Generation R Study. Continued maternal smoking during pregnancy was associated with higher diastolic blood pressure and fractional shortening at the age of 6 years. We observed stronger effect estimates for maternal smoking compared to paternal smoking, suggesting that direct intrauterine mechanisms might be involved. However, effect estimates were small and paternal smoking was associated with childhood aortic root diameter. This suggests a possible influence of unmeasured shared social and environmental factors, rather than direct intrauterine effects. To investigate the impact of smoking of both parents in pregnancy on the risk of adult hypertension, we assessed the associations of maternal and paternal smoking in pregnancy with hypertension in adulthood in the Nurses' Health Study II (**Chapter 3.2**). We observed positive associations of both maternal and paternal smoking during pregnancy, with the risk of hypertension in their adult daughters. However, these associations were largely attenuated after adjustment for body weight throughout life, with BMI closest to the diagnosis of hypertension having the greatest impact. In the same ongoing longitudinal cohort study, we investigated the associations of maternal and paternal smoking in pregnancy with the risks of type 2 diabetes in **Chapter 3.3**. We found positive associations between maternal or paternal smoking during pregnancy and the risk of type 2 diabetes in adulthood, independent of birth weight and adult risk factors for type 2 diabetes. However, the effect estimates attenuated after adjustment for body weight throughout life. Early nutritional exposures may chronically affect cardiovascular development and lead to cardiovascular disease in later life. The effect of early nutritional exposures on the risk of cardiovascular disease in later life might be explained by early cardiovascular structural and functional adaptations due to breastfeeding. Therefore, the purpose of **Chapter 3.4** was to evaluate the influence infant feeding patterns on cardiovascular development in the first 24 months of life. Never breastfed children tended to have a smaller left atrial diameter during the first 24 months of life compared to breastfed children, although results were nonsignificant. At the age of 6 years, children who had never been breastfed had a higher carotid-femoral pulse wave velocity, a smaller left atrial diameter and a lower left

ventricular mass compared to breastfed children (**Chapter 3.5**). In addition, age of introduction of solid foods was negatively related to systolic and diastolic blood pressure at the age of 6 years. We concluded that early nutrition may influence cardiovascular structural and functional development in childhood.

In **Chapter 4,** the associations of inflammation in pregnancy with maternal and neonatal complications were evaluated. **Chapter 4.1** describes the associations between first trimester C-reactive protein levels in pregnancy, maternal blood pressure development and gestational hypertensive disorders. We observed that the associations between first trimester maternal C-reactive protein levels and maternal systolic and diastolic blood pressure measured throughout pregnancy, attenuated after adjustment for maternal BMI. Similarly, the associations between first-trimester maternal C-reactive protein levels and the risks of pregnancy-induced hypertension and preeclampsia were largely explained by maternal BMI. This study provides further ground for the hypothesis of a mediating role of inflammation in the association between BMI and preeclampsia. In **Chapter 4.2** we investigated whether elevated C-reactive protein levels during pregnancy were associated with increased risks of fetal growth restriction and neonatal complications. We observed associations of elevated first trimester maternal C-reactive protein levels (≥25.0 mg/L) with fetal growth restriction and increased risks of preterm birth and SGA, independent of maternal BMI.

Finally, **Chapter 5** provides a general discussion on the studies described in this thesis and considers the results in the context of the current literature. In addition, general methodological issues are described and perspectives for future research are suggested.

In conclusion, the studies described in this thesis suggest that fetal, infant and childhood growth and its determinants influence cardiovascular development from early life onwards. Structural and functional adaptations may possibly have a lasting influence on cardiovascular risk in later life. However, the long-term clinical importance of the cardiovascular changes is largely unknown and further follow-up investigations of this and other cohorts are urgently needed.

SAMENVATTING

Cardiovasculaire aandoeningen zijn wereldwijd een belangrijke oorzaak van zowel morbiditeit als mortaliteit bij volwassenen. Verschillende epidemiologische studies hebben verbanden aangetoond tussen de vroege leefomgeving en het risico op ziekte op latere leeftijd. Deze studies hebben geleid tot de "Developmental plasticity" hypothese, welke beschrijft dat een organisme zich op verschillende manieren kan ontwikkelen, afhankelijk van de specifieke omgevingsinvloeden. Deze plasticiteit kan bijdragen aan de overleving op korte termijn, maar kan negatieve consequenties hebben voor de gezondheid op lange termijn.

