Social inequalities in cardiovascular health among mothers and their offspring

SELMA H. BOUTHOORN

Social Inequalities in Cardiovascular Health Among Mothers and Their Offspring

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Social Inequalities in Cardiovascular Health Among Mothers and Their Offspring

Sociale ongelijkheden in cardiovasculaire gezondheid bij moeders en hun kinderen

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Voor mijn ouders

Voor Matthijs

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1 General introduction

General introduction

Social inequalities and cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of disability and mortality worldwide and has become a major public health concern.[1] Globally, the number of people dying from CVD has been estimated to increase from more than 17 million in 2008, representing 30% of all global deaths, to more than 23 million in 2030.[1] The largest contribution to the cardiovascular burden comes from coronary heart disease and stroke.[1] The main cardiovascular risk factors have been identified nowadays, and they can be divided into behavioral risk factors (smoking, physical inactivity, unhealthy diet and excessive alcohol consumption), metabolic risk factors (high blood pressure, diabetes, overweight/obesity and hypercholesterolemia) and other risk factors (ageing, gender and stress).[2]

Risk of CVD is not equally distributed among populations, but strongly associated with social disadvantage. Social disadvantage refers to a low socioeconomic position (SEP), based on education, occupation or income, or non-native ethnicity (the latter being strongly associated with SEP). There is ample evidence of both socioeconomic and ethnic inequalities in health, particularly in CVD, and in cardiovascular risk factors preceding CVD.[3, 4] CVD and its risk factors were originally more common in higher socioeconomic groups in the developed world,[5] but over the last 50 years there is overwhelming evidence of a reversal of this pattern which led to an inverse association of SEP with morbidity and mortality from CVD in the developed world.[5-8] In the Netherlands, the risk of dying from CVD is 70% higher among low educated individuals as compared to high educated individuals; higher rates of smoking, excessive alcohol use and physical inactivity contribute substantially to inequalities in CVD.[9] Also, ethnic inequalities in morbidity and mortality, particularly from CVD, have been reported, which could partly be explained by a lower SEP and by cardiovascular risk factors among non-native ethnic groups.[10, 11]

Reducing inequalities in CVD and its underlying risk factors, often considered a priority in public health policies, requires an appropriate understanding of the explanation of these inequalities. For this purpose, several perspectives have been proposed.[12, 13] The 'selection perspective' assumes that CVD or cardiovascular risk factors determine SEP. The 'specific determinants perspective' implies that socioeconomic inequalities arise from the unequal distribution of risk factors, mainly material factors, psychosocial factors and health-related behavioral factors. The 'life course perspective' integrates the latter two perspectives and suggests that CVD inequalities in adult life are partly determined by differential exposure to biological and social factors earlier in life, and this differential exposure may also determine adult SEP.[13] Given the evidence of CVD as diseases that

develop over the life course, understanding the development of inequalities in CVD and risk factors early in life or even during pregnancy is crucial.

Inequalities in maternal cardiovascular health during pregnancy

Cardiovascular health of women during pregnancy is associated with adverse outcomes in both mother and child. Women who suffered from gestational hypertensive disorders, obesity and diabetes during pregnancy are much more likely to develop type 2 diabetes and CVD in the long term.[14, 15] Children born from mothers with excessive gestational weight gain or diabetes during pregnancy are found to have a higher risk of macrosomia and childhood obesity,[16, 17] whereas gestational hypertensive disorders increase the risk of delivering preterm and small size for gestational age infants.[18] Furthermore, the role of abnormal placentation, which may cause higher uterine and umbilical artery resistance patterns and is thought to reflect impaired utero-placental and feto-placental blood flow, has been emphasized in the development of gestational hypertensive disorders, preterm birth and small size for gestational age births.[19, 20] Barker et al. found that low birth weight was associated with an increased risk of CVD in adult life which led to the idea that an adverse intrauterine environment may have long-term cardiovascular consequences for the offspring.[21, 22] Thereafter, several other epidemiological studies replicated the finding that low birth weight was associated with an increased risk of CVD, as well as with diabetes type 2.[23, 24] These findings led to the formulation of the 'Barker' hypothesis later termed the 'Developmental Origins of Health and Disease (DOHaD)' hypothesis.[25] This hypothesis suggests that the increased risk of CVD is due to adverse exposures in fetal and early postnatal life, which may lead to persistent metabolic, physiological and structural adaptations in order to favor short-term survival, and as such 'programmes' adult CVD.[26] Consequently, the offspring of mothers who suffer from pregnancy complications have an increased risk of developing CVD due to adverse intrauterine circumstances. The latter implies that social inequalities in CVD may already arise in prenatal life and emphasizes the importance of studying social inequalities in this particular period.

Social inequalities in fetal growth retardation and preterm birth are well reported.[27] These inequalities lead to children with different prospects of a healthy development already at the onset of their live. Hence, pregnancy can be a critical period in the genesis and development of social inequalities in long-term CVD. It has therefore been proposed that the reduction of social inequalities in this particular period of life should get top priority in the European strategy to tackle inequalities in health.[28] However, still much is unknown about the association of social disadvantage with cardiovascular risk factors in pregnancy, and even less is known about the underlying mechanisms. Therefore, the aim of this thesis is to get more insight in social inequalities in cardiovascular health

during pregnancy, and to better understand the underlying pathways. We hypothesize that women from non-western ethnic groups and women with a low SEP may have an adverse cardiovascular risk profile during pregnancy more frequently than native ethnic groups and women with a high SEP. Identifying social inequalities in cardiovascular risk factors during pregnancy and understanding the underlying mechanisms, one of the main purposes of this thesis, is essential to find entry points for prevention and intervention strategies.

Inequalities in growth and cardiovascular health in childhood

Growth

Socioeconomic inequalities in height, an important indicator of children's health, have been well reported.[29] Children of parents with low SEP are found to be smaller than children of high socioeconomic families, but less is known about other aspects of growth. Only few studies investigated SEP and its relation with growth of the head. Head circumference is an important indicator of growth and development of the brain, especially in early childhood, and a small head circumference is associated with a lower intelligence quotient.[30] As such, investigating the association of SEP and head circumference contributes to the understanding how many different aspects of growth are affected by having a low SEP, and which potential mediating factors may explain this effect.

Overweight and obesity

Many studies have shown that cardiovascular risk factors, such as obesity and hypertension, may already be present in early childhood and track into adulthood where they might contribute to an increased risk of CVD.[31, 32] Previous findings suggest that socioeconomic inequalities in CVD partly arise in early childhood through the unequal distribution of cardiovascular risk factors among children from low socioeconomic families. Indeed, there is consistent evidence showing that school-aged children from low socioeconomic families are more likely to be overweight and obese compared to children from high socioeconomic families.[33] This consistent inverse association is remarkable against the background of research showing that mothers from high SEP give birth to heavier babies as compared to mothers from low SEP.[27] These previous findings suggest that the inverse association between SEP and child weight/body mass index (BMI) emerges in the period after birth and before school age, thus somewhere during the preschool period. To improve the understanding of the exact onset of the inverse socioeconomic gradient in childhood overweight and obesity, it is important to conduct longitudinal studies to the effects of SEP and weight/BMI during the period from birth to the school period. Furthermore, in order to reduce the burden of childhood overweight

and obesity and its associated cardiovascular diseases, it is imperative to understand why some children become overweight and obese, while others do not. Several studies tried to unravel the different pathways leading to overweight and obesity, and although much has been clarified, many questions remain unanswered. For example, it has been suggested that taste may influence energy intake and subsequently effect BMI. In particular the taste of the bitter substance 6-n-propylthiouracil (PROP) is found to be associated with BMI, but studies are inconsistent.[34, 35] These studies have an observational design and inferring causality from observational studies can be problematic. Therefore, more studies are necessary to better understand the causal role of the ability to taste PROP in the pathophysiology of overweight and obesity by using a different kind of study design. Furthermore, in recent years evidence emerged that maternal smoking during pregnancy is associated with obesity and higher blood pressure in the offspring, possibly through the phenomenon of 'fetal programming', but studies are inconclusive.[36-38] Thus far, it is unclear whether these associations are explained by biological mechanisms, as confounding by social patterning is a leading alternative. Studies on prenatal smoking and longitudinally measured childhood BMI and blood pressure are scarce. Thus, to unravel the pathways leading to overweight/obesity and high blood pressure, it is important to investigate whether maternal smoking during pregnancy is associated with the development of childhood BMI and blood pressure, and to confirm previous findings that showed an association of prenatal smoking with BMI and blood pressure in the offspring.

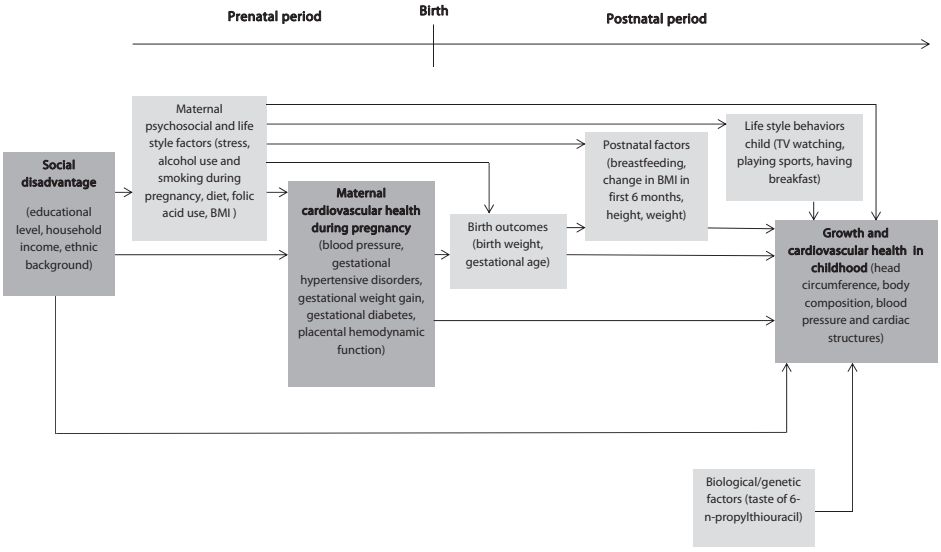

Figure 1. Conceptual framework of potential mechanisms by which social disadvantage and biological/genetic factors might influence growth and cardiovascular health in mothers and their offspring studied in this thesis

Blood pressure and cardiac structures

Recently, socioeconomic inequalities in blood pressure have been shown to already exist in early childhood.[39] High blood pressure and obesity are recognized to influence cardiac structures, such as aortic root size, left atrial and left ventricular enlargement, which in turn contributes to the risk of cardiovascular disease among adults.[40, 41] Since obesity and high blood pressure already exist in childhood and are more common in children from low socioeconomic families, it is important to investigate whether there are already socioeconomic inequalities in cardiac structures in early childhood and what the underlying mechanisms are in order to further improve the understanding of the origins of socioeconomic inequalities in CVD. A conceptual framework of studies presented in this thesis is presented in figure 1.

Research questions

The main purpose of this thesis was to improve the understanding of the pre-, peri- and postnatal development of socioeconomic and ethnic inequalities in CVD risk factors. For this purpose, studies were conducted on two main topics:

Part I. Inequalities in maternal cardiovascular health during pregnancy

The research questions were:

1. Is there an association between ethnic background and blood pressure levels in pregnancy and in gestational hypertensive disorders, and which factors explain this association?
2. Is there an association between socioeconomic position and maternal cardiovascular health during pregnancy, and which factors explain this association?

Part II. Inequalities in growth and cardiovascular health in childhood

3. Is maternal socioeconomic position associated with the child's head growth in the first year of life, and which factors explain this association?

4a. Are there socioeconomic inequalities in child's body composition at the age of 6, and which factors explain this association?

b. At what age does the inverse socioeconomic gradient in body mass index emerge?

5. Is there an association of socioeconomic position with childhood blood pressure and cardiac structures at the age of 6, and which factors explain this relation?
6. Is there a causal relation between the ability to taste the bitter substance 6-n-propylthiouracil (PROP) and child's body composition at the age of 6?

7a. Is maternal smoking during pregnancy associated with the development of child's body mass index and systolic blood pressure in the first 7-10 years of life?

b. Is there an association of maternal smoking during pregnancy with body mass index, fat mass index and systolic blood pressure at the age of 7-10?

Methods

The studies conducted in this thesis were embedded in two prospective cohort studies.

The Generation R Study

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, The Netherlands.[42] The Generation R Study has been designed to identify early, environmental and genetic causes of normal and abnormal growth, development and health during fetal life, childhood and adulthood (www.generationr.nl). The cohort included 9778 mothers and their children living in the study area. While enrolment ideally took place in early pregnancy, it was possible until the birth of the child. In total, 8879 women were enrolled in pregnancy. All children were born between April 2002 and January 2006. Assessments during pregnancy included physical examinations, ultrasound assessments and questionnaires, and were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Postnatal assessments were conducted in the preschool period, from birth to 4 years of age, through regular questionnaires and through routine visits to the child health care centers. At the age of 6 years, all children were invited to a dedicated research center in the Erasmus MS – Sophia Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements. The studies described in Part I were primarily focussed on data collected from the pregnant women, and the studies described in Part II were focussed on the children.

Project Viva

Project Viva is prospective cohort study of pregnant women and their offspring, following them from fetal life into young adulthood.[43] The goal of Project Viva is to find ways to improve health of mothers and their children by looking at the effects of mother's diet as well as other factors during pregnancy and after birth (dacp.org/viva/). Women who were attending their initial prenatal visit at one of 8 urban and suburban obstetrical offices of a multi-specialty group practice located in eastern Massachusetts were recruited between April 1999 and July 2002. In total, 2342 women were enrolled during pregnancy, of whom 9% withdrew or were lost to follow-up, leaving 2128 subjects who delivered a live singleton infant. Mothers completed regular interviews and questionnaires. Children were followed with in-person visits just after delivery, in infancy (6-10 months), early childhood (3-5 years), and mid-childhood (7-10 years) in a research office or at home, and with annual mailed questionnaires. Several measurements were taken during these visits, including anthropometric measurements and blood pressure measurements.

Outline

The objectives of this thesis are addressed in several studies presented in the following chapters. Part I is devoted to social inequalities in maternal cardiovascular health during pregnancy. In Chapter 2 we investigated whether ethnic background was associated with systolic and diastolic blood pressure in each trimester of pregnancy and with gestational hypertensive disorders. Also, potential underlying mechanisms were examined. Chapter 3 and 4 are devoted to socioeconomic inequalities in placental hemodynamic function and gestational diabetes. In addition, the contribution of more proximal determinants of placental hemodynamic function and gestational diabetes leading to these inequalities were investigated. Chapter 5 describes the association of socioeconomic position with excessive gestational weight gain and inadequate gestational weight gain. Also, gestational weight gain in each trimester of pregnancy was examined across the different socioeconomic subgroups. Part II is devoted to inequalities in growth and cardiovascular health in childhood. Chapter 6 describes the association and the underlying pathways of maternal socioeconomic position with their offspring's head circumference during the first year of life. Chapter 7 is focused on the emergence of the inverse associa-

Table 1. Overview of studies presented in this thesis

Chapter	Sample	Population for analysis	Determinant	Outcome	Focus
2	Generation R cohort	N=6215	Maternal ethnicity	Blood pressure and gestational hypertensive disorders	mothers
3	Generation R cohort	N=7033	Maternal educational level	Placental resistance indices	mothers
4	Generation R cohort	N=7511	Maternal educational level	Gestational diabetes	mothers
5	Generation R cohort	N=6979	Maternal educational level	Gestational weight gain	mothers
6	Generation R cohort (Dutch only)	N=3383	Maternal educational level	Head circumference	0-1 year
7	Generation R cohort (Dutch only)	N=3656	Maternal educational level and household income	BMI and fat mass	0-6 years
8	Generation R cohort	N=5843	Maternal educational level	Blood pressure and cardiac structures	6 years
9	Generation R cohort	N=3778	Taste of the bitter substance 6-n-propylthiouracil	BMI and fat mass	6 years
10	Project Viva	N=1755	Maternal smoking during pregnancy	BMI, fat mass and blood pressure	0-10 years

tion of socioeconomic position in overweight and obesity among preschool children. Additionally, we investigated socioeconomic inequalities in BMI and fat mass, and underlying mechanisms, at 6 years of age. In chapter 8 we present the relation between maternal socioeconomic position and child's blood pressure and cardiac structures at 6 years of age. Furthermore, we investigated the contribution of explanatory factors in this relation. In chapter 9 a Mendelian Randomization design is used to investigate the causal relation of PROP-taster status with BMI and fat mass at 6 years of age. Chapter 10 describes the association of maternal smoking during pregnancy and the development of BMI and systolic blood pressure in the offspring in the first 7-10 years of life. Finally, chapter 11 provides a more general discussion of the main findings from previous chapters, in which they are described in a broader context, and implications and suggestions for future research are discussed. An overview of the studies described in this thesis is shown in table 1.

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PART I

Inequalities in maternal cardiovascular health during pregnancy

2

Ethnic differences in blood pressure and hypertensive complications during pregnancy

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Adapted from Hypertension. 2012;60(1):198-205

Abstract

Objective: The aim was to investigate ethnic differences in blood pressure levels in each trimester of pregnancy and the risk of gestational hypertensive disorders and the degree to which such differences can be explained by education and lifestyle-related factors.

Methods: The study included 6215 women participating in a population-based prospective cohort study from early pregnancy onward in Rotterdam. Ethnicity was assessed at enrollment. Blood pressure was measured in each trimester. Information about gestational hypertensive disorders was available from medical charts. Lifestyle factors included smoking, alcohol, caffeine intake, folic acid supplementation, sodium and energy intake, body mass index, and maternal stress. Associations and explanatory pathways were investigated using linear and logistic regression analysis.

Results: Dutch pregnant women had higher systolic blood pressure levels as compared with women in other ethnic groups in each trimester of pregnancy. Compared with Dutch women, Turkish and Moroccan women had lower diastolic blood pressure levels in each trimester. These differences remained after adjusting for education and lifestyle factors. Turkish and Moroccan women had a lower risk of gestational hypertension as compared with Dutch women (odds ratio, 0.32 [95% CI, 0.18–0.58] and odds ratio, 0.28 [95% CI, 0.14–0.58]), and Cape Verdean women had an elevated risk of preeclampsia (odds ratio, 2.22 [95% CI, 1.22–4.07]). Differences could not be explained by education or lifestyle.

Conclusions: Substantial ethnic differences were observed in blood pressure levels and risk of gestational hypertensive disorders in each trimester of pregnancy, and a wide range of variables could not explain these differences.

Introduction

Hypertensive disorders during pregnancy complicate about 7% of all pregnancies and are important causes of maternal and perinatal morbidity and mortality worldwide.[1,2] In the Netherlands, eclampsia/preeclampsia is the leading cause of maternal mortality.[3] The risk of gestational hypertension and preeclampsia has been demonstrated to differ by ethnic background.[3,4] Black descent has been suggested as risk factor for pregnancy-related hypertensive disorders.[4–6]

Blood pressure levels during pregnancy are important risk factors for gestational hypertensive disorders.[7] Little is known about variation in blood pressure development during pregnancy across different ethnic groups. One study found white pregnant women to have the highest blood pressure levels and women with West Indian decent to have the lowest blood pressure levels.[8] Another study showed that Nigerian women had higher diastolic blood pressure (DBP) levels as compared with white women.[9] The underlying pathways explaining the ethnic differences in blood pressure and gestational hypertensive disorders are largely unknown. Some of these ethnic differences may result from differences in socioeconomic position or in prevalence rates of cardiovascular risk factors, such as obesity, smoking, or physical inactivity.[6,10]

Ethnic minority groups of non-European origin now form about 11% of the total population of the Netherlands.[11] Studies from the United States, the United Kingdom, and the Netherlands found nonpregnant women from ethnic minority groups to have elevated blood pressure levels and more cardiovascular risk factors compared with white women.[10,12,13] Therefore, we hypothesized women from ethnic minority groups to also have higher blood pressure levels during pregnancy and a higher risk of gestational hypertension and preeclampsia. In this study, we assessed the associations between ethnic background and systolic blood pressure (SBP) and DBP levels in each trimester of pregnancy and whether socioeconomic position and lifestyle-related determinants could explain these differences among 6215 pregnant women in Rotterdam. In addition, we examined whether ethnic background was associated with the risk of gestational hypertension and preeclampsia.

Methods

Study Design

This study was embedded within the Generation R Study, a population-based prospective cohort study from early pregnancy onward. Details have been described elsewhere.[14–16]

Briefly, the cohort includes 9778 mothers and their children living in Rotterdam. All of the children were born between April 2002 and January 2006. Assessments during pregnancy included physical examinations, ultrasound assessments, and questionnaires and were planned in early pregnancy (gestational age < 18 weeks), midpregnancy (gestational age 18–25 weeks), and late pregnancy (gestational age < 25 weeks). The study was conducted in accordance with the guidelines proposed in the World Medical Association of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Centre Rotterdam. Written consent was obtained from all of the participating parents.[17]

Ethnic Background

Ethnicity was assessed by country of birth of the participating women and their parents and was obtained by questionnaires. Ethnicity was defined according to the classification of Statistics Netherlands. The participant was of non-Dutch origin if one of her parents was born in another country than the Netherlands.[18] If both parents were born abroad, the country of the participant's mother decided on her ethnic background. Next, a distinction was made between women of Dutch ethnic background and the non-Dutch minority groups in this study, Turkish, Moroccan, Antillean, Surinamese, and Cape Verdean. Women with a Surinamese ethnicity were further classified into Surinamese-Hindustani, Surinamese-Creole, or Surinamese-other.

Population for Analysis

In total, 8880 women were enrolled during pregnancy. For the present study, we excluded women without information on ethnic background (n=678). Pregnant women with an "other-Western" and "other non-Western" ethnic background were excluded because of small numbers or mixed composition of these populations (n=1715). Of the remaining 6487 women, those without any blood pressure measurement (n=15) and with preexisting hypertension (n=114) were excluded. Furthermore, we excluded pregnancies leading to fetal death (n=66), loss to follow-up (n=9), and twin pregnancies (n=68), because main interest was in low-risk pregnancies. Thus, the cohort for analysis was composed of 6215 women of Dutch, Turkish, Moroccan, Surinamese-Creole, Surinamese-Hindustani, Antillean, and Cape Verdean ethnic backgrounds. A participant flowchart is given in the Figure S1.

Blood Pressure

Blood pressure was measured with the Omron 907 automated digital oscillometric sphygmanometer, which was validated in nonpregnant adults (OMRON Healthcare Europe BV, Hoofddorp, the Nether- lands).[19] All of the participants were seated in upright position with back support and were asked to relax for 5 minutes. 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. In case of an upper arm >33 cm, a larger cuff (32– 42 cm) was used. The mean value of 2 blood pressure readings over a 60-second interval was documented for each participant.

Pregnancy-Induced Hypertension and Preeclampsia

Information on pregnancy complications was obtained from medical charts. Women suspected of pregnancy complications, based on these records, were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere.[14] Briefly, the following criteria were used to identify women with pregnancy-induced hypertension, development of SBP >140 mm Hg and/or DBP >90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as >2 dipstick readings of >2+, 1 catheter sample reading of >1+, or a 24-hour urine collection containing >300 mg of protein) were used to identify women with preeclampsia.[20]

Explanatory Variables

Most effects of ethnicity on blood pressure are probably indirect ones, acting through more proximal determinants of blood pressure.[21] We considered the following factors to be such potential explanatory variables in the pathway between ethnicity and blood pressure.

Educational Level

Information on educational level, as one indicator of socioeconomic position, was obtained using questionnaires at enrollment. Highest completed educational level was classified into the following 4 categories: (1) low (no education, primary school, or lower vocational training); (2) midlow (>3 years general secondary school or intermediate vocational training); (3) midhigh (higher vocational training or Bachelor's degree); and (4) high (university degree).

Lifestyle-Related Determinants

Smoking, alcohol consumption, and caffeine intake were assessed by questionnaires in each trimester. From the first questionnaire, infor- mation about folic acid supplementation use was obtained. Prepreg- nancy weight was established at enrollment through a questionnaire. On the basis of height (in centimeters), measured at enrollment without shoes, and prepregnancy weight, we calculated prepregnancy body mass index (in weight/height^2). Maternal distress was measured by a questionnaire in midpregnancy using the Brief Symptom Inventory, which gives a Global Severity Index. Higher Global Severity Index reflected pregnant women to experience more stress. Daily sodium and

energy intake during pregnancy were assessed at enrollment using a quantitative food frequency questionnaire.[22] Portion sizes in grams per day were estimated using standardized household measures.[23] To calculate average daily nutritional values, the Dutch food composition table 2006 was used.[24]

Confounding Variables

We treated maternal age at enrollment, parity, and gestational age at enrollment as potential confounders, because they cannot be consid- ered indisputable explanatory variables.[21,25] Parity was obtained through a questionnaire at enrollment. Gestational age was estab- lished by fetal ultrasound examination during the first ultrasound visit.[15]

Statistical Analysis

First, the associations of ethnic background with repeatedly measured SBP and DBP were analyzed using unbalanced repeated- measurement regression analysis.[26] These models take the correlation between repeated measurements of the same subject into account and allow for incomplete outcome data and are described in detail in the online-only Data Supplement. Second, we used multivariate linear regression analysis to study the associations of ethnic background with SBP and DBP in each trimester of pregnancy. To study the overall effect of ethnicity on blood pressure, we started with a model that included the potential confounders (model 1). Subsequently, the potential explanatory variables were added to the model, first separately and then simultaneously (adjusted model). Interaction terms between ethnic background and the explanatory variables were tested for significance. If the test was significant, we also stratified the analysis by the variables. Furthermore, we exam ined the associations of ethnic background with the risks of pregnancy-induced hypertension and preeclampsia using multiple logistic regression models. These models were also adjusted for confounders and the potential explanatory variables. Multiple imputation was used to deal with the missing values in the explaining covariates. Five imputed data sets were created and analyzed together. The repeated-measurement analysis was performed using the Statistical Analysis System version 9.2 (SAS, Institute Inc, Cary, NC). All of the other analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL).

Results

Subject Characteristics

Compared with Dutch women, women of the non-Dutch minority populations were younger, more frequently overweight, more frequently lower educated, less frequently

consumed alcohol, less often used folic acid supplementation, and had a higher sodium intake ($P<0.001$; Table 1). Mean SBP levels were significantly higher in Dutch women compared with non-Dutch women in all 3 trimesters of pregnancy, except for Surinamese-Creole women in the first and second trimesters and for Antillean women in the first trimester. Mean DBP levels were significantly higher in Dutch women as compared with Turkish and Moroccan women, except for Turkish women in the first and second trimester. In total, there were 251 cases (4.2%) of pregnancy-induced hypertension and 120 cases (2.1%) of preeclampsia. The highest prevalence of pregnancy-induced hypertension was among Dutch and Surinamese-Creole women (5.2% and 5.5%) and for preeclampsia among Surinamese-Hindustani and Cape Verdean women (3.8% and 4.2%; $P<0.001$; Table 1).

Longitudinal Measurements

Figure S2 shows the results of the repeated-measurement analyses of ethnic background and SBP and DBP patterns, respectively. SBP was highest among Dutch pregnant women compared with the non-Dutch pregnant women, except for Surinamese-Creole and Antillean women. In all of the ethnic groups, SBP increased throughout pregnancy (Figure S2A), but the lowest increase was among Surinamese-Creole women ($P<0.05$). For all of the ethnic groups, DBP showed a midpregnancy dip, with an increase afterward (Figure S2B). The lowest increase in DBP was observed in Moroccan women ($P<0.05$). The regression coefficients for gestational age–independent (intercept) and gestational age–dependent differences (interaction, ethnic background and gestational age) are given in Table S1.

Ethnicity, Blood Pressure, and Gestational Hypertensive Disorders

Age, parity, gestational age at intake, educational level, body mass index, smoking, alcohol use, caffeine intake, and folic acid use appeared to be significant factors associated with blood pressure levels during pregnancy (Table S2). The amount of women who drank >1 glass alcohol per day was 5.4%, 1.6%, and 1.3%, respectively, in first, second, and third trimesters. Analysis per trimester showed that, as compared with Dutch women, SBP was lower in the non-Dutch minority groups in each trimester of pregnancy. Turkish and Moroccan pregnant women had lower DBP levels as compared with Dutch women. These differences slightly increased after adjusting for our explanatory variables, mainly after inclusion of education (Table 2). Significant interaction terms were found between ethnic background and both parity and age in the association with blood pressure levels. Stratified analyses, according to parity and age, are shown in Table S3. In addition, the stratified models were adjusted for the other explanatory variables. This did not alter our previous findings that the association between ethnic background and blood pressure levels during pregnancy remained unexplained (data not shown).

Table 1. Subject characteristics (N=6215)*

General characteristics	Dutch	Surinamese-creoles	Surinamese-hindustani	Turkish	Moroccan	Cape Verdean	Antillean	P-value‡
	(N =3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)	
Age (yrs)	31.2(4.5)	27.9(6.5)	27.6(4.9)	27.2(5.0)	27.9(5.3)	27.2(6.0)	26.1(5.4)	P<0.001
Heigth (cm)	170.8(6.4)	166.4(6.9)	160.0(5.4)	161.6(5.8)	162.7(5.8)	164.9(6.6)	164.8(6.1)	P<0.001
Weight (kg)	70.7(12.3)	71.9(16.4)	61.6(12.8)	68.2(13.1)	70.4(13.4)	67.7(13.2)	70.8(14.1)	P<0.001
Body mass index (kg/m$^{2)}$	23.2(3.7)	24.6(5.1)	23.2(4.5)	24.7(4.7)	25.1(4.3)	23.6(3.7)	24.5(4.7)	P<0.001
Parity (%)								
Nulliparous	59.4	56.0	57.2	45.4	39.7	57.1	58.0	P<0.001
Missing	0.2	0.0	0.0	0.1	0.2	0.9	0.4	
Gestational age at intake (wks)†	13.6(10.2,23.9)	15.1(10.7,29.4)	14.4(10.1,23.2)	15.4(10.5,30.5)	16.2(10.6,31.2)	15.4(11.1,29.2)	15.5(8.7,28.8)	P<0.001
Educational level (%)								
Low	16.8	36.2	38.0	52.2	53.7	49.8	43.6	P<0.001
Mid-low	25.6	50.9	46.4	34.7	33.9	41.1	41.3	
Mid-high	24.6	10.8	10.4	8.4	9.2	7.9	11.0	
High	32.9	2.2	5.2	4.7	3.2	1.2	4.2	
Missing	0.5	1.7	1.2	4.5	7.5	3.6	2.3	
Maternal stress index‡	0.13(0.00,0.88)	0.29(0.00,1.32)	0.29(0.00,1.36)	0.44(0.00,2.12)	0.37(0.00,1.95)	0.37(0.00,1.90)	0.33(0.00,1.40)	P<0.001
Missing	13.9	29.3	26.0	31.5	37.3	30.2	26.5	
Alcohol consumption (%)								
None	48.9	47.8	70.4	89.7	95.1	53.2	55.3	P<0.001
First trimester only	16.0	15.5	14.0	3.2	0.9	15.5	15.5	
Continued	35.0	36.6	15.6	7.1	3.9	28.7	29.2	
Missing	7.6	6.5	6.0	8.9	9.2	10.0	5.3	

Table 1. Subject characteristics (N=6215)* **(continued)**

General characteristics	Dutch	Surinamese-creoles	Surinamese-hindustani	Turkish	Moroccan	Cape Verdean	Antillean	P-value‡
	(N =3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)	
Smoking habits (%)								
None	74.1	66.4	78.4	62.7	92.3	68.0	72.3	P<0.001
First trimester only	8.9	13.4	8.0	7.5	2.1	8.8	7.6	
Continued	17.0	20.3	13.6	29.8	5.6	23.3	20.1	
Missing	7.9	6.9	6.8	9.1	10.3	11.2	6.8	
Folic acid supplement use (%)								
Preconceptional use	53.8	17.2	20.8	16.2	14.8	12.7	20.1	P<0.001
First 10 weeks use	33.3	35.3	33.2	24.9	19.1	26.9	26.9	
No use	12.9	47.4	46.0	58.9	66.1	60.4	53.0	
Missing	17.6	22.4	20.8	23.1	22.5	23.6	25.0	
Caffeine intake (%)								
None	3.7	9.5	5.6	2.9	3.9	4.5	9.1	P<0.001
<2 units per day	46.9	70.7	66.4	51.7	61.2	73.1	76.9	
2-3.9 units per day	38.4	16.4	22.0	35.8	29.8	20.5	11.0	
4-5.9 units per day	8.9	2.6	2.4	6.1	3.6	1.5	1.9	
≥6 units per day	2.2	0.9	3.6	3.5	1.5	0.3	1.1	
Missing	2.4	8.2	7.2	8.1	8.4	9.1	5.3	
Daily sodium intake (mg)	3281.1(1491.4)	3511.5(2268.7)	3456.1(2414.4)	3685.0(2604.9)	3487.0(2231.6)	3421.1(2285.3)	3612.7(2566.1)	P<0.001
Daily energy intake (kcal)	2135.9(479.3)	1881.1(492.4)	1876.6(526.8)	1945.3(468.3)	1982.7(463.9)	1947.8(497.6)	1973.0(494.7)	P<0.001
Missing	12.8	32.7	35.2	46.7	45.7	37.5	37.1	

Table 1. Subject characteristics (N=6215)* **(continued)**

General characteristics	Dutch	Surinamese-creoles	Surinamese-hindustani	Turkish	Moroccan	Cape Verdean	Antillean	P-value‡
	(N =3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)	
Mean systolic blood pressure (mmHg)								
1st trimester	117.3(11.9)	116.0(10.9)	110.4(11.6)§	112.1(11.3)§	112.1(11.6)§	114.3(11.2)§	116.1(11.0)	P<0.001
2nd trimester	118.4(11.6)	117.1(12.0)	110.9(11.6)§	114.2(11.7)§	113.5(11.4)§	115.8(12.5)§	116.4(11.6)	P<0.001
3rd trimester	120.2(11.4)	117.3(11.4)§	113.1(12.1)§	115.8(11.5)§	115.0(12.3)§	117.0(11.9)§	117.9(12.3)§	P<0.001
Mean diastolic blood pressure (mmHg)								
1st trimester	68.4(9.2)	67.9(9.4)	68.8(9.6)	67.4(9.7)	66.5(9.4)§	67.9(8.8)	68.7(9.6)	P=0.004
2nd trimester	67.1(9.3)	67.9(10.2)	67.5(9.0)	66.4(9.3)	65.5(8.8)§	67.0(9.0)	67.4(8.9)	P=0.004
3rd trimester	69.4(9.2)	68.9(10.1)	69.5(9.1)	68.1(9.3)§	67.1(8.8)§	69.3(9.4)	69.3(9.9)	P<0.001
Hypertensive pregnancy complications (%)								
Pregnancy induced hypertension	5.2	5.5	3.4	1.7	1.5	3.2	2.9	P<0.001
Preeclampsia	1.9	2.4	3.8	1.6	0.8	4.2	3.7	P<0.001
Missing	9.2	11.2	9.6	5.3	4.1	9.7	11.4	

*Values are means (standard deviation) or percentages.

†Median (95% range).

‡P-values are calculated with the chi-square test for categorical variables and ANOVA for continuous variables.

§ Significantly different means compared to the Dutch (reference) group calculated with Bonferroni post hoc tests.

Table 2. Cross-sectional associations of ethnic background with systolic and diastolic blood pressure*

Models	Dutch	Surinamese-creoles	Surinamese-hindustani	Turkish	Moroccan	Cape Verdean	Antillean
	(N=3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)
First trimester Systolic blood pressure (mm Hg)							
Model 1†	Reference	-1.6(-3.5,0.3)	-7.2(-9.0,-5.4)#	-5.5(-6.7,-4.4)#	-5.2(-6.5,-3.8)#	-3.2(-4.7,-1.6)#	-1.6(-3.4,0.2)
Model 1 + educational level	Reference	-2.1(-4.0,-0.2)‖	-7.6(-9.3,-5.9)#	-6.0(-7.2,-4.8)#	-5.7(-7.1,-4.4)#	-3.8(-5.4,-2.2)#	-2.0(-3.8,-0.2)‖
Model 1 + lifestyle related variables‡	Reference	-1.9(-3.7,0.001)	-6.8(-8.5,-5.1)#	-6.3(-7.5,-5.0)#	-6.3(-7.7,-4.9)#	-3.0(-4.5,-1.4)#	-2.1(-3.9,-0.3)‖
Fully adjusted model§	Reference	-2.0(-3.9,-0.1)‖	-6.9(-8.6,-5.3)#	-6.4(-7.6,-5.1)#	-6.4(-7.8,-5.0)#	-3.2(-4.7,-1.6)#	-2.2(-4.0,-0.4)‖
Second trimester Systolic blood pressure (mm Hg)							
Model 1†	Reference	-1.6(-3.2,0.1)	-7.9(-8.7,-7.1)#	-4.5(-5.5,-3.4)#	-4.8(-6.0,-3.7)#	-2.9(-4.3,-1.6)¶	-2.5(-4.0,-1.0)¶
Model 1 + educational level	Reference	-2.1(-3.8,-0.5)¶	-8.5(-10.0,-6.9)#	-5.1(-6.2,-4.1)#	-5.5(-6.7,-4.3)#	-3.7(-5.1,-2.3)#	-3.0(-4.5,-1.4)#
Model 1 + lifestyle related variables‡	Reference	-2.3(-3.9,-0.7)¶	-7.6(-9.1,-6.1)#	-5.4(-6.5,-4.3)#	-6.0(-7.3,-4.8)#	-2.8(-4.2,-1.4)#	-3.1(-4.6,-1.6)#
Fully adjusted model§	Reference	-2.5(-4.1,-0.9)¶	-7.8(-9.3,-6.2)#	-5.6(-6.7,-4.5)#	-6.2(-7.5,-5.0)#	-3.1(-4.5,-1.7)#	-3.3(-4.8,-1.7)#
Third trimester Systolic blood pressure (mm Hg)							
Model 1†	Reference	-3.4(-4.2,-2.6)#	-7.7(-8.5,-6.9)#	-4.8(-5.9,-3.8)#	-5.4(-6.6,-4.3)#	-3.8(-5.1,-2.4)#	-3.0(-4.5,-1.5)#
Model 1 + educational level	Reference	-4.0(-5.6,-2.3)#	-8.2(-9.7,-6.6)#	-5.5(-6.6,-4.5)#	-6.0(-7.2,-4.9)#	-4.5(-5.9,-3.1)#	-3.5(-5.0,-1.9)#
Model 1 + lifestyle related variables‡	Reference	-4.1(-5.7,-2.5)#	-7.3(-8.9,-5.8)#	-5.9(-7.0,-4.8)#	-6.5(-7.7,-5.2)#	-3.8(-5.2,-2.4)#	-3.6(-5.1,-2.0)#
Fully adjusted model§	Reference	-4.3(-5.9,-2.7)#	-7.5(-9.1,-6.0)#	-6.0(-7.2,-5.0)#	-6.6(-7.9,-5.4)#	-4.1(-5.4,-2.7)#	-3.7(-5.2,-2.2)#
First trimester Diastolic blood pressure (mm Hg)							
Model 1†	Reference	-0.4(-1.2,1.1)	0.7(-0.7,2.0)	-0.6(-1.6,0.3)	-1.3(-2.4,-0.2)‖	-0.1(-1.4,1.2)	0.6(-0.8,2.1)
Model 1 + educational level	Reference	-0.6(-2.1,0.8)	0.5(-0.9,1.8)	-0.9(-1.8,0.1)	-1.6(-2.7,-0.5)¶	-0.4(-1.7,0.8)	0.4(-1.0,1.9)
Model 1 + lifestyle related variables‡	Reference	-0.9(-2.3,0.6)	0.7(-0.6,2.0)	-1.2(-2.1,-0.2)‖	-2.6(-3.7,-1.5)#	-0.1(-1.4,1.1)	-0.01(-1.4,1.4)
Fully adjusted model§	Reference	-0.9(-2.4,0.5)	0.7(-0.7,2.0)	-1.2(-2.1,-0.2)‖	-2.7(-3.8,-1.5)#	-0.2(-1.5,1.0)	-0.1(-1.5,1.3)

Table 2. Cross-sectional associations of ethnic background with systolic and diastolic blood pressure* **(continued)**

Models	Dutch	Surinamese-creoles	Surinamese-hindustani	Turkish	Moroccan	Cape Verdean	Antillean
	(N=3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)
Second trimester Diastolic blood pressure (mm Hg)							
Model 1†	Reference	1.0(-0.3,2.2)	0.5(-0.1,1.1)	-0.4(-1.2,0.4)	-1.1(-2.1,-0.2)‖	0.04(-1.0,1.1)	0.5(-0.6,1.6)
Model 1 + educational level	Reference	0.6(-0.7,1.9)	0.2(-1.1,1.4)	-0.9(-1.7,-0.02)‖	-1.6(-2.5,-0.6)¶	-0.4(-1.6,0.7)	0.2(-1.0,1.5)
Model 1 + lifestyle related variables‡	Reference	0.1(-1.2,1.3)	0.4(-0.8,1.6)	-1.4(-2.2,-0.5)¶	-2.7(-3.7,-1.7)#	-0.1(-1.2,1.0)	-0.3(-1.5,0.9)
Fully adjusted model§	Reference	-0.1(-1.3,1.2)	0.3(-0.9,1.5)	-1.5(-2.3,-0.6)¶	-2.8(-3.8,-1.8)#	-0.2(-1.3,0.9)	-0.4(-1.6,0.8)
Third trimester Diastolic blood pressure (mm Hg)							
Model 1†	Reference	-0.3(-0.9,0.4)	0.3(-0.3,0.9)	-0.9(-1.7,-0.1)‖	-1.6(-2.5,-0.7)¶	0.1(-1.0,1.2)	0.3(-1.0,1.5)
Model 1 + educational level	Reference	-0.5(-1.8,0.7)	0.002(-1.2,1.3)	-1.2(-2.1,-0.4)¶	-1.9(-2.9,-1.0)#	-0.3(-1.4,0.8)	-0.001(-1.2,1.2)
Model 1 + lifestyle related variables‡	Reference	-1.1(-2.4,0.2)	0.2(-1.0,1.4)	-2.0(-2.9,-1.1)#	-3.0(-4.0,-2.0)#	-0.1(-1.2,1.0)	-0.5(-1.7,0.7)
Fully adjusted model§	Reference	-1.1(-2.4,0.2)	0.1(-1.1,1.4)	-2.0(-2.9,-1.1)#	-3.0(-4.1,-2.0)#	-0.2(-1.3,1.0)	-0.5(-1.7,0.7)

*Values are regression coefficients (95% confidence interval) that reflect the difference in blood pressure in mmHg per ethnic background compared to the Dutch (reference) group. Estimates are from multiple imputed data.

† Model 1: adjusted for age, parity and gestational age at enrolment

‡ Model 1 + adjusted for body mass index, smoking habits, alcohol consumption, caffeine intake, daily sodium intake, daily energy intake, folic acid supplement and maternal stress.

§ Model 1 + adjusted for body mass index, smoking habits, alcohol consumption, caffeine intake, daily sodium intake, daily energy intake, folic acid supplement, maternal stress, educational level and gestational age at visit.

‖ p-value < 0.05, ¶ p-value < 0.01, # p-value < 0.001

Table 3. Ethnic differences in hypertensive complications during pregnancy*

Hypertensive complication	Dutch (ref)	Surinamese-creoles	Surinamese-Hindustani	Turkish	Moroccan	Cape Verdean	Antillean
	(N=3886)	(N=232)	(N=250)	(N=718)	(N=534)	(N=331)	(N=264)
	OR	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Preeclampsia							
Unadjusted model	1.00	1.24(0.50,3.11)	2.04(1.00,4.13)‡	0.83(0.44,1.57)	0.40(0.14,1.10)	2.22(1.22,4.07)‡	1.97(0.97,3.99)
Adjusted model†	1.00	1.13(0.43,3.06)	1.80(0.84,3.87)	0.75(0.36,1.55)	0.38(0.13,1.11)	2.06(1.04,4.09)‡	1.87(0.86,4.06)
Pregnancy induced hypertension							
Unadjusted model	1.00	1.06(0.58,1.93)	0.64(0.31,1.32)	0.32(0.18,0.58)‖	0.28(0.14,0.58)§	0.61(0.32,1.16)	0.54(0.25,1.17)
Adjusted model†	1.00	0.86(0.45,1.56)	0.63(0.29,1.36)	0.31(0.16,0.59)‖	0.28(0.13,0.61)§	0.64(0.32,1.29)	0.47(0.21,1.06)

* Values are odds ratios (95% confidence interval) per ethnic background compared to the Dutch (reference) group. Odds ratios are from multiple imputed data and derived from logistic regression models.

† Models are adjusted for gestational age at visit, education, age, parity, smoking during pregnancy, alcohol use during pregnancy, caffeine intake during pregnancy, sodium intake, energy intake, folium acid use, body mass index and maternal stress index.

‡ p-value < 0.05, § p-value < 0.01, ‖ p-value < 0.00

Table 3 shows the associations of ethnic background with gestational hypertensive disorders. Cape Verdean and Surinamese-Hindustani women had a higher risk of preeclampsia as compared with Dutch women in the unadjusted model. In the adjusted model, this higher risk was only observed among Cape Verdean women (odds ratio, 2.06 [95% CI, 1.04 – 4.09]). As compared with Dutch women, Turkish and Moroccan women had a lower risk of pregnancy- induced hypertension (odds ratio, 0.32 [95% CI, 0.18 – 0.58]; odds ratio, 0.28 [95% CI, 0.14 – 0.58]), which did not attenuate after inclusion of the potential explanatory variables.

Discussion

Our hypothesis that women from ethnic minority groups would have higher blood pressure levels during pregnancy as compared with Dutch women was not confirmed. In contrast, Dutch pregnant women had higher SBP levels than non-Dutch women in each trimester of pregnancy despite more favorable characteristics, such as a higher level of education, lower body mass index, and a lower sodium intake. Turkish and Moroccan women had lower DBP levels and a lower risk of pregnancy-induced hypertension as compared with Dutch women, which could also not be explained by education or lifestyle. Cape Verdean women had an increased risk of preeclampsia as compared with Dutch women.

Methodologic Considerations

The strengths of this study are the prospective population-based design and the availability of many important determinants that may explain the association among ethnic background, blood pressure levels, and pregnancy hypertensive complications. In addition, we had a large sample size with 6215 participants with 16 615 blood pressure measurements and included the largest ethnic minority groups in the Netherlands.

To various extents, our results may have been influenced by the following limitations. We evaluated a different number of women in each trimester. Furthermore, women with treatment of gestational hypertensive disorders could also have influenced our results. Therefore, we repeated our analyses including only women with BP measured in all 3 trimesters (n=4478), and, second, we repeated the analyses excluding women with gestational hypertensive disorders. Essentially similar results were found as compared with the models with all of the women included (data not shown). The response at baseline for participation in the Generation R cohort was 61%. Pregnant women who participated were higher educated, more healthy, and more frequently of European origin than those who did not participate.[16] This selective nonresponse may have resulted in

biased effect estimates if the associations would be different between those included and not included in the analyses. Information on many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle-related determinants. Furthermore, because of the observational design, residual confounding attributed to unmeasured factors might still be an issue. Another possible limitation is that blood pressure varies during the day according to a circadian rhythm.[27] We were unable to account for this, because our study did not include ambulatory blood pressure measurements. This probably introduced some random mea surement error. The presence of systematic bias, however, is unlikely, because we do not assume that inaccurate measurements or the influence of the circadian rhythm on blood pressure change differed systematically by ethnic background. Finally, the food frequency questionnaire was used, which is only validated in nonpregnant white women.[22] This could have led to an underestimation of the effect of energy and sodium intake on blood pressure levels during pregnancy.

Ethnicity, Blood Pressure, and Gestational Hypertensive Disorders

We observed substantial differences in blood pressure levels in each trimester of pregnancy and the risk of gestational hypertensive disorders between various ethnic groups. In contrast to our study, most studies found black women to have higher blood pressure levels and higher prevalence of hypertension than white women.[9,10,12,13,28] These studies, however, were mostly about nonpregnant, older women or conducted in other countries with other cultures, lifestyles, and healthcare systems. In line with our study, another Dutch study among 2413 pregnant women found Dutch women to have the highest SBP levels in pregnancy and the lowest DBP levels for Mediterranean women.[6] This may suggest blood pressure differences to reverse during pregnancy and to be higher among white women compared with other ethnic groups. One possible explanation might be that the maternal cardiovascular system of the various ethnic groups adapts differently during pregnancy because of genetic differences or different environmental factors.

Several studies identified black descent as a risk factor for gestational hypertensive disorders.[4–6] In our study we found Cape Verdean women but not Surinamese-Creole and Antillean women to have a higher risk of preeclampsia. This is in contrast to the study of Knuist et al,[6] who found Surinamese-Creole, Antillean, and West-African women to have a higher risk of preeclampsia. Previously, we have shown that DBP is more strongly associated with the risk of gestational hypertensive disorders and might be a better predictor of gestational hypertensive disorders than SBP.[7] In line with these findings, we observed in this study that, although Dutch women had the highest SBP levels, they did not have the highest risk of preeclampsia or pregnancy-induced hypertension. In

addition, we found low DBP to be associated with a lower risk of pregnancy-induced hypertension. Several studies described a fall in BP in midpregnancy.[27] We observed a midpregnancy dip in DBP but not in SBP. In line with our findings, the study of Nama et al.[29] also did not find this midpregnancy dip in SBP.

The ethnic blood pressure differences and difference in risks of pregnancy-hypertensive complications found in our study remained largely unexplained, although we included a wide range of potential explanatory variables. It has been suggested that gestational diabetes might partly explain the difference in the risk of gestational hypertensive disorders between ethnic groups. Some studies found that gestational diabetes increased the risk of preeclampsia and that this effect was stronger among black women.[4] In our study, there were no significant differences in the prevalence of gestational diabetes per ethnic background, and the effects of gestational diabetes on blood pressure and hypertensive complications were the same among the various ethnic groups (data not shown). Other potential determinants that were not available for the current study, such as physical activity, metabolic factors (eg, cholesterol and fatty acid levels), parameters of endothelial function, genetic factors, or currently unknown risk factors, may contribute to the explanation.[30–32] Of note, the percentage of Dutch women using folic acid before pregnancy was higher than the percentage of women who used folic acid during pregnancy. A possible explanation could be a lack of knowledge about adequate folic acid use. Physicians and midwives should be aware of this and should provide information to these women.

In our fully adjusted model we found multiparity, high educational level, and continued alcohol use during pregnancy to be significantly associated with lower blood pressure levels. Most women who used alcohol during pregnancy were low-to-moderate alcohol drinkers (<1 glass of alcohol per day). Previous research also showed that moderate alcohol drinkers had lower BP levels compared with nondrinkers and heavy drinkers.[33] However, alcohol use during pregnancy might harm the unborn child and is, therefore, not recommended. Higher body mass index was significantly associated with higher blood pressure levels. These findings are in line with other studies, except for the association between parity and blood pressure, where findings are inconsistent.[34,35] We found significant interaction terms between ethnicity and parity. Thus, the effect of parity varied among the different ethnic groups. Repeated pregnancies may negatively influence the risk for cardiovascular disease in the long term;[36] our findings suggest that this may differ per ethnic group. Furthermore, we observed that older maternal age was associated with a lower SBP among all ethnic groups, but no association was found between maternal age and DBP. However, the effect of maternal age on DBP differed per

ethnic group, because Turkish and Moroccan women <35 years of age were observed to have lower DBP levels as compared with Dutch women of the same age.

Conclusion

Our study demonstrated substantial differences in blood pressure levels and pregnancy hypertensive disorders in a multiethnic society. Remarkably, these differences remain largely unexplained, although we included a wide range of known risk factors. Underlying mechanisms for these ethnic differences in blood pressure levels and gestational hypertensive disorders need to be identified, and genes may be considered to play a role. Understanding of these ethnic disparities may lead to entry points for prevention and treatment and ultimately improved maternal and fetal pregnancy outcomes.

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Supplemental Material

Unbalanced repeated measurement regression models

The associations of ethnic background with repeatedly measured systolic and diastolic blood pressure were analyzed using unbalanced repeated measurement regression models. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data. Using fractional polynomials of gestational age, the best fitting models were constructed. For this analysis, ethnic background was categorized into 7 groups and included in these models. An interaction term with ethnicity*gestational age was also included. These models can be written as:

Systolic blood pressure = $ß_0$ + $ß_1$*ethnic background + $ß_2$*gestational age + $ß_3$*gestational age^{-2} + $ß_4$*ethnic background*gestational age + $ß_5$*parity + $ß_6$*age + $ß_7$*gestational age at enrolment

Diastolic blood pressure = $ß_0$ + $ß_1$*ethnic background + $ß_2$*gestational age + $ß_3$*gestational age$^{0.5}$ + $ß_4$*ethnic background*gestational age + $ß_5$*parity + $ß_6$*age + $ß_7$*gestational age at enrolment

In these models, '$ß_0$ + $ß_1$ *ethnic background' reflects the blood pressure levels per ethnic background and '$ß_2$*gestational age + $ß_3$*gestational age^{-2}'reflects the slope of change in blood pressure per week of gestational age for systolic blood pressure (SBP), and '$ß_2$*gestational age + $ß_3$*gestational age$^{0.5}$'reflects the slope of change in blood pressure per week of gestational age for DBP. Main interest was in the term '$ß_4$*ethnic background*gestational age', which reflects the difference in change in blood pressure per week of gestational age between the different ethnic background categories for SBP and DBP. We also included the confounders: $ß_5$*parity + $ß_6$*age + $ß_7$*gestational age at enrolment.

Supplementary Table S1. Longitudinal associations between ethnic background and systolic and diastolic blood pressure*

Ethnicity	Difference in systolic blood pressure			
	Intercept	P-value†	Slope (mm Hg (95% CI))	P-value†
Dutch	119.70	<0.001	Reference	
Surinamese-creole	120.04	0.81	-0.12(-0.23,-0.01)	0.03
Surinamese-hindustani	112.75	<0.001	-0.03(-0.13,0.07)	0.53
Turkish	114.35	<0.001	0.02(-0.04,0.08)	0.51
Moroccan	114.97	<0.001	-0.02(-0.09,0.05)	0.60
Cape Verdean	116.52	<0.01	-0.01(-0.10,0.08)	0.83
Antillean	119.24	0.73	-0.09(-0.19,0.01)	0.08

Ethnicity	Difference in diastolic blood pressure			
	Intercept	P-value†	Slope (mm Hg (95% CI)	P-value†
Dutch	99.06	<0.001	Reference	
Surinamese-creole	99.66	0.59	-0.02(-0.11,0.06)	0.56
Surinamese-hindustani	100.17	0.27	-0.03(-0.11,0.05)	0.47
Turkish	99.32	0.69	-0.04(-0.09,0.01)	0.12
Moroccan	98.99	0.92	-0.06(-0.12,-0.0004)	0.049
Cape Verdean	98.89	0.86	0.01(-0.06,0.08)	0.83
Antillean	100.69	0.12	-0.05(-0.13,0.03)	0.21

*Values are based on repeated non-linear regression models (based on 16615 measurements) and reflect the change in blood pressure in mm Hg per ethnic background compared to Dutch women (reference group). The models are adjusted for confounders: maternal age, parity and gestational age at enrolment.
†P-value reflects the significance level of the estimate.

Supplementary Table S2. Effect estimates of the association between ethnic background and blood pressure during pregnancy*

	Systolic blood pressure					
Covariate	**B (95%CI) 1st trimester**	**P-value**	**B (95% CI) 2nd trimester**	**P-value**	**B (95% CI) 3rd trimester**	**P-value**
Age	-0.11 (-0.19,-0.03)	0.009	-0.11 (-0.19,-0.04)	0.002	-0.11 (-0.18,-0.03)	0.004
Parity	-0.78 (-1.22,-0.31)	0.001	-1.06 (-1.45,-0.67)	<0.001	-1.21 (-1.60,-0.83)	<0.001
Gestational age at visit	-0.23 (-0.39,-0.07)	0.006	-0.17 (-0.25,-0.08)	<0.001	-0.003 (-0.08.0.07)	0.936
Educational level	-0.29 (-0.58,-0.004)	0.047	-0.44 (-0.70,-0.18)	0.001	-0.37 (-0.62,-0.12)	0.004
BMI	0.87 (0.79,0.96)	<0.001	0.80 (0.72,0.87)	<0.001	0.71 (0.63,0.79)	<0.001
Smoking	-0.11 (-0.63,0.41)	0.675	0.15 (-0.29,0.59)	0.504	0.60 (0.15,1.04)	0.008
Alcohol use	-0.65 (-1.09,-0.29)	0.001	-0.47 (-0.85,-0.08)	0.017	-0.49 (-0.86,-0.12)	0.010
Caffeine intake	0.42 (-0.09,0.92)	0.102	0.40 (0.01,0.78)	0.043	0.25 (-0.23,0.73)	0.297
Folic acid supplementation	0.54 (-0.01,1.10)	0.056	0.36 (-0.10,0.83)	0.125	0.08 (-0.36,0.53)	0.714
Maternal stress	-1.00 (-2.01,0.02)	0.055	-0.90 (-1.91,0.12)	0.083	-0.90 (-2.12,0.33)	0.140
Sodium intake	0	0.821	0	0.532	0	0.462
Energy intake	0.001 (0.001,0.002)	<0.001	0.001 (0.00,0.002)	0.005	0.001 (0.00,0.001)	0.008
	Diastolic blood pressure					
Covariate	**B (95%CI) 1st trimester**	**P-value**	**B (95% CI) 2nd trimester**	**P-value**	**B (95% CI) 3rd trimester**	**P-value**
Age	0.01 (-0.06,0.07)	0.814	0.02 (-0.03,0.08)	0.408	0.04 (-0.02,0.09)	0.234
Parity	-0.74 (-1.1, -0.38)	<0.001	-1.05 (-1.36,-0.74)	<0.001	-1.45 (-1.76,-1.14)	<0.001
Gestational age at visit	-0.31 (-0.44,-0.19)	<0.001	-0.04 (-0.11,0.03)	0.239	-0.01 (-0.06,0.05)	0.835
Educational level	-0.16 (-0.39,0.06)	0.152	-0.28 (-0.48,-0.08)	0.005	-0.11 (-0.31,0.09)	0.279
BMI	0.73 (0.67,0.80)	<0.001	0.71 (0.65,0.77)	<0.001	0.63 (0.56,0.69)	<0.001
Smoking	-0.92 (-1.30,-0.53)	<0.001	-0.59 (-0.96,-0.23)	0.002	-0.03 (-0.38,0.32)	0.858
Alcohol use	-0.37 (-0.68,-0.05)	0.023	-0.56 (-0.87,-0.25)	<0.001	-0.62 (-0.93,-0.32)	<0.001
Caffeine intake	0.11 (-0.25,0.46)	0.545	0.06 (-0.27,0.039)	0.733	0.01 (-0.32,0.34)	0.946
Folic acid supplementation	0.44 (0.06,0.82)	0.022	0.33 (-0.02,0.69)	0.067	0.09 (-0.26,0.43)	0.629
Maternal stress	-0.47 (-1.29,0.34)	0.412	-0.78 (-1.85,0.28)	0.137	-0.57 (-1.86,0.72)	0.346
Sodium intake	0	0.622	0	0.928	0	0.802
Energy intake	0	0.158	0	0.683	0	0.704

B = effect estimate, 95% CI = 95 % confidence interval

*Covariates' effect estimates in the fully adjusted model

Supplementary Table S3. Stratified associations of ethnicity with blood pressure levels during pregnancy according to parity and age*

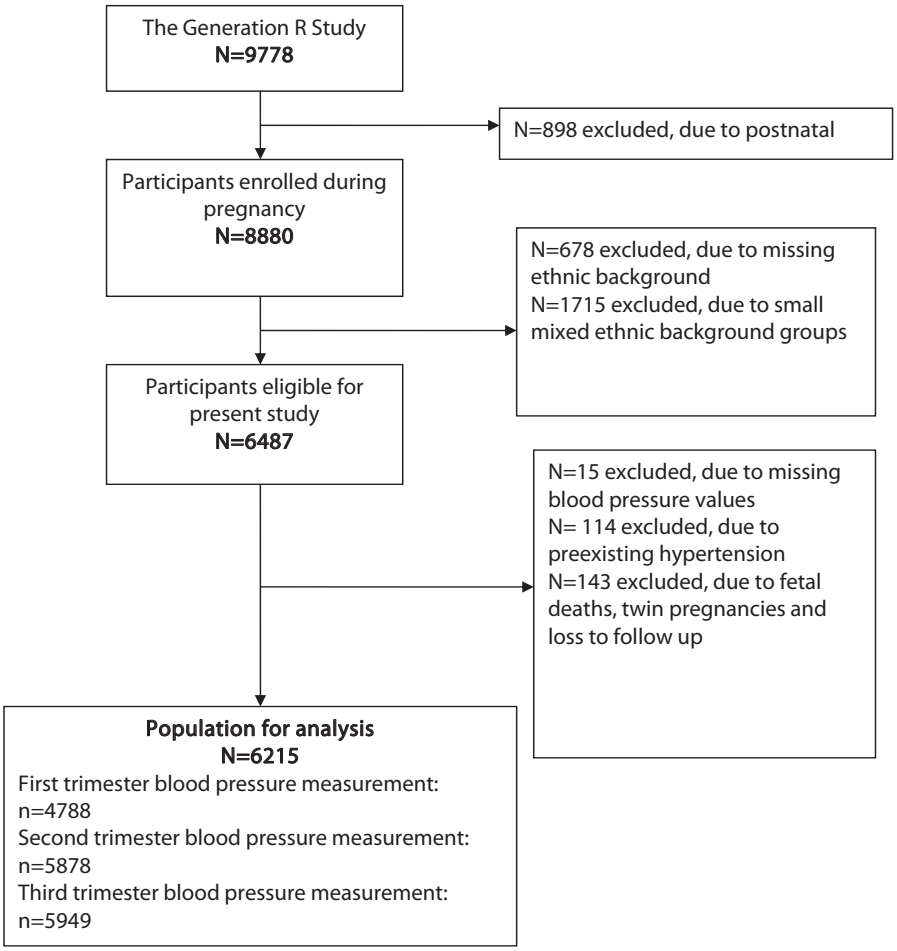
Covariate	Dutch (N=3886)	Surinamese-creoles (N=232)	Surinamese-hindustani (N=250)	Turkish (N=718)	Moroccan (N=534)	Cape Verdean (N=331)	Antillean (N=264)
First trimester							
Systolic Blood pressure (mm Hg)							
Parity							
Nulliparous (n=3462)	Reference	-0.8(-3.3,1.8)	-7.4(-9.6,-5.3)§	-6.0(-7.5,-4.4)§	-6.7(-8.6,-4.7)§	-4.6(-6.6,-2.6)§	-0.9(-3.5,1.4)
Multiparous (n=2739)	Reference	-1.7(-4.5,1.0)	-6.0(-8.5,-3.4)§	-4.0(-5.6,-2.4)§	-3.4(-5.1,-1.7)§	-0.5(-2.8,1.9)	-1.5(-4.1,1.2)
Second trimester							
Systolic Blood pressure (mm Hg)							
Parity							
Nulliparous	Reference	-1.8(-3.9,0.3)	-7.5(-9.5,-5.5)§	-5.0(-6.4,-3.6)§	-5.3(-7.0,-3.6)§	-3.8(-5.5,-2.0)§	-2.3(-4.2,-0.3)†
Multiparous	Reference	-0.5(-2.9,1.8)	-7.4(-9.7,-5.0)§	-2.8(-4.1,-1.5)§	-3.8(-5.2,-2.4)§	-0.9(-3.0,1.1)	-1.7(-4.0,0.6)
Third trimester							
Systolic Blood pressure (mm Hg)							
Parity							
Nulliparous	Reference	-1.6(-3.7,0.5)	-6.8(-8.7,-4.8)§	-5.3(-6.7,-3.9)§	-5.6(-7.3,-4.0)§	-3.3(-5.0,-1.6)§	-3.0(-4.9,-1.1)‡
Multiparous	Reference	-4.5(-6.9,-2.0)§	-7.7(-10.1,-5.2)§	-3.0(-4.2,-1.7)§	-4.1(-5.5,-2.7)§	-3.0(-5.0,-1.0)‡	-1.4(-3.7,1.0)
First trimester							
Diastolic Blood pressure (mm Hg)							
Age (years)							
< 25 (n=1262)	Reference	-1.5(-4.2,1.3)	-0.5(-2.9,2.0)	-2.0(-3.7,-0.3)†	-2.8(-4.8,-0.9)‡	-1.0(-3.1,1.0)	1.1(-1.2,3.5)
25-35 (n=4030)	Reference	0.2(-1.9,2.3)	0.8(-0.9,2.5)	-0.7(-1.8,0.5)	-1.8(-3.2,-0.4)†	-1.0(-2.7,0.7)	-0.5(-2.5,1.5)
>35 (n=923)	Reference	-0.7(-4.4,3.1)	0.6(-5.1,6.2)	2.1(-1.8,6.0)	0.5(-2.5,3.6)	4.8(0.7,8.8)†	0.7(-4.8,6.1)

Supplementary Table S3. Stratified associations of ethnicity with blood pressure levels during pregnancy according to parity and age* (continued)

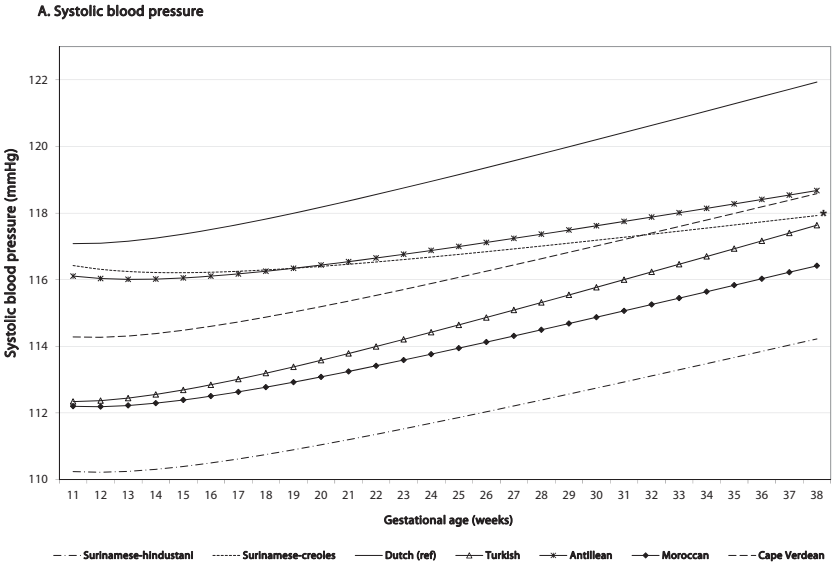
Covariate	Dutch (N=3886)	Surinamese-creoles (N=232)	Surinamese-hindustani (N=250)	Turkish (N=718)	Moroccan (N=534)	Cape Verdean (N=331)	Antillean (N=264)
Parity							
Nulliparous	Reference	-0.2(-2.2,1.8)	-0.4(-2.1,1.3)	-1.9(-3.1,-0.7)‡	-2.8(-4.4,-1.3)§	-1.8(-3.4,-0.2)	0.9(-0.9,2.8)
Multiparous	Reference	-0.8(-3.1,1.5)	1.8(-0.4,3.9)	0.2(-1.1,1.5)	-0.8(-2.1,0.6)	1.3(-0.6,3.2)	-0.4(-2.6,1.8)
Second trimester Diastolic Blood pressure (mm Hg)							
Age (years)							
< 25	Reference	-0.4(-2.6,1.8)	-0.6(-2.8,1.6)	-2.6(-4.1,-1.2)§	-2.8(-4.5,-1.1)‡	-1.1(-3.0,0.7)	0.1(-1.8,1.9)
25-35	Reference	0.7(-1.1,2.6)	0.5(-1.0,2.1)	0.04(-1.0,1.0)	-1.4(-2.5,-0.2)†	-0.8(-2.3,0.7)	-0.3(-2.0,1.5)
>35	Reference	2.8(-0.4,6.0)	0.2(-5.0,5.4)	-0.3(-3.2,2.6)	-1.1(-3.8,1.6)	4.3(1.0,7.6)†	1.1(-4.1,6.2)
Parity							
Nulliparous	Reference	0.9(-0.8,2.6)	0.04(-1.5,1.6)	-1.3(-2.4,-0.2)†	-2.0(-3.4,-0.7)‡	-1.4(-2.7,0.05)	-0.1(-1.7,1.4)
Multiparous	Reference	0.8(-1.1,2.7)	0.9(-0.9,2.8)	0.3(-0.8,1.4)	-0.6(-1.8,0.5)	1.6(-0.04,3.3)	0.9(-0.9,2.8)
Third trimester Diastolic Blood pressure (mm Hg)							
Age (years)							
< 25	Reference	-1.8(-4.1,0.5)	-2.1(-4.4,0.2)	-3.0(-4.5,-1.5)§	-4.0(-5.7,-2.3)§	-2.0(-3.9,-0.1)†	-1.1(-3.0,0.9)
25-35	Reference	-0.6(-2.4,1.2)	0.5(-1.0,2.1)	-1.1(-2.0,-0.1)†	-1.9(-3.0,-0.8)‡	-0.02(-1.5,1.5)	-0.8(-2.5,0.9)
>35	Reference	0.8(-2.4,4.1)	2.7(-2.4,8.1)	-0.3(-3.1,2.5)	-2.4(-5.2,0.3)	2.3(-1.1,5.8)	3.3(-1.4,8.0)
Parity							
Nulliparous	Reference	-0.2(-1.9,1.5)	-0.1(-1.6,1.5)	-2.0(-3.1,-0.9)§	-2.1(-3.4,-0.7)‡	-0.9(-2.3,0.5)	-1.1(-2.7,0.4)
Multiparous	Reference	-0.5(-2.4,1.4)	0.5(-1.4,2.4)	0.1(-1.0,1.1)	-1.5(-2.6,-0.3)†	1.1(-0.5,2.7)	1.6(-0.2,3.4)

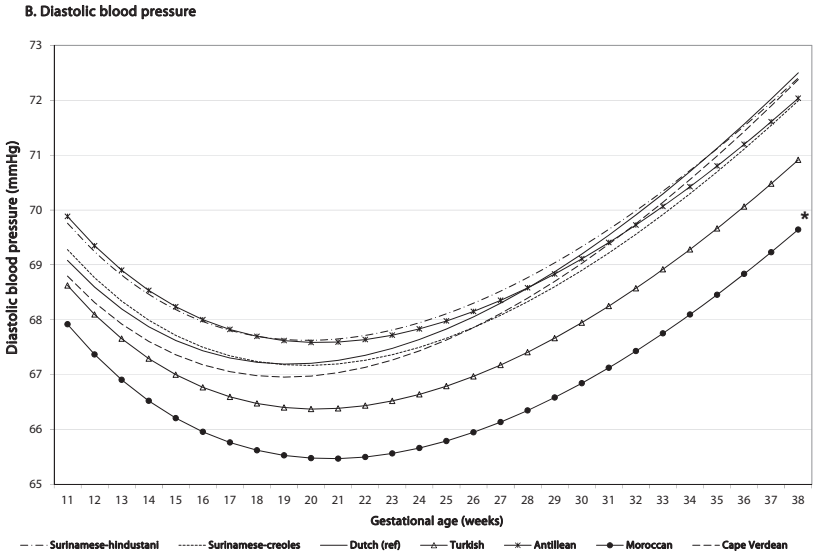
*Values are regression coefficients (95% confidence interval) that reflect the change in blood pressure in mm Hg among ethnic minority groups compared to Dutch women stratified according to parity and age (interaction terms with ethnicity p<0.01). Estimates are from multiple imputed data.

† p-value < 0.05, ‡ p-value < 0.01, § p-value < 0.001



Supplementary Figure S1. Flow chart of the study population





Supplementary Figure S2. Blood pressure patterns of different ethnic groups compared to the Dutch population

*****P-value<0.05. Change in blood pressure in mmHg for women with different ethnic backgrounds compared to Dutch women as reference group based on repeated regression models (systolic blood pressure= ß0 + ß1*ethnic background + ß2*gestational age + ß3*gestational age-2 + ß4*ethnic background*gestational age + ß5*parity + ß6*age + ß7*gestational age at enrolment and diastolic blood pressure = ß0 + ß1*ethnic background + ß2*gestational age + ß3*gestational age0.5 + ß4*ethnic background*gestational age + ß5*parity + ß6*age + ß7*gestational age at enrolment). P-values reflect the difference in change in blood pressure per week of gestational age per ethnic population. Estimates are given in Supplementary Table S1.

3

Socioeconomic inequalities in placental vascular resistance

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Abstract

Objective: To examine the association between socioeconomic position (SEP) and umbilical and uterine placental resistance indices in the second and third trimester, and to what extent this could be explained by lifestyle-related behaviors.

Design: Prospective cohort study.

Setting: Rotterdam, the Netherlands.

Patients: 7,033 pregnant women of mean age (± standard deviation) 29.9 (±5.2) years.

Interventions: None.

Main Outcome Measures: Uterine artery resistance index (UARI) and umbilical artery pulsatility index (UAPI) in second and third trimester measured with Doppler ultrasound.

Results: Third-trimester UARI and both second- and third-trimester UAPI were statistically significantly higher for women with lower educational levels as compared with those with higher educational levels. Educational level was strongly associated with the risk of continuously high levels of UARI and UAPI from second to third trimester of pregnancy. Notching was not associated with SEP. Smoking was a significant contributor to the association of SEP and increased placental resistance indices; body mass index, folic acid supplementation use, and alcohol use were not.

Conclusions: Women from low socioeconomic subgroups have higher placental resistance indices, which may cause a higher prevalence of pregnancy complications. This was mainly explained by maternal smoking during pregnancy.

Introduction

The relationship of socioeconomic inequality to pregnancy complications and adverse birth outcomes, including preeclampsia,[1,2] fetal growth restriction,[3–5] and preterm birth[6], has been well reported. Such inequalities affect a child's prospects for healthy development at the start of his or her life. Thus, reducing socioeconomic disparities during this particular period of life has been proposed as a top priority in the European strategy to tackle health inequalities.[7] However, the origins of these inequalities may be rooted in prenatal life, and understanding the relevant causal prenatal mechanisms may help to develop effective interventions toward reducing these disparities.

Continued smoking and other unhealthy behaviors during pregnancy explain a substantial part of the socioeconomic inequalities in birth outcomes.[8] Recently, population-based studies have emphasized the role of abnormal early placentation in the development of pregnancy complications and adverse birth outcomes, probably to some extent caused by unhealthy lifestyle-related behaviors such as smoking.[9,10] Abnormal placentation may lead to higher uterine and umbilical artery resistance patterns, which are thought to reflect impaired uteroplacental and fetoplacental blood flow.[11,12] Higher uterine and umbilical artery resistance patterns have been associated with the risk of preeclampsia, fetal growth restriction, and preterm birth.[9, 10, 13] Also, consistently higher placental vascular resistance throughout pregnancy has been shown to be associated with a risk of adverse pregnancy outcomes.[9]

We hypothesize that women from lower socioeconomic subgroups have higher uterine and umbilical artery resistance patterns, to some extent due to their higher levels of unhealthy behavior, including smoking in particular. We therefore investigated whether educational levels of women are associated with uterine and umbilical artery resistance indices, and whether lifestyle-related determinants could explain these associations in a population-based prospective cohort study among 7,033 pregnant women. Furthermore, we examined whether women with lower educational levels are at an increased risk of continuously high levels of placental resistance indices from the second to third trimester of pregnancy.

Methods

Study Design

This study was embedded within the Generation R Study, a population-based prospective cohort study from early pregnancy onwards. Details have been described

elsewhere.[14, 15] Briefly, the cohort includes 9,778 mothers and their children living in Rotterdam, the Netherlands. Although enrollment ideally took place in early pregnancy, it was possible until the birth of the child. In total, 8,879 women were enrolled during pregnancy. All children were born between April 2002 and January 2006. Assessments during pregnancy included physical examinations, ultrasound assessments, and questionnaires and were planned in early pregnancy (gestational age <18 weeks), midpregnancy (gestational age 18–25 weeks), and late pregnancy (gestational age ≥25 weeks). The study was conducted in accordance with the guidelines proposed by the World Medical Association of Helsinki and was approved by the medical ethics committee of the Erasmus MC University Medical Centre of Rotterdam. Written consent was obtained from all participating parents.[16]

Population for Analysis

The data of all participating prenatal women were available (n=8,879). For the present study, we excluded the women who had no placental resistance indices measurements (n=1,156). Additionally, we excluded pregnancies with the following outcomes: fetal death (n=25), induced abortion (n=8), loss to follow up (n=28), and twin pregnancies (n=3). Finally, we excluded women for whom there was no information about educational level (n=626). Thus, the cohort for analysis comprised 7,033 pregnant women.