De onderliggende biologische mechanismen die het verband tussen de vroege groei, omgeving en cardiovasculaire ziekten verklaren, zijn grotendeels onbekend. Een mogelijke oorzaak zou een suboptimale cardiovasculaire ontwikkeling als gevolg van groeivertraging en een ongunstige vroege omgeving kunnen zijn. Daarom zijn de studies in dit proefschrift gericht op het effect van prospectief gemeten groeipatronen en omgevingsinvloeden op de cardiovasculaire ontwikkeling van zowel kinderen als volwassenen.

De meeste studies beschreven in dit proefschrift zijn uitgevoerd binnen de Generation R Studie, een populatie-gebaseerd prospectief cohort onderzoek onder bijna 10.000 kinderen vanaf de vroege foetale fase tot in de jongvolwassenheid. De Generation R Studie is opgezet om de factoren van invloed op de groei, ontwikkeling en gezondheid van kinderen te onderzoeken. Daarnaast is voor twee studies gebruik gemaakt van gegevens verzameld in de Nurses' Health Study II, een prospectief cohort onderzoek onder vrouwelijke geregistreerde verpleegsters, gestart in 1989.

In **Hoofdstuk 2** is de invloed van vroege groeipatronen op de cardiovasculaire ontwikkeling in de kindertijd onderzocht. In **Hoofdstuk 2.1** hebben we referentiecurven gecreëerd voor de groei van verschillende cardiovasculaire structuren tot de leeftijd van 24 maanden in een Nederlandse subgroep van het Generation R cohort. Daarnaast hebben we Z-scores geformuleerd, wat de klinische toepasbaarheid van de curven verhoogd. In **Hoofdstuk 2.2** zijn de associaties van antropometrie en cardiovasculaire structuren en functie bij Nederlandse kinderen in de eerste 24 maanden van het leven beschreven. We hebben een positief verband gevonden tussen lengte, gewicht, body mass index en body surface area en linker cardiovasculaire structuren. Daarnaast hebben we geconstateerd dat overgewicht en obesitas mogelijk invloed hebben op de cardiovasculaire ontwikkeling bij kinderen op de leeftijd van 24 maanden. In **Hoofdstuk 2.3** is de hypothese dat suboptimale groei in de eerste levensfasen een effect heeft op de cardiovasculaire ontwikkeling in de kindertijd onderzocht. Vroeggeboorte en een te laag gewicht voor de zwangerschapsduur waren geassocieerd met een hogere systolische en diastolische bloeddruk, en met een kleinere linker atrium diameter, diameter van de aortabasis, en linker ventrikel massa op de leeftijd van 6 jaar. De toename van foetale beenlengte en geschat

foetaal gewicht tussen het tweede en derde trimester van de zwangerschap, alsmede tussen het derde trimester en geboorte, lieten eveneens een verband zien met de diameter van de aortabasis en linker ventrikel massa. In de studie beschreven in **Hoofdstuk 2.4** is nagegaan of aan groeivertraging gerelateerde veranderingen in de bloedstroom profielen van de placenta of foetus geassocieerd waren met het cardiovasculaire systeem in de kindertijd. We hebben geen relatie gevonden tussen placenta of foetale haemodynamiek in het derde trimester van de zwangerschap en bloeddruk of arteriële vaatwandstijfheid op de leeftijd van 6 jaar. Wel werden associaties aangetoond tussen bloedstroom profielen van de placenta of foetus en structurele en functionele hartmetingen in het derde trimester, zoals de diameter van de foetale aorta ascendens, en linker hartstructuren in de kindertijd. Dit verband bleek onafhankelijk te zijn van geschat foetaal gewicht.