Socioeconomic Position

Our indicator of socioeconomic position (SEP) was the educational level of the pregnant woman. Each woman's level of education was established using a questionnaire at enrollment. The Dutch Standard Classification of Education was used to categorize four subsequent levels of education: [1] high (university degree), [2] mid-high (higher vocational training, bachelor's degree), [3] mid-low (>3 years general secondary school, intermediate vocational training), and [4] low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general second- ary school).[17]

Placental Hemodynamic Function

Placental vascular resistance was evaluated with recorded flow velocity wave forms from the uterine and umbilical arteries in second and third trimester (18). A raised uterine artery resistance index (UARI) and umbilical artery pulsatility index (UAPI) indicate increased placental resistance.[19]The indices are calculated as ratios between peak systolic velocity (A), end-diastolic peak velocity (B), and mean velocity (mean). The pulsatility index is calculated as (A - B)/mean and the resistance index as (A - B)/A (20). UARI was measured in the uterine arteries near the crossover with the external iliac artery. UAPI was measured in a free-floating loop of the umbilical cord. For each measurement,

three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound during fetal apnea and without fetal movement. The mean of three measurements was used for further analysis. The presence of notching was assessed in the uterine arteries and reflects an abnormal waveform resulting from increased downstream blood flow resistance. Ultrasound measurements were performed in a blinded fashion with regard to previous measurements and pregnancy outcomes. Placental resistance index measurements were performed in 87% of the 8,879 prenatally enrolled women because the placental resistance indices were measured at only one of the two research centers.

Mediators

Based on previous literature,[9,21,22] the following factors were considered to be potential explanatory factors in the pathway between SEP and placental perfusion. Smoking and alcohol consumption were assessed by questionnaires in each trimester. From the first questionnaire, information about folic acid supplementation use was obtained. The prepregnancy weight was established at enrollment through a questionnaire. On the basis of height (cm), measured at enrollment without shoes, and prepregnancy weight, we calculated the prepregnancy body mass index (BMI; weight/ height^2).

Confounders

We treated maternal age at enrollment, parity, gestational age at enrollment, ethnicity, and gestational age at time of measurement as potential confounders. Maternal age was assessed at enrollment. Parity was obtained through a questionnaire at enrollment. Gestational age was established by fetal ultrasound examination during the first ultrasound visit.[14] Ethnicity was assessed by country of birth of the participating women and their parents. Information about country of birth was obtained by questionnaires. A distinction was made between women of Dutch ethnic background and the following non-Dutch minority groups in this study: Turkish, Moroccan, Antillean, Surinamese Creole, Surinamese Hindustani, and Cape Verdean. Ethnicity was defined according to the classification of Statistics Netherlands.[23] The participant was considered to be of non-Dutch origin if one of her parents was born in a country other than the Netherlands. If both parents had been born abroad, the country of the participant's mother was used to decide her ethnic background.

Statistical Analyses

Associations between educational level and all covariates, UAPI, and UARI were explored using chi-square tests and analysis of variance (ANOVA). Linear regression models were used to assess the association of educational level with UAPI and UARI (model 1). Next, potential mediators were added to these models. To assess their mediating effects, the corresponding percentages of attenuation of effect estimates were calculated by

comparing differences between model 1 and the models including the mediators: 100 x (B model 1 with explanatory factor - B model 1)/(B model 1). Finally, a full model containing the educational level and all the explanatory factors assessed the joint effects of the explanatory factors. For these analyses, we standardized UARI and UAPI values by dividing the original values by their corresponding standard deviations.

Logistic regression models were used to examine the association between educational level and notching. Sensitivity analyses were conducted with net household income as the indicator of SEP. To examine the association between educational level and tracking of placental resistance indices, UARI and UAPI were categorized in tertiles. Logistic regression models were used to calculate the odds ratio of remaining in the third tertile from second to third trimester for the different educational levels, as this was previously found to be associated with preeclampsia, preterm birth, and small- for-gestational-age at birth in the same study cohort (9). Interactions between ethnicity and SEP on UARI and UAPI were tested (P< .05). We additionally used linear mixed models (the PROC MIXED procedure in the SAS software) to assess the association between maternal educational level and placental resistance indices. These models are described in detail in the supplemental material (available online).

Multiple imputation was used to deal with missing values in the covariates. Five imputed data sets were created and analyzed together. A 95% confidence interval (CI) was calculated around the mediating effects using a bootstrap method with 1,000 resamplings per imputed data set in the statistical program R.[24] The other statistical analyses were performed using Statistical Package of Social Science (SPSS) version 20.0 for Windows (SPSS Inc.).

Results

From the total study population, 23.4% of the women had a high educational level, and 26.5% had a low educational level (Table 1). Compared with the high educated women, the low- educated women were heavier, more frequently had a non-Dutch ethnic background, more frequently smoked during pregnancy, less frequently used alcohol during pregnancy, and less frequently used folic acid supplementation (P< .001). The mean UAPI and UARI were higher among women with low education as compared with women with high education in both the second and third trimesters (Table 1). Nonresponse analyses showed that women without placental resistance index measurements were lower educated, more frequently of non-Dutch ethnicity, and had higher BMI scores (Supplemental Table 1).

Table 1. Characteristics of the study population

		Maternal educational level				
	Total N=7033	**High n=1649**	**Mid-high n=1356**	**Mid-low n=2162**	**Low n=1866**	**P-value***
Maternal characteristics†						
Age (years)	29.9 (5.2)	33.0 (3.3)	31.4 (4.1)	28.7 (5.1)	27.3 (5.8)	<0.001
Body mass index before pregnancy (kg/m^2)	23.5 (4.3)	22.5 (3.0)	23.0 (3.6)	23.8 (4.6)	24.5 (5.0)	<0.001
Parity (% nulliparous)	57.1	58.3	62.9	60.4	48.1	<0.001
Gestational age at enrolment (weeks)	14.1 (10.9,22.5)	13.6 (10.9,21.6)	13.6 (10.6,21.6)	14.2 (10.9,22.8)	14.9 (11.1,23.4)	<0.001
Ethnicity (%)						
Dutch	64.2	93.9	83.2	53.4	38.4	<0.001
Cape Verdean	5.2	0.4	1.9	7.1	9.2	
Dutch Antilles	4.0	0.8	2.2	5.9	5.8	
Moroccan	7.8	1.3	3.7	9.0	14.7	
Turkish	11.4	2.5	4.9	12.4	22.0	
Surinamese Creole	3.6	0.4	2.1	6.2	4.6	
Surinamese Hindustani	3.9	0.8	1.9	6.1	5.3	
Smoking (%)						
Yes	26.9	13.4	21.3	28.5	40.7	<0.001
No	73.1	86.6	78.7	71.5	59.3	
Alcohol use (%)						
Yes	51.7	76.5	63.4	44.0	30.4	<0.001
No	48.3	23.5	36.6	56.0	69.6	
Folic acid supplement use (%)						
Yes	72.5	92.0	85.4	70.9	46.1	<0.001
No	27.5	8.0	14.6	29.1	53.9	
Mean uterine artery resistance index						
Second trimester	0.54 (0.09)	0.53 (0.09)	0.54 (0.09)	0.54 (0.09)	0.55 (0.09)	0.023
Third trimester	0.49 (0.08)	0.48 (0.08)	0.48 (0.08)	0.48 (0.08)	0.49 (0.08)	0.001
Mean umbilical artery pulsatility index						
Second trimester	1.20 (0.19)	1.18 (0.18)	1.20 (0.18)	1.21 (0.19)	1.22 (0.19)	<0.001
Third trimester	0.98 (0.17)	0.97 (0.17)	0.97 (0.17)	0.99 (0.17)	0.99 (0.17)	0.001

Values are percentages, means (SD) or medians (90% range) for the total population and by level of maternal education.

*P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.

†Data was missing for BMI before pregnancy (14.7%), parity (0.2%), ethnicity (20.7%), smoking (7.0%), alcohol use (7.5%) and folic acid supplement use (20.4%).

Multivariable linear regression analyses adjusted for confounders showed that third-trimester UARI, but not second trimester UARI, is higher for low educated women (P< .01)

as compared with high educated women. UAPI is higher for mid-low educated women in the second trimester (P< .05) and for low educated women in both the second (P< .001) and third (P< .05) trimester as compared with high educated women. A negative socioeconomic gradient was found for third-trimester UARI (P= .002 for trend) and for UAPI in the second trimester (P< .001 for trend) and third trimester (P= .006 for trend). No different risk of notching was observed for the different educational levels (Table 2). Two statistically significant interaction terms were found for educational level (reference group: high education) and ethnic background (reference group: Dutch) on UARI and UAPI. These interactions were low education * Dutch Antilles for third-trimester UAPI (P= .028) and low education * Surinamese Hindustani for second-trimester UARI (P= .026). No other statistically significant interactions were found for education or ethnic background on UAPI and UARI (P>.05). Stratified results according to ethnic background are shown in Supplemental Table 2.

Table 2. Educational level and placental resistance indices (n=7033)

	Uterine artery RI (SD)*		**Umbilical artery PI (SD)***		**Notching (OR)†**
	2nd trimester N=4220	**3th trimester N=4175**	**2nd trimester N=5656**	**3th trimester N=6173**	**3th trimester N=4405**
Educational level					
High	Ref	Ref	Ref	Ref	Ref
Mid-high	0.03 (-0.07,0.12)	0.02 (-0.07,0.11)	0.04 (-0.03,0.12)	-0.01 (-0.09,0.06)	1.02 (0.75,1.41)
Mid-low	0.07 (-0.02,0.15)	0.07 (-0.02,0.16)	0.09 (0.01,0.16)‡	0.05 (-0.02,0.13)	0.93 (0.68,1.26)
Low	0.06 (-0.04,0.16)	0.17 (0.07,0.27)§	0.20 (0.11, 0.28)‖	0.11 (0.02,0.19)‡	1.04 (0.74,1.46)
P for trend	0.180	0.002	<0.001	0.006	0.893

Abbreviations: OR: odds ratio, SD: standard deviation

*Values are linear regression coefficients (95% confidence interval) derived from multivariable linear regression. The effect estimates represent the difference in placental resistance indices, compared to reference group (high education). All models are adjusted for age, parity, gestational age at intake, ethnicity and gestational age at measurement. Estimates are based on multiple imputed data.

†Values are odds ratios (95% confidence interval) derived from logistic regression models. The effect estimates reflect difference in risk of notching, compared to reference group (high education). All models are adjusted for age, parity, gestational age at intake, ethnicity and gestational age at measurement. Estimates are based on multiple imputed data.

‡ P<0.05 §P<0.01 ‖P<0.001

Regarding the proportion of explanation by each risk factor (Table 3), smoking during pregnancy and prepregnancy BMI were statistically significant contributors to the association of education and third-trimester UARI in the low educational subgroup: 13% attenuation (95% CI, -41% to -0.1%) and 9% attenuation (95% CI, -28% to -0.2%) respectively. Overall, 30% (95% CI, -85% to -6%) of the association of education and UARI was explained for low education by including the explanatory factors (see Table 3). Smoking

during pregnancy was the greatest contributing factor in the association between education and second-trimester UAPI in the mid-low and low educational subgroups, explaining 27% (95% CI, -121% to -10%) and 22% (95% CI, -44% to -11%), respectively.

Table 3. Attenuation of placental resistance indices for the different levels of education after individual adjustment for explanatory factors

	Maternal educational level			
	Mid-low B (95% CI)	**Attenuation a* (95% CI)**	**Low B (95% CI)**	**Attenuation b* (95% CI)**
Uterine artery RI (SD) Third trimester				
Model 1 (includes confounders)			0.17 (0.07,0.27)	
Model 1 + pre-pregnancy BMI			0.15 (0.05,0.27)	-9% (-28,-0.2)
Model 1 + smoking			0.15 (0.04,0.25)	-13% (-41,-0.1)
Model 1 + alcohol use			0.17 (0.07,0.27)	+1% (-11,13)
Model 1 + folic acid use			0.16 (0.05,0.26)	-8% (-29,2)
Fully adjusted model			0.12 (0.01,0.23)	-30% (-85,-6)
Umbilical artery PI (SD) Second trimester				
Model 1 (includes confounders)	0.09 (0.01,0.16)		0.20 (0.11, 0.28)	
Model 1 + pre-pregnancy BMI	0.08 (0.0,00.15)	-8% (-40,1)	0.18 (0.10,0.27)	-5% (-14,0.3)
Model 1 + smoking	0.06 (-0.2,0.14)	-27% (-121,-10)	0.15 (0.06,0.24)	-22% (-44,-11)
Model 1 + alcohol use	0.08 (0.00,0.16)	-7% (-41,7)	0.19 (0.10,0.27)	-4% (-14,3)
Model 1 + folic acid use	0.08 (0.01,0.16)	-4% (-19,2)	0.18 (0.10,0.27)	-6% (-16,2)
Fully adjusted model	0.04 (-0.04,0.12)	-53% (-233,-22)	0.11 (0.02,0.20)	-43% (-80,-23)
Umbilical artery PI (SD) Third trimester				
Model 1 (includes confounders)			0.11 (0.02,0.19)	
Model 1 + pre-pregnancy BMI			0.10 (0.02,0.19)	-7% (-34,7)
Model 1 + smoking			0.07 (-0.02,0.15)	-38% (-142,-16)
Model 1 + alcohol use			0.10 (0.01,0.19)	-7% (-39,9)
Model 1 + folic acid use			0.10 (0.02,0.18)	-8% (-39,8)
Fully adjusted model			0.04 (-0.05,0.13)	-66% (-246,-28)

Abbreviations: B: effect estimate, CI: confidence interval, BMI: body mass index

*Attenuation a and attenuation b represent the attenuations of effect estimates for mid-low and low education relative to model 1 (includes confounders) after individual adjustment for explanatory factors (100 x ($B_{\text{model 1}} - B_{\text{model 1 with explanatory factor}}$) / ($B_{\text{model 1}}$)). High maternal educational level is the reference group.

No differences in placental hemodynamics were observed for the mid-high educational subgroup and for the mid-low group in third trimester as compared to the high educational subgroup, therefore attenuations in effect estimates for these subgroups are not presented.

In the lowest educational subgroup, smoking explained 38% (95% CI, -142% to -16%) of the association between third-trimester UAPI and education. The overall explanation was 53% (95% CI, -233% to -22%) for the mid-low educational subgroup, and 43% (95% CI, -80% to -23%) for the lowest educational subgroup in second trimester. In third trimester, the overall explanation was 66% (95% CI, -246% to -28%) in the lowest educational subgroup (see Table 3).

Educational level was associated with the risk of remaining in the third tertile from second to third trimester for both UARI and UAPI (Table 4): the lower the education, the higher the risk (UARI; P= .003 for trend, UAPI; P< .001 for trend). Linear mixed models also showed that both UARI and UAPI were higher among lower educated women as compared with high educated women during pregnancy (Supplemental Figure 1).

Table 4. Association of educational level with tracking of placental resistance indices from second to third trimester

	Tracking in third tertile from second to third trimester			
	UARI N=2647		**UAPI N=4850**	
Educational level	**% (N)**	**OR (95% CI)***	**% (N)**	**OR (95% CI)***
High	14.8 (103)	Ref	11.1 (134)	Ref
Mid-high	14.7 (79)	0.99 (0.70,1.41)	14.7 (143)	1.32 (1.02,1.70) †
Mid-low	19.3 (154)	1.45 (1.04,2.01) †	17.0 (249)	1.54 (1.21,1.98) ‡
Low	22.3 (137)	1.63 (1.13,2.35) ‡	18.3 (220)	1.74 (1.32,2.28) §
P for trend		0.003		<0.001

Abbreviations: OR: odds ratio, CI: confidence interval

*Values are logistic regression coefficients derived from multivariable logistic regression. The effect estimates represent the difference in risk of remaining in the third tertile of UARI and UAPI from second to third trimester, compared to reference group (high education). All models are adjusted for age, parity, gestational age at intake, ethnicity and gestational age in each pregnancy period. Estimates are based on multiple imputed data.

† P<0.05 ‡P<0.01 §P<0.001

Discussion

Our hypothesis that women from low socioeconomic subgroups have higher uterine and umbilical artery resistance patterns as compared to women from high socioeconomic subgroups was confirmed by this study. Smoking during pregnancy explained important parts of the socioeconomic inequalities in uteroplacental and feto-placental blood flow. To the best of our knowledge, this is the first study showing socioeconomic inequalities in placental resistance indices in mid- and late pregnancy and that this was mainly explained by smoking. Also, level of education was associated with tracking of

placental resistance indices in the third tertile from second to third trimester. No associations between educational level and notching were observed.

Methodological considerations

The strengths of this study are the prospective population-based design and the availability of many important covariates that may explain the association between educational level and placental resistance indices. In addition, we had a large sample size of 7033 participants and the availability of 8395 UARI measurements and 11829 UAPI measurements.

To various extents, our results may have been influenced by the following limitations. We used maternal educational level as indicator of SEP. Maternal education may reflect general and health-related knowledge, health behaviour, health literacy and problem-solving skills.[25, 26] It is less clear to what extent it captures the material and financial aspects of the household. We therefore repeated the analyses using net household income as determinant, and we found similar results. The lowest income group was significantly associated with higher third-trimester UARI and higher second- and third-trimester UAPI (Supplementary Table S3). Information on various covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle related determinants. Nonresponse analyses showed that there was some selection towards a relatively high educated and more healthy study population. Non-participation would have led to selection bias if the associations of maternal educational level with placental resistance index measurements differed between participants and non-participants. Previous research showed that this bias is minimal,[27, 28] since selection on outcome is unlikely because of the prospective design of the study. However, it cannot be ruled out completely. Finally, part of the effect of education on placental resistance indices remained unexplained. Other lifestyle-related behaviours which were not available for the current study, such as physical activity, may contribute to the explanation. However, physical activity might influence placental resistance indices partially through its contribution to BMI. Furthermore, stress-related factors, shown to be associated with both SEP and placenta resistance, might explain some of the remaining effect.[29, 30] Inclusion of stress, measured by a questionnaire in mid-pregnancy using the Brief Symptom Inventory, to our models contributed slightly to the explanation between education and placental resistance patterns, and an unexplained part remained (data not shown, available upon request).

Educational level and placental resistance indices

During first and second trimester trophoblast invades the spiral arteries and replaces the muscular and elastic arterial layer by collagen, which ensures a decrease in vascular

resistance in the uterine and umbilical artery.[31] Failure of the physiological trophoblast invasion may lead to increased vascular resistance, which may cause some of the most important obstetrical complications such as preeclampsia and fetal growth restriction.[9, 12, 13, 32, 33] In our study, we showed that women with low education have higher second and third trimester vascular resistance patterns in both the uterine and umbilical artery as compared to high educated women. Several previous studies, including studies from our own study cohort, found that women with low SEP have an increased risk of developing pregnancy complications,[1, 2, 5, 8, 34] but much is unknown about the pathways underlying these socioeconomic inequalities. Our findings suggest that these inequalities may be partly explained by impaired uteroplacental and feto-placental blood flow. This is further underlined by our results showing that lower education is associated with increased risk of placental resistance indices to remain in the third tertile from second to third trimester, which also has been associated with adverse pregnancy outcomes.[9] However, we didn't find an association between SEP and notching. The clinical importance of variations in placental blood flow is not yet fully understood, but previously it has been found that already small variations in second and third trimester placental vascular resistance are associated with the risk of adverse pregnancy outcomes.[9] This suggests that our findings, although they might seem small, may contribute to socioeconomic inequalities in health of mothers and their offspring. However, since the clinical importance is not yet fully understood our results require careful interpretation and future studies are recommended.

We found smoking to be the factor contributing most to the association between education and placental resistance indices. Previous research showed that smoking is the most important explanatory factor concerning socioeconomic inequalities in birth weight.[8, 35] Maternal smoking may alter placental development in several ways which in turn might affect fetal growth.[36] Our findings suggest that the effect of smoking on IUGR may be partly caused by increased resistance in the uterine and umbilical artery. This is in line with previous research which showed that women who smoke have, on average, a higher umbilical artery RI, a surrogate measure for an abnormal placental villous vascular tree.[21] Our findings add to the evidence that reducing smoking rates among women should get top priority during preconception and antenatal care, especially among women from low socioeconomic subgroups. Evidence-based intervention strategies should be further developed and implemented to help women to stop smoking.[37] Since smoking has been found in previous studies to be protective for preeclampsia,[38] other underlying pathways may lead from low SEP to preeclampsia. One possible explanation might be that BMI, which has been found to be associated with both SEP and preeclampsia, or other unknown factors overrule the 'protective' effect of smoking.[38] Another possibility

might be that other pathways than increased placental resistance indices influence the risk of preeclampsia among different educational subgroups.

Previous research showed that Doppler examinations in high-risk pregnancies might be useful to identify a group of women in need of increased surveillance,[20] while low-risk pregnancies do not benefit from those examinations.[39] High-risk pregnancies include conditions such as diabetes, hypertensive disorders, cardiac disorders, IUGR and multiple pregnancy.[20] Thus far, low SEP has not been identified as 'high-risk status' in itself, although pregnancy complications are more common among women from low socioeconomic subgroups. Since our results suggest that women with a low SEP may have an increased risk of impaired placental blood flow, future research is necessary to evaluate the clinical effectiveness of using Doppler examinations among this particular group of women. These examinations may lead to early detection of abnormal placental vasculature, and would allow for interventions to reduce maternal and fetal morbidity and mortality among women from low socioeconomic subgroups.

Previously, it has been demonstrated that the effect of SEP on health may differ by ethnic background, and that some ethnic groups do not have the same health benefits from higher levels of SEP than Caucasians.[40, 41] We found that the effect of SEP on placental blood flow differed by ethnic background. Significant interactions were found between SEP and Dutch Antillean women on third-trimester UARI and between SEP and Surinamese Hindustanic women on second-trimester UAPI. However, chance findings cannot be excluded, as interactions were only significant for these two ethnic groups on one outcome. Therefore, conclusions must be drawn carefully and future research is needed.

Conclusion

This study shows that women from low socioeconomic subgroups have higher placental resistance indices, which was mainly explained by maternal smoking during pregnancy. They also have an increased risk of placental resistance indices to track in the third tertile during pregnancy, which has been associated with the risk of adverse pregnancy outcomes. Alterations in utero-placental and feto-placental blood flow might be one of the underlying pathways leading to a higher prevalence of pregnancy complications, such as preterm birth and fetal growth restriction, among women with a low SEP. Further research is needed to examine the clinical importance of variations in placental blood flow and the use of standard Doppler examinations among women from low socioeconomic subgroups.

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Supplemental Material

Supplementary Table S1. Characteristics of women with and without placental resistance indices measurements

	Women with placental resistance indices measurements N=7723	Women without placental resistance indices measurements N=1156	p-value*
Maternal characteristics			
Educational level			
High	23.4	20.0	0.001
Mid-high	19.2	16.5	
Mid-low	30.8	32.1	
Low	26.6	31.3	
Age (years)	29.7 (5.3)	29.3 (5.6)	0.014
Body mass index before pregnancy (kg/m^2)	23.6 (4.3)	24.2 (4.8)	<0.001
Parity (% nulliparous)	56.2	51.0	0.008
Gestational age at enrolment (weeks)	14.2 (10.9, 22.9)	15.9 (11.2, 24.8)	<0.001
Ethnicity (%)			
Dutch	62.8	56.2	<0.001
Cape Verdean	5.4	5.3	
Dutch Antilles	4.1	7.1	
Moroccan	8.6	10.2	
Turkish	11.8	10.9	
Surinamese Creole	3.6	*5.2*	
Surinamese Hindustani	3.8	5.2	
Smoking (%)			
Yes	27,6	25.2	0.131
No	74.2	74.8	
Alcohol use (%)			
Yes	51.0	44.3	<0.001
No	49.0	55.7	
Folic acid supplement use (%)			
Yes	71.4	64.6	<0.001
No	28.6	35.4	

Values are percentages, means (SD) or medians (90% range).
*P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.

Supplementary Table S2. Educational level and placental resistance indices stratified for ethnicity

		Maternal educational level			
High		**Mid-high**	**Mid-low**	**Low**	**P-value of interaction†**
Uterina artery RI (SD)*					
Third trimester					
Dutch	Ref	0.02 (-0.08,0.110	0.09 (-0.01,0.19)	0.15 (0.03,0.28) ‡	
Cape Verdean	Ref	0.71 (-0.46,1.87)	0.50 (-0.66,1.65)	0.52 (-0.63,1.67)	0.611
Dutch Antilles	Ref	0.81 (-0.15,1.77)	0.74 (-0.16,1.65)	1.19 (0.24,2.1) ‡	0.028
Moroccan	Ref	-0.31 (-1.0,0.39)	-0.25 (-1.07,0.57)	-0.05 (-0.78,0.68)	0.474
Turkish	Ref	0.32 (-0.34,0.98)	0.31 (-0.29,0.92)	0.43 (-0.18,1.05)	0.759
Surinamese Creole	Ref	0.69 (-0.58,1.97)	0.75 (-0.32,1.82)	0.78 (-0.34,1.89)	0.672
Surinamese Hindustani	Ref	-0.45 (-1.43,0.53)	-0.56 (-1.42,0.30)	-0.30 (-1.20,0.59)	0.635
Umbilical artery PI (SD)*					
Second trimester					
Dutch	Ref	0.04 (-0.04,0.13)	0.10 (0.01,0.19) ‡	0.28 (0.17,0.39) §	
Cape Verdean	Ref	-0.68 (-2.00,0.64)	-0.48 (-1.81,0.85)	-0.52 (-1.79,0.75)	0.143
Dutch Antilles	Ref	0.15 (-0.66,0.97)	-0.04 (-0.75,0.66)	-0.17 (-0.98,0.64)	0.221
Moroccan	Ref	-0.11 (-0.72,0.51)	0.01 (-0.51,0.54)	0.08 (-0.46,0.61)	0.653
Turkish	Ref	-0.05 (-0.55,0.45)	-0.11 (-0.57,0.35)	0.07 (-0.42,0.56)	0.338
Surinamese Creole	Ref	0.53 (-0.63,1.70)	0.27 (-0.80,1.33)	0.50 (-58,1.58)	0.853
Surinamese Hindustani	Ref	-0.18 (-0.96,0.61)	-0.51 (-1.17,0.16)	-0.53 (-1.25,0.19)	0.026

Abbreviations: SD, standard deviation

*Values are linear regression coefficients (95% confidence interval) derived from multivariable linear regression. The effect estimates represent the difference in placental resistance indices, compared to reference group (high education) per ethnic background. All models are adjusted for age, parity, gestational age at intake and gestational age at measurement. Estimates are based on multiple imputed data.

†P-value reflects the significance level of the interaction term low educational level (ref high educational level) * ethnic background (ref Dutch). No significant interactions were found for mid-low and mid-high education * ethnic background on UARI and UAPI. ‡ P<0.05 §P<0.001

Supplementary Table S3. Net household income and placental resistance indices (n=5703)

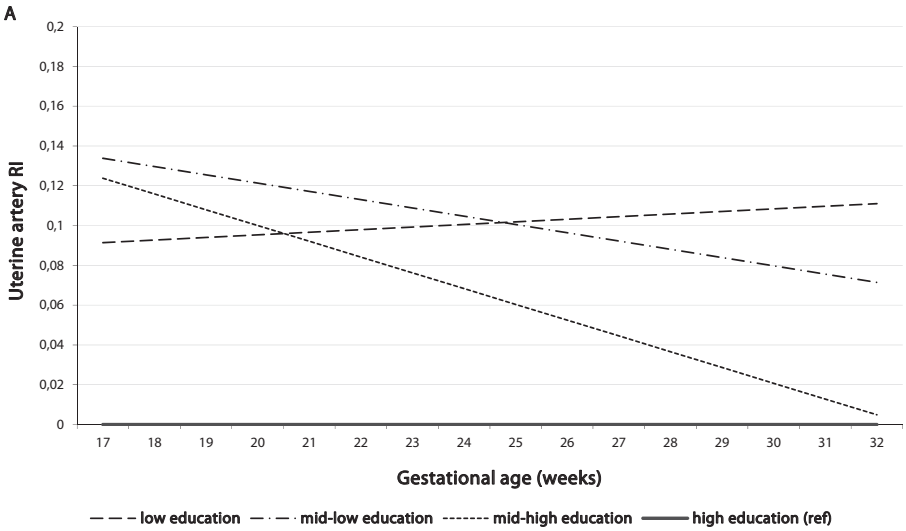
	Uterine artery RI (SD)*		Umbilical artery PI (SD)*		Notching (OR)†
	2nd trimester N=3476	3th trimester N=3485	2nd trimester N=4599	3th trimester N=5085	3th trimester N=3676
Net household income					
> € 2200	Ref	Ref	Ref	Ref	Ref
€ 1600 - € 2200	0.12 (0.02,0.22) ‡	0.11 (0.01,0.21) ‡	0.06 (-0.02,0.15)	0.06 (-0.02,0.15)	0.96 (0.67,1.36)
< € 1600	0.10 (-0.001,0.19)	0.14 (0.04,0.24) §	0.09 (0.01,0.18) ‡	0.10 (0.02,0.19) ‡	1.27 (0.92,1.74)
P for trend	0.030	0.004	0.029	0.012	0.172

Abbreviations: OR; odds ratio, SD, standard deviation

*Values are linear regression coefficients (95% confidence interval) derived from multivariable linear regression. The effect estimates represent the difference in placental resistance indices, compared to reference group (net household income > € 2200). All models are adjusted for age, parity, gestational age at intake, ethnicity and gestational age at measurement. Estimates are based on multiple imputed data.

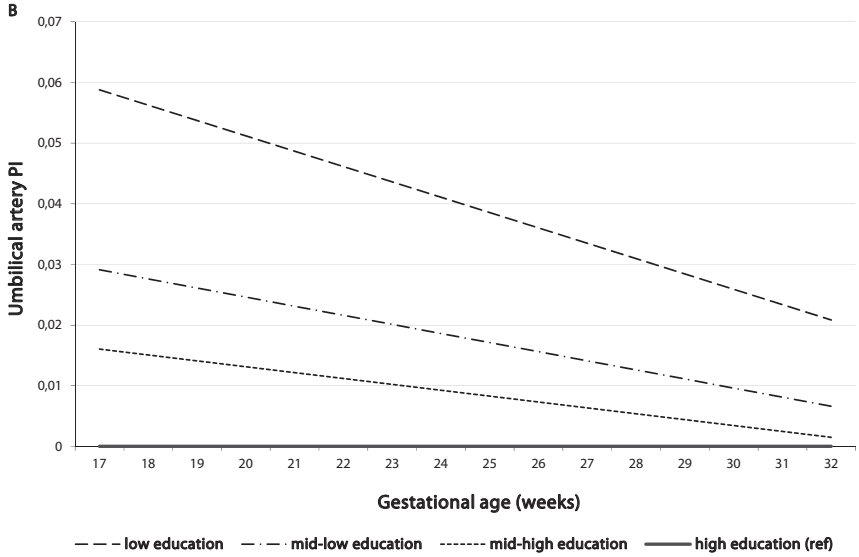
†Values are odds ratios (95% confidence interval) derived from logistic regression models. The effect estimates reflect difference in risk of notching, compared to reference group (net household income > € 2200). All models are adjusted for age, parity, gestational age at intake, ethnicity and gestational age at measurement. Estimates are based on multiple imputed data.

‡ P<0.05 §P<0.01



1A. Uterine artery resistance index development among women with different educational levels. Difference in placental resistance indices measurements for women with low, mid-low and mid-high educational levels as compared to high education (reference) based on repeated measurement analysis. Uterine artery resistance index = $ß_0$ + $ß_1$*educational level + $ß_2$*gestational age + $ß_3$*educational level*gestational age + $ß_4$*ethnicity + $ß_5$*gestational age at intake + $ß_6$*age + $ß_7$*parity

Supplemental Figure S1. Placental resistance indices among women with different educational levels
Linear mixed models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data.
Linear models, without adding polynomials, appeared to be the best-fitting models for placental resistance indices and can be written as:
Uterine artery resistance index= $ß_0$ + $ß_1$*educational level + $ß_2$*gestational age + $ß_3$*educational level*gestational age + $ß_4$*ethnicity + $ß_5$*gestational age at intake + $ß_6$*age + $ß_7$*parity
Umbilical artery pulsatility index = $ß_0$ + $ß_1$*educational level + $ß_2$*gestational age + $ß_3$*educational level*gestational age + $ß_4$*ethnicity + $ß_5$*gestational age at intake + $ß_6$*age + $ß_7$*parity
In these models, '$ß_0$ + $ß_1$*educational level' reflects the intercept and '$ß_2$*gestational age reflects the slope of change in placental resistance index per week. The uterine artery and umbilical artery resistance patterns among women with different educational levels are shown in Supplemental Figure 1a and 1b.



1B. Umbilical artery pulsatility index development among women with different educational levels. Difference in placental resistance indices measurements for women with low, mid-low and mid-high educational levels as compared to high education (reference) based on repeated measurement analysis. Uterine artery resistance index = $ß_0$ + $ß_1$*educational level + $ß_2$*gestational age + $ß_3$*educational level*gestational age + $ß_4$*ethnicity + $ß_5$*gestational age at intake + $ß_6$*age + $ß_7$*parity

4

Low educated women have an increased risk of gestational diabetes mellitus

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Abstract

Objective: To investigate whether maternal educational level is associated with gestational diabetes mellitus (GDM), and to what extent risk factors for GDM mediate the effect of educational level.

Methods: We examined data of 7511 pregnant women participating in a population-based cohort study in Rotterdam, the Netherlands. Highest achieved education was categorized into four levels. Diagnosis of GDM was retrieved from delivery records. Odds ratios (OR) of GDM were calculated for levels of education, adjusting for confounders and potential mediators. Mediators were selected according to Baron and Kenny's causal step approach.

Results: Adjusted for ethnicity, age, family history of diabetes and parity, women in the lowest educational level were three times more likely to develop GDM than women in the highest level (OR 3.07; 95% CI: 1.37, 6.89). Selected mediators were alcohol use and BMI. Additional adjustment for alcohol use attenuated the OR to 2.54 (95% CI: 1.11, 5.78). The individual addition of body mass index (BMI) further attenuated the OR to 2.35 (95 % CI: 1.03, 5.35). All mediators together explained 51% (95% CI:-122,-25) of the association between low education and GDM.

Conclusions: Low maternal educational level is associated with GDM, which is mainly due to higher rates of overweight and obesity. In order to reduce the higher rates of GDM, and consequently type 2 diabetes among women in low socioeconomic subgroups, prevention and intervention strategies need to be focused on reducing the rates of overweight and obesity before pregnancy.

Introduction

Gestational diabetes mellitus (GDM) is associated with several adverse outcomes in both mother and child. Women who suffered from GDM are much more likely to develop type 2 diabetes than women without GDM, probably GDM serves as a metabolic stress test that uncovers women who are prone to develop type 2 diabetes in future.[1] Children born from mothers with GDM are found to have a higher risk of macrosomia, childhood obesity and childhood metabolic syndrome.[2, 3] Consequently, all of the adverse outcomes following GDM put both mother and child at a strongly increased risk for developing long-term cardiovascular disease. [4, 5] As the worldwide prevalence of diabetes, including GDM, is predicted to rise from 2.8% in 2000 to 4.4% in 2030, [6] health complications associated with exposure to GDM will also increase.

Moreover, low socioeconomic position (SEP), as indicated by educational level, occupational class or income level, has been identified by previous studies as a major risk factor in the development of type 2 diabetes.[7, 8] However, markedly fewer studies have examined the association between measures of SEP and GDM. One such study conducted in Turin, Italy found that low SEP, assessed by educational level and employment, was a risk factor in the development of GDM.[9] The results, however, were based on a relatively small case-control study, and therefore further studies are needed to confirm the results of such findings within a larger population based study. Furthermore, it is unclear to what extent other risk factors for GDM contribute to the association between SEP and GDM. Several risk factors for diabetes which are found to vary by SEP may play a role in the development of potential socioeconomic inequalities in GDM, including alcohol use, smoking, unhealthy diet, obesity and stress.[10-12] A better understanding of the relation between SEP and GDM and its underlying pathways may provide novel opportunities to prevent GDM and its adverse health outcomes in later life, including type 2 diabetes.

Therefore, within the Generation R study, a prospective cohort study with extensive assessments during pregnancy,[13] we investigated whether educational level as indicator of SEP is associated with the risk of GDM. We also examined to what extent risk factors for GDM, i.e. substance use, diet, stress and body mass index (BMI), contribute to the explanation of any association between educational level and GDM.

Materials and Methods

The Generation R Study

This study was embedded in the Generation R Study, a population-based prospective co-

hort study from fetal life until young adulthood. The Generation R Study has been described previously in detail.[14, 15] Briefly, the cohort includes 9778 (response rate 61%) mothers and their children of different ethnicities living in Rotterdam, the Netherlands.[13] Enrolment was aimed in early pregnancy but was possible until birth of the child. All children were born between April 2002 and January 2006. Assessments in pregnancy, including physical examinations, ultrasound assessments and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam.[16] Written consent was obtained from all participating parents.

Study Population

Of the 9778 women, 8879 were enrolled in pregnancy and eligible for present analysis.[13] We excluded from the analyses women with missing information on educational level (n=817) and on diagnosis of GDM (n=365). We also excluded women with self-reported pre-existing diabetes (n=31), twin pregnancies (n=88), induced abortions (n=18) and fetal death (n=49), leaving a study population of 7511 subjects.

Educational level

Educational level was used as indicator of SEP. The highest educational level was established by a questionnaire at enrollment. The Dutch Standard Classification of Education was used to categorize 4 subsequent levels of education: high (university degree), mid-high (higher vocational training, Bachelor's degree), mid-low (>3 years general secondary school, intermediate vocational training) and low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general secondary school).[17]

Diagnosis of Gestational Diabetes Mellitus

GDM was diagnosed by a community midwife or an obstetrician according to Dutch midwifery and obstetric guidelines using the following criteria: either a random glucose level >11.0 mmol/L, fasting glucose ≥7.0 mmol/L or a fasting glucose between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test. In clinical practice and for this study sample, an abnormal glucose tolerance test was defined as a glucose level greater than 7.8 mmol/L after glucose intake.

Potential mediators

Level of maternal education cannot affect the risk for GDM directly but is likely to act through more proximal risk factors, so-called mediators.[18] We considered the following fac-

tors to be potential mediators in the pathway between maternal education and GDM.[10-12]

Substance use during pregnancy: Smoking and alcohol consumption (no, until pregnancy was known, continued during pregnancy) was assessed by questionnaire in early, mid- and late pregnancy.

Nutritional information: First trimester nutritional information (total energy intake [kcal], carbohydrates [energy %], fat [energy %]) was obtained by a food frequency questionnaire at enrolment.

Stress: Maternal distress was measured by a questionnaire in mid-pregnancy using the Brief Symptom Inventory, which gives a Global Severity Index (GSI). Higher GSI reflected pregnant women to experience more stress.

Pre-pregnancy body mass index: Information about maternal weight just before pregnancy was obtained by questionnaires at enrolment. On the basis of height (cm), measured at enrolment without shoes, and pre-pregnancy weight, we calculated pre-pregnancy body mass index (BMI; weight/height^2).

Potential confounders

The following factors were treated as potential confounders:[10] maternal age, parity, family history of diabetes (no, yes, do not know) in a first degree relative and ethnicity, since these factors cannot be considered indisputable mediators.[18] These variables were obtained through questionnaires at enrolment. The following ethnic groups were defined: Dutch, Turkish, Moroccan, Surinamese Creole, Surinamese Hindustani, Antillean and Cape Verdean.[19]

Statistical Analyses

Associations between educational level and all covariates were explored using Chi-square tests, ANOVAs and Kruskal-Wallis tests. Multiple logistic regression was used to calculate odds ratios (OR) for GDM and the corresponding 95% confidence intervals (CI) for levels of education, adjusted for the potential confounders ethnicity, age, family history of diabetes and parity (model 1). Next, potential mediators were added to these models. To evaluate the mediating effects of all potential explanatory factors Baron and Kenny's causal step approach was used.[20] Only those factors that were significantly associated with the outcome (independent of maternal educational level) and unequally distributed across SEP groups (Table 1) were added separately to model 1. [20] To assess their mediating effects, the corresponding percentages of attenuation of the odds ratios were calculated by comparing differences of model 1 with the adjusted ones (100 x

$(OR_{+mediator} - OR_{model\ 1}) / (OR_{model\ 1} - 1))$. Finally, a full model containing educational level and all selected mediators assessed the joint effects of the explanatory factors.

Multiple imputation was used to deal with missing values in the covariates. Five imputed datasets were created and analysed together. A 95% confidence interval (CI) was calculated around the mediating effects using a bootstrap method with 1000 re-samplings per imputed dataset in the statistical program .[21] The other statistical analyses were performed using Statistical Package of Social Science (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, IL, USA). A p-value of 0.05 was taken to indicate statistical significance.

Results

Of the 7511 women in the study, the mean age was 29.8 years (SD: 5.2) and 57.0% were nulliparous. From the total study population, 27.0% were in the lowest educational level and 23.1% were in the highest educational level (Table 1). GDM was diagnosed in 71 women (0.9%). Stratified by educational level, the cumulative incidence of GDM was 0.6% (n=10), 0.9% (n=13), 1.0% (n=23) and 1.2% (n=25) for women of high, mid-high, mid-low and low education respectively. Age, alcohol use during pregnancy and energy intake were positively associated with level of education ($p < 0.001$) while parity, family history of diabetes, smoking during pregnancy, intake of carbohydrates, BMI and maternal stress ($p < 0.001$) were negatively associated with level of education.

Table 2 shows which potential mediators were associated (independently form educational level and confounders) with GDM. Continued alcohol use during pregnancy and BMI were the mediators which were selected according to Baron's and Kenny's causal step approach. Smoking, diet and stress were not selected, since they were not associated with GDM independently from educational level.

Compared to women with high education, women with low education had a significantly increased risk of GDM after adjustment for ethnicity, age, family history of diabetes and parity (OR 3.07; 95% CI: 1.37, 6.89) (Table 3). Additional individual adjustment for alcohol use resulted in an attenuation of the OR to 2.54 (95% CI: 1.11, 5.78) for low educated women, explaining 26% (95% CI: -68%,-3%) of the association between educational level and GDM. The greatest contributor to the explanation of low educational level and GDM was BMI, which explained 35% (95% CI: -90.-18). Full adjustment for all confounders and mediators resulted in an explanation of 51% (-122,-25). Similar contributions of alcohol use and BMI were observed for mid-low education and GDM (Table 3).

Table 1. Characteristics of the study population (n=7511)

		Maternal education level				
	Total N=7511	**High N=1735**	**Mid-high N=1426**	**Mid-low N=2319**	**Low N=2031**	**P value[a]**
Potential confounders[b]						
Age (years)	29.8 (5.2)	33.0 (3.3)	31.4 (4.1)	28.7 (5.1)	27.4 (5.7)	<0.001
Parity (% nulliparous)	57.0	58.7	63.1	60.2	47.4	<0.001
Ethnicity (%)						
Dutch	63.0	94.0	82.0	52.6	37.1	<0.001
Cape Verdean	5.2	0.4	2.1	6.9	9.1	
Dutch Antilles	4.2	0.8	2.4	5.9	6.1	
Moroccan	8.0	1.3	3.7	8.9	15.2	
Turkish	11.3	2.3	4.9	12.5	21.3	
Surinamese Creole	4.1	0.5	2.8	6.4	5.3	
Surinamese Hindustani	4.3	0.8	2.2	6.7	5.8	
Family history of diabetes						
No (%)	80.3	87.3	86.1	78.4	72.4	<0.001
Yes (%)	17.1	11.7	12.7	18.4	23.4	
Do not know (%)	2.6	1.0	1.3	3.3	4.2	
Potential mediators[b]						
Smoking						
No (%)	73.4	86.7	78.9	71.3	60.7	<0.001
Until pregnancy was known (%)	8.7	8.1	9.5	9.3	8.0	
Continued during pregnancy (%)	17.9	5.2	11.6	19.3	31.3	
Alcohol use						
No (%)	49.0	23.5	37.2	57.0	69.9	<0.001
Until pregnancy was known (%)	13.5	13.7	15.7	15.3	9.9	
Continued during pregnancy (%)	37.5	62.8	47.2	27.8	20.2	
Total energy intake (kcal)	2046 (565)	2097 (500)	2129 (540)	2002 (569)	1979 (633)	<0.001
Carbohydrates (energy%)	48.7 (6.5)	47.6 (5.7)	48.7 (6.2)	49.2 (6.7)	49.4 (7.4)	<0.001
Fat intake (energy%)	36.2 (5.7)	36.5 (5.0)	36.0 (5.4)	36.0 (5.8)	36.2 (6.4)	0.038
Pre-pregnancy BMI(kg/m^2)	23.6 (4.3)	22.5 (3.0)	23.0 (3.6)	23.9 (4.6)	24.6 (5.0)	<0.001
Maternal stress index	0.17 (0.00,1.46)	0.10 (0.00,0.78)	0.13 (0.00,0.92)	0.21 (0.00,1.58)	0.25 (0.00,1.91)	<0.001
Outcome						
Gestational Diabetes	0.9	0.6	0.9	1.0	1.2	0.23

Values are percentages , means (SD) or medians (95% range) for the total population and by level of maternal education.

[a] P-values are calculated with the Chi-square test for categorical variables, ANOVA for continuous variables and Kruskal-Wallis test for non-normally distributed continuous variables.

[b] Data was missing for parity (0,1%), ethnicity (20.0%), family history of diabetes (7.4%), smoking (6.8%), alcohol use (7.3%), total energy intake (23.7%), fat intake (23.7%), carbohydrates (23.7%), maternal distress (25.6%) and BMI before pregnancy (16.6%

Table 2. Associations between potential mediators and gestational diabetes (n=7511)

	Gestational diabetes	
	OR (95% CI)[a]	**P-value**
Potential mediators		
Smoking		
No (%)	1.00	
Until pregnancy was known (%)	0.33 (0.08,1.42)	0.14
Continued during pregnancy (%)	0.85 (0.44,1.62)	0.61
Alcohol use		
No (%)	1.00	
Until pregnancy was known (%)	0.50 (0.19,1.32)	0.16
Continued during pregnancy (%)	0.51 (0.28,0.93)	0.03
Total energy intake (kcal)	1.00 (1.00,1.00)	0.84
Carbohydrates (energy%)	0.98 (0.95,1.03)	0.45
Fat intake (energy%)	1.00 (0.95,1.05)	0.96
Pre-pregnancy BMI(kg/m^2)	1.12 (1.08,1.17)	<0.001
Maternal stress index	0.88 (0.44,1.78)	0.73

Table is based on imputed dataset.

[a]Values are derived from logistic regression models and represent OR (95% confidence intervals), adjusted for maternal educational level and the potential confounders (gender, age, ethnicity and family history of diabetes)

Table 3. Odds ratios and change in odds ratios of gestational diabetes for the different levels of maternal education after individual adjustment for each selected mediator (n=7511).

	Maternal educational level			
Models[a]	**High**	**Mid-high**	**Mid-low**	**Low**
Model 1	1.00	1.80 (0.78,4.14)	2.29 (1.05,4.96)	3.07 (1.37,6.89)
Model 2	1.00	1.69 (0.73,3.89)	1.98 (0.90,4.36)	2.54 (1.11,5.78)
Change 1*			-23% (-107,3)	-26% (-68,-3)
Model 3	1.00	1.69 (0.74,3.90)	1.87 (0.85,4.10)	2.35 (1.03,5.35)
Change 2*			-32% (-127,-13)	-35% (-90,-18)
Fully adjusted	1.00	1.59 (0.69,3.67)	1.66 (0.75,3.67)	2.02 (0.87,4.65)
Change 3*			-47% (-187,-14)	-51% (-122,-25)

[a]Model 1: Baseline model adjusted for ethnicity, age, family history of diabetes and parity

Model 2: Model 1 + alcohol use

Model 3: Model 1 + body mass index

Fully adjusted model adjusted for ethnicity, age family history of diabetes, parity, alcohol use and body mass index

Discussion

This study showed that low educational level is associated with a three times higher risk for developing GDM compared with a high educational level. The mediating effects of alcohol use and BMI explained a great part of the increased risk. However, smoking, diet and stress did not contribute to the explanation of an increased risk of GDM among low educated women.

Methodological considerations

The main strength of this study lies in the population-based prospective design, in which a large number of women were enrolled early in pregnancy, and information on relevant potential confounders and mediators was available. Therefore it was possible to include indicators of known risk factors for GDM in the explanatory models.[22-24]

Some limitations should also be addressed. First, while the diagnostic criteria used to identify cases of GDM in this study compare well to those used by the American Diabetes Association,[25] some cases of GDM may have been missed, as suggested by the relatively low incidence of GDM.[26] This was because measurement of blood glucose levels was not a standard prenatal procedure. Although presence of glucosuria is routinely tested, measurements of blood glucose levels are usually only performed when glucose intolerance is suspected based on symptoms of GDM e.g. polydipsia, polyuria or macrosomia. Cases of GDM without overt symptoms might have remained unrecognized by the prenatal caregiver and consequently not been included in our study. Second, information on educational attainment and most of the included risk factors were collected using questionnaires, which might have induced some misclassification. Finally, the selected mediators BMI and alcohol use explained about half of the association between low educational level and GDM, but the remaining risk of low educated women to develop GDM was still two times higher as compared to high educated women. The fully adjusted OR was not significant anymore, but this might be due to a lack of statistical power because of the relatively small number of women suffering from GDM. We were unable to completely explain the association between low educational level and GDM. Unmeasured factors related to both SEP and GDM, such as physical activity, could not be taken into account and may explain some of the remaining effects of SEP on GDM.

Educational level and gestational diabetes mellitus

Although the association between SEP and type 2 diabetes is well established in previous research,[7, 8] conflicting results exist regarding the relation between SEP and GDM. Our results are comparable with findings from a case-control study performed in Italy,[9] which reported that women with primary school education had an increased risk of GDM (OR

1.87; 95% CI: 1.1-3.2) compared to women of a higher educational level. Furthermore, our findings are in line with a large population-based study in Australia, in which women in the lowest socioeconomic subgroups, defined by area of residence, had an OR of 1.65 (95% CI: 1.60-1.70) as compared to women in the highest socioeconomic subgroup.[27] The authors hypothesized that obesity might be one of the explanatory factors in the association between SEP and GDM, as they did not have information on body weight. Our study confirms the hypothesis that overweight and obesity are mediating factors in this relation. However, there are also studies which did not find an association between SEP and GDM.[28, 29] A possible explanation for the different findings might be the use of different indicators of SEP.

A large part of the increased risk of GDM in low educated women was explained by relatively high rates of overweight and obesity in this subgroup. Excess adipose tissue has been demonstrated to lead to the release of unsaturated fatty acids, glycerol, hormones and proinflammatory cytokines which are involved in the development of insulin resistance.[30] Unsaturated fatty acids are recognized as the most critical factor in modulating insulin sensitivity. Pancreatic cells (β-cells) regulate the quantity of insulin released, and in response to insulin resistance, the release of insulin will be increased to maintain normal glucose levels. Chronic exposure to high levels of unsaturated fatty acids may result in dysfunction of pancreatic cells, which in turn may result in type 2 diabetes.[30] A similar mechanism may link obesity, insulin resistance and GDM. This illustrates the need to reduce the burden of overweight and obesity through lifestyle changes in lower socioeconomic groups.

Relatively low rates of alcohol consumption in lower educated subgroups contributed significantly to the explanation of the increased risk GDM among low educated women. This was because alcohol consumption was protective in the development of GDM in our data. Alcohol consumption has been found to be associated with diabetes type 2 in a U-shaped fashion.[31, 32] It has been suggested that low to moderate alcohol consumption, in contrast to excessive alcohol consumption, might have a protective effect on the development of type 2 diabetes.[31, 33] In two randomised trials, moderate alcohol consumption improved insulin sensitivity and lowered triglyceride concentrations, which might explain the protective effect of moderate alcohol use on type 2 diabetes.[34, 35] Also, moderate alcohol consumption might have an anti-inflammatory effect.[36] Low to moderate alcohol consumption has also been reported to lower the risk of other cardiovascular outcomes, including stroke and coronary heart disease.[37, 38] We found no published studies describing a similar effect of alcohol consumption on GDM, but similar processes as in type 2 diabetes may underlie the effect of moderate alcohol use in the development of GDM. This is supported by the fact that in our study the vast

majority of women were not heavy drinkers, but low to moderate drinkers (<1 glass a day during their pregnancy). A recent study found that <1 drink per occasion regularly 6 times throughout a week was associated with the lowest risk of the development of type 2 diabetes.[32] It is, however, important to note that alcohol use during pregnancy is associated with teratogenic effects on the unborn child and alcohol use during pregnancy is therefore not recommended. Furthermore, our results need very careful interpretation because we cannot ascertain a causal relation as residual confounding by other unmeasured lifestyle factors such as dietary habits might have driven the reduction in risk of GDM with alcohol consumption. Therefore, we recommend to replicate our findings in future studies with large and varied datasets.

Although smoking was more prevalent among lower educated women than higher educated women in our study, it did not contribute to the explanation between low education and GDM. Previous studies are conflicting about the role of smoking in the development of GDM. The Nurses' Health Study found a 40% increased risk of GDM among smokers compared with never smokers.[24] In contrast, a large Swedish study of 212.190 women did not find an association between smoking and GDM,[39] which confirms our results. These inconsistencies might be explained by differences in characteristics of the study population, including age, ethnicity and case subjects, and percentage of long-term smokers. Stress was in our study not associated with GDM. This is in contrast to the study of Hosler et al.,[12] which found that stressful events were associated with GDM. However, stress is a complex construct and stressful events might reflect a different aspect of stress and consequently have a different effect on GDM than the measure of stress used in our study. Thus, the specific role of smoking and stress in the development of GDM has yet to be clarified.

Furthermore, there is no consistent evidence between dietary intake and GDM. A previous study showed that higher intake of fat and a lower intake of carbohydrates was associated with increased risk of GDM,[11] while other studies, including our study, did not.[40, 41] In our study the association between educational level and nutrient intake was the opposite of what was expected, i.e. low educated women had the lowest energy intake. Possibly this is due to underreporting specifically among low educated women. Another possibility might be that low educated pregnant women, who are more frequently overweight/obese before pregnancy, are advised by their prenatal caregiver to reduce their energy and fat intake to achieve an optimal gestational weight gain.

Conclusions

Several previous studies have demonstrated the link between higher degrees of social deprivation and adverse health outcomes, including the development of type 2

diabetes. Our study extends these findings by demonstrating that women from lower socioeconomic subgroups have also a higher incidence of GDM, which is mainly due to higher rates of overweight and obesity. In order to reduce the higher rates of GDM, and consequently type 2 diabetes among women in low socioeconomic subgroups, prevention and intervention strategies need to be focused on reducing the rates of overweight and obesity before pregnancy.

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Low education is associated with inadequate and excessive gestational weight gain

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Submitted

Abstract

Background: Excessive and inadequate gestational weight gain (GWG) impact on health of mothers and their children. The aim of this study was to investigate the association between maternal education and maximum GWG, as well as educational inequalities in the development of GWG during pregnancy.

Methods: The study included 6979 women participating in a prospective cohort study in the Netherlands. Maternal educational was related to maximum GWG (according to the IOM criteria) and to trimester- specific GWG in multivariable logistic regression analyses. In the analyses, age, parity and ethnicity were included as confounders.

Results: A significant interaction between maternal education and pre-pregnancy BMI was found. As compared to high educated normal weight women an increased risk of excessive GWG was found among low educated normal weight women (OR 2.00 (95% CI: 1.49,2.68)). Overweight and obese women had an increased risk of excessive GWG, regardless of their level of education. High educated overweight and obese women had a higher risk of excessive GWG than low educated women with normal weights (OR 5.11 95% CI:3.39,7.71 vs. OR 2.2 95% CI: 1.56-3.12). Low educated women had a higher GWG in early pregnancy (0.09 kg/week (95% CI: 0.06,0.11) than higher educated women. Low education was also associated with a higher risk of inadequate GWG (OR 1.73 (95% CI: 1.22,2.45)).

Conclusions: Low maternal education is associated with excessive GWG among normal weight women, and developed mainly in early pregnancy In addition, low education was also associated with an increased risk of inadequate GWG.

Introduction

Excessive gestational weight gain (GWG) has been found to be associated with short-term and long-term adverse maternal and childhood health outcomes, including maternal adiposity after pregnancy, large for gestational age births, and childhood obesity.[1-4] Inadequate GWG has been associated with small for gestational age births, which in turn has been associated with cardiovascular diseases in the long term.[1, 5] According to the Institute of Medicine (IOM) guidelines, which were revised in 2009, optimal GWG is based on pre-pregnancy BMI, whereby overweight and obese women are recommended to gain less weight during pregnancy than normal and underweight women.[6]

Studies suggest socioeconomic inequalities in gestational weight gain, although the scarce evidence is mixed.[7-10] A recent Swedish study found that women with low education and a normal pre-pregnancy BMI were at greater risk of excessive GWG as compared to their high educated normal weight counterparts.[7] Available evidence also suggest inequalities in inadequate GWG, although this association is not consistent across studies.[7, 10, 11] An improved understanding is important, because socioeconomic inequalities in GWG may play a role in the development of socioeconomic inequalities in small for gestational age births, maternal and childhood overweight and cardiovascular diseases in later life.[12-14]

The specific period in which women gain weight might influence the development of adverse outcomes. Previous research showed that weight gain in early pregnancy, but not in later pregnancy, was associated with postpartum weight retention and childhood overweight.[15, 16] No previous research however, investigated socioeconomic inequalities in trimester specific GWG, despite this being crucial information for an appropriate intervention strategy. Therefore, the aim of this study is to investigate the association between maternal educational level, as an indicator of socioeconomic position (SEP), and excessive and inadequate GWG, as well as the association between maternal educational level and GWG in different trimesters of pregnancy in a prospective cohort study.

Methods

Study design and population

This study was embedded within the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood that has previously been described in detail.[17] Enrolment was aimed at early pregnancy, but was allowed until the birth of the child. Pregnant women were enrolled between 2001 and 2005. Off all eligible children in the study area, 61 % participated at birth in the study. The study was conducted in

accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center Rotterdam. Written consent was obtained from all participating mothers.[18] Information about educational level was available among 8152 women who were enrolled during pregnancy, of whom at least one maternal weight measurement during pregnancy was available in 7223 subjects. We excluded pregnancies not leading to singleton live births (n=244). In total, 6979 women were eligible for the present study.

Educational level

Maternal educational level was used as indicator of SEP. The highest educational level was asked for in a questionnaire at enrolment. The Dutch Standard Classification of Education was used to categorize 4 subsequent levels of education: high (university degree), mid-high (higher vocational training, Bachelor's degree), mid-low (>3 years general secondary school, intermediate vocational training) and low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general secondary school).[19]

Pre-pregnancy BMI and gestational weight gain

At enrolment, we measured maternal height (cm) and obtained information about maternal weight just before pregnancy by questionnaire. We calculated pre-pregnancy body mass index (BMI; weight/height^2). Pre-pregnancy BMI was categorized into 4 categories (underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (≥30 kg/m^2)). Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery and available in 3260 mothers. Among this group of mothers, we defined inadequate, adequate or excessive gestational weight gain according to the Institute of Medicine (IOM) guidelines (for underweight mothers: 12.5-18 kg; for normal weight mothers: 11.5-16 kg; for overweight mothers: 7-11.5 kg; for obese mothers: 5-9 kg).[6] Maternal weight was further assessed one to three times during pregnancy depending on the gestational age at enrolment; in early-, mid- and late-pregnancy. We measured maternal weight without shoes and heavy clothing. Based on the timing of maternal weight measurements within our study cohort, we defined early-, mid- and late GWG, using self-reported and measured maternal weight data, as; the start of pregnancy until 13 weeks of gestation (median 13.4 weeks, 95% range 9.9-18.9); from 13 weeks until 26 weeks of gestation (median 29.9 weeks, 95% range 20.5, 31.4); and from 26 weeks until 40 weeks of gestation (median 39.0 weeks, 95% range 32.8, 42.0), respectively. Data was available for 5528, 6183 and 3452 mothers, respectively.

Potential confounders

Age, parity and ethnicity were treated as potential confounding variables. Information about these variables was obtained through questionnaires at enrolment. The following

ethnic groups were define: [20] Dutch, Turkish, Moroccan, Surinamese-Creole, Surinamese-Hindustani, Antillean and Cape Verdean.

Statistical analyses

Mean levels of GWG and of covariates by levels of maternal education were explored using Chi-square tests and ANOVAs. Univariable and multivariable logistic regression models were used to examine associations between maternal educational level and inadequate and excessive GWG. Additionally, linear regression models were used to examine the association of maternal educational level with GWG in early, mid and late pregnancy. Interactions were tested between maternal education and pre-pregnancy BMI and between maternal education and ethnic background. In these analyses, ethnic background was categorized into 3 categories (Dutch, non-western, other western) due to power considerations. There were no significant interactions between education and ethnic background on GWG ($P>0.05$). A significant interaction, however, was found between education and pre-pregnancy BMI on GWG according to the IOM criteria ($P<0.05$). Therefore, odds ratios of the association between maternal education and GWG are presented for the total study population, for normal weight women and for overweight/obese women with high educated normal weight women as the reference category. Multiple imputation was used to deal with missing values in the covariates.[21] Five imputed datasets were created and analysed together. All statistical analyses were performed using Statistical Package of Social Science (SPSS) version 20.0.

Results

Table 1 shows the characteristics of the study population. Of the 6979 participating women, 24.5% had a high education and 25.2% had a low education. Low educated women were younger, less frequently of Dutch origin, more often nulliparous and more frequently overweight and obese before pregnancy as compared to high educated women ($P<0.001$). Mean maximum GWG was not significantly different across the educational subgroups ($P=0.07$). Both an inadequate and an excessive GWG according to the IOM guidelines were more prevalent among the low educated women ($P<0.001$) (Table 1).

Figure 1 shows the odds ratios of excessive and inadequate GWG for different educational subgroups, adjusted for age, parity and ethnicity. Results are shown for the total study population, for normal weight women and for overweight/obese women, because of a significant interaction ($P<0.05$). Results are not shown for underweight women, because statistical power was too low to investigate educational inequalities in GWG among these women. Figure 1a shows the association between SEP and excessive

Table 1. Characteristics of the study population by maternal education

Characteristics[b]	Total N=6979	Maternal education High N=1709	Mid-high N=1386	Mid-low N=2128	Low N=1756	P-value[a]
Age (years)	30.0 (5.1)	32.9 (3.3)	31.4 (4.0)	28.8 (5.0)	27.3 (5.7)	<0.001
Parity (% nulliparous)	57.5	58.6	63.0	60.3	48.5	<0.001
Ethnicity (%)						
Dutch	65.9	94.2	83.3	55.2	39.6	<0.001
Cape Verdean	4.8	0.2	2.1	6.3	9.5	
Dutch Antilles	3.9	0.8	2.1	5.3	6.4	
Moroccan	7.0	1.2	3.3	8.3	13.6	
Turkish	10.2	2.5	4.4	11.8	19.7	
Surinamese Creole	3.8	0.5	2.6	6.0	4.9	
Surinamese Hindustani	4.4	0.8	2.2	7.0	6.2	
BMI before pregnancy (kg/m^2)	23.5 (4.2)	22.5 (3.1)	23.0 (3.6)	23.9 (4.6)	24.5 (5.0)	<0.001
BMI in categories (%)						
Underweight	4.4	3.5	3.6	4.7	5.4	<0.001
Normal weight	68.8	79.6	75.0	65.1	57.3	
Overweight	18.8	14.3	16.4	20.1	23.6	
Obese	8.1	2.5	5.0	10.1	13.7	
Outcomes						
Maximum gestational weight gain (kg)	14.9 (5.8)	14.9 (4.9)	15.2 (5.5)	14.9 (6.2)	14.3 (7.3)	0.07
Gestational weight gain in (kg/week)						
Early pregnancy	0.17 (0.25)	0.15 (0.19)	0.17 (0.21)	018 (0.28)	0.19 (0.30)	<0.001
Mid-pregnancy	0.49 (0.25)	0.51 (0.21)	0.51 (0.23)	0.49 (0.25)	0.47 (0.28)	0.004
Late pregnancy	0.55 (0.39)	0.55 (0.35)	0.55 (0.37)	0.57 (0.42)	0.51 (0.45)	0.39
Gestational weight gain according to IOM criteria (%)						
Inadequate	20.3	19.0	17.3	21.4	25.9	<0.001
Adequate	35.4	40.6	37.1	32.2	26.9	
Excessive	44.3	40.4	45.6	46.4	47.2	

Values are percentages or means (SD) for the total population and by level of maternal education.
[a] P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.
[b] Data was missing for parity (0.2%), ethnicity (20.7%) and BMI before pregnancy (14.7%)

GWG. An inverse educational gradient was found in the total population and among normal weight women, i.e. the lower the educational level the higher the odds ratio of excessive GWG. Low educated normal weight women had more than a doubled risk of excessive GWG as compared to their high educated normal weight counterparts (OR

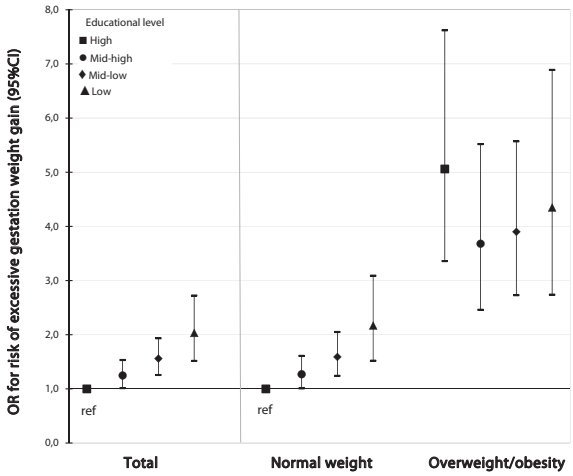
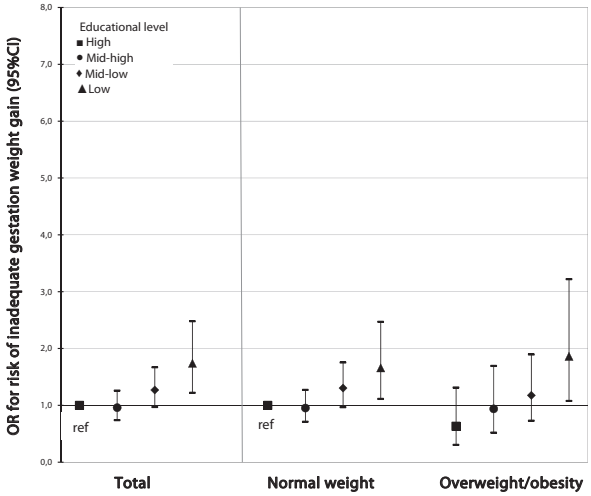


Figure 1A. Educational level and the risk of excessive gestational weight gain according to IOM criteria in the total study population and stratified by pre-pregnancy BMI



1B. Educational level and the risk of inadequate gestational weight gain according to IOM criteria in the total study population and stratified by pre-pregnancy BMI

Values are odds ratio's (95% confidence interval) derived from logistic regression models. The OR represent the risk of excessive and inadequate GWG per educational subgroup in the total study population and stratified on pre-pregnancy BMI (high educated (normal weight) women are the reference group; P for interaction <0.05). Models were adjusted for ethnicity, age and parity. Estimates are based on multiple imputed data. Statistical power was too low to investigate differences in GWG among underweight women. Results are stratified due to a significant interaction of educational level*pre-pregnancy BMI on GWG (P<0.05).

2.20 (95% CI: 1.56,3.12). No educational gradient in excessive GWG was observed among overweight and obese women. In fact, these women all showed a higher odd ratio of excessive GWG as compared to the high educated normal weight women, regardless of their level of education. The odds ratio of high educated overweight and obese women appeared to be higher than the risk of low educated normal weight women (OR 5.11 (95% CI: 3.39,7.71) vs. OR 2.20 (95% CI: 1.56,3.12)). Figure 1b shows the association between maternal education and inadequate GWG. An inverse educational gradient was found in the total study population and in the normal weight and overweight and obese subgroup; i.e. the lower the educational level the higher the risk of inadequate GWG. Low educated women showed a 1.73 (95% CI 1.22,2.45) times higher odds ratio of inadequate GWG as compared to high educated women.

Table 2 shows that lower educated women gained more weight in early pregnancy as compared to high educated women (P for trend <0.001), but not in mid and late pregnancy, after adjustment for age, parity and ethnicity. No interaction was found between education and pre-pregnancy BMI on GWG in early, mid and late pregnancy.

Table 2. Maternal education and gestational weight gain in early, mid and late pregnancy

	Gestational weight gain[a]		
	Early pregnancy (kg/week) (n=5528)	**Mid pregnancy (kg/week) (n=6183)**	**Late pregnancy (kg/week) (n=3452)**
Educational level			
High	Ref	Ref	Ref
Mid-high	0.02 (0.01,0.03)*	0.00 (-0.01,0.01)	-0.01 (-0.03,0.01)
Mid-low	0.06 (0.05,0.07)***	-0.01 (-0.02,0.01)	0.02 (-0.02,0.06)
Low	0.09 (0.06,0.11)***	-0.01 (-0.04,0.01)	-0.02 (-0.06,0.03)
P for trend	<0.001	0.21	0.92

[a] Values are linear regression coefficients (95% confidence interval) derived from multivariable linear regression. The effect estimates represent the difference in GWG in early, mid and late pregnancy, compared to reference group (high education). Models are adjusted for confounders (age, parity and ethnicity). Estimates are based on multiple imputed data.
* P<0.05 **P<0.01***P<0.001

Discussion

This study shows that low maternal educational level is associated with excessive GWG among women with a normal pre-pregnancy BMI, but not among women with pre-pregnancy overweight or obesity. High educated overweight and obese women were at a higher risk of excessive GWG than low educated normal weight women. Furthermore,

low maternal education was also associated with inadequate GWG. We observed that women in the lowest educational group had a higher GWG in early pregnancy, but not in later pregnancy.

Methodological considerations

The strengths of this study are the population-based design and multiple maternal weight measurements throughout pregnancy. In addition, we had a large sample size and were able to adjusted for potentially important confounders. We did encounter however, also some limitations which need to be taken into account when interpreting the results.

In contrast to several other studies, we included both nulliparous and multiparous women.[7, 8] Although we adjusted for parity, it is still possible that there is residual confounding due to the fact that additional factors may influence BMI before a subsequent pregnancy, such as weight retention and length of inter-birth interval, which may not be fully captured by adjusting for parity. Therefore we repeated our analyses in a subgroup of our study population and included only nulliparous women. The results of these sub-analyses were very similar as compared to the results of the total study population (data not shown). Information on pre-pregnancy weight and maximum GWG was self-reported. Self-reported weight tends to be underestimated, especially among women with higher weights, and this might have resulted in some misclassification. However, self-reported pregnancy weight and objectively measured weight at intake (r=0.85) and self-reported maximum weight and weight measured at 30 weeks of gestation (r=0.80) were strongly correlated. Additionally, we performed a sensitivity analyses to assess the association of maternal education level and GWG measured at 30 weeks. Results were very similar as compared to the results for self-reported maximum weight (data not shown). We had information on GWG according to the IOM criteria only in a subgroup of our total study population (46.7%). Non-response analyses, comparing women with and without information on GWG according to the IOM criteria, showed that there was some selection towards a relatively high educated, Dutch and more healthy study population (Supplemental Table S1). This might have led to an underestimation of the association between educational level and GWG according to the IOM criteria.

Education and gestational weight gain

Excessive GWG may have adverse consequences for maternal and fetal outcomes.[22] Pregnancy might be a potential key stage in the development of maternal overweight and obesity [4] and may pose the offspring at an increased risk of developing overweight and obesity in childhood.[3, 23, 24] Consequently, socioeconomic inequalities in excessive GWG may contribute to socioeconomic inequalities in overweight and obesity among

women of reproductive age and their offspring. However, only few studies investigated the relation between SEP and GWG.[7, 8, 10, 25] In line with previous studies, we showed that women with a low SEP had a higher risk of excessive GWG as compared to women with a high SEP.[7, 8, 10] However, one study among 55.608 women did not find an association between SEP and excessive GWG, but between SEP and inadequate GWG only.[25] This might be explained by the fact that the latter study was conducted in the US, with different ethnic groups included such as black women, who are found to be at a higher risk for inadequate GWG than white women.[26] They also used a different SEP indicator; neighbourhood socioeconomic disadvantage. Possibly, pathways by which neighbourhood socioeconomic disadvantage influences GWG may predominantly act on inadequate GWG. For example neighborhood stressors such as crime and violence may mainly influence inadequate GWG, but are less likely to influence excessive GWG.

Preeclampsia, which is shown to affect women with a low SEP more frequently, might have influenced weight gain in pregnancy and might have explained part of the higher risk of excessive GWG among low educated women.[27] However, results were similar after excluding women with preeclampsia (data not shown, available upon request).

In agreement with Holowko et al.[7] we found an inverse association between maternal education and excessive GWG among normal weight women only. Among overweight and obese women, we observed no association between maternal education and excessive GWG. This might be plausible since high education did also not 'protect' these women of becoming overweight and obese before pregnancy, which strongly indicates that they differ from their normal weight counterparts. This might be explained by the fact that some factors overrule the positive effects of education among this particular subgroup of women, such as a stronger genetic predisposition or being more frequently exposed to obesogenic factors as compared to their normal weight counterparts, which may make them more prone to both overweight and obesity and excessive GWG. Another explanation might be that overweight and obese women are allowed to gain considerable less weight than normal weight women according to the guidelines, which makes it much easier in this subgroup to exceed the allowed amount of weight gain independent of educational level. We found that high educated women who were overweight/obese had a higher risk of excessive GWG than low educated women with a normal weight. Thus, pre-pregnancy overweight/obesity appeared to be a very strong determinant of GWG which overruled the positive effects of a high educational level.

We also found that lower-educated women were at an increased risk of inadequate GWG and also had a slightly higher prevalence of underweight before pregnancy. This is in line with a study conducted in the U.S. which found that women with low educational

levels had a higher risk of inadequate GWG.[11, 28] A Japanese study, however, did not find an association between SEP and inadequate GWG. [9] These conflicting results might be explained by the fact that the studies were conducted in various countries where they have different knowledge and beliefs about pregnancy and different health care systems.

It is unclear why low educated women have a higher risk of inadequate GWG. Possibly, they adapt more 'poorly' to their pregnancy due to less knowledge about a healthy pregnancy or more limited access to obstetric care. Another possibility might be that high stress levels, more frequently described by women with a low SEP, negatively influence GWG.[29] Inadequate GWG has been associated with increased risk of children born small for gestational age and recently also with decreased neonatal fat mass, lean mass and head circumference.[1, 30] Adverse fetal outcomes have been found to be more frequent among women with a low SEP, and inadequate GWG might be one of the underlying mechanisms linking low SEP to adverse fetal outcomes such as small for gestational age birth.[14, 31] However, conclusions need to be drawn carefully as reversed causality cannot be excluded, i.e. fetal growth restriction might also be the cause of the lower weight gain among low educated women. Replication of our findings are necessary to confirm our results.

To the best of our knowledge, this is the first study showing that socioeconomic inequalities in excessive GWG may arise in early pregnancy. In early pregnancy GWG includes mainly maternal fat deposition, whereas weight gain in mid- and late pregnancy mainly reflects maternal and amniotic fluid expansion, and growth of the fetus, placenta and uterus.[6] Previous research showed that specifically early GWG (< 20 weeks) was a strong predictor for postpartum weight retention and childhood obesity.[15, 16] Another study found that greater GWG in early pregnancy, but not in later pregnancy, was independently associated with an increased risk of gestational hypertension.[32] These finding have important implications for prevention and intervention strategies, because they suggest that strategies should be aimed primarily before pregnancy and focus especially on women from low socioeconomic subgroups and perhaps even more important on overweight/obese women in all socioeconomic subgroups. To prevent inadequate and excessive GWG more research is necessary to develop effective evidence-based prevention strategies, because to date only small effects of interventions were found in poor quality studies.[33]

Conclusion

Our study shows that low educated normal weight women are at an increased risk of excessive weight gain and that low educated women are also at an increased risk of

an inadequate weight gain during pregnancy. Given the potential short and long term adverse health consequences associated with inadequate and excessive GWG, future research is necessary to investigate the exact underlying pathways in order to develop effective preventive strategies. Special attention should be drawn to women from low socioeconomic subgroups, as well as on overweight and obese women from all socio-economic subgroups.