In **Hoofdstuk 3** is onderzoek gedaan naar de verschillende omgevingsfactoren die van invloed kunnen zijn op de cardiovasculaire ontwikkeling van kinderen. **Hoofdstuk 3.1** behandelt het verband tussen roken van de ouders tijdens de zwangerschap en cardiovasculaire structuren en functie op de leeftijd van 6 jaar in de Generation R Studie. We hebben aangetoond dat doorroken van de moeder tijdens de zwangerschap leidt tot een hogere diastolische bloeddruk en fractional shortening. Daarnaast hebben we geconstateerd dat roken van de vader tijdens de zwangerschap geassocieerd is met een grotere diameter van de aortabasis. Dit duidt op een mogelijke invloed van onbekende gedeelde sociale- en omgevingsfactoren, in plaats van een direct intra-uterien effect van roken. Gebruik makend van de data van de Nurses' Health Study II, is de relatie van roken van beide ouders tijdens de zwangerschap met het risico op hypertensie op volwassen leeftijd onderzocht in **Hoofdstuk 3.2**. In deze studie is een klein effect gevonden van roken van zowel moeder als vader, en daarnaast zijn aanwijzingen gevonden dat deze associaties werden gemedieerd door de huidige body mass index van de dochters. In dezelfde cohort studie is aangetoond dat dochters van ouders die gerookt hebben tijdens de zwangerschap, een licht verhoogd risico hebben op het ontwikkelen van diabetes mellitus type 2 (**Hoofdstuk 3.3**). Echter, ook dit verhoogde risico wordt waarschijnlijk gemedieerd door de huidige body mass index. Voeding in de eerste levensfasen is geassocieerd met een veranderd cardiovasculair risicoprofiel op de latere leeftijd. Daarom onderzochten we in een Nederlands subcohort van de Generation R Studie (**Hoofdstuk 3.4**) de hypothese dat borstvoeding in het eerste levensjaar de cardiovasculaire ontwikkeling van kinderen kan beïnvloeden. Linker atrium diameter, aortabasis diameter, linker ventrikel massa en fractional shortening waren in de eerste twee levensjaren niet verschillend tussen kinderen die wel of geen borstvoeding hadden gehad. Ook zijn er geen associaties gevonden tussen de duur of exclusiviteit van borstvoeding en linker hartstructuren of functie. In aanvulling op de resultaten beschreven in hoofdstuk 3.4, is in **Hoofdstuk 3.5** het effect van borstvoeding en de introductie van bijvoeding op de cardiovasculaire ontwikkeling op de leeftijd van 6 jaar onderzocht. Deze studie liet zien dat het geven van borstvoeding in het eerste levensjaar geassocieerd is met een lagere arteriële vaatwandstijfheid, en een grotere linker atrium diameter en linker

ventrikelmassa. Daarnaast was de leeftijd van introductie van bijvoeding negatief geassocieerd met zowel systolische als diastolische bloeddruk. We concludeerden hieruit dat voeding in de kindertijd mogelijk langdurige gevolgen heeft voor de cardiovasculaire ontwikkeling.

In **Hoofdstuk 4** is de invloed van ontsteking tijdens de zwangerschap op maternale en neonatale complicaties beschreven, aan de hand van de ontstekingsparameter C-reactive protein. **Hoofdstuk 4.1** rapporteert over de positieve associatie tussen C-reactive protein concentraties in het eerste trimester van de zwangerschap, en maternale bloeddruk en het risico op zwangerschapshypertensie of preeclampsie. Echter, de associaties verminderden wanneer rekening gehouden werd met maternale body mass index. Deze studie versterkt de hypothese dat ontsteking een mediërende rol speelt in de eerder vastgestelde associatie tussen maternale body mass index en preeclampsie. In **Hoofdstuk 4.2** onderzochten we of maternale C-reactive protein concentraties in het eerste trimester van de zwangerschap geassocieerd waren met foetale groeipatronen en het risico op neonatale complicaties. We toonden een verband aan tussen een C-reactive protein concentratie van minstens 25 mg/L en een verlaagd geschat foetaal gewicht en geboortegewicht. Tevens vonden we dat het risico op een laag geboortegewicht voor de zwangerschapsduur hoger was bij kinderen van moeders met verhoogde C-reactive protein concentraties.

Tot slot zijn in **Hoofdstuk 5** de belangrijkste resultaten beschreven in dit proefschrift in de context van recent gepubliceerde studies beschouwd. Daarnaast worden methodologische beperkingen en de interpretatie van de bevindingen behandeld.

Concluderend, de studies beschreven in dit proefschrift suggereren dat vroege groei en verschillende omgevingsfactoren verband kunnen houden met de cardiovasculaire ontwikkeling in de kindertijd. Structurele en functionele aanpassingen hebben mogelijk permanente gevolgen voor het cardiovasculaire risicoprofiel op latere leeftijd. Echter, het klinisch belang van de huidige bevindingen op lange termijn is tot op heden onbekend, wat de waarde van vervolgonderzoek onderschrijft.