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Supplemental Material

Supplemental Table S1. Non response analyses comparing women with and without information on GWG according to the IOM criteria (n=6979)

	Information on GWG according to IOM criteria		
Characteristics	**No N=3719**	**Yes N=3260**	**P-value[a]**
Maternal education			
High	17.1	32.9	<0.001
Mid-high	16.1	24.1	
Mid-low	32.7	28.0	
Low	34.1	15.0	
Age (years)	29.0 (5.3)	31.0 (4.7)	<0.001
Parity (% nulliparous)	55.1	60.1	<0.001
Ethnicity (%)			
Dutch	54.4	78.9	<0.001
Cape Verdean	6.9	2.6	
Dutch Antilles	5.1	2.5	
Moroccan	9.9	3.8	
Turkish	13.1	6.9	
Surinamese Creole	4.8	2.6	
Surinamese Hindustani	5.9	2.7	
BMI before pregnancy (kg/m^2)	23.8 (4.1)	23.2 (3.9)	<0.001
BMI in categories (%)			
Underweight	4.9	3.9	<0.001
Normal weight	63.7	73.1	
Overweight	21.0	16.9	
Obese	10.5	6.0	

PART II

Inequalities in growth and cardiovascular health in childhood

6

Head circumference of infants born to mothers with different educational levels

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Abstract

Objective: Head circumference (HC) reflect growth and development of the brain in early childhood. It is unknown whether socioeconomic differences in HC are present in early childhood. Therefore, we investigated the association between socioeconomic position (SEP) and HC in early childhood, and potential underlying factors.

Methods: The study focused on Dutch children born between April 2002 and January 2006 who participated in The Generation R Study, a population-based prospective cohort study in Rotterdam, the Netherlands. Maternal educational level was used as indicator of SEP. HC measures were concentrated around 1, 3, 6 and 11 months. Associations and explanatory factors were investigated using linear regression analysis, adjusted for potential mediators.

Results: The study included 3383 children. At 1, 3 and 6 months of age, children of mothers with a low education had a smaller HC than those with a high education (difference at 1 month: -0.42 SD; 95% CI: -0.54,-0.30; at 3 months: -0.27 SD; 95% CI -0.40,-0.15; and at 6 months: -0.13 SD; 95% CI -0.24,-0.02). Child's length and weight could only partially explain the smaller HC at 1 and 3 months of age. At 6 months, birth weight, gestational age and parental height explained the HC differences. At 11 months, no HC differences were found.

Conclusion: Educational inequalities in HC in the first 6 months of life can be mainly explained by pregnancy-related factors, such as birth weight and gestational age. These findings further support public health policies to prevent negative birth outcomes in lower socioeconomic groups.

Introduction

Growth in childhood is an important indicator of children's health.[1, 2] Children of parents with low socioeconomic positions (SEP) are found to be smaller, both pre- and postnatal, than children of high SEP parents.[2-5] Nutrition, genetic and environmental factors, e.g. maternal smoking, birth weight and maternal height are important mediating factors that may explain these inequalities.[4, 6] There is, however, a need to further improve the understanding of the mechanisms through which SEP affects growth in childhood.

Several studies recognized head circumference (HC) as an important reflection of growth and development of the brain, especially in early childhood.[7, 8] Smaller HC may be associated with a lower intelligence quotient (IQ) and learning problems.[8, 9] This association was even found for HC values immediately under the mean.[8] Lower IQ is related to higher mortality, and SEP has been suggested to be a mediator of this IQ-related mortality.[10] HC is also a sensitive anthropometric indicator of prolonged malnutrition during infancy, so clinicians use HC as a measure of failure to thrive.[11, 12]

The effect of SEP on growth of HC has been previously described.[4, 13, 14] A British study found a higher SEP to be associated with greater HC growth in children from 9 months to 9 years.[14] A study from Pakistan also found HC to vary directly with SEP in infancy.[13] Maternal educational level is one of the most frequently used indicators of SEP and has been shown to be a consistent socioeconomic predictor of health.[15-17] Furthermore, educational level has been shown to be a good predictor of pregnancy outcomes.[18] Therefore, we hypothesized that a low maternal educational level, as indicator of SEP, is associated with smaller HC in childhood. The underlying pathways through which SEP affects HC are not well considered in studies including a broad range of explanatory variables. Thus, the aim of this study was to investigate the association between maternal educational level and HC from birth up to the first year of life. Our second aim was to investigate the possible explanatory mechanisms underlying this association using multivariate regression models.

Methods

Study design and population

This study was embedded within the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood that has previously been described in detail.[19, 20] Enrollment was aimed in early pregnancy (gestational age <18.0 weeks) at the routine fetal ultrasound examination but was allowed until birth of the child. All

children were born between April 2002 and January 2006 and lived in the study area of Rotterdam, The Netherlands (participation rate 61%).[20] The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam. Written consent was obtained from all participating parents.

Consent of postnatal follow-up was available for 7893 children. We restricted our analyses to the subgroup of children of Dutch ethnicity, because SEP may interact with ethnicity regarding their effects on growth and health.[21-23] We excluded twins (n=114), and the second or third child (n=409) of the same mother, since data were correlated.

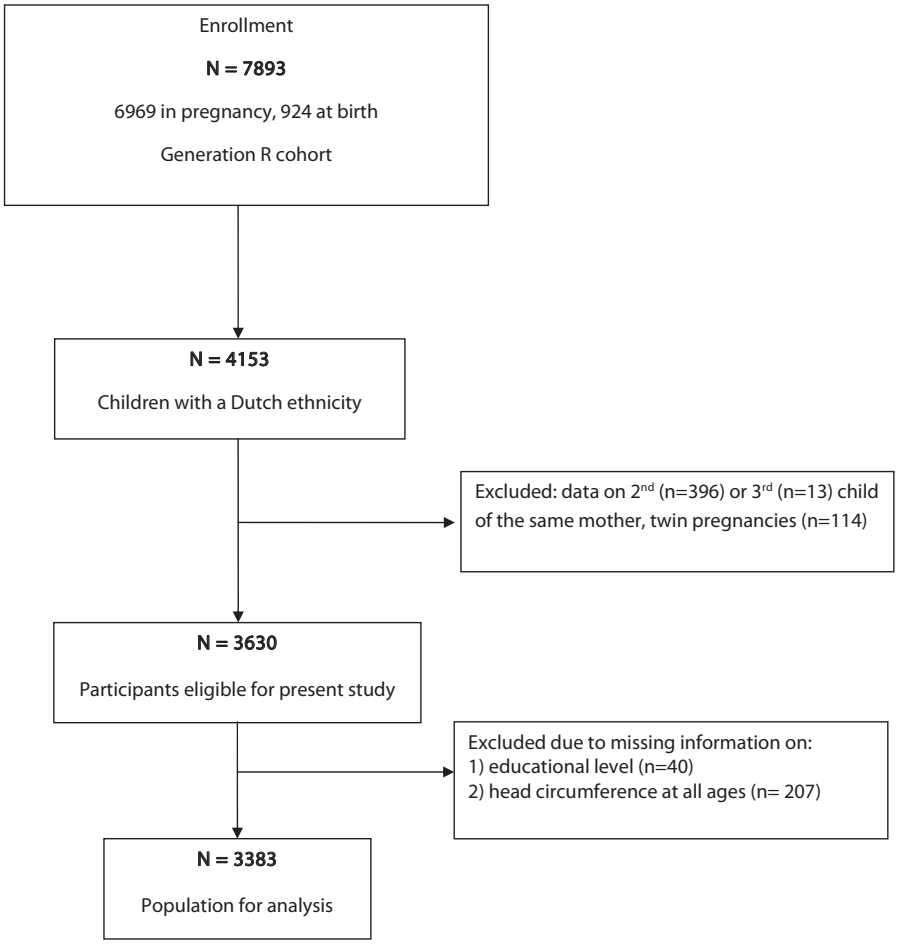

Figure 1 Flowchart of the study population

We also excluded participants without information on maternal educational level (n=40) and those without HC measurements (n=207), leaving a study population of 3383 children (Figure 1). Women whose children were excluded from our study were older, more frequently nulliparous at enrollment, less inclined to smoke during pregnancy and had lower psychopathology scores as compared to women whose children were included. The excluded children had a shorter gestational age, were smaller in length, less heavy and had smaller HC's at 1 and 3 months of age (Table S1).

Maternal educational level

Level of maternal education was established using questionnaires at enrollment. The Dutch Standard Classification of Education was used to categorize 4 subsequent levels of education: 1. high (university degree), 2. mid-high (higher vocational training, Bachelor's degree), 3. mid-low (>3 years general secondary school, intermediate vocational training) and 4. low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general secondary school).[24]

Measurement of head circumference

HC measurements were taken during routine screenings at 1, 2, 3, 4, 6 and 11 months by well-trained staff. HC was measured to the nearest 0.1 cm by a tape line. Values were expressed as age- and gender-adjusted standard-deviation scores (SDS) using Dutch reference growth curves.[25, 26] The difference of 1 HC SDS in children under the age of 1 reflects about 1 centimeter.[26]

Potential mediators

Any effect of maternal educational level on child's HC is probably an indirect one, acting through more proximal determinants of early growth, so-called mediators.[27, 28] We considered the following factors to be such potential mediators in the pathway between maternal educational level and HC. These were chosen based on previous literature of determinants of child's HC.[5, 18, 29-33]

Pregnancy and birth characteristics

Birth weight and gestational age at birth were obtained from midwife and hospital registries. We used gestational-age adjusted standard deviation scores for birth weight.

Gestational diabetes was diagnosed according to Dutch midwifery and obstetric guidelines using the following criteria: random glucose level >11.0 mmol/L, fasting glucose >7.0 mmol/l or a fasting glucose between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test, in women with no pre-existing diabetes.

Information about smoking and alcohol consumption was assessed in first, second and third trimester. Smoking and alcohol use at enrollment were assessed by a single closed question with three answer options (no, first trimester only, continued in pregnancy). To assess smoking in second and third trimester, mothers were asked whether they smoked in the past 2 months (no, yes) in the second and third questionnaire.

Parental anthropometrics

Maternal and paternal heights were measured at our research centers. Pre-pregnancy weight was established at enrollment through questionnaire. On the basis of height and pre-pregnancy weight (weight/height^2), we calculated pre-pregnancy body mass index (BMI).

Psychosocial and material factors

Using questionnaires in early pregnancy, we established whether the pregnancy was planned (yes/no) and the presence of financial difficulties (yes/no).

Child characteristics

Because HC, length and weight are related to each other, we evaluated the contribution of the children's weight and length at time of HC measurement.[29] Standard-deviation scores (SDS) adjusted for age and gender were constructed for all these growth measurements.[26] Information on breastfeeding (yes/no) was derived from questionnaires at the child's age of 2, 6 and 12 months.

Confounding variables

We treated maternal age at enrollment and parity as potential confounders, since they cannot be considered indisputable mediators.[27] Parity was obtained through a questionnaire at enrollment.

Statistical analyses

Because head circumference measurements were concentrated around the ages of 1, 3, 6 and 11 months, we assessed the association between mother's educational level and child's head circumference at 1 (mid-90% range 0.9-1.4), 3 (mid-90% range 3.0-3.8), 6 (mid-90% range 5.8-6.9) and 11 (mid-90% range 10.4-11.9) months of age using multiple linear regression. Unstandardized regression coefficients, reflecting the difference in HC (in SDS), and 95% confidence intervals (CI) were reported for each educational level compared to the reference category (highest educational level). We started with a model that included the confounders (model 1). Next, this model was additionally adjusted for the potential mediators. For each adjustment, the corresponding percentages of change in HC differences (effect estimates) were calculated by comparing the HC differences of

model 1 with the adjusted ones (100 x ($B_{model\ 1} - B_{model\ 1\ with\ mediator}$) / ($B_{model\ 1}$)) (Table S2).[34] Only those variables that individually produced at least 10% change were added to linear regression models, first separately, then simultaneously (full model). We repeated the analysis including all covariates, and found essentially similar results as compared to the models with only the 10% change covariates included (data not shown). For each covariate, an interaction term with educational level was tested for significance, none of them were significant (data not shown).

Linear mixed models ('PROC MIXED' procedure in SAS) were used to assess the association between maternal educational level and longitudinally measured SD scores of HC in the first year of life. In total, we had 16958 measurements of SD scores of HC. The best fitting model structure was: head circumference (in SDS) = $ß_0$ + ß1 * educational level + $ß_2$ * age + $ß_3$* educational level * age. In this model the interaction term educational level * age was added with a significance of $p < 0.001$. Age reflects the time of HC measurement.

Percentages of missing values in the covariates ranged from 0% to 36.2% (Table 1). Because the missing values were not completely at random, the multiple imputation procedure in SPSS 17.0 was used.[35] No differences in results were observed between analyses with imputed missing data or complete cases only. Statistical analyses were performed using Statistical Package of Social Science (SPSS) version 17.0 for Windows (SPSS Inc, Chicago, IL, USA) and Statistical Analysis Software (SAS) version 9.2 for Windows (SAS Institute, Cary, NC, USA). A p-value of <0.05 was taken to indicate statistical significance.

Results

Study population characteristics

Of the 3383 children, 33.2% of their mothers had a high educational level and 14.6% had a low educational level. Compared with mothers with a high education, those with a low education were on average younger, shorter, more likely to smoke and less likely to drink alcohol during pregnancy. Fewer of them started and continued breastfeeding, they had higher psychopathology scores, more of them had financial difficulties, more had unplanned pregnancies ($p<0.001$) and more of them suffered from preeclampsia ($p=0.030$). Their children were on average lighter at birth and had a shorter gestational duration, a shorter height at 1 month and a lower weight at 1 month ($p<0.001$) and at 3 months ($p=0.033$) of age (Table 1).

Table 1. General characteristics of the study population (n = 3383)[a]

		Maternal educational level				
	Total n=3383	**High n=1122 (33.2%)**	**Mid-high n=871 (25.7%)**	**Mid-low n=895 (26.4%)**	**Low n=495 (14.6%)**	**P-value[b]**
Pregnancy and birth characteristics[c]						
Maternal age (years)	31.3 (4.6)	33.1 (3.2)	32.1 (3.9)	30.1 (4.8)	28.0 (5.7)	<0.001
Parity (% nullipara)	66.1	63.8	68.3	68.7	63.0	0.072
Infant gender (% girls)	49.8	49.5	50.4	50.6	48.1	0.801
Gestational age at birth (weeks)	39.9 (1.7)	40.1 (1.6)	40.0 (1.6)	39.8 (2.0)	39.7 (1.6)	<0.001
Birth weight (grams)	3482.6 (554.3)	3553.9 (534.5)	3514.9 (537.8)	3433.3 (581.1)	3353.5 (548.0)	<0.001
Gestational diabetes (% yes)	0.8	0.8	0.6	0.7	1.2	0.607
Maternal smoking during pregnancy (%)						
None	75.7	85.7	79.0	71.7	54.5	<0.001
Until confirmed pregnancy	10.0	7.9	11.1	10.8	10.9	
Continued during pregnancy	14.3	6.3	9.9	17.4	34.5	
Maternal alcohol use during pregnancy (%)						
None	32.3	18.6	28.4	42.0	52.5	<0.001
Until confirmed pregnancy	16.8	14.3	18.7	18.8	15.6	
Continued during pregnancy	50.9	67.1	52.9	39.2	31.7	
Parental anthropometrics[c]						
Maternal height (cm)	170.4 (6.5)	171.1 (6.1)	171.0 (6.4)	170.0 (6.6)	168.1 (6.8)	<0.001
Pre-pregnancy BMI mother (kg/m^2)	23.1 (3.8)	22.7 (3.1)	22.7 (3.4)	23.6 (4.4)	23.9 (4.7)	<0.001
Paternal height (cm)	183.9 (7.0)	185.0 (6.7)	184.1 (6.9)	183.6 (7.1)	181.8 (7.3)	<0.001
Psychosocial and material factors[c]						
Financial difficulties (% yes)	14.4	6.1	12.5	18.4	29.3	<0.001
Pregnancy planned (% no)	19.1	11.4	14.8	23.1	36.8	<0.001
Child characteristics[c]						
Length SDS at 1 month of age	-0.15 (1.1)	-0.02 (1.0)	-0.07 (1.0)	-0.21 (1.1)	-0.47 (1.2)	<0.001
Length SDS at 3 months of age	0.05 (1.0)	0.10 (1.0)	0.04 (1.0)	0.06 (1.0)	-0.08 (1.1)	0.058
Length SDS at 6 months of age	-0.02 (0.9)	0.01 (0.9)	-0.05 (0.9)	-0.02 (1.0)	-0.01 (2.8)	0.618
Length SDS at 11 months of age	-0.14 (0.9)	-0.15 (0.9)	-0.17 (0.9)	-0.11 (0.9)	-0.08 (0.9)	0.216
Weight SDS at 1 month of age	0.09 (1.2)	0.21 (1.1)	0.18 (1.3)	0.0 (1.3)	-0.20 (1.2)	<0.001
Weight SDS at 3 months of age	0.25 (1.0)	0.30 (1.0)	0.27 (1.0)	0.22 (1.1)	0.15 (1.0)	0.033
Weight SDS at 6 months of age	0.07 (0.9)	0.07 (0.9)	0.04 (0.9)	0.08 (1.0)	0.10 (0.9)	0.605
Weight SDS at 11 months of age	-0.02 (0.9)	-0.02 (0.8)	-0.04 (0.9)	-0.02 (0.9)	0.0 (1.0)	0.824
Breastfeeding (yes %)	90	96	93.7	84.8	79.6	<0.001

BMI=body mass index, SDS=standard deviation scores.

[a]Values are percentages or means (SD) for the total population and by level of maternal education.

[b]P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.

[c]Data were missing for parity (2.4%), gestational age (0.1%), smoking during pregnancy (14.5%), alcohol use during pregnancy (14.1%), gestational diabetes (3.3%), maternal height (7.9%), pre-pregnancy BMI (20%), paternal height (17.0%), psychopathology (18.2%), financial difficulties (9.9%), pregnancy planned (5.2%),height SDS at 1 (30.9%), 3 (36.2%), 6 (17.0%) and 11(14.4%) months, weight at 1 (18.0%), 3 (25.9%), 6 (6.7%) and 11 (14.2%) months and breastfeeding (5.7%).

Significant differences were observed in mean HC SDS per educational level at 1, 3, 6 and 11 months. Post hoc analyses showed that children from mothers with the highest educational level had significantly larger HCs than children from mothers with mid-low and low educational levels at all ages (Table 2).

Table 2. Head circumference SDS characteristics in the total study population and by level of maternal education (n=3383)

		Maternal educational level				
	Total (n=3383)	**High (n= 1122)**	**Mid-high (n=871)**	**Mid-low (n=895)**	**Low (n=495)**	**P-value[a]**
Mean head circumference SDS (SD)						
1 month	0.19 (0.95)	0.32 (0.90)	0.25 (0.90)	0.13 (0.95)	-0.11 (1.07)	< 0.001
missings (%)	20.2	19.5	18.3	23.8	18.8	
3 months	0.04 (0.88)	0.15 (0.89)	0.05 (0.80)	0.01 (0.92)	-0.15 (0.92)	< 0.001
missings (%)	27.4	26.0	25.4	31.3	27.1	
6 months	-0.07 (0.89)	0 (0.86)	-0.06 (0.85)	-0.13 (0.92)	-0.17 (0.95)	0.001
missings (%)	8.5	7.9	8.6	8.4	9.9	
11 months	-0.07 (0.89)	0 (0.86)	-0.06 (0.85)	-0.10 (0.88)	-0.13 (0.99)	0.024
missings (%)	16.3	14.5	15.8	16.5	20.6	

HC = head circumference, SDS = standard deviation score

[a] Values are calculated with ANOVA for continuous variables and reflect means (SD) per educational level.

Contribution of potential mediators

At 1 and 3 months of age, the differences in HC for the low and/or the mid-low education group were attenuated with more than 10% by individual adjustment for maternal and paternal height, pre-pregnancy BMI, birth weight, gestational age, child's weight and height and breastfeeding (Table S2). Birth weight and gestational age, when added together to model 1, explained about half of the effect of low education. Adjustment for length and weight explained approximately 60% and 30%, respectively. In the full model, children in the lowest educational subgroup still had significantly smaller HCs at these ages (p=0.003 and p=0.012). The association between mid-low education and HC disappeared due to mediation of birth weight and gestational age. At 3 months, adjustment for parental and maternal height had the same effect in the mid-low subgroup (Table 3).

At 6 months, an attenuation of 10% was observed after individual adjustment for maternal and paternal height, pre-pregnancy BMI, smoking during pregnancy, birth weight and gestational age (Table S2). When added to model 1, complete elimination of the association of low and mid-low education was observed after adjustment for birth weight, gestational age and smoking during pregnancy and after adjusting for paternal and maternal height. High pre-pregnancy BMI was positively associated with HC SDS

Table 3. Differences in child's head circumference at 1, 3, 6 and 11 months of age between maternal educational levels[a]

	Maternal educational level			
Models	**High education**	**Mid-high education**	**Mid-low education**	**Low education**
1 month of age (n=2699)				
Model 1[b]	Reference	-0.07 (-0.17,0.02)	-0.17 (-0.27,-0.07)**	-0.42 (-0.54,-0.30)**
Model 1 + paternal and maternal height	Reference	-0.06 (-0.15,0.04)	-0.12 (-0.22,-0.03)*	-0.32 (-0.44,-0.20)**
Model 1 + pre-pregnancy BMI	Reference	-0.08 (-0.17,0.02)	-0.19 (-0.29,-0.09)**	-0.44 (-0.57,-0.32)**
Model 1 + birth weight SDS + gestational age	Reference	-0.04 (-0.11,0.04)	-0.05 (-0.12,0.03)	-0.18 (-0.27,-0.08)**
Model 1 + child's height and weight SDS at 1 month	Reference	-0.06 (-0.14,0.01)	-0.08 (-0.16,-0.001)*	-0.16 (-0.26,-0.07)**
Model 1 + breastfeeding	Reference	-0.07 (-0.16,0.03)	-0.15 (-0.25,-0.05)**	-0.38 (-0.50,-0.26)**
Fully adjusted model[c]	Reference	-0.06 (-0.13,0.01)	-0.06 (-0.13,0.02)	-0.15 (-0.24, -0.05)**
3 months of age (n=2455)				
Model 1[b]	Reference	-0.10 (-0.19,-0.01)*	-0.11 (-0.21,-0.02)*	-0.27 (-0.40,-0.15)**
Model 1 + paternal and maternal height	Reference	-0.08 (-0.17,0.01)	-0.08 (-0.18,0.01)	-0.20 (-0.31,-0.08)**
Model 1 + pre-pregnancy BMI	Reference	-0.10 (-0.19,-0.01)*	-0.13 (-0.22,-0.03)*	-0.29 (-0.41,-0.17)**
Model 1 + birth weight SDS + gestational age	Reference	-0.08 (-0.16,-0.003)*	-0.05 (-0.14,0.03)	-0.13 (-0.23,-0.02)*
Model 1 + child's height and weight SDS at 3 months	Reference	-0.07 (-0.14,0.003)	-0.08 (-0.16,-0.001)*	-0.18 (-0.28,-0.08)**
Model 1 + breastfeeding	Reference	-0.09 (-0.19,-0.003)*	-0.10 (-0.20,-0.003)*	-0.25 (-0.37,-0.13)**
Fully adjusted model[c]	Reference	-0.07 (-0.14,0.01)	-0.06 (-0.14,0.02)	-0.13 (-0.23,-0.03)*
6 months of age (n=3095)				
Model 1[b]	Reference	-0.06 (-0.15,0.02)	-0.11 (-0.19,-0.02)*	-0.13 (-0.24,-0.02)*
Model 1 + paternal and maternal height	Reference	-0.05 (-0.13,0.03)	-0.07 (-0.16,0.01)	-0.04 (-0.15,0.07)
Model 1 + pre-pregnancy BMI	Reference	-0.07 (-0.15,0.02)	-0.12 (-0.21,-0.03)**	-0.15 (-0.25,-0.04)**
Model 1 + birth weight SDS + gestational age + smoking in pregnancy	Reference	-0.04 (-0.12,0.03)	-0.04 (-0.12,0.04)	0.01 (-0.09,0.11)
Fully adjusted model[d]	Reference	-0.04 (-0.12,0.04)	-0.04 (-0.12,0.04)	0.03 (-0.08,0.13)
11 months of age (n=2832)				
Model 1[b]	Reference	-0.06 (-0.15,0.03)	-0.08 (-0.17,0.01)	-0.10 (-0.22,0.01)

* p-value <0.05, ** p-value < 0.01, SDS = standard-deviation score, BMI = body mass index

[a]Values are regression coefficients (95% confidence interval) and reflect the differences in head circumference (in standard deviation scores) in offspring of mothers with mid-high, mid-low and low educational level relative to children of women with high educational level. The values are derived from linear regression analyses performed on the data after multiple imputation of the covariates.

[b]Model 1: adjusted for maternal age and parity

[c]Fully adjusted model: adjusted for parity, maternal age, child's height and weight (in SDS) at measurement of HC, birth weight SDS, gestational age, paternal and maternal height, pre-pregnancy BMI and breastfeeding (yes/no).

[d]Fully adjusted model: adjusted for parity, maternal age, birth weight SDS, gestational age, smoking during pregnancy, paternal and maternal height and pre-pregnancy BMI

at all ages (Table 3 and Table S2). At 11 months of age, there were no differences in HC between the educational subgroups after adjusting for confounders (p>0.05) (Table 3).

Results from our linear mixed models showed that HC differences between the various educational subgroups became smaller with increasing age due to declining HC growth in the higher educational subgroups. Compared with children of mothers with high educational levels, those whose mothers had mid-low and low educational levels showed a relatively faster growth of head circumference (P for educational level * age ≤ 0.001) (Figure 2 and Table S3).

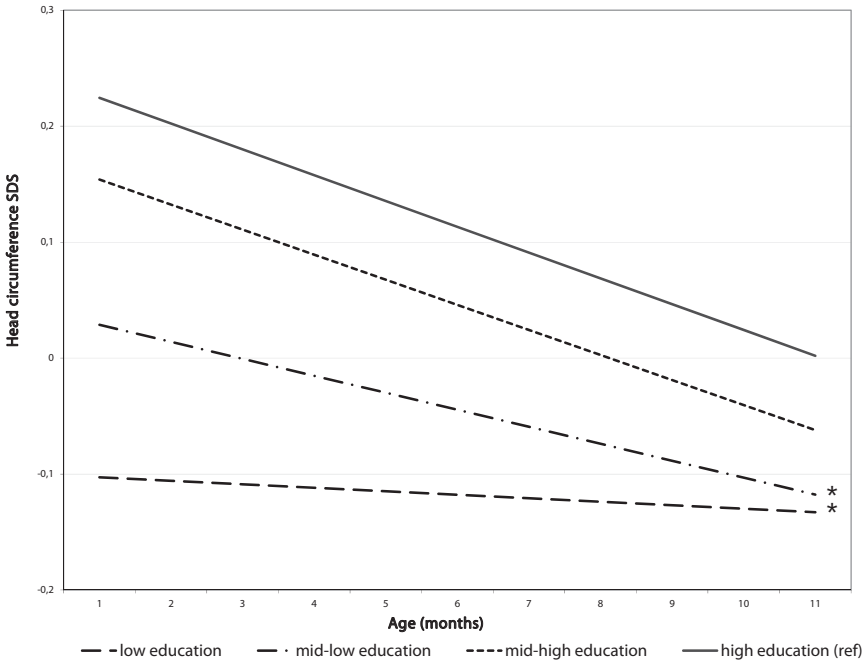

Figure 2 Association between maternal educational level and longitudinally measured head circumference growth

Results are based on repeated linear regression model and reflect the standard deviation scores of head circumference (based on 16958 measurements) growth in the first postnatal year in the offspring of mothers with low, mid-low, mid-high educational levels and high educational level. High education is reference group. Estimates are adjusted for maternal age at enrollment and parity. * P for educational level * age ≤ 0.001.

Discussion

This study found children of mothers with a low and mid-low educational level to have significantly smaller HCs at the age of 1, 3 and 6 months compared to children of mothers with a high educational level. The factors which mainly explained the socioeconomic inequalities in HC were found to be birth weight, gestational age and the child's length and weight.

The HC differences became smaller with increasing age. It has been suggested that infants whose intrauterine growth was constrained tend to grow faster postnatally to compensate.[31, 32, 36] Although we found HC differences to become smaller with age, this did not seem to be due to catch-up growth in the lower socioeconomic subgroup. We found that relative to the high socioeconomic subgroup, the low socioeconomic subgroup showed a continued high relative postnatal growth trajectory which reduced the difference between groups. One possible explanation is that children will grow to their genetic growth potential, which we assume is, on average, similar in all socioeconomic subgroups.[37] In the first months of life growth is largely a continuation of intrauterine growth, independent of genes, which appeared to be better in the higher socioeconomic subgroups.[4, 32, 37] Thus, one would expect HCs of children from higher subgroups to be larger compared to children from lower subgroups in the first months. With increasing age, when genetic factors may become more important, one would expect HC differences to decrease, because children grow to their genetic growth potential and the importance of genes are assumed to be equal for all socioeconomic subgroups.[37] Some studies found adults within lower socioeconomic subgroups to have smaller head sizes.[38] So, while our study showed marked inequalities in HC after birth, which declined during the first year, it is possible that inequalities in HC arise again later in life. For example, Gale et al. showed a relative increase in HC growth in the higher socioeconomic subgroup after the age of one.[14]

In our study, the HC differences could mainly be explained by shorter gestational age and lower birth weight in the lower educational subgroups. At 1 and 3 months of age, the child's weight and length only partially contributed to the HC differences. This suggests that socioeconomic inequalities in HC arise prenatally. It underlines the importance of preventing inequalities of birth outcomes arising in pregnancy, as they influence not only fetal,[4] but also postnatal HC.

The potential mediators included in this study partially explained the educational differences in HC at 1 and 3 months. The remaining effect may be due to other factors, such as environmental and genetic factors.[32, 37] One such factor might be size and shape of the

mother's bony pelvis, since it has been reported that women with flat pelvises tend to have smaller babies with a smaller HC.[39] Flat pelvis is more common in women who have short stature and poor general physique which is more likely to appear among women with low SEP.[33, 39] Parental HC has been found to be a predictor of neonatal HC.[8] However, we did not have data on HC of the parents. This merits further investigation.

Parental height also contributed to HC differences between the different socioeconomic subgroups. The effect of parental height was strongest at 6 months of age. This is in agreement with the study of Smit et al., who found that heritability of head size is very low or absent in infants younger than 3 months and heritability estimates were 90% at 4 to 5 months.[37] Maternal height seemed to be of more importance on growth of HC than paternal height, which is in line with other studies.[32]

Smoking during pregnancy contributed to larger HC differences at 6 months. This is in line with other studies which found impaired postnatal growth of infants prenatally exposed to cigarette smoking.[32] However, it is unclear why we only found an effect of smoking at the age of 6 months. Our findings confirm that reducing smoking rates among pregnant women is very important, since smoking not only impairs fetal HC growth,[4] but also postnatal HC growth. Creating awareness among pregnant women, e.g. through midwifes, that smoking might be associated with smaller brain volume of their children in infancy, might increase their motivation to stop smoking.

Our study also showed that lower educated subgroups have factors that have suppressive effects on a small HC. For instance, high maternal BMI was positively associated with HC. This finding was also observed for neonatal HC and maternal BMI.[40] However, high BMI represents risks to a pregnant woman and her unborn child, and is therefore not recommended.[41, 42]

The differences in HC between the various maternal educational levels could not be explained by psychosocial factors, drinking alcohol during pregnancy, smoking during pregnancy in the first 3 months, or having gestational diabetes. Since these factors are found to be negatively associated with birth weight and gestational age, they might act on HC via indirect pathways.[43, 44]

The study of Silva et al. showed that a lower maternal educational level was associated with slower fetal growth and this effect appeared to be strongest for fetal brain, although this was not significant.[4] Therefore, we additionally explored whether HC was proportional to length per educational subgroup at 1, 3, 6 and 11 months. Regression analysis adjusting for maternal age and parity was used to calculate mean HC SDS minus

mean length SDS. At 11 months, we found that HC was relatively smaller in relation to length in the low education subgroup than in the high education subgroup ($p<0.05$) (Figure S1). However, conclusions must be drawn carefully, since this could also indicate ease of moving weight and long bones rather than brain and skull. Further research is needed.

Methodological considerations

The main strength of this study lies in its population-based prospective design, in which a large number of women were enrolled early in pregnancy, and in the fact that information on relevant potential confounders and mediators was available. Limitation of this type of design is the sensitivity to selection bias, information bias and residual confounding due to unmeasured covariates. The choice whether to consider a factor a confounder or a mediator was based on pre-existing knowledge about social and biological determinants of growth. It is not always a straightforward one, though, and is sometimes arbitrary. Another source of discussion when defining a factor as a mediator, is the causal relationship that is inferred between SEP and that factor. Because actual establishment of causality is only possible with experimental data, one cannot exclude the possibility that the association between SEP and the mediator is not causal.

Although there are other measures of SEP, we used maternal educational level as indicator of SEP. Education is an important determinant of employment and economic circumstances, and thus reflects material resources. It also reflects non-economic social characteristics, such as general and health-related knowledge, which influences health behavior, literacy, problem-solving skills and prestige.[15, 45] Level of education has also been linked to a greater differentiation in health outcomes than other socioeconomic indicators.[46] Although educational level is a useful indicator of SEP, it may not entirely capture the material and financial aspects of SEP. Therefore, we repeated the analyses using household income level as determinant, and we found comparable results. There was one exception: income-related differences in HC were statistically significant at 11 months of age after adjustment for confounders. These HC differences could be explained with the same mediators as found at 6 months of age (data not shown).

To various extents, our results may have been influenced by the following limitations. Information on pre-pregnancy BMI, smoking and alcohol consumption during pregnancy and psychosocial determinants was derived from questionnaires. This may have induced some misclassification. Misclassification of potential mediating risk factors may have contributed to the lack of explanation of the observed association between maternal education and HC. Fetal growth restriction can lead to an underestimation of the effect of SEP on HC, because the gestation may be less certain for disadvantaged

women if they have a higher proportion of unplanned pregnancies. Ultrasound correction of gestational age may then induce a bias if there is fetal growth restriction. In our study, however, there were no differences in the prevalence of children born with fetal growth restriction by educational subgroups (data not shown, available upon request). The response rate among Dutch pregnant women in The Generation R Study was relatively high (68%), but there was some selection towards a relatively highly educated and somewhat healthier study population, which may have led to some underestimation of the estimated effect of low maternal education.[20] Another possible limitation is that we excluded 770 children for several reasons. This could have led to selection bias. Finally, the relative impact of pre- and postnatal factors will depend on environmental conditions which may differ per country. The generalizability of our findings to other populations may therefore be limited.

Conclusion

This study adds to the small body of literature concerning socioeconomic inequalities of HC in infancy. Our results add to the evidence of the negative impact of a low SEP on different aspects of growth in childhood. These findings warrant a public health strategy aimed at tackling these inequalities, a strategy that should already start during the preconception period and should include the prevention of a low birth weight and short gestational age. Furthermore, midwives, obstetricians and pediatricians should be aware of the impact of socioeconomic disadvantage on a child's growth. Finally, our findings and the long-term consequences need to be confirmed in other studies.

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Supplemental Material

Supplemental Table S1 General characteristics of the excluded population (n = 770)[a]

	Total n=770	P-value[b]
Pregnancy and birth characteristics		
Maternal age (years)	32.7 (4.0)	<0.001
Parity (% nullipara)	21.3%	<0.001
Infant gender (% girls)	48.3%	0.456
Gestational age at birth (weeks)	38.8 (2.7)	<0.001
Birth weight (grams)	3196.5 (805.4)	<0.001
Gestational diabetes (% yes)	1.0	0.576
Maternal smoking during pregnancy (%)		
None	82.0	0.007
Maternal alcohol use during pregnancy (%)		
None	33.4	0.626
Parental anthropometrics		
Maternal height (cm)	170.9 (6.8)	0.081
Pre-pregnancy BMI mother (kg/m^2)	23.2 (3.6)	0.884
Paternal height (cm)	184.1 (7.4)	0.930
Psychosocial and material factors		
Financial difficulties (% yes)	14.0	0.352
Pregnancy planned (% no)	18.9	0.898
Child characteristics		
Length SDS at 1 month of age	-0.31 (1.3)	0.006
Length SDS at 3 months of age	-0.15 (1.1)	0.002
Length SDS at 6 months of age	-0.10 (1.0)	0.058
Length SDS at 11 months of age	-0.20 (1.0)	0.189
Weight SDS at 1 month of age	0.03 (1.4)	0.002
Weight SDS at 3 months of age	0.05 (1.2)	0.002
Weight SDS at 6 months of age	-0.07 (1.0)	0.002
Weight SDS at 11 months of age	-0.14 (1.0)	0.011
HC SDS at 1 month of age	0.03 (1.1)	0.002
HC at 3 months of age	-0.10 (0.9)	0.003
HC at 6 months of age	-0.13 (0.9)	0.180
HC at 11 months of age	-0.09 (0.9)	0.431
Breastfeeding (yes %)	88.7	0.430

BMI=body mass index, SDS=standard deviation scores, HC=head circumference.
[a]Values are percentages or means (SD) for the total excluded population.
[b]P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables compared to the variables of the total included population. Values are calculated with non-imputed data.

Supplementary Table S2 Change in head circumference differences (in SDS) for maternal educational level after adjustment for potential mediators

Level of maternal education	High (n=1122)	Mid-high (n=871) B (95% CI)	Change a[a]	Mid-low (n=895) B (95% CI)	Change b[a]	Low (n=495) B (95% CI)	Change c[a]
1 month							
Model 1 (includes maternal age and parity)	Reference	-0.07 (-0.17,0.02)		-0.17 (-0.27,-0.07)		-0.42 (-0.54,-0.30)	
Pregnancy characteristics							
Model 1 + birth weight SDS	Reference	-0.05 (-0.13,0.03)	**-31.1%**	-0.10 (-0.18,-0.01)	**-56.2%**	-0.27 (-0.38,-0.16)	**-35.5%**
Model 1 + gestational age	Reference	-0.05 (-0.13,0.03)	**-27.0%**	-0.11 (-0.19, -0.03)	**-34.9%**	-0.30 (-0.40,-0.19)	**-28.8%**
Model 1 + gestational diabetes	Reference	-0.07 (-0.17,0.02)	-1.4%	-0.17 (-0.27,-0.07)	0%	-0.42 (-0.54,-0.30)	0%
Model 1+ smoking during pregnancy	Reference	-0.07 (-0.16,0.03)	-8.1%	-0.15 (-0.25,-0.06)	-9.5%	-0.38 (-0.50,-0.25)	-9.8%
Model 1 + alcohol consumption during pregnancy	Reference	-0.08 (-0.17,0.02)	+1.4%	-0.17 (-0.27,-0.07)	+2.4%	-0.42 (-0.54,-0.30)	+1.2%
Parental anthropometrics							
Model 1 + maternal height	Reference	-0.07 (-0.16,0.02)	-6.8%	-0.14 (-0.24,-0.04)	**-17.8%**	- 0.35 (-0.47,-0.23)	**-16.1%**
Model 1 + paternal height	Reference	-0.06 (-0.15,0.04)	**-22.9%**	-0.15 (-0.24,-0.05)	**-13.0%**	-0.37 (-0.49,-0.23)	**-10.8%**
Model 1 + pre-pregnancy BMI	Reference	-0.08 (-0.17,0.02)	+5.4%	-0.19 (-0.29,-0.09)	**+11.2%**	-0.44 (-0.57,-0.32)	+6.5%
Psychosocial and material factors							
Model 1 + pregnancy planned	Reference	-0.07 (-0.17,0.02)	-2.7%	-0.17 (-0.26,-0.07)	-2.4%	-0.41 (-0.53,-0.29)	-2.2%
Model 1 + financial difficulties	Reference	-0.07 (-0.16,0.02)	-4.1%	-0.16 (-0.26,-0.07)	-3.6%	-0.41 (-0.53,-0.28)	-2.6%
Child determinants							
Model 1 + child's weight SDS at 1 month	Reference	-0.06 (-0.13,0.01)	**-24.3%**	-0.07 (-0.15,-0.002)	**-56.2%**	-0.16 (-0.25,-0.07)	**-62.8%**
Model 1 + child's height SDS at 1 month	Reference	-0.05 (-0.13,0.03)	**-32.4%**	-0.10 (-0.19,-0.02)	**-39.6%**	-0.22 (-0.32,-0.11)	**-47.5%**
Model 1 + breastfeeding (yes/no)	Reference	-0.07 (-0.16,0.03)	-9.5%	-0.15 (-0.25,-0.05)	**-10.7%**	-0.38 (-0.50,-0.26)	-8.6%

Supplementary Table S2 Change in head circumference differences (in SDS) for maternal educational level after adjustment for potential mediators (continued)

Level of maternal education	High (n=1122)	Mid-high (n=871) B (95% CI)	Change a[a]	Mid-low (n=895) B (95% CI)	Change b[a]	Low (n=495) B (95% CI)	Change c[a]
3 months							
Model 1 (includes maternal age and parity)	Reference	-0.10 (-0.19,-0.01)		-0.11 (-0.21,-0.02)		-0.27 (-0.40,-0.15)	
Pregnancy characteristics							
Model 1 + birth weight SDS	Reference	-0.10 (-0.18,-0.01)	-3.1%	-0.08 (-0.17,0.01)	**-26.5%**	-0.18 (-0.29,-0.07)	**-34.2%**
Model 1 + gestational age	Reference	-0.08 (-0.17,0.002)	**-16.3%**	-0.08 (-0.17,0.01)	**-31.9%**	-0.20 (-0.31,-0.10)	**-25.0%**
Model 1 + gestational diabetes	Reference	-0.10 (-0.19,-0.01)	-1.0%	-0.11 (-0.21,-0.02)	-0.9%	-0.27 (-0.39,-0.16)	+0.7%
Model 1+ smoking during pregnancy	Reference	-0.10 (-0.19,-0.003)	-3.1%	-0.11 (-0.20,-0.01)	-5.3%	-0.26 (-0.38,-0.13)	-5.9%
Model 1 + alcohol consumption during pregnancy	Reference	-0.10 (-0.19,-0.01)	+1.0%	-0.12 (-0.21,-0.02)	+3.5%	-0.28 (-0.40,-0.16)	+1.8%
Parental anthropometrics							
Model 1 + maternal height	Reference	-0.09 (-0.18,-0.003)	-4.1%	-0.09 (-0.18,0.01)	**-21.2%**	-0.22 (-0.34,-0.10)	**-19.1%**
Model 1 + paternal height	Reference	-0.09 (-0.18,0.004)	**-12.2%**	-0.10 (-0.20,-0.01)	**-10.6%**	-0.24 (-0.35,-0.12)	**-13.2%**
Model 1 + pre-pregnancy BMI	Reference	-0.10 (-0.19,-0.01)	+1.0%	-0.13 (-0.22,-0.03)	**+11.5%**	-0.29 (-0.41,-0.17)	+7.7%
Psychosocial and material factors							
Model 1 + pregnancy planned	Reference	-0.10 (-0.19,-0.01)	0%	-0.12 (-0.21,-0.02)	+1.8%	-0.28 (-0.40,-0.16)	+1.5%
Model 1 + financial difficulties	Reference	-0.10 (-0.19, -0.003)	-3.1%	-0.11 (-0.21,-0.01)	-3.5%	-0.26 (-0.38,-0.14)	-3.3%
Child determinants							
Model 1 + child's weight SDS at 3 months	Reference	-0.09 (-0.17,-0.02)	-6.1%	-0.10 (-0.17,-0.02)	**-14.2%**	-0.19 (-0.28,-0.09)	**-31.3%**
Model 1 + child's height SDS at 3 months	Reference	-0.06 (-0.14,0.02)	**-37.8%**	-0.08 (-0.16,0.01)	**-31.0%**	-0.19 (-0.30,-0.08)	**-30.5%**
Model 1 + breastfeeding (yes/no)	Reference	-0.9 (-0.19,-0.003)	-4.1%	-0.10 (-0.20,-0.003)	**-12.4%**	-0.25 (-0.37,-0.13)	8.8%

Supplementary Table S2 Change in head circumference differences (in SDS) for maternal educational level after adjustment for potential mediators (continued)

Level of maternal education	High (n=1122)	Mid-high (n=871) B (95% CI)	Change a[a]	Mid-low (n=895) B (95% CI)	Change b[a]	Low (n=495) B (95% CI)	Change c[a]
6 months							
Model 1 (includes maternal age and parity)	Reference	-0.06 (-0.15,0.02)		-0.11 (-0.19,-0.02)		-0.13 (-0.24,-0.02)	
Pregnancy characteristics							
Model 1 + birth weight SDS	Reference	-0.05 (-0.13,0.03)	**-19.0%**	-0.07 (-0.15,0.02)	**-39.3%**	-0.02 (-0.13,0.08)	**-81.4%**
Model 1 + gestational age	Reference	-0.05 (-0.13,0.03)	**-17.5%**	-0.07 (-0.15,0.01)	**-34.6%**	-0.07 (-0.18,0.03)	**-42.6%**
Model 1 + gestational diabetes	Reference	-0.06 (-0.15,0.02)	0%	-0.11 (-0.19,-0.02)	-0.9%	-0.13 (-0.24,-0.02)	+1.6%
Model 1+ smoking during pregancy	Reference	-0.06 (-0.14,0.02)	-4.8%	-0.10 (-0.18,-0.01)	-7.5%	-0.11 (-0.22,0.002)	**-16.2%**
Model 1 + alcohol consumption during pregnancy	Reference	-0.07 (-0.15,0.02)	+3.2%	-0.11 (-0.20,-0.02)	+1.9%	-0.13 (-0.24,-0.02)	+2.3%
Parental anthropometrics							
Model 1 + maternal height	Reference	-0.06 (-0.17,0.04)	-6.3%	-0.08 (-0.17,0.002)	**-23.4%**	-0.06 (-0.17,0.04)	**-50.4%**
Model 1 + paternal height	Reference	-0.05 (-0.13,0.03)	-19.0%	-0.09 (-0.17,-0.01)	**-15.9%**	-0.09 (-0.20,0.01)	**-27.9%**
Model 1 + pre-pregnancy BMI	Reference	-0.07 (-0.15,0.02)	+3.2%	-0.12 (-0.21,-0.03)	**+11.2%**	-0.15 (-0.25,-0.04)	**+13.2%**
Psychosocial and material factors							
Model 1 + pregnancy planned	Reference	-0.06 (-0.15,0.02)	0%	-0.11 (-0.19,-0.02)	0%	-0.13 (-0.24,-0.02)	-0.8%
Model 1 + financial difficulties	Reference	-0.06 (-0.15,0.02)	0%	-0.11 (-0.19,-0.02)	-0.9%	-0.13 (-0.24,-0.02)	-1.6%
Child determinants							
Model 1 + child's weight SDS at 6 months	Reference	-0.05 (-0.12,0.03)	**-28.6%**	-0.10 (-0.17,-0.03)	-8.4%	-0.12 (-0.21,-0.03)	-8.5%
Model 1 + child's height SDS at 6 months	Reference	-0.04 (-0.12,0.03)	**-30.2%**	-0.10 (-0.18,-0.02)	-6.5%	-0.12 (-0.22,-0.02)	-7.8%
Model 1 + breastfeeding (yes/no)	Reference	-0.06 9-0.15,0.02)	-1.6%	-0.10 (-0.19,-0.02)	-4.7%	-0.12 (-0.23,-0.01)	-6.2%

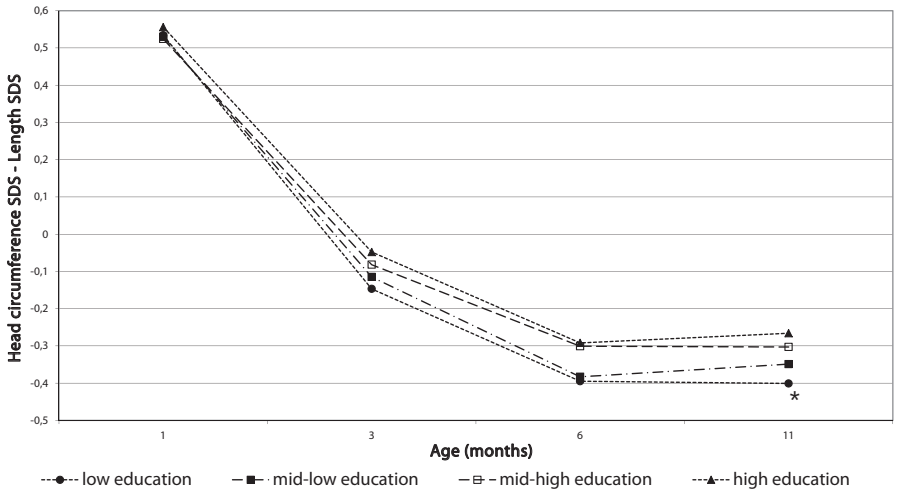
B=effect estimate, CI=confidence interval, BMI=body mass index.

[a] Change a, b and c represent the changes in effect estimates for mid-high, mid-low and low education relative to model 1 (includes confounders) after individual adjustment for potential mediators (100 x ($B_{model\ 1} - B_{model\ 1\ with\ mediator}$) / ($B_{model\ 1}$)). The percentages in bold attenuate with ≥ 10%.

Supplementary Table S3 Longitudinal associations between maternal educational level and child's head circumference[a]

	Difference in growth rate of head circumference			
Maternal educational level	**Intercept**	**P-value[b]**	**Slope (SDS (95% CI))**	**P-value[b]**
High	0.2467	<0.001	Reference	
Mid-high	0.1756	0.069	0.001 (-0.003,0.005)	0.767
Mid-low	0.0433	<0.001	0.008(0.003,0.012)	<0.001
Low	-0.1000	<0.001	0.019(0.014,0.024)	<0.001

[a]Values are based on linear mixed models (based on 16958 measurements) and reflect the difference in growth in standard deviation score (SDS) of head circumference per educational subgroup compared to the high subgroup, which is the reference group.
[b]P-value reflects the significance level of the estimate



Supplementary Figure S1 Mean head circumference SDS minus mean length SDS, stratified by maternal educational level at 1, 3, 6 and 11 months of age
SDS = standard-deviation score
*Mean head circumference SDS - mean length SDS is significant different in the low educational subgroup from that in the subgroup with high education at level p≤0.05. All values are adjusted for maternal age and parity.

7

Development of socioeconomic inequalities in obesity among Dutch pre-school and school-aged children

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Abstract

Objective: To investigate the emergence of the inverse socioeconomic gradient in body mass index (BMI) in the first 6 years of life. Furthermore, associations of socioeconomic position (SEP) with BMI and total fat mass (%) were assessed at age 6, and potential mediating factors in the pathway between SEP and children's body composition were investigated.

Design and Methods: 3656 Dutch children participating in a prospective cohort study in Rotterdam, the Netherlands, were included from 2002-2006. Maternal educational level and net household income were used as indicators of SEP. BMI and fat mass were both outcome measures. Associations and mediation analyses were investigated using linear mixed models and linear regression analyses.

Results: The lowest SEP groups showed a larger increase in BMI over time as compared to the highest SEP groups ($p<0.001$), which resulted in the emergence of the inverse SEP gradient around 3 to 4.5 years of age. In 6-year-old children, both BMI and total fat mass were significantly higher for children of low educated mothers (difference in BMI SDS: 0.24; 95% CI 0.15,0.33; and in total fat mass (%): 2.68; 95% CI 2.19,3.17), which was also shown for children with a low household income. This was mainly explained by parental BMI and prenatal smoking.

Conclusions: The inverse socioeconomic gradient in obesity emerges during the pre-school period, and widens with increasing age. A public health strategy aimed at tackling the development of inequalities in obesity in early childhood needs to start before birth and should include the prevention of prenatal smoking and obesity of parents.

Introduction

Childhood obesity and its associated adverse health effects is a major public health concern.[1] There is consistent evidence showing that school-aged children from low socioeconomic position (SEP) are more likely to be overweight and obese compared with children from high SEP,[2] and these disparities may even be growing.[3] This consistent inverse association is remarkable against the background of research showing that mothers from high SEP give birth to heavier babies as compared to mothers from low SEP.[4, 5] These findings suggest that the inverse association between SEP and child weight/BMI emerges in the period after birth and before school-age, that is during the preschool period. Indeed, two studies conducted in the United Kingdom and Germany, suggest that the inverse SEP gradient became manifest between 2 and 6 years of age.[6, 7] In a previous study, we found that 2-3 year-old children from lower educated mothers had lower body mass index (BMI) z-scores and were at decreased risk for childhood overweight compared with children from high educated mothers.[8] To improve our understanding of the exact onset of the inverse socioeconomic gradient in childhood overweight and obesity, the current study extends the follow up period until the school period.

Moreover, SEP probably not directly affects the risk of childhood obesity, but is likely to act through more proximal risk factors, or mediators.[9, 10] Mediating factors contributing to social inequalities in obesity in children are still largely unknown. Several factors, such as prenatal smoking, paternal BMI, birth weight, physical activity and television viewing, vary by SEP and are associated with childhood obesity.[9, 11, 12] These factors might mediate the relationship between SEP and childhood obesity.

The aims of this study were threefold. First, we aimed to investigate the association between SEP and BMI from early childhood (1 month) to the school period (6 years). Since associations between SEP and BMI vary by indicator of SEP,[2] maternal education and family income are used as indicators of SEP. Second, we aimed to assess the association between SEP and children's body composition (i.e. BMI and total fat mass) at the age of 6 years. We included body fat mass as additional measure of children's body composition since BMI may underestimate the educational gradient of childhood adiposity.[13] Third, we conducted mediation analysis to identify factors in the causal pathway from SEP to children's body composition at the age of 6 years, and we investigated to what extent these mediators explained the association between SEP and body composition.

Methods and procedures

Study design and population

This study was embedded within the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood that has previously been described in detail.[14] All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently being followed-up until young adulthood. The study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam. Written consent was obtained from all participating parents.[15]

We restricted our analyses to the subgroup of children with a native Dutch mother, because the association between SEP and overweight may differ between ethnic subpopulations.[16] Consent for postnatal follow-up during the preschool period (0-4 years) or the school period (6 years) was available for 4331 children with a native Dutch mother.[14] Twins (n=140) were excluded from analyses to avoid clustering and because they more often have impaired fetal and postnatal growth patterns. Also, to avoid clustering, data on the second and third pregnancy of any woman participating in The Generation R Study with more than one child (n= 396) were excluded. Also excluded were participants without information on educational level (n= 113), as well as children without any information on BMI in preschool and school period (n=26). In total, 3656 children were eligible for the present study.

Socioeconomic position

Maternal educational level and net household income were used as indicators of SEP. The highest educational level attained by the mother was established by questionnaire at enrollment using the Dutch Standard Classification of Education, categorized in 4 levels: high (university degree), mid-high (higher vocational training, Bachelor's degree), mid-low (>3 years general secondary school, intermediate vocational training) and low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general secondary school).[17] Data on monthly net household income was obtained at enrollment and categorized into 3 groups (< € 1600/month, € 1600 - € 2200/month, > € 2200/month).

Body composition

In the preschool period, height and weight were measured with standardized methods at each visit to the Child Health Centers. Standard visits at the Child Health Centers take place at 1, 2, 3, 4, 6, 11, 14, 18, 24, 36 and 46 months. At the age of 6 years, weight

and height were measured at a well-equipped and dedicated research center in the Erasmus Medical Center - Sophia Children's Hospital.[14] BMI was calculated using the formula: weight (kg) / height $(m)^2$. BMI values were expressed as age- and gender-adjusted standard-deviation scores (SDS) from Dutch reference growth curves, using the Growth Analyzer program.[18, 19] Percentage body fat was measured by DXA scan and calculated as total body fat mass divided by total body mass (lean mass + fat mass + bone mass of total body) × 100. A more detailed description of the measurements is given in the Supplementary Material.

Potential mediators

The following factors were considered to be potential mediators in the pathway between SEP and body composition at the age of 6 years, based on previous literature on early determinants of childhood overweight and obesity.[20-22]

Prenatal factors

Information on pre-pregnancy weight and smoking during pregnancy (no, until confirmed pregnancy and continued during pregnancy) was obtained by questionnaires. Maternal height was measured during visits at our research center. On the basis of height and pre-pregnancy weight, we calculated mother's pre-pregnancy body mass index (BMI) (weight/$height^2$). Father's BMI was calculated from measured height and weight at enrollment.

Perinatal factors

Birth weight and gestational age at birth were obtained from midwife and hospital registries.

Postnatal factors

Information on breastfeeding (ever/never) was obtained by questionnaires at 2, 6 and 12 months. Change in BMI SDS between 1 and 6 months after birth was calculated as: BMI SDS at 6 months after birth – BMI SDS at 1 month after birth.

Lifestyle factors

Information on television viewing time (< 2 hours/day, ≥ 2 hours/day) as indicator of sedentary behavior, playing sports (yes/no) as indicator of physical activity, and having breakfast daily (yes/no) were obtained from questionnaires at the age of 6 years.

Potential confounders

Child's sex and exact age at measurement were treated as confounders.

Statistical analyses

Associations between maternal educational level and all covariates, BMI, and body fat were explored using Chi-square tests and ANOVAs. Linear mixed models ('PROC MIXED' procedure in SAS) were used to assess the association between maternal educational level with longitudinally measured BMI SDS from 1 month to age 6. Models are described in detail in the Supplementary Material.

Linear regression models were used to assess the associations of maternal educational level with BMI-for-age adjusted SDS and total fat mass (%) at 6 years of age (90% range: 5.7-6.8 years) (model 1). To evaluate the mediating effects of all potential mediators Baron and Kenny's causal step approach was used.[23] Only those factors that were significantly associated with the outcome (independent of maternal educational level; Supplemental Table S1) and unequally distributed across SEP groups (Table 1) were added to model 1. The order in which selected mediators were added to the previous model was based on a hierarchical approach, accounting for the hierarchical relationships between these factors (Supplemental Figure S1), starting with the most distal mediators.[24] To assess their explanatory effects, the corresponding percentages of attenuation of effect estimates were calculated by comparing differences between model 1 and the models including the mediators ($100 \times (B_{\text{model 1}} - B_{\text{model with mediators}}) / (B_{\text{model 1}})$). Finally, a full model containing maternal educational level and all mediators assessed the joint effects of the mediators. In this way, the total effect of maternal educational level on the outcome is defined as the effect of maternal educational level on the outcome that is explained by the mediators (indirect effect) and the effect of the exposure unexplained by those mediators (direct effect).[10] All analyses were repeated with household income as indicator of SEP. Interaction terms between maternal educational level and child's sex were not significant; therefore analyses for BMI SDS and total fat mass (%) were not stratified for sex.

Multiple imputation was used to deal with missing values in the covariates. Five imputed datasets were created and analysed together.[25] A 95% confidence interval (CI) was calculated around the mediating effects using a bootstrap method with 1000 resamplings per imputed dataset in the statistical program R.[26] The remaining statistical analyses were performed using Statistical Package of Social Science (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, IL, USA) and Statistical Analysis Software (SAS) version 9.3 for Windows (SAS Institute, Cary, NC, USA).

Table 1. Characteristics of the study population (n=3656)

		Maternal educational level				
	Total n=3656	**High n=1187**	**Mid-high n=937**	**Mid-low n=968**	**Low n=564**	**P-value[a]**
Net household income (%)						
<1600 euro	11.9	2.0	8.7	15.3	38.3	<0.001
1600-2200 euro	14.2	5.1	14.5	21.3	23.9	
>2200 euro	73.9	92.9	76.8	63.4	37.8	
Prenatal factors						
Mother's pre-pregnancy BMI (kg/m^2)	23.2 (4.0)	22.6 (2.9)	22.7 (3.4)	23.7 (4.4)	24.4 (5.2)	<0.001
Father's BMI (kg/m^2)	25.2 (3.3)	24.8 (3.0)	24.8 (3.0)	25.5 (3.5)	26.0 (4.1)	<0.001
Maternal smoking during pregnancy (%)						
None	74.4	85.8	80.0	69.5	50.4	<0.001
Until confirmed pregnancy	9.5	8.7	9.8	10.5	8.9	
Continued during pregnancy	16.1	5.5	10.2	20.0	40.8	
Perinatal factors						
Gestational age (weeks)	40.1 (37.0-42.1)	40.3 (37.1-42.1)	40.3 (37.1-42.1)	40.1 (37.0-42.0)	39.9 (36.0-41.9)	<0.001
Birth weight (grams)	3479 (558)	3541 (538)	3510 (572)	3443 (572)	3354 (582)	<0.001
Postnatal factors						
Breastfeeding (% yes)	90.0	95.8	94.3	84.1	76.1	<0.001
Change in BMI SDS between 1-6 months	-0.32 (1.0)	-0.38 (1.0)	-0.34 (1.0)	-0.26 (1.1)	-0.22 (1.0)	0.077
Life style factors at 6 years of age						
Watching television (% ≥2 hours/day)	11.0	5.3	8.8	14.7	24.1	<0.001
Playing sports (% yes)	49.1	57.4	46.8	45.0	39.3	<0.001
Having breakfast daily (% yes)	97.1	98.2	97.4	96.8	93.9	<0.001
Childhood characteristics						
Sex (% girls)	49.6	49.0	49.9	50.7	48.0	0.746
Childhood characteristics at 6 years						
Height (cm)	119.5 (5.7)	119.6 (5.3)	119.0 (5.5)	119.5 (5.8)	120.0 (6.9)	0.030
BMI (kg/m^2)	15.9 (1.5)	15.8 (1.3)	15.8 (1.3)	16.0 (1.6)	16.4 (2.0)	<0.001
Total fat mass (%)	23.9 (4.8)	23.1 (4.2)	23.4 (4.4)	24.6 (5.0)	25.8 (5.7)	<0.001

Values are percentages, means (SD) or medians (90% range) for the total population and by level of maternal education.

[a]P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.

[b]Data was missing for mother's pre-pregnancy BMI (21.1%), father's BMI (19.0%), smoking during pregnancy (6.1%), gestational age (0.1%), birth weight (0.1%), breastfeeding (14.1%), change in BMI SDS between 1 and 6 months (46.5%), watching television (19.7%), playing sports (15.3%), having breakfast daily (15.2%) and height (17.7%).

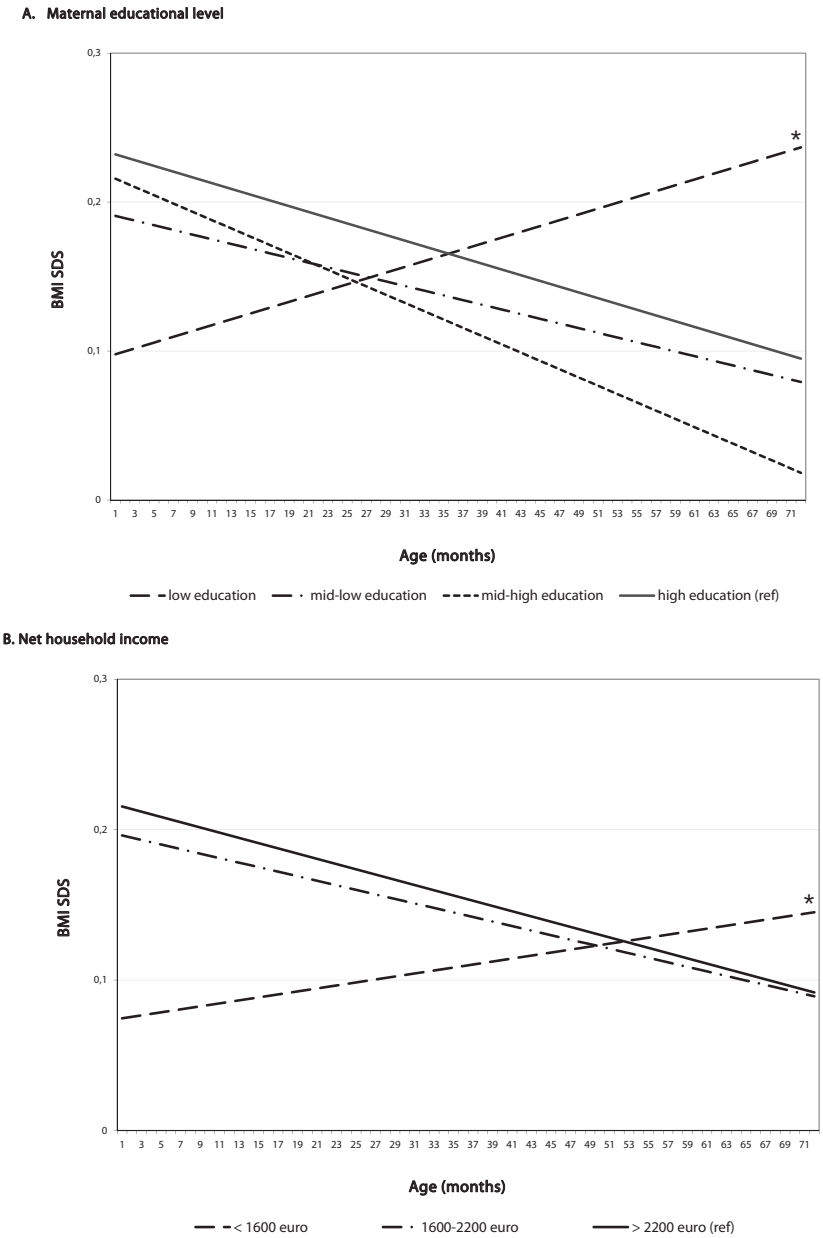

Figure 1. Association between maternal educational level and net household income and longitudinally measured BMI (in SDS)

Results are based on linear mixed models and reflect the standard deviation scores of BMI (based on 28027 measurements for educational level and 23459 measurements for net household income) in the first 6 years of life in A. Children from mothers with low, mid-low, mid-high and high educational level and in B. Children from families with a household income of < 1600 euro, 1600-2200 euro and > 2200 euro. High educational level and a household income of >2200 euro are the reference groups. *P value for educational level*age and for household income*age is <0.0001.

Results

Table 1 shows maternal and child characteristics of the study population. Of the 3656 children, 32.5% of their mothers had a high educational level and 15.4% had a low educational level. Low educated mothers were heavier, had heavier partners and more frequently smoked during pregnancy than high educated mothers ($p<0.001$). Their children had lower birth weights and a shorter gestational duration ($p<0.001$).

Linear mixed models with maternal educational level and BMI SDS patterns (figure 1A) and with household income and BMI SDS patterns (figure 1B) showed that BMI SDS of children from low educated and low income families is lower than high educated and high income families in the first months of life ($p<0.001$). In all the educational and income subgroups there was a decrease of BMI SDS over time, except for the lowest educational and the lowest income subgroup, where a significant increase in BMI SDS was observed ($p<0.001$). As a result, BMI SDS values of the lowest and highest subgroups cross between the ages of 34-38 months for educational level and around 50-54 months for household income. From then on, an inverse socioeconomic gradient in BMI emerges and widens with increasing age. Regression coefficients for age-independent (intercept) and age-dependent differences (interaction educational level/household income * age) are given in Supplemental Table S2.

Multivariable linear regression analyses showed that both BMI SDS and total fat mass at age 6 are higher for children of lower educated mothers than children of high educated mothers (Tables 2 and 3). The correlation between BMI and total fat mass was moderate ($r= 0.58$; $p<0.001$) (Supplemental Figure S1). Of all potential mediators, the following were selected in the association between maternal education and BMI SDS at age 6 based on Baron and Kenny's approach: parental BMI, maternal smoking during pregnancy, birth weight, gestational age and having breakfast daily (Supplemental Table S1). Hierarchical linear models fitted on BMI and total fat mass are shown in Table 2 and Table 3. Parental BMI and smoking during pregnancy contributed most to educational inequalities in BMI (attenuation 54% (95% CI: -98% to -33% in the lowest educational group). All selected mediators together contributed 42% (95% CI: -77% to -21%) to the educational inequalities in BMI SDS (Table 2). For educational inequalities in total fat mass, the following mediators were selected: parental BMI, birth weight, watching television, playing sports and having breakfast daily. Parental BMI and life style factors appeared to contribute most to the educational inequalities in fat mass, and all mediators together explained 25% (95%CI: -36,-17) of these educational inequalities (Table 3). After inclusion of all selected mediators in the model with either BMI and fat mass as outcome, all mediators remained significant (data not shown).

Table 2. Multiple linear regression analyses for associations between maternal educational level and child's BMI SDS at 6 years of age (n=3010)

	Maternal educational level					
Models[b]	**High**	**Mid-high B (95%CI)**	**Mid-low B (95%CI)**	**Attenuation a[a] (95%CI)**	**Low B (95% CI)**	**Attenuation b[a] (95%CI)**
Model 1	Ref	-0.01 (-0.09,0.07)	0.08 (0.004, 0.16)		0.24 (0.15,0.33)	
Model 2	Ref	-0.02 (-0.10,0.05)	0.02 (-0.06,0.09)	-77% (-436,-32)	0.15 (0.05,0.24)	-38% (-69,-23)
Model 3	Ref	-0.03 (-0.10,0.05)	0.003 (-0.07,0.08)	-97% (-511,-41)	0.11 (0.01,0.21)	-54% (-98,-33)
Model 4	Ref	-0.02 (-0.09,0.05)	0.03 (-0.05,0.10)	-65% (-339,-20)	0.15 (0.06,0.24)	-38% (-71,-18)
Fully adjusted model	Ref	-0.02 (-0.09,0.05)	0.03 (-0.05,0.10)	-68% (-354,-22)	0.14 (0.05,0.24)	-42% (-77,-21)

B=effect estimate, CI=confidence interval, BMI=body mass index.

[a]Attenuation a and attenuation b represent the attenuations of effect estimates for mid-low and low education relative to model 1 after additional adjustment for the selected mediators (100 x ($B_{model\ 1} - B_{model\ with\ mediators}$) / ($B_{model\ 1}$)). High maternal educational level is the reference group. No BMI SDS differences were observed for the mid-high educational subgroup as compared to the high educational subgroup, therefore attenuations in effect estimates for mid-high education are not presented.

[b] Model 1: unadjusted

Model 2 : model 1+ mother's pre-pregnancy BMI and father's BMI

Model 3: model 2 + maternal smoking during pregnancy

Model 4: model 3 + birth weight and gestational age

Fully adjusted: model 4 + having breakfast daily

Table 3. Multiple linear regression analyses for associations between maternal educational level and child's total fat mass (%) at 6 years of age (n=2934)

	Maternal educational level						
Models[b]	**High**	**Mid-high B (95%CI)**	**Attenuation a[a] (95%CI)**	**Mid-low B (95%CI)**	**Attenuation b[a] (95%CI)**	**Low B (95%CI)**	**Attenuation c[a] (95%CI)**
Model 1	Ref	0.49 (0.10,0.89)		1.50 (1.11,1.90)		2.68 (2.19,3.17)	
Model 2	Ref	0.43 (0.04,0.82)	-12% (-45,3)	1.24 (0.85,1.63)	-17% (-26,-11)	2.29 (1.80,2.79)	-14% (-22,-9)
Model 3	Ref	0.43 (0.04,0.82)	-13% (-46,3)	1.23 (0.84, 1.62)	-18% (-27,-11)	2.27 (1.77,2.77)	-15% (-23,-9)
Fully adjusted model	Ref	0.35 (-0.04,0.74)	-29% (-93,-10)	1.11 (0.72,1.50)	-26% (-38,-18)	2.00 (1.50,2.50)	-25% (-36,-17)

B=effect estimate, CI=confidence interval, BMI=body mass index.

[a]Attenuation a, b and c represent the attenuations of effect estimates for mid-low and low education relative to model 1 (includes confounders: age, sex and height) after additional adjustment for the selected mediators (100 x ($B_{model\ 1} - B_{model\ with\ mediators}$) / ($B_{model\ 1}$)). High maternal educational level is the reference group.

[b] Model 1: adjusted for confounders age at measurement, sex and height

Model 2 : model 1+ mother's pre-pregnancy BMI and father's BMI

Model 3: model 2 + birth weight

Fully adjusted: model 3 + life style factors at 6 years of age (watching television, playing sports and having breakfast daily)

Supplementary Tables S3 and S4 show the results from the linear regression analyses using household income as SEP indicator. Smoking during pregnancy explained most of the associations of household income with children's BMI , but after adjustment for all selected mediators the association between household income and BMI remained unexplained. For fat mass the selected mediators contributed 34% (95% CI: -67,-13) to the income inequalities, with smoking during pregnancy and life style factors being the most important contributors.

Discussion

This study adds to the small body of literature showing the inverse SEP gradient in body composition to emerge in the preschool period around 3 to 4.5 years of age. At 6 years of age, marked socioeconomic inequalities were observed in both children's BMI and total body fat mass (%). Moreover, the pathway between SEP and body composition depended slightly on SEP indicator and measure of body composition.

Socioeconomic position and body composition

In line with previous research we found less educated mothers to give birth to children with lower birth weights as compared to more educated mothers,[5] which may be caused by more frequent smoking among less educated women.[27] This might also explain the lower BMI until the age of 3 years among low SEP children.[8] However, children from low SEP families had a higher rate of weight gain in the first 6 years of life than children from high SEP families, which resulted in the emergence of the inverse SEP gradient around 3 to 4.5 years of age. This is in line with previous research which also showed the inverse SEP gradient to emerge between 2 and 6 years of age.[6, 7] Also, in line with our study, a previous Dutch study found a higher BMI and fat mass among 5/6 year old children of low educated mothers, as well as among children in low income families. They also found that maternal smoking during pregnancy and maternal pre-pregnancy BMI were the most important contributors to these inequalities.[28] Our findings also agree with results from a recent nationwide survey, investigating the prevalence of overweight and obesity in Dutch children (0-21 years), which showed an inverse association between overweight/obesity and parental educational level.[29] Thus, our study adds to the evidence of the emergence and existence of socioeconomic inequalities in body composition in early childhood in the Netherlands. Furthermore, our results suggest that socioeconomic inequalities in BMI are widening with increasing age, which may result in even wider socioeconomic inequalities in adolescence and adulthood.

For both education and income, we found similar longitudinal BMI patterns across different SEP groups during the first 6 years of life. It has been hypothesized that SEP indicators may operate through different pathways to influence the development of adiposity, with education influencing knowledge and beliefs and income influencing access to resources.[30] Our results support this hypothesis; while BMI was an important explanation in the pathway from maternal educational level to body composition, it was not important in the pathway from household income to body composition. This may be due to a lack of variation in paternal BMI as a function of household income (data not shown). Life style factors appeared to play a more important role in the association between SEP and total fat mass as compared to the association between SEP and BMI. This might be explained by the fact that total fat mass is a more direct and precise measure of adiposity, because it can discriminate between fat mass and lean body mass.[31]

The contribution of parental BMI to childhood adiposity has been found in previous literature as well and may act through the inheritance of genes or through a shared family eating and activity environment.[32, 33] Smoking during pregnancy was one of the most important and consistent contributors in the associations of both SEP indicators with children's BMI and fat mass. This is in line with previous literature showing smoking during pregnancy to be associated with childhood obesity.[20, 34, 35] A major issue regarding maternal smoking and offspring obesity is whether the association is causal; confounding by socio-demographic and environmental factors is a leading alternative. Some studies suggest that smoking may influence childhood obesity through its contribution to lower birth weights, which are followed by periods of rapid weight gain in the first months of life.[20] However, this could not be confirmed in our study, since smoking during pregnancy was not associated with change in BMI between 1 and 6 months (data not shown). Alternative pathways leading to obesity may be an influence of prenatal smoking on neural regulation, which may cause increased appetite and reduced physical activity in the offspring.[36] Other studies suggest that smoking during pregnancy is a proxy for an unhealthy lifestyle in the child's postnatal environment, such as unfavorable dietary behaviors and low physical activity levels.[37] So far, the exact underlying mechanisms are still far from clear and need to be investigated in future research.[34] Our results with respect to the protective effects of healthy lifestyles such as playing sports, television watching < 2hours/day and daily breakfast are supported by previous research.[6]

Methodological considerations

The strengths of this study are the availability of repeated measurements of height and weight in the first 6 years of life which allowed us to investigate BMI development across different socioeconomic groups in a crucial time period, and the measurement of

total body fat mass (%) at age 6. The latter is a more sensitive measure to discriminate between fat mass and lean mass as compared to BMI.

Several limitations should be considered. Although the initial participation rate in The Generation R Study was relatively high (61%), there was some selection towards a relatively higher educated and healthier study population.[38] As a result, socioeconomic inequalities reported in the study may be an underestimation of socioeconomic inequalities in the total population. Information on many covariates in this study was self-reported, which may have resulted in underreporting of adverse lifestyle-related determinants. If underreporting was more severe in the lower socioeconomic groups, this may have biased our results towards an underestimation of the contribution of these factors to the observed socioeconomic inequalities. Unmeasured factors related to both SEP and body composition, such as sleep patterns and consumption of energy-dense foods, could not be taken into account and may explain some of the remaining effects of SEP on body composition.[39, 40] Finally, some assumptions are necessary for our approach of mediation analysis in order to be valid, which include assumptions of causality, absence of mediator-outcome confounding and absence of exposure-mediator interaction.[10] In our study, interactions were tested between exposure and all the mediators and were not statistically significant. Measured confounders have been taken into account in the relation between mediators and outcomes; unmeasured confounders, and particularly unmeasured confounders unrelated to education or income were difficult to imagine, but their existence cannot be excluded.