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PHD PORTFOLIO

Summary of PhD training and teaching activities

Name PhD student : Layla L. de Jonge
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PhD period : 16th of March 2009 – 16th of September 2012
Promotores : Prof. dr. A. Hofman and Prof. dr. W. A. Helbing
Supervisor : Dr. V. W. V. Jaddoe

	Year	Workload (ECTS)
1. PhD training		
Research skills		
Master's degree Epidemiology, NIHES, Erasmus University	2009-2011	
Principles of research in medicine	2009	0.7
Methods of public health research	2009	0.7
Health economics	2009	0.7
Cohort studies	2009	0.7
Introduction to public health	2009	0.7
Primary and secondary prevention research	2009	0.7
Classical methods for data-analysis	2009	5.7
Modern statistical methods	2009	4.3
Genome wide association analysis	2010	1.4
Conceptual foundation of epidemiologic study design	2010	0.7
Case-control studies	2010	0.7
Principles of genetic epidemiology	2010	0.7
Markers of prognostic research	2010	0.7
Study design	2010	4.3
Clinical epidemiology	2010	5.7
Methodological topics in epidemiological research	2010	1.4
General academic skills		
Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC	2009	0.7
Scientific English, Erasmus MC	2010	2.0
In-depth courses		
General ultrasound course Fontys	2009	1.0
Courses for the quantitative researcher	2009	1.4
Clinical cardiovascular epidemiology, COEUR, Erasmus MC	2010	1.5
Missing values in clinical research	2010	0.7
Diagnostic research	2011	0.9
Introduction to clinical and public health genomics	2011	1.9
Symposia and workshops		

40 Years of epidemiology at Erasmus MC. Rotterdam, The Netherlands	2009	0.3
Generation R symposium: Genetics in child cohort studies. Rotterdam, The Netherlands	2010	0.3
Dag voor de jonge onderzoeker. Velthoven, The Netherlands	2011	0.3
(Inter)national conferences and presentations		
European Society for Paediatric Research Annual Meeting. Hamburg, Germany. Poster presentation	2009	0.7
DOHaD. Santiago de Chile, Chile. Oral and poster presentation	2009	1.4
Werkgroep Epidemiologisch Onderzoek Nederland. Nijmegen, The Netherlands. Poster presentation	2010	0.7
Conference of Epidemiological Longitudinal Studies in Europe. Paphos, Cyprus. Oral and post presentation	2010	1.4
Sophia Children's Hospital Research Day. Rotterdam, The Netherlands. Oral and poster presentation	2010	1.4
Sophia Children's Hospital Pediatric Cardiology Research Meeting. Rotterdam, The Netherlands. Oral presentation	2011	1.4
International Conference on Nutrition and Growth. Paris, France. Poster presentation	2012	0.7
DOHaD Satellite Meeting: New Developments in Developmental Epidemiology. Rotterdam, The Netherlands. Oral presentation.	2012	0.7
Reviewing papers		
Paediatric and perinatal epidemiology	2011	0.2
PLoS One	2012	0.6
European Journal of Epidemiology	2012	0.2

2. Teaching

Supervising Master's theses		
Supervisor Geeske Ernst, student Clinical epidemiology, NIHES. Manuscript: *C-reactive protein levels in early pregnancy, fetal growth patterns and the risks of neonatal complications.*	2010	2.0
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3. International research projects

International research project at Harvard School of Public Health. Boston, Massachusetts, USA. Manuscript: *Parental smoking during pregnancy and the risk of adult onset hypertension.*	2011	

4. Other

Participation in the organizing committee planning the weekly seminars of the Department of Epidemiology	2010-2012	

ABOUT THE AUTHOR

Layla de Jonge was born on September 3, 1983 in Leiden, The Netherlands. She graduated from secondary school in 2001 at the Stedelijk Gymnasium Leiden. In the same year, she commenced her studies in Medicine at the Leiden University Medical Center. In 2004, she postponed her studies for one year to accept her appointment as the "Student Assessor" to the board of directors of the Leiden University Medical Center. During her various medical internships, Layla obtained clinical training in Ophthalmology, Dermatology and Otolaryngology at the Academic Hospital Paramaribo in Suriname. She received the LAG Award for the best scientific manuscript during the academic year 2008 - 2009. After obtaining her Medical degree in 2009, Layla began the work described in this thesis at the Department of Epidemiology, Erasmus MC, Rotterdam (Promotores Prof. dr. A. Hofman and Prof. dr. W. A. Helbing, copromotor dr. V. W. V. Jaddoe). During this project, she worked as a research fellow at the Harvard School of Public Health in Boston, Massachusetts, USA, under supervision of dr. K. B. Michels. She obtained her Master of Science degree in Epidemiology at the Netherlands Institute for Health Sciences in 2011. In June 2013, Layla will start as a resident (AIOS) at the Department of Radiology at the Erasmus MC.

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