Conclusion

The inverse association between SEP and childhood BMI emerges during the preschool period, and significant socioeconomic inequalities in body composition are observed in 6-year-old children. A public health strategy aimed at low SEP families should already start during the preconception period and should include the prevention of prenatal smoking and promoting healthy life styles among parents-to-be, such as increasing physical activity. Promoting healthy lifestyles prior to conception will not only lower parental BMI, but will also affect their offspring's BMI, since this may result in creating a healthy eating and activity environment for their future children.

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Supplemental Material

Body composition

At the age of 6 years, participating children and their mothers were invited to a well-equipped and dedicated research center in the Erasmus Medical Center - Sophia Children's Hospital between March 2008 and January 2012. At the research center, weight was measured in lightweight clothes and without shoes using a mechanical personal scale (SECA) and height was measured in children by a Harpenden stadiometer (Holtain Limited) in standing position, which were both calibrated on a regular basis.

The DXA scans provided quantifications of bone and soft tissue measurements for the total body and sub regions, including bone mineral content (g), fat mass (g), and lean mass (g) (iDXA; General Electric, formerly Lunar Corp., Madison, WI). Children were scanned in a supine position with their feet together in a neutral position and hands flat by their sides. All scans were performed by well-trained and certified research staff who repositioned the regions of interest when appropriate. All DXA scans were obtained using the same device and software (enCORE2010).

Unbalanced repeated measurement regression models

The best fitting model structure was: BMI (in SDS) = $ß_0$ + ß1 * educational level + $ß_2$ * age + $ß_3$* educational level * age. In this model, the interaction term educational level * age was added with a significance of $p < 0.001$. In this model, age reflects the time of BMI measurement.

Supplemental Table S1. Associations between potential mediators and BMI (SDS) and fat mass (%) at 6 years of age

	BMI (SDS)		Fat mass (%)	
	B (95% CI)	P-value	B (95% CI)	P-value
	Maternal educational level			
Prenatal factors				
Mother's pre-pregnancy BMI (kg/m^2)	0.04 (0.03,0.04)	<0.001	0.19 (0.15,0.24)	<0.001
Father's BMI (kg/m^2)	0.04 (0.03,0.06)	<0.001	0.19 (0.13,0.25)	<0.001
Maternal smoking during pregnancy (%)				
None	Ref		Ref	
Until confirmed pregnancy	-0.04 (-0.14,0.06)	0.46	0.15 (-0.44,0.74)	0.63
Continued during pregnancy	0.12 (0.03, 0.21)	0.007	0.35 (-0.18,0.89)	0.20
Perinatal factors				
Gestational age (weeks)	0.03 (0.01,0.05)	0.001	0.00 (-0.10,0.10)	0.99
Birth weight (grams)	0.00 (0.00,0.00)	<0.001	0.00 (0.00,0.00)	<0.001
Postnatal factors				
Breastfeeding (0=no, 1=yes)	-0.06 (-0.17,0.06)	0.31	-0.63 (-1.28,0.03)	0.06
Change in BMI SDS between 1-6 months	0.02 (-0.02,0.06)	0.35	0.26 (0.07,0.45)	0.009
Life style factors at 6 years of age				
Watching television (0= <2 hours/day, 1 = ≥2 hours/day)	0.08 (-0.03,0.19)	0.16	0.74 (0.16,1.32)	0.01
Playing sports (0=no, 1=yes)	0.02 (-0.04,0.08)	0.54	-0.37 (-0.72,-0.02)	0.04
Having breakfast daily (0=yes, 1=no)	0.29 (0.11,0.48)	0.002	1.60 (0.41,2.79)	0.009
	Household income			
Prenatal factors				
Mother's pre-pregnancy BMI (kg/m^2)	0.04 (0.03,0.04)	<0.001	0.22 (0.18,0.27)	<0.001
Father's BMI (kg/m^2)	0.05 (0.04,0.06)	<0.001	0.22 (0.16,0.29)	<0.001
Maternal smoking during pregnancy (%)				
None	Ref		Ref	
Until confirmed pregnancy	-0.04 (-0.14,0.07)	0.48	0.21 (-0.43,0.85)	0.52
Continued during pregnancy	0.16 (0.07, 0.25)	<0.001	0.86 (0.31,1.42)	0.002
Perinatal factors				
Gestational age (weeks)	0.03 (0.01,0.04)	0.003	-0.04 (-0.14,0.06)	0.45
Birth weight (grams)	0.00 (0.00,0.00)	<0.001	0.00 (0.00,0.00)	<0.001
Postnatal factors				
Breastfeeding (0=no, 1=yes)	-0.10 (-0.21,0.01)	0.08	-1.12 (-1.75,-0.50)	<0.001
Change in BMI SDS between 1-6 months	0.02 (-0.02,0.06)	0.35	0.28 (0.08,0.49)	0.007
Life style factors at 6 years of age				
Watching television (0= <2 hours/day, 1 = ≥2 hours/day)	0.11 (-0.01,0.22)	0.07	1.10 (0.47,1.72)	0.001
Playing sports (0=no, 1=yes)	0.01 (-0.05,0.07)	0.73	-0.49 (-0.84,-0.13)	0.007
Having breakfast daily (0=yes, 1=no)	0.31 (0.12,0.50)	0.001	1.76 (0.42,3.10)	0.012

B=effect estimate, CI=confidence interval, BMI=body mass index.
Table is based on imputed dataset.
Values are derived from linear regression models and represent effect estimates (95% confidence intervals), adjusted for maternal educational level

Supplemental Table S2. Longitudinal associations between maternal educational level, net household income and child's BMI[a]

	Difference in growth rate of BMI SDS			
Maternal educational level	**Intercept**	**P-value[b]**	**Slope (SDS (95% CI))**	**P-value[b]**
High	0.2339	<0.001	Reference	
Mid-high	-0.0155	0.653	-0.001 (-0.002,0.0001)	0.071
Mid-low	-0.0416	0.233	0.0004 (-0.001,0.001)	0.453
Low	-0.1380	0.001	0.004 (0.003,0.005)	<0.0001
Net household income				
> 2200 euro	0.2171	<0.001	Reference	
1600-2200 euro	-0.0195	0.647	0.0002 (-0.0009,0.0014)	0.685
<1600 euro	-0.1435	0.002	0.003 (0.001,0.004)	<0.0001

[a]Values are based on linear mixed models (based on 28027 measurements for educational level and 23459 measurements for net household income) and reflect the difference in BMI (in SDS) per educational and household income group and compared to the high and > 2200 euro group, which are the reference groups.
[b]P-value reflects the significance level of the estimate

Supplemental Table S3. Multiple linear regression analyses for associations between net household income and child's BMI SDS at 6 years of age (n=3009)

	Net household income			
Models[b]	**>2200 euro**	**1600-2200 euro B (95%CI)**	**<1600 euro B (95%CI)**	**Attenuation a[a] (95%CI)**
Model 1	Ref	0.04 (-0.06,0.13)	0.12 (0.02,0.21)	
Model 2	Ref	0.01 (-0.08,0.11)	0.13 (0.03,0.22)	8% (-27,79)
Model 3	Ref	-0.00 (-0.09,0.09)	0.10 (-0.01,0.20)	-16% (-107,39)
Model 4	Ref	0.00 (-0.08,0.09)	0.14 (0.04,0.23)	18% (-20,136)
Fully adjusted model	Ref	0.00 (-0.08,0.09)	0.12 (0.02,0.21)	2% (-50,98)

B=effect estimate, CI=confidence interval, BMI=body mass index.
[a]Attenuation a represents the attenuations of effect estimates for net household income <1600 euro relative to model 1 after additional adjustment for selected mediators (100 x ($B_{model\ 1} - B_{model\ with\ mediators}$) / ($B_{model\ 1}$)). Net household income >2200 euro is the reference group. No BMI SDS differences were observed for the 1600-2200 euro subgroup as compared to the >2200 euro subgroup, therefore attenuations in effect estimates for the 1600-2200 euro subgroup are not presented.
[b] Model 1: unadjusted
Model 2 : model 1+ mother's pre-pregnancy BMI and father's BMI
Model 3: model 2 + maternal smoking during pregnancy
Model 4: model 3 + birth weight and gestational age
Fully adjusted: model 4 + having breakfast daily

Supplemental Table S4. Multiple linear regression analyses for associations between net household income and child's total fat mass (%) at 6 years of age (n=2928)

	Net household income				
Models[b]	**>2200 euro**	**1600-2200 euro B (95%CI)**	**Attenuation a[a] (95%CI)**	**<1600 euro B (95%CI)**	**Attenuation b[a] (95%CI)**
Model 1	Ref	0.74 (0.27,1.22)		1.36 (0.84, 1.89)	
Model 2	Ref	0.62 (0.16,1.09)	-16% (-50,-2)	1.39 (0.86,1.93)	2% (-10,20)
Model 3	Ref	0.50 (0.04,0.96)	-32% (-92,-15)	1.20 (0.64,1.76)	-12% (-32,5)
Model 4	Ref	0.50 (0.04,0.96)	-33% (-97,-15)	1.18 (0.62,1.74)	-14% (-35,4)
Model 5	Ref	0.50 (0.03,0.96)	-43% (-90,-15)	1.13 (0.57,1.70)	-17% (-40,1)
Fully adjusted model	Ref	0.46 (0.00,0.92)	-38% (-109,-19)	0.90 (0.30,1.50)	-34% (-67,-13)

B=effect estimate, CI=confidence interval, BMI=body mass index.

[a]Attenuation a and b represent the attenuations of effect estimates for net household income 1600-2200 euro and <1600 euro relative to model 1 (includes confounders: age, sex and height) after additional adjustment for selected mediators ($100 \times (B_{\text{model 1}} - B_{\text{model with mediators}}) / (B_{\text{model 1}})$). Net household income >2200 euro is the reference group.

[b] Model 1: adjusted for confounders age at measurement, sex and height

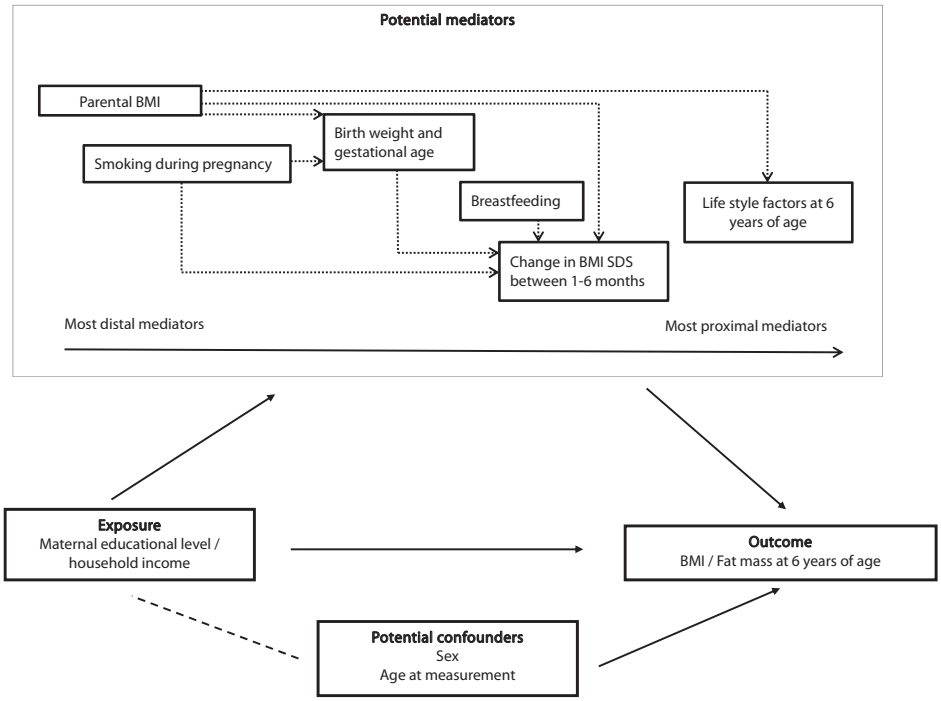
Model 2 : model 1+ mother's pre-pregnancy BMI and father's BMI

Model 3: model 2 + maternal smoking during pregnancy

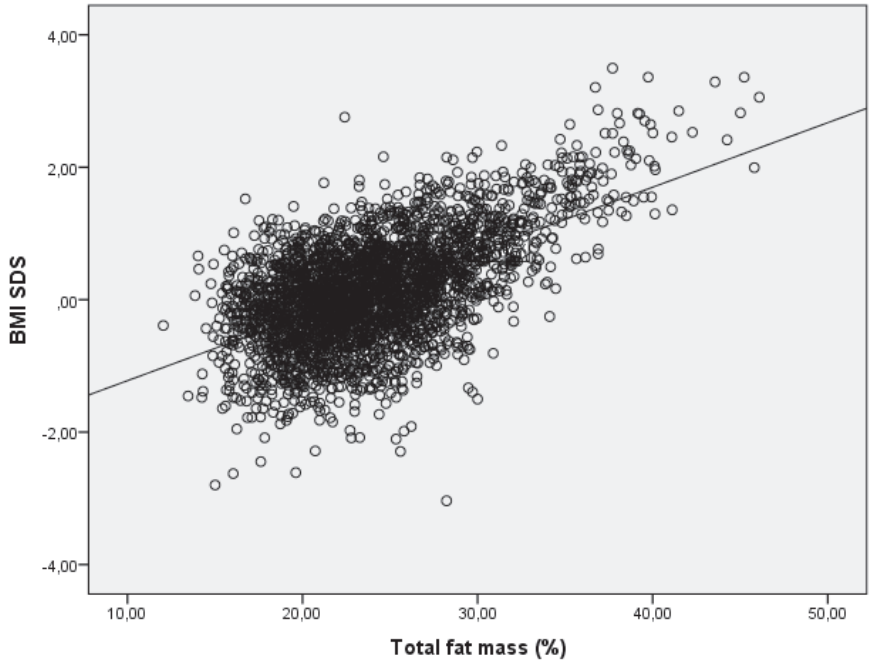
Model 4: model 3 + birth weight

Model 5: model 4 + breastfeeding

Fully adjusted: model 5 + life style factors at 6 years of age (watching television, playing sports and having breakfast daily)



Supplemental Figure S1. Conceptual framework for the association between maternal educational level/ household income and BMI/fat mass at 6 years of age



Supplemental Figure S2. Correlation between BMI SDS and total fat mass (%)

8

Maternal educational level and blood pressure, aortic stiffness, cardiovascular structure and functioning in childhood

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Abstract

Background: In adults, low level of education was shown to be associated with higher blood pressure levels and alterations in cardiac structures and function. It is currently unknown whether socioeconomic inequalities in arterial and cardiac alterations originate in childhood. Therefore, we investigated the association of maternal education with blood pressure levels, arterial stiffness and cardiac structures and function at the age of 6 years, and potential underlying factors.

Methods: The study included 5843 children participating in a prospective cohort study in the Netherlands. Maternal education was assessed at enrollment. Blood pressure, carotid-femoral pulse wave velocity, left atrial diameter, aortic root diameter, left ventricular mass and fractional shortening were measured at the age of 6 years.

Results: Children with low educated (category 1) mothers had higher systolic (2.80 mm Hg, 95% CI: 1.62-2.94) and diastolic (1.80 mm Hg, 95% CI: 1.25-2.35) blood pressure levels as compared to children with high educated (category 4) mothers. The main explanatory factors were the child's BMI, maternal BMI and physical activity. Maternal education was negatively associated with fractional shortening (p for trend 0.008), to which blood pressure and child's BMI contributed the most. No socioeconomic gradient was observed in other arterial and cardiac measurements.

Conclusion: Socioeconomic inequalities in blood pressure are already present in childhood. Higher fractional shortening among children from low socioeconomic families might be a first cardiac adaptation to higher blood pressure and higher BMI. Interventions should be aimed at lowering child BMI and increasing physical activity among children from low socioeconomic families.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in most western parts of the world. Low socioeconomic position (SEP) has been recognized as a marked risk factor in the occurrence of CVD among adults.[1, 2] A better understanding of the origins and underlying mechanisms of socioeconomic inequalities in CVD is essential to develop effective strategies aimed at preventing or reducing these inequalities. Growing evidence suggest that CVD inequalities arise already in childhood, since several CVD risk factors including, low birth weight, short gestational age, physical inactivity, childhood obesity and familial hypertension, affect children from low socioeconomic families more frequently.[3-5] These risk factors may cause inequalities in blood pressure levels in childhood, and may track into adulthood.[6] Indeed, a recent study showed socioeconomic inequalities in blood pressure to appear at the age of 5-6 years old already, mediated by known CVD risk factors including birth weight, childhood BMI and maternal BMI.[7]

Alterations in arterial and cardiac structures such as aortic stiffness, aortic root size, left atrial and left ventricular enlargement, contribute to the risk of CVD among adults.[8-11] In adults, it has been shown previously that a low level of education was associated with left ventricular hypertrophy, left ventricular dilatation and cardiac dysfunction.[12] Factors recognized to influence these structures include elevated blood pressure levels and obesity.[12-15] These factors are more common in children from low SEP families and were also found to cause a higher left ventricular mass in childhood already.[16, 17] However, no previous study examined the socioeconomic gradient in early structural changes of the heart among children. Therefore, to improve the understanding of the origins and underlying mechanisms of CVD inequalities, we investigated in a population-based, prospective cohort study, the association between maternal educational level and blood pressure levels, arterial stiffness and cardiac structures and function at the age of 6 years. Furthermore, we examined whether adiposity measures, lifestyle-related determinants and birth characteristics could explain these associations.

Methods

Study design

This study is embedded within the Generation R Study, a population-based prospective cohort study from early pregnancy onwards described in detail elsewhere.[18] Enrolment was aimed at early pregnancy, but was allowed until the birth of the child. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently being followed-up until young adulthood. The study was con-

ducted in accordance with the guidelines proposed in the World Medical Association of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam. Written consent was obtained from all participating parents.[19]

Study group

In total, 8305 children still participate in the study from the age of 5 years.[18] The participating children and their mothers were invited to a well-equipped and dedicated research center in the Erasmus Medical Center - Sophia Children's Hospital between March 2008 and January 2012. Measurements were focused on several health outcomes including body composition, obesity, heart and vascular development and behaviour and cognition.[18] In total, 6690 children visited the research center. Mothers of children who didn't visit the research center had a lower educational level, more frequently smoked during pregnancy ($p<0.001$) and fewer of them gave breastfeeding ($p=0.001$) as compared to mothers of children who visited the research center. No differences were found in other characteristics between these groups (Supplementary Table S1). We excluded twins (n=167) and participants who lacked information on maternal educational level (n=594) and on cardiovascular measurements (n=50). Furthermore, children with echocardiographic evidence of congenital heart disease or kidney disease were excluded (n=36), leaving a study population of 5843 children (Supplementary Figure S1).

Maternal educational level

Our indicator of SEP was educational level of the mother. Level of maternal education was established using questionnaires at enrollment. The Dutch Standard Classification of Education was used to categorize 4 subsequent levels of education: 1. high (university degree), 2. mid-high (higher vocational training, Bachelor's degree), 3. mid-low (>3 years general secondary school, intermediate vocational training) and 4. low (no education, primary school, lower vocational training, intermediate general school, or 3 years or less general secondary school).[20]

Child cardiovascular structures and function

We measured blood pressure with the child in supine position quietly awake. Systolic and diastolic blood pressure (SBP and DBP) was measured at the right brachial artery, four times with one minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus ™ (Paramus, NJ, USA).[21] A cuff was selected with a cuff width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference. More than 90% of the children who visited the research center had four successful blood pressure measurements available. SBP and DBP were determined by excluding the first measurement and averaging the other measurements.

Carotid-femoral pulse wave velocity, the reference method to assess aortic stiffness [11], was assessed using the automatic Complior device (Complior; Artech Medical, Pantin, France) with participants in supine position. The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured over the surface of the body to the nearest centimeter. Through piezoelectric sensors placed on the skin, the device collected signals to assess the time delay between the pressure upstrokes in the carotid artery and the femoral artery. Carotid-femoral pulse wave velocity was calculated as the ratio of the distance travelled by the pulse wave and the time delay between the feet of the carotid and femoral pressure waveforms, as expressed in meters per second.[22] To cover a complete respiratory cycle, the mean of at least 10 consecutive pressure waveforms was used in the analyses. Recently, it has been shown that pulse wave velocity can be measured reliably, with good reproducibility in a large pediatric population-based cohort.[23]

Two-dimensional M-mode echocardiographic measurements were performed using the ATL-Philips Model HDI 5000 (Seattle, WA, USA) or the Logiq E9 (GE Medical Systems, Wauwatosa, WI, USA) devices. The children were examined in a quiet room with the child awake in supine position. Missing echocardiograms were mainly due to restlessness of the child or unavailability of equipment or sonographer. Left atrial diameter, interventricular end-diastolic septal thickness (IVSTD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-diastolic posterior wall thickness (LVPWTD), interventricular end-systolic septal thickness, left ventricular end-systolic diameter, left ventricular end-systolic posterior wall thickness, aortic root diameter, and fractional shortening were measured using methods recommended by the American Society of Echocardiography.[24] Left ventricular mass (LV mass) was computed using the formula derived by Devereux et al:[25] $LV\ mass = 0.80 \times 1.04((IVSTD + LVEDD + LVPWTD)^3 - (LVEDD)^3) + 0.6$. To assess reproducibility of ultrasound measurements, the intraobserver and interobserver intraclass correlation coefficients were calculated previously for left atrial diameter, aortic root diameter, IVSTD, LVEDD and LVPWTD in 28 subjects (median age 7.5 years, interquartile range 3.0-11.0) and varied between 0.91 to 0.99 and 0.78 to 0.96, respectively.[26]

Explanatory variables

The following factors were considered to be potential explanatory factors in the pathway between SEP and blood pressure, cardiovascular structures and function. These were chosen based on previous literature.[7, 27-30]

Maternal characteristics

Information on pre-pregnancy weight, pre-pregnancy hypertension (yes, no) and smoking during pregnancy (no, until confirmed pregnancy and continued during pregnancy) and financial difficulties, as indicator of family stress, was obtained by questionnaires. Also, another measurement of family stress was assessed with the family assessment device score at age 5. A higher score reflects more 'stress'. Information on pregnancy-induced hypertension and preeclampsia was obtained from medical records. Women suspected of pregnancy hypertensive complications, based on these records, were crosschecked with the original hospital charts. Details of these procedures have been described elsewhere.[31] Maternal height was measured during visits at our research center. On the basis of height and pre-pregnancy weight, we calculated pre-pregnancy body mass index (BMI) (weight/height^2).

Birth characteristics

Birth weight and gestational age at birth were obtained from midwife and hospital registries. Information on breastfeeding during infancy (ever/never) was obtained by questionnaires.

Child characteristics

Weight of the child was measured while wearing lightweight clothes and without shoes by using a mechanical personal scale (SECA), and height was measured by a Harpenden stadiometer (Holtain Limited) in standing position, which were both calibrated on a regular basis. BMI was calculated using the formula; weight (kg) / height (m)^2. Standard-deviation scores (SDS) adjusted for age and gender were constructed for these growth measurements.[32] Watching television (< 2 hours/day, ≥ 2 hours/day), as indicator of sedentary behaviour, and playing sports (yes/no), as indicator of physical activity, were obtained from questionnaires at the age of 5 years.

Confounding variables

Gender and date of birth of the child were obtained from midwife and hospital registries. Ethnicity (Dutch, other western, non-western) was obtained from the first questionnaire at enrollment in the study. These factors were considered as potential confounding factors.

Statistical analyses

First, univariate associations of maternal educational level to all covariates, blood pressure, carotid-femoral pulse wave velocity, cardiac structures and function were explored using Chi-square tests and ANOVAs. Second, we assessed the association of maternal educational level with blood pressure levels, carotid-femoral pulse wave velocity, car-

diac structures and function using linear regression models adjusted for the potential confounders (model 1). To evaluate the mediating effects of all potential explanatory factors Baron and Kenny's causal step approach was used [33]. Only those factors that were significantly associated with the outcome (independent of maternal educational level; data available upon request) and unequally distributed across SEP groups (Table 1) were added separately to model 1.[33] To assess their mediating effects, the corresponding percentages of attenuation of effect estimates were calculated by comparing differences of model 1 with the adjusted ones ($100 \times (B_{\text{model 1}} - B_{\text{model 1 with explanatory factor}}) / (B_{\text{model 1}})$). Finally, a full model containing maternal educational level and all the explanatory factors assessed the joint effects of the explanatory factors. The explanatory mechanisms were investigated only for outcomes (blood pressure, pulse wave velocity, cardiac structures and function) that differed by maternal educational level after adjustment for confounders (model 1). Interaction terms between maternal educational level and child's sex on all the outcomes were not significant ($p>0.1$); therefore analyses were not stratified for sex. Multiple imputation was used to deal with the missing values in the covariates. Five imputed datasets were created and analysed together. A 95% confidence interval (CI) was calculated around the percentage attenuation using a bootstrap method with 1000 re-samplings per imputed dataset in the statistical program R.[34] All the other statistical analyses were performed using Statistical Package of Social Science (SPSS) version 20.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Table 1 shows the characteristics of the study population. Of all participating children 22.4% (n= 1308) had mothers with a low educational level and 25.3% (n=1483) of the mothers were high educated. Compared with mothers with a high education, those with a low education had a higher pre-pregnancy BMI, suffered more frequently from pre-pregnancy hypertension, had more financial difficulties and a higher family assessment device score ($p<0.001$), indicating more stress within their families, fewer of them gave breastfeeding, and they more frequently smoked during pregnancy ($p=0.002$). Their children were on average lighter at birth and had a shorter gestational duration ($p<0.001$). Mothers with a high education were more frequently Dutch, while mothers with a low education had more frequently a non-western ethnic background ($p<0.001$). Children of low educated mothers less frequently played sports, watched more frequently ≥ 2 hours television per day and were heavier as compared with children of high educated mothers ($p<0.001$). Mean systolic (SBP) and diastolic blood pressure (DBP) levels ($p<0.001$) and carotid-femoral pulse wave velocity ($p=0.02$) were negatively

Table 1. Characteristics of the study population (N=5843)

		Maternal educational level				
	Total N=5843	**High n=1483**	**Mid-high n=1246**	**Mid-low n=1806**	**Low n=1308**	**P-value[a]**
Maternal characteristics[b]						
Body mass index before pregnancy (kg/m^2)	23.6 (4.2)	22.6 (3.0)	23.1 (3.6)	23.9 (4.4)	24.7 (5.1)	<0.001
Pre-pregnancy hypertension (% yes)	1.5	0.9	1.4	1.7	2.3	0.002
Pregnancy-induced hypertension (% yes)	4.3	4.4	4.0	5.0	3.7	0.35
Preeclampsia (% yes)	2.0	1.7	1.2	2.3	2.6	0.07
Smoking during pregnancy (%)						
No	75.2	86.7	79.5	72.4	62.3	<0.001
Until confirmed pregnancy	9.0	8.2	9.7	10.2	7.8	
Continued during pregnancy	15.7	5.2	10.8	17.4	29.9	
Financial difficulties (% yes)	16.7	5.9	12.2	22.7	38.0	<0.001
Family assessment device score	1.52 (0.50)	1.42 (0.38)	1.49 (0.41)	1.56 (0.46)	1.66 (0.46)	<0.001
Birth characteristics[b]						
Gestational age at birth (weeks)	40.1 (37.0-42.0)	40.3 (37.1-42.1)	40.2 (37.1-42.1)	40.0 (37.0-42.0)	40.0 (36.4-42.0)	<0.001
Birth weight (grams)	3433 (552)	3533 (530)	3477 (538)	3388 (560)	3344 (560)	<0.001
Breastfeeding during infancy (%)						
Yes	92.6	96.6	94.4	89.6	88.8	<0.001
No	7.4	3.4	5.6	10.4	11.2	
Child characteristics at 6 years[b]						
Watching television (%)						
< 2 hours/day	81.6	92.5	87.1	78.2	62.8	<0.001
≥ 2 hours/day	18.4	7.5	12.9	21.7	37.2	
Playing sports (% yes)	45.7	57.3	47.6	42.3	32.2	<0.001
Height (cm)	119.4 (6.0)	119.3 (5.4)	118.9 (5.5)	119.5 (6.2)	120.0 (6.8)	<0.001
Body mass index (m/kg^2)	16.2 (1.9)	15.8 (1.3)	15.9 (1.5)	16.3 (1.9)	16.9 (2.4)	<0.001
Heart rate (beats/min)	82 (12)	81 (12)	82 (11)	82 (12)	83 (12)	<0.001
Confounding variables						
Male sex (%)	50.1	50.7	51.4	49.8	48.3	0.41
Age (years)	6.2 (0.5)	6.1 (0.4)	6.1 (0.4)	6.2 (0.5)	6.3 (0.6)	<0.001
Ethnicity (%)						
Dutch	58.7	76.4	69.7	52.5	36.6	<0.001
Other western	8.3	11.9	8.9	7.3	5.0	
Non western	33.0	11.7	21.3	40.2	58.4	
Outcomes						
Systolic blood pressure (mmHg)	102.6 (8.1)	101.2 (7.6)	101.9 (7.7)	103.0 (8.2)	104.4 (8.6)	<0.001
Diastolic blood pressure (mmHg)	60.6 (6.7)	59.5 (6.3)	60.2 (6.5)	61.0 (6.8)	61.8 (7.0)	<0.001
Carotid-femoral pulse wave velocity (m/s)	5.5 (0.9)	5.4 (0.9)	5.5 (0.9)	5.5 (0.9)	5.6 (0.9)	0.02
Aortic root diameter (mm)	19.3 (1.8)	19.2 (1.7)	19.2 (1.8)	19.3 (1.8)	19.4 (1.8)	0.12
Left atrial diameter (mm)	25.2 (2.7)	25.1 (2.7)	25.1 (2.7)	25.1 (2.7)	25.5 (2.8)	<0.001
Left ventricular mass (g)	53.4 (11.6)	54.1 (11.5)	52.9 (11.0)	52.9 (11.5)	54.1 (12.4)	0.003
Fractional shortening (%)	35.3 (4.5)	35.1 (4.4)	35.3 (4.3)	35.4 (4.6)	35.3 (4.5)	0.49

Values are percentages, means (SD) or medians (95% range) for the total population and by level of maternal education.

[a]P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables.

[b]Data was missing for pre-pregnancy BMI (19.9%), pre-pregnancy hypertension (14.4%), pregnancy-induced hypertension (11.1%), preeclampsia (13.2%), smoking during pregnancy (7.3%), financial difficulties (35.4%), family assessment device score (14.5%), parity (1.9%), birth weight (0.1%), breastfeeding (19.9%), watching television (20.0%), playing sports (13.4%), height (0.1%) and BMI (0.1%).

associated with level of education. Also, left atrial diameter (p<0.001) and left ventricular mass (p=0.003) differed according to educational level (Table 1).

Multivariable linear regression analyses adjusted for the potential confounders showed that the lower the education of the mother, the higher the systolic and diastolic blood pressure of their children (p for trend <0.001) (Table 2). No consistent associations of maternal education were observed with carotid-femoral pulse wave velocity, aortic root diameter, left atrial diameter, and left ventricular mass. Fractional shortening was negatively associated with maternal educational level (p for trend 0.008). There was no interaction between ethnicity and maternal education on these outcomes (p > 0.05).

Table 2. Associations between net household income, blood pressure and cardiac structures at the age of 6[a]

Maternal education level	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Pulse wave velocity (m/s)	Left atrial diameter (mm)	Aortic root diameter (mm)	Left ventricular mass (g)	Fractional shortening (%)
High	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Mid-high	0.62 (-0.001,1.24)	0.69** (0.18,1.21)	0.03 (-0.05,0.11)	-0.08 (-0.28,0.13)	-0.06 (-0.19,0.07)	-1.05* (-1.86,-0.24)	0.18 (-0.15,0.52)
Mid-low	1.29*** (0.71,1.87)	1.27*** (0.79,1.76)	0.02 (-0.05,0.09)	-0.15 (-0.34,0.03)	-0.06 (-0.18,0.06)	-1.30** (-2.06,-0.54)	0.32* (0.01,0.64)
Low	2.28*** (1.62,2.94)	1.80*** (1.25,2.35)	0.06 (-0.02,0.14)	0.04 (-0.17,0.25)	-0.03 (-0.16,0.11)	-0.50 (-1.36,0.36)	0.45* (0.10,0.80)
P for trend	< 0.001	< 0.001	0.22	0.93	0.64	0.11	0.008

* p-value <0.05, ** p-value < 0.01, *** p-value<0.001

[a]Values represent differences in blood pressure levels, carotid-femoral pulse wave velocity, left cardiac structures and fractional shortening (with 95% confidence interval) relative to children of mothers with high educational level, and are derived from linear regression analyses performed on the data after multiple imputation of the covariates. All models are adjusted for child sex, current age and ethnicity. Models with ultrasound outcomes are additionally adjusted for ultrasound device and performing sonographer.

Maternal pre-pregnancy BMI, maternal pre-pregnancy hypertension, gestational age, and the child's height and BMI were related both to maternal educational level and SBP. The selected explanatory factors for DBP included maternal pre-pregnancy hypertension, birth weight, watching television, playing sports, and the child's height and BMI. Birth weight, child's BMI, SBP and DBP were the selected explanatory factors in the association between maternal education and fractional shortening. For each selected factor, an interaction with maternal education was tested for significance, none of them were significant (p > 0.05).

Table 3. Attenuation of the increase of systolic blood pressure (mm Hg) for the different levels of maternal education after individual adjustment for explanatory factors

	Maternal educational level			
	Mid-low B (95% CI) [a]	Attenuation a [b] (95% CI)	Low B (95% CI) [a]	Attenuation b [b] (95% CI)
Systolic blood pressure				
Model 1 (includes child sex, current age and ethnicity)	1.29 (0.71,1.87)		2.28 (1.62,2.94)	
Maternal characteristics				
Model 1 + pre-pregnancy BMI	1.13 (0.54,1.72)	-11% (-22,-6)	2.00 (1.33,2.67)	-12% (-18,-7)
Model 1 + pre-pregnancy hypertension	1.28 (0.69,1.86)	-2% (-5,0.0)	2.27 (1.60,2.93)	-1% (-4,-0.1)
Birth characteristics				
Model 1 + gestational age	1.24 (0.95,1.54)	-4% (-8,-1)	2.23 (1.89,2.57)	-2% (-5,-1)
Child characteristics				
Model 1 + BMI	1.11 (0.54,1.68)	-15% (-29,-7)	1.67 (1.02,2.32)	-29% (-42,-20)
Model 1 + height	1.43 (0.86,2.00)	+11% (3,27)	2.46 (1.81,3.10)	+8% (3,15)
Fully adjusted model	1.14 (0.57,1.71)	-12% (-26,0.3)	1.74 (1.09,2.39)	-24% (-36,-14)

B=effect estimate, CI=confidence interval, BMI=body mass index.

[a]The effect estimates represent the attenuated effect estimates and their 95% CI's relative to model 1 after individual adjustment for explanatory factors .

[b] Attenuation a and attenuation b represent the attenuations of effect estimates for mid-low and low education relative to model 1 (includes confounders) after individual adjustment for explanatory factors (100 x ($B_{\text{model 1}} - B_{\text{model 1 with explanatory factor}}$) / ($B_{\text{model 1}}$)). High maternal educational level is the reference group. No systolic blood pressure differences were observed for the mid-high educational subgroup as compared to the high educational subgroup, therefore attenuations in effect estimates for mid-high education are not presented.

Regarding the proportion of explanation by each risk factor (Table 3 and Table 4), the child's BMI was the most important contributor to the association of maternal education to SBP in the mid-low and low educational subgroup (15% attenuation (95% CI: -29% to -7%) and 29% (95% CI: -42% to -20%)). Secondly, maternal pre-pregnancy BMI contributed to the association between maternal education and SBP accounting for 11% (95% CI: -22% to -6%) and 12% (95% CI: -18% to -7%) respectively. Overall, 12% (95% CI: -26% to 0.3%) and 24% (95% CI: -36% to -14%) of the association of maternal education and SBP was explained for mid-low and low education by including the explanatory factors (Table 3). For DBP, the child's BMI and playing sports were the most contributing factors in the association between maternal education and DBP, explaining 8% (95% CI: -14% to -3%) and 7% (95% CI: -13% to -3%) in the lowest educational subgroup. The overall explanation was 12% (95% CI: -47% to -3%), 16% (95% CI: -30% to -8%) and 21% (95% CI: -35% to -12%) in the mid-high, mid-low and low educational subgroup respectively (Table 4). For both SBP and DBP the socioeconomic differences remained significant after including all the explanatory factors (Table 3 and Table 4). Complete elimination of

Table 4. Attenuation of the increase of diastolic blood pressure (mm Hg) for the different levels of maternal education after individual adjustment for explanatory factors

	Maternal educational level					
	Mid-high B (95% CI)[a]	Attenuation a[b]	Mid-low B (95% CI)[a]	Attenuation b[b]	Low B (95% CI)[a]	Attenuation c[b]
Diastolic blood pressure						
Model 1 (includes child sex, current age and ethnicity)	0.69 (0.18,1.21)		1.27 (0.79,1.76)		1.80 (1.25,2.35)	
Maternal characteristics						
Model 1 + pre-pregnancy hypertension	0.69 (0.17,1.20)	0% (-4,2)	1.26 (0.78,1.75)	-1% (-3,0.1)	1.79 (1.24,2.34)	-1% (-3,-0.01)
Birth characteristics						
Model 1 + birth weight	0.67 (0.40,0.93)	-4% (-16,-0.4)	1.22 (0.97,1.46)	-4% (-9,-1)	1.75 (1.46,2.03)	-3% (-7,-1)
Child characteristics						
Model 1 + BMI	0.68 (0.41,0.94)	-2% (-10,1)	1.23 (0.98,1.48)	-3% (-7,-1)	1.67 (1.13,2.20)	-8% (-14,-3)
Model 1 + height	0.73 (0.47,1.00)	+6% (1,23)	1.32 (0.86,1.77)	+4% (1,8)	1.86 (1.58,2.14)	+3% (1,6)
Model 1 + watching television	0.67 (0.15,1.19)	-2% (-9,0.1)	1.22 (0.73,1.71)	-3% (-7,-0.1)	1.70 (1.14,2.26)	-5% (-12,-0.2)
Model 1 + playing sports	0.64 (0.12,1.16)	-8% (-31,-3)	1.20 (0.71,1.68)	-7% (-13,-3)	1.68 (1.13,2.24)	-7% (-13,-3)
Fully adjusted model	0.61 (0.09,1.12)	-12% (-47,-3)	1.07 (0.58,1.56)	-16% (-30,-8)	1.43 (0.86,1.99)	-21% (-35,-12)

B=effect estimate, CI=confidence interval, BMI=body mass index.

[a]The effect estimates represent the attenuated effect estimates and their 95% CI's relative to model 1 after individual adjustment for explanatory factors .

[b]Attenuation a, attenuation b and attenuation c represent the attenuations of effect estimates for mid-high, mid-low and low education relative to model 1 (includes confounders) after individual adjustment for explanatory factors ($100 \times (B_{\text{model 1}} - B_{\text{model 1 with explanatory factor}}) / (B_{\text{model 1}})$). High maternal educational level is the reference group.

Table 5. Attenuation of the increase of fractional shortening (%) for the different levels of maternal education after individual adjustment for explanatory factors

	Maternal educational level			
	Mid-low B (95% CI)[a]	**Attenuation a[b] (95% CI)**	**Low B (95% CI)[a]**	**Attenuation b[b] (95% CI)**
Fractional shortening				
Model 1 (includes child sex, current age, ethnicity, ultrasound device and performing sonographer)	0.32 (0.01,0.64)		0.45 (0.10,0.80)	
Birth characteristics				
Model 1 + birth weight	0.30 (-0.01,0.61)	-8 % (-44,1)	0.42 (0.07,0.78)	-7% (-29,-1.0)
Child characteristics				
Model 1 + BMI	0.29 (-0.01,0.59)	-11% (-63,-2)	0.35 (-0.01,0.70)	-23% (-85,-9)
Model 1 + SBP	0.24 (-0.08,0.56)	-27% (-142,25)	0.26 (-0.10,0.63)	-42% (-159, -12)
Model 1 + DBP	0.27 (-0.02,0.70)	-16% (-106,35)	0.34 (-0.02,0.70)	-25% (-105,3.8)
Fully adjusted model	0.21 (-0.11,0.52)	-36% (-222,41)	0.19 (-0.17,0.56)	-58% (-229,-22)

B=effect estimate, CI=confidence interval, BMI=body mass index, SPB=systolic blood pressure, DBP=diastolic blood pressure.

[a]The effect estimates represent the attenuated effect estimates and their 95% CI's relative to model 1 after individual adjustment for explanatory factors .

[b] Attenuation a and attenuation b represent the attenuations of effect estimates for mid-low and low education relative to model 1 (includes confounders) after individual adjustment for explanatory factors (100 x ($B_{\text{model 1}} - B_{\text{model 1 with explanatory factor}}$) / ($B_{\text{model 1}}$)). High maternal educational level is the reference group. No differences in fractional shortening were observed for the mid-high educational subgroup as compared to the high educational subgroup, therefore attenuations in effect estimates for mid-high education are not presented.

the association of mid-low and low education with fractional shortening was observed after individual adjustment for BMI, SBP and DBP. SBP was the most contributing factor, explaining 27% (95% CI: -142% to 25%) and 42% (95% CI: -159% to -12%) respectively (Table 5).

Discussion

This study shows that socioeconomic inequalities in adult CVD may have their origins in childhood. We found socioeconomic inequalities in systolic and diastolic blood pressure to occur already at the age of 6 years. Furthermore, a positive association was found between maternal education and fractional shortening, which was explained by higher blood pressure levels and a higher BMI among children of lower educated mothers. We did not find evidence for socioeconomic inequalities in carotid- femoral pulse wave

velocity, aortic root diameter, left atrial diameter, and left ventricular mass at the age of 6 years.

Methodological considerations

The strengths of this study are the prospective population-based design and the availability of many important covariates that may explain the association between maternal education, blood pressure levels, and cardiac structures and function. In addition, we had a large sample size of 5843 participants and the availability of several cardiovascular measurements in most of these children.

To various extents, our results may have been influenced by the following limitations. We used maternal educational level as indicator of SEP. Maternal education may reflect general and health-related knowledge, health behavior, health literacy and problem-solving skills.[35, 36] Furthermore, it has been shown that education is the strongest and most consistent socioeconomic predictor of cardiovascular risk factors.[37] It is less clear to what extent it captures the material and financial aspects of the household. We therefore repeated the analyses using household income level as determinant, and we found comparable results with the highest SBP and DBP and the highest fractional shortening in the lowest income group (Supplementary Table S2). Information on various covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle related determinants. In our blood pressure measurements, we were not able to account for circadian rhythm. Yet, while this may have resulted in random error, it seems less likely that bias varied systematically across socioeconomic groups. Although the participation rate in The Generation R Study was relatively high (61%), there was some selection towards a relatively highly educated and more healthy study population.[38] Non-participation would have led to selection bias if the associations of maternal educational level with the outcome differed between participants and non-participants. Previous research showed that this bias is minimal.[39, 40] However, it cannot be ruled out completely. Finally, unmeasured factors related to both SEP and blood pressure, such as salt intake and psychosocial factors, might explain some of the remaining effect of SEP on blood pressure. Sleep patterns may also explain some of the remaining effect, since this has been associated with cardiovascular health in previous studies.[41]

Maternal education, blood pressure and cardiac structures and function

Previous literature concerning the association between socioeconomic circumstances and blood pressure in childhood is conflicting.[7, 42] This might be due to the use of different study designs and different study populations. In line with previous literature we found BMI to be the factor contributing most to the association between maternal education and blood pressure.[7] Obesity may cause disturbances in autonomic function,

insulin resistance and abnormalities in vascular structure and functions, which in turn may cause elevated blood pressure levels.[43] Maternal pre-pregnancy BMI was also an important explanation of the socioeconomic gradient in SBP. Maternal BMI and a child's BMI are correlated; the effect of maternal BMI may act for an important part through its effect on the child's BMI. This is supported by our findings which showed that after adjustment for child's BMI and maternal BMI, only child's BMI remained significant (data not shown). A novel finding of this study is that indicators of sedentary behavior and physical activity, including watching television and playing sports, mediate the association between maternal education on DBP. Although these effects may pass through the effects of the child's BMI on DBP, its significant association in the fully adjusted model suggests that there is probably also an independent effect of physical activity on DBP, which explains the socioeconomic inequalities in DBP. This is supported by a recent study of Knowles et al. who found higher physical activity to be associated with lower DBP levels among 5-7 year old children, independent of BMI and other markers of adiposity.[44] These findings suggest that intervention should be aimed at increasing physical activity, especially among children with a lower SEP.

Although children from low SEP families had higher BP levels and a higher BMI which is previously shown to be associated with larger left ventricular mass and atrial enlargement,[12-15] we did not find a socioeconomic gradient in cardiac structures at the age of 6 years. We found, however, left ventricular mass to be lower in the mid-high and the mid-low educational subgroup as compared to the high educational subgroup. The direction of the association was not entirely equal to what was expected. Also, no socioeconomic gradient was observed and therefore commonly used cardiovascular risk factors explaining socioeconomic inequalities are not likely to be an explanation. Future research is necessary to replicate these findings. Another remarkable finding was the higher fractional shortening among children with mid-low and low educated mothers. One explanation might be that these children experience more stress during the echocardiographic assessment due to a lower lack of parental support because they, as well as their parents, are more impressed by the measurement setting, resulting in a higher fractional shortening. However, stress would also have increased their heart rate and adjustment for heart rate would then have explained, at least to some extent, the association between maternal education and fractional shortening. In our study, this explanation is less likely since heart rate, as indicator of stress, was not associated with fractional shortening. Since an increased fractional shortening was explained by higher blood pressure levels and a higher BMI, another explanation might be that it is the first indication of cardiac adaptation to higher blood pressure levels and higher BMI among low SEP children. However, in a later stage higher blood pressure levels and a higher BMI result in alterations of cardiac structures such as left ventricular and left

atrial enlargement,[45] which are associated with a lower fractional shortening. Thus our findings that lower education leads to a higher fractional shortening at the age of 6 are not fully understood and a change finding can also not be excluded. Confirmation in future studies is therefore highly recommended and replication would suggest that cardiac structural alterations can be prevented at this age by lowering blood pressure levels and body weight, especially among children from low socioeconomic families.

Conclusion

Our study adds to the small body of literature concerning socioeconomic differences in blood pressure and shows that inequalities arise in early childhood which were mainly explained by socio-economic inequalities in the child's BMI and physical activity. No differences in arterial stiffness or cardiac structures were observed per educational subgroup. However, a socioeconomic gradient in fractional shortening was observed, with children from low SEP families having a higher fractional shortening as compared to children from high SEP families. This might be a first cardiac adaptation to a higher blood pressure and a higher BMI. The results require careful interpretation since the associations were relatively small and the clinical importance not yet fully understood.

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Supplemental Material

Supplementary Table S1. Characteristics of children visiting and not visiting the research center at 6 years of age (n = 8305)[a]

	Children not visiting the research center (n=1615)	Children visiting the research center (n=6690)	P-value[b]
Maternal characteristics[b]			
Maternal educational level (%)			
High	22.1	25.2	<0.001
Mid-high	16.0	21.6	
Mid-low	26.9	31.0	
Low	35.1	22.1	
Body mass index before pregnancy (kg/m^2)	23.5 (4.3)	23.6 (4.2)	0.58
Pre-pregnancy hypertension (% yes)	1.8	1.9	0.91
Pregnancy-induced hypertension (% yes)	3.1	4.3	0.059
Preeclampsia (% yes)	2.4	2.2	0.62
Smoking during pregnancy (%)			
No	71.6	74.6	<0.001
Until confirmed pregnancy	7.3	8.9	
Continued during pregnancy	21.2	16.5	
Financial difficulties (% yes)	16.5	17.5	0.56
Family assessment device score	1.51 (0.41)	1.53 (0.43)	0.26
Birth characteristics[b]			
Gestational age at birth (weeks)	39.1 (1.9)	39.8 (1.9)	0.15
Birth weight (grams)	3388 (593)	3401 (573)	0.42
Breastfeeding during infancy (%)			
Yes	89.1	92.3	0.001
No	10.9	7.7	
Confounding variables			
Male sex (%)	51.9	50.1	0.02
Ethnicity (%)			
Dutch	54.7	56.9	0.09
Other western	7.7	8.5	
Non western	37.6	34.6	

[a]Values are percentages or means (SD).

[b]P-values are calculated with the Chi-square test for categorical variables and ANOVA for continuous variables for children who didn't visit the research center (n= 1615) compared to children who visited the research center (n=6690). Values are calculated with non-imputed data.

Supplementary Table S2. Associations between net household income, blood pressure and cardiac structures at the age of 6 (n=4682)[a]

Net household income	Systolic blood pressure (mm Hg)	Diastolic blood pressure (mm Hg)	Pulse wave velocity (m/s)	Left atrial diameter (mm)	Aortic root diameter (mm)	Left ventricular mass (g)	Fractional shortening (%)
> € 2200 (n=2777)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
€ 1600 - € 2200 (n=716)	0.70* (0.03,1.36)	0.46 (-0.09,1.00)	0.03 (-0.05,0.11)	-0.05 (-0.25,0.16)	0.01 (-0.12,0.15)	-0.67 (-1.49,0.14)	-0.001 (-0.38,0.37)
< € 1600 (n=1189)	1.11** (0.47,1.78)	1.17*** (0.66,1.69)	0.05 (-0.03,0.13)	-0.17 (-0.36,0.03)	-0.13 (-0.26,0.003)	-0.25 (-1.01,0.59)	0.49** (0.16,0.82)
P for trend	< 0.001	< 0.001	0.20	0.10	0.07	0.45	0.007

* p-value <0.05, ** p-value < 0.01, *** p-value<0.001

[a]Values represent differences in blood pressure levels, carotid-femoral pulse wave velocity, left cardiac structures and fractional shortening (with 95% confidence interval) relative to children of mothers with net household income of > €2200, and are derived from linear regression analyses performed on the data after multiple imputation of the covariates. All models are adjusted for child sex, current age and ethnicity. Models with ultrasound outcomes are additionally adjusted for ultrasound device and performing sonographer.

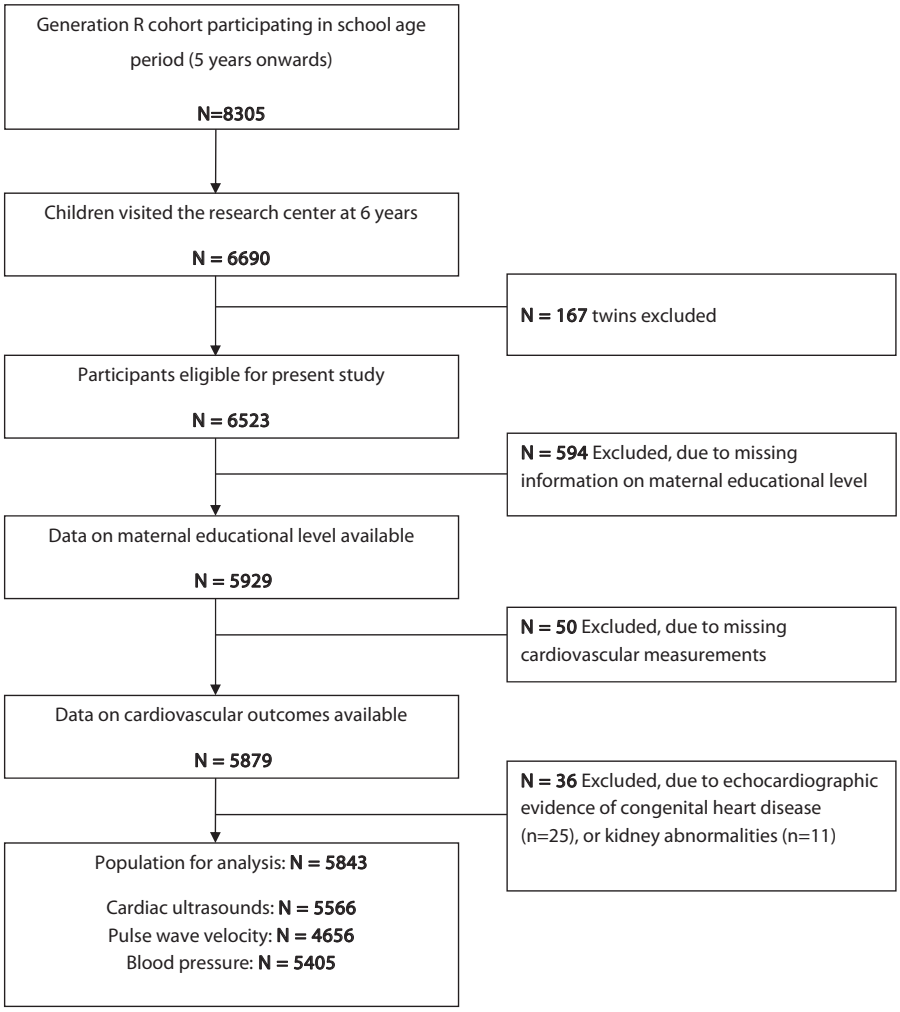

Figure S1 Flow chart of participants included in the analysis

9

Genetic taste blindness to bitter and body composition in childhood

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Abstract

Background: The ability to taste 6-n-propylthiouracil (PROP) may be associated with body composition, but previous findings from observational studies are conflicting and cannot be interpreted causally. The aim of this study was to estimate the causal association between PROP taster status and body composition in a population-based cohort study.

Methods: The study was embedded in a population-based prospective birth cohort study. *TAS2R38* genotype (rs713598) was used as an instrumental variable (IV) to obtain unbiased effect estimates of the relation between PROP taster status and body weight (n = 3778). Adiposity measures included body mass index and fat mass measured by DXA scan at the child's age of 6 years. Associations were investigated using both ordinary linear regression (OLS) and two-stage least squares regression (2SLS).

Results: Non-taster girls had higher BMI standard deviation scores (SDS) and higher body fat as compared to taster girls (results from linear regression BMI SDS: -0.09, P = 0.023, body fat mass (%): -0.49, P = 0.028). *TAS2R38* genotype predicted PROP phenotype (F=240), indicating a strong IV. The 2SLS effect estimates were imprecise but similar to the observational estimates (-0.08 for BMI SDS and -0.46 for body fat mass %) and were not significantly different from the OLS results (Hausman test: P>0.10). For boys there were no differences observed between tasters and non-tasters.

Conclusions: Our findings suggest a causal relation between PROP taster status and body weight among 6 year-old girls; Mendelian Randomization were consistent with conventional estimates. In contrast, body weight among boys appeared to be independent of PROP taster status. Further research should focus on possible underlying pathways, such as dietary behaviour.

Introduction

The ability to taste the bitter substance 6-n-propylthiouracil (PROP), which is chemically related to phenylthiocarbamide (PTC), is genetically determined.[1] People who are genetically insensitive to the bitter taste of PROP have been termed "non-tasters" as compared to the PROP sensitive "tasters". In the US, approximately 30% of the Caucasian population is insensitive to the bitter taste of PROP, but wide variation has been shown among different ethnic groups.[2]

In the past years, some studies[3-7] suggested a relationship between the ability to taste the bitter substance PROP and BMI, but results are inconsistent.[8-12] Underlying mechanisms are unknown, but it has been speculated that as compared to tasters, non-tasters have an appetite for greater variety of foods, especially high-fat foods.[13, 14] As a result, non-tasters may consume more energy and develop higher body weights than tasters.[15] However, previous studies have focused on BMI as an indicator of overweight and obesity whereas body fat mass measured by dual- energy X-ray absorptiometry (DXA) is a more sensitive measure; it discriminates between fat mass and lean body mass and, therefore, may better reflect nutritional status than BMI. Also, earlier studies were observational in design and inferring causality from observational studies is problematic.[16, 17] In addition, the association between phenotype and outcome might be due to residual confounding particular by socioeconomic position (SEP), ethnic or lifestyle factors, or due to reverse causation.[16]

A Mendelian randomization design, using gene(s) randomly allocated at conception as an instrumental variable, provides an alternative way to circumvent problems with causal inference in observational studies.[18] The perception of bitter taste is mediated by G-protein coupled receptors, located in taste cells within taste bud of the tongue.[19] Variants in *TAS2R38*, encoding a G-protein-coupled bitter receptor located on chromosome 7q, have been strongly associated with PROP/PTC sensitivity.[1]. Three common single nucleotide polymorphisms (SNPs), all of which result in amino acid changes in the protein (A49P, V262A and I296V), give rise to the two main haplotypes which are commonly found in human populations; the 'taster' haplotype PAV (proline-alanine-valine), and the 'non-taster' haplotype AVI (alanine-valine-isoleucine).[1] These variants explain 60%-70% of the variation in PROP taster status.[1, 15] This makes these genetic variants very useful as instrumental variables since the method of Mendelian Randomization works better the stronger the association between gene and phenotype.[18, 20] Therefore, we assessed in a large prospective birth cohort study whether PROP phenotype is causally related with BMI and body fat mass among 6-year-old children using *TAS2R38* genotype as instrumental variable (IV) for PROP phenotype.

Subjects and Methods

Study design

This study is embedded within the Generation R Study, a population-based prospective cohort study from early pregnancy onwards described in detail elsewhere.[21-23] Enrollment was aimed at early pregnancy, but was allowed until the birth of the child. All children were born between April 2002 and January 2006 and form a prenatally enrolled birth-cohort that is currently being followed-up until young adulthood. The study was conducted in accordance with the guidelines proposed in the World Medical Association of Helsinki and has been approved by the Medical Ethical Committee of the Erasmus MC, University Medical Centre Rotterdam. Written consent was obtained from all participating parents.[24]

Study group

Consent for postnatal follow-up was available for 7893 children. The participating children and their mothers were invited to a well-equipped and dedicated research center in the Erasmus Medical Center - Sophia Children's Hospital between March 2008 and January 2012. Measurements were focused on several health outcomes including body composition, obesity, heart and vascular development and behaviour and cognition.[23] In total, 6690 children visited the research center. We excluded twins (n=167) and participants who lacked information on PROP taster status (n=629), leaving a study population of 5894 children (Supplementary Figure S1).

Genotyping

In the Generation R Study, cord blood for DNA isolation was available in 59% (n=5495) of all live-born participating children. Sex-mismatch rate between genome-based sex and midwife record-based sex was low (<0.5%), indicating that possible contamination of maternal DNA was extremely low. Missing cord blood samples were mainly due to logistical constraints at the delivery. Individual genotype data of *TAS2R38* variants were extracted from the genome-wide Illumina 610 Quad Array for the vast majority of the population. Additional DNA of 314 children was isolated from peripheral blood which was collected at the research center and genotyped using the Illumina 660 Quad Array. There are 3 common variants in the *TAS2R38* gene that have been reported to be associated with bitter sensitivity. The A49P variant (rs713598) is in high linkage disequilibrium (LD) with the A262V variant (rs1726866) (r^2=0.81 in our study population). The third variant I296V (rs10246939) is in almost perfect LD with the V262A variant (r^2=0.92 in our study population). We present results for the A49P (rs713598) SNP only, since they are in strong linkage disequilibrium.[1] The allele and genotype coding was based on the amino acid substitution alanine/proline substitution at position 49 of the protein (A49P)

as reported in previous studies.[4, 25] Children homozygous for the bitter-insensitive allele (GG) were classified as Ala/Ala (AA), children heterozygous (GC) for the bitter-insensitive allele as Ala/Pro (AP), and children homozygous (CC) for the bitter-sensitive allele as Pro/Pro (PP). Genotype data for the A49P polymorphisms was available for 3778 (64.1%) of the children with complete phenotypic information.

PROP test

The PROP test was conducted around the age of six years (median 6.0, mid-90% range 5.8-6.8) by well-trained staff in the research center. PROP taster status was determined by using a method developed by Keller et al.[13] Children were classified as "tasters" or "non-tasters" by giving them 5 mL of 0.56mmol/l solution of PROP (6-propyl-2-thiouracil; pharmacy of Erasmus MC, Rotterdam) after which they were asked the question: 'Do you taste anything?' Children who reported "no" or "like water" were classified as non-tasters. Children who reported "no" or "water" but showed classic rejection sign, such as grimacing or frowning, were classified as tasters. Those who reported a taste for the solution were further questioned as to what the solution tasted like. Responses of "bad", "bitter", "sour", "yucky" and "spicy" were all recorded as tasters. Children who reported that the solution tasted "good" were classified as non-tasters. Some children (n=118) gave discordant answers such as "good" and "bitter". These children were classified as tasters.

Body composition

Weight was measured in lightweight clothes and without shoes by using a mechanical personal scale (SECA) and height was measured in children by a Harpenden stadiometer (Holtain Limited) in standing position, which were both calibrated on a regular basis. BMI was calculated using the formula; weight (kg) / height $(m)^2$. BMI was measured in 5887 children. Standard deviation scores (SDS) adjusted for age and gender were constructed for these growth measurements (height, weight and BMI).[26] The DXA scans provided quantifications of bone and soft tissue measurements for the total body and sub regions, including bone mineral content (g), fat mass (g), and lean mass (g) (iDXA; General Electric, formerly Lunar Corp., Madison, WI). Percentage body fat was calculated as total body fat mass divided by total body mass which were obtained from DXA scan (lean mass + fat mass + bone mass of total body) $\times$ 100. Children were scanned in a supine position with their feet together in a neutral position and hands flat by their sides. In total, DXA scans were performed in 5794 children. All scans were performed by well-trained and certified research staff who repositioned the regions of interest when appropriate. All DXA scans were obtained using the same device and software (enCORE2010).

Confounding variables

Information on maternal educational level (highest education finished), net parental household income (<€1600, ≥ €1600) was obtained from the first questionnaire at enrollment in the study and were used as indicators of socioeconomic position. Caucasian ethnicity was defined as having principal components within four SD values of the CEU cluster (Northerwestern European ancestry) of HapMap[27], the others were classified as non-Caucasian. Gender of the child was obtained from midwife and hospital registries. Maternal height and paternal weight and height were measured during visits at our research center. Pre-pregnancy weight was established at enrollment through a questionnaire. On the basis of height and pre-pregnancy weight (weight/height^2), we calculated pre-pregnancy body mass index (BMI). Playing outside (≤ 2 days, > 2 days), as indicator of physical activity, was obtained from a questionnaire at the age of 5 years.

Statistical analysis

Chi-square tests, two-sample T-test and ANOVA were used to assess whether PROP phenotype and *TAS2R38* genotype were associated with potential confounders, such as socio-demographic, lifestyle related and parental characteristics. We assessed the association of PROP taster status with BMI and body fat mass using linear regression models adjusted for all the potential confounders. Mendelian randomization analyses may avoid confounding or reverse causation that may bias conventional observational effect estimates, but MR is also less statistically efficient than conventional analyses.[28] Therefore, if the conventional approach is unbiased, parameter estimates from conventional models are preferred. We use the MR analysis to assess evidence for bias in the conventional effect estimates. For the Mendelian randomization based estimates, we used two-stage least squares analysis with *TAS2R38* genotype (rs713598) as an instrumental variable using two indicator variables (AP and PP) [28]. Two-stage least square estimation proceeds by first fitting the regression of PROP phenotype (exposure) on *TAS2R38* genotype (instrument), and the second step assesses the association of PROP phenotype with BMI and body fat mass (outcome) on the fitted values from the first stage regression. Since the A49P variant and the V262A variant were in high, but not in perfect LD (r^2=0.81), we investigated whether adding the V262A variant as 'extra' instrument would increase the explained variance and improve power, but results were similar as compared with using A49P only. To compare the estimates from the ordinal regression and the two-stage least squares regression the Durbin-Hausman test was used. The two-stage least squares regression was only adjusted for potential confounders associated with *TAS2R38* genotype, since alleles of *TAS2R38* genotype are randomly allocated at conception and unlikely to be confounded by SEP, lifestyle or other factors. Multiple imputation was used to deal with the missing values in the covariates (ranging from 6.6% to 35.9%). Five imputed datasets were created and analyzed together. Analyses were performed using

the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA). For the Mendelian randomization approach STATA 12 (StataCorp LP, College Station, Texas) was used.

Results

Characteristics of the children grouped by PROP phenotype status are presented in Table 1. Overall, 77.4% of the children were classified as PROP "tasters", and 22.6% were "non-tasters". Boys were more frequently non-tasters (52.8%, N=702) as compared to girls (47.2%, N=628) (P = 0.014). No differences in the distribution of age, physical activity, ethnic background, socioeconomic position and parental BMI were found by PROP taster status (Table 1). Table 2 shows that *TAS2R38* genotype was not associated with

Table 1. Socio-demographic characteristics by PROP taster status

	N	Non-tasters (N = 1330)	Tasters (N = 4564)
Age (years)	5894	6.2 (0.5)	6.2 (0.5)
Gender (%)*			
Boys	2936	52.8	48.9
Girls	2958	47.2	51.1
Child's ethnicity (%)			
Caucasian	2007	54.6	52.7
Non Caucasian	1772	45.4	47.3
Playing outside (%)			
≤ 2 days/week	351	9.3	8.1
> 2 days/week	3848	90.7	91.9
Maternal education (%)			
High	1344	26.0	24.8
Mid high	1141	20.4	21.6
Mid low	1675	30.2	31.5
Low	1201	23.4	22.1
Household income (%)			
Low income	1191	27.4	26.6
Higher income	3257	72.6	73.4
Maternal BMI (kg/m^2)	4404	23.6(4.3)	23.6(4.2)
Paternal BMI (kg/m^2)	4065	25.3(3.5)	25.3(3.4)

* P<0.05

Values are percentages or mean (SD). P-values are for Chi-square test for categorical variables, and two-sample T test for continuous variables.

gender, physical activity, socioeconomic position and parental BMI for both Caucasians and non-Caucasians.

The minor allele frequency (MAF) of the C allele (coding for Proline (P)) of *TAS2R38* was found to be 0.43 in our study sample. Splitting the population in two ethnic groups showed some difference with MAF of 0.40 in Caucasian children and 0.48 in non-Caucasian children (Table 2). Children who carried the PP or AP genotypes were much more likely to be PROP "tasters" by phenotype (OR: 19.1; 95% CI: 13.8, 26.6 and OR: 13.5; 95% CI: 11.0, 16.4). According to participants PROP taster status, 80.4% would have been classified the same status according to their *TAS2R38* genotype. The overall contribution of genotype to phenotype was 35.6% (Nagelkerke $R^2 = 0.356$, $P < 0.001$).

Table 2. Socio-demographic characteristics by *TAS2R38* genotype stratified on Caucasian and non Caucasian background

		Caucasian				Non Caucasian		
***TAS2R38* genotype**	**N**	**AA (N = 707)**	**AP (N = 997)**	**PP (N = 299)**	**N**	**AA (N = 497)**	**AP (N = 861)**	**PP (N = 412)**
Prop taster status*								
Non-tasters (%)	471	55.6	6.7	3.7	392	54.7	10.2	7.8
Tasters (%)	1532	44.4	93.3	96.3	1378	45.3	89.8	92.2
Age (years)	2003	6.1 (0.4)	6.1 (0.4)	6.1 (0.4)	1770	6.3 (0.6)	6.3 (0.6)	6.3 (0.6)
Gender (%)								
Boys	994	52.6	47.1	50.8	882	49.3	50.2	49.8
Girls	1009	47.4	52.9	49.2	888	50.7	49.8	50.2
Playing outside (%)								
≤ 2 days/week	70	3.8	4.5	3.4	160	12.3	12.9	16.8
> 2 days/week	1634	96.2	95.5	96.6	1012	87.7	66.1	83.2
Maternal education (%)								
High	745	40.5	34.8	41.8	178	10.7	11.0	12.1
Mid high	547	27.7	28.4	26.0	259	15.8	17.1	15.1
Mid low	469	21.6	26.1	21.2	597	38.5	36.8	37.6
Low	208	10.2	10.7	11.0	559	35.0	35.1	35.2
Household income (%)								
Low income	149	8.3	8.1	8.1	651	49.0	49.6	47.2
Higher income	1670	91.7	91.9	91.9	680	51.0	50.4	52.8
Maternal BMI (kg/m^2)	1690	23.6 (3.6)	23.0 (3.6)	23.0 (3.7)	1395	23.8 (4.5)	24.0 (4.2)	23.6 (4.7)
Paternal BMI (kg/m^2)	1812	25.0 (3.2)	25.1 (3.3)	25.2 (3.4)	1095	25.3 (3.8)	25.4 (3.5)	25.4 (3.5)

* P<0.001

Values are percentages or mean (S.D.). P-values are for Chi-square test for categorical variables, and ANOVA for continuous variables. A is coding for the G allele, P is coding for the C allele.

Table 3. Association between adiposity measures and PROP phenotype derived from multivariable linear regression[a]

		Unadjusted		Adjusted[b]			Unadjusted		Adjusted[b]			Unadjusted		Adjusted[b]	
	N	ß	P value	ß	P value	N	ß	P value	ß	P value	N	ß	P value	ß	P value
BMI SDS				**Overall**					**Boys**					**Girls**	
PROP taster															
Taster	1329	-0.04	0.177	-0.04	0.163	702	0.02	0.621	0.01	0.340	627	-0.10	0.019	-0.09	0.023
Non-taster	4558	Ref		Ref		2232	Ref		Ref		2326	Ref		Ref	
Fat mass (%)[c]															
PROP taster															
Taster	1313	-0.20	0.255	-0.18	0.278	690	-0.19	0.384	-0.19	0.355	623	-0.53	0.025	-0.49	0.028
Non-taster	4481	Ref		Ref		2188	Ref		Ref		2293	Ref		Ref	

SDS = standard deviation score

[a]Values reflect the effect estimates (ß) and their p-values

[b]Adjusted for parental BMI, maternal education, household income, ethnicity child and playing outside.

[c]All models for fat mass are also adjusted for exact age and height at measurement.

Multivariable regression analyses were performed with PROP phenotype being the independent variable and BMI (in SDS) and body fat mass (% of total body mass) the dependent variable (Table 3). A significant interaction between PROP phenotype and gender on BMI SDS was found (P=0.040). Therefore, the models were also calculated for boys and girls separately. All models were adjusted for parental BMI, socioeconomic position, ethnicity child and playing outside. The models for fat mass were additionally adjusted for exact age and height at measurement. Taster girls were found to have significantly lower BMI standard deviation scores (difference: -0.09 SDS; 95% CI: -0.17,-0.01; P=0.023) and a lower body fat mass (difference: -0.49%; 95% CI: -0.93,-0.05; P=0.028) as compared to non-taster girls. No association between PROP phenotype and BMI (difference: 0.01 SDS; 95% CI: -0.06, 0.09; P=0.340) or body fat mass (difference: -0.19%; 95% CI: -0.60, 0.21; P=0.355) was found in boys (Table 3). Maternal educational level, household income and ethnicity did not interact with PROP phenotype to influence BMI or body fat mass (P>0.05). A second linear regression was performed with *TAS2R38* genotype being the independent variable. BMI and fat mass were not significantly different according to *TAS2R38* genotype (P>0.10).

Table 4. Association of adiposity measures with PROP phenotype in a Mendelian Randomization Design in the overall population and among boys and girls separately

	Ordinary Linear Regression			2-Stage Least Square			Hausman P value[a]
	N	ß (95% CI)	P value	N	ß (95% CI)	P value	
Overall							
BMI SDS[b]	5887	-0.04 (-0.09,0.02)	0.163	3773	-0.01 (-0.14,0.12)	0.930	0.649
Fat mass (%)[c]	5794	-0.18(-0.51,0.15)	0.278	3716	-0.003 (-0.79,0.79)	0.995	0.686
Boys							
BMI SDS[b]	2953	0.01 (-0.06,0.09)	0.340	1876	0.07 (-0.11,0.25)	0.453	0.575
Fat mass (%)[c]	2878	-0.19(-0.60,0.21)	0.355	1841	0.11(-0.87,1.09)	0.822	0.580
Girls							
BMI SDS[b]	2934	-0.09(-0.17,-0.01)	0.023	1897	-0.08(-0.27,0.11)	0.390	0.933
Fat mass (%)[c]	2916	-0.49 (-0.93,-0.05)	0.028	1875	-0.46(-1.53,0.60)	0.394	0.965

SDS = standard deviation score

[a]The Hausman P value indicates whether the 2-stage least squares and fully adjusted ordinary linear regression estimates differ. Results are presented as standardized regression coefficients (ß) along with 95% confidence intervals (CI). The 2-stage least square analysis is adjusted for ethnicity child and derived from non-imputed data.

[b]Adjusted for parental BMI, maternal education, household income, ethnicity child and playing outside in the ordinary linear regression.

[c]Adjusted for parental BMI, maternal education, household income, ethnicity child, playing outside, exact age and height at measurement in the ordinary linear regression.

Table 4 shows the result of the Mendelian Randomization approach. The regression of the taster phenotype on TAS2R38 genotype (F=240) indicated the genotype provided a strong instrumental variable. The effect estimates derived from ordinary linear regression (OLS) (BMI difference: -0.04 SDS; 95% CI: -0.09, 0.02; P=0.163 and fat mass difference: -0.18%; 95% CI -0.51, 0.15; P=0.316) and 2SLS regressions (BMI difference: -0.01 SDS; 95% CI: -0.14, 0.12; P=0.930 and fat mass difference: -0.003%; 95% CI -0.79, 0.79; P=0.995) were not significant in the overall population. For boys the results of the 2SLS regression were also not significant (BMI difference: 0.07 SDS; 95% CI: -0.11, 0.25; P=0.453 and fat mass difference: 0.11%; 95% CI -0.87, 1.09.15; P=0.822). For girls, the OLS effect estimates were significant for both BMI SDS (P=0.023) and fat mass (P=0.028). The Hausman test indicated no significant differences between the OLS and 2SLS effect estimates (P>0.10), although the 2SLS effect estimates were imprecise and the CIs included the null (Table 4). Point estimates from OLS and 2SLS models were similar, providing no evidence of bias in the more efficient OLS models.

Discussion

To the best of our knowledge, this is the first population-based study to use a Mendelian randomization (MR) approach regarding PROP phenotype and adiposity measures. This study suggests that PROP taster status is causally related to body composition measures in girls. We found non-taster girls to have a higher BMI and a higher body fat mass as compared to taster girls. For boys, no differences were observed in BMI and body fat mass between PROP tasters and non-tasters.

Methodological considerations

The main strength of this study lies in the fact that we used a MR design which is less susceptible to confounding as compared to an observational design since the genes used as instrumental variables are unlikely to be systematically related to socio-demographic factors or lifestyle.[17, 18] Second, MR analysis rules out the possibility of reversed causation which can play a role in an observational study.[17] This is particularly important in the association between PROP status and body composition, since there is evidence for altered taste sensitivity in obese subjects, but it is not clear whether this causes obesity or whether obesity secondarily alters taste.[29, 30] Furthermore, this is the first study showing a relation between PROP status and fat mass in girls measured by DXA scan.

MR analyses can be used to estimate the causal effect of taster status on adiposity if three key assumptions are fulfilled.[18, 31] First, there must be no unmeasured common causes of *TAS2R38* genotype (A49P) and the adiposity measures. Our possible confound-

ers and *TAS2R38* were not associated, and generally there are few plausible causes of genotype other than parental genotype or population group. Second, we assume that *TAS2R38* is associated with PROP taster status; this assumption was confirmed in our data. Third, we assume that there is no direct association between TAS2R38 and body composition and that every directed pathway from *TAS2R38* to BMI and fat mass passes through PROP taster status. Pleiotropy would violate this assumption, but unfortunately this cannot be tested statistically.[31] In the literature, however, the A49P variant of the *TAS2R38* genotype has not been linked to other phenotypes which might be associated with adiposity measures. Eating behaviour disinhibition might be an exception, since this was found to be associated with the V262A variant in one study.[32] Previous literature, however, showed that variation in taste function influences eating behaviour, suggesting that we fulfilled the third assumption of MR as well.[33]

PROP phenotype, *TAS2R38* genotype and body composition

The prevalence of non-tasters in our study population is somewhat lower compared to the prevalence found in the Caucasian population in the U.S.[2, 34] and that found in other studies.[4, 15, 35] The allele frequencies found in our population were comparable with previously reported frequencies for both the Caucasian and non-Caucasian population.[36] The ability to taste PROP was more common in girls than in boys, which is in line with previous research.[2, 37] *TAS2R38* genotype explained 35.6% of the variation in PROP phenotype. This is comparable with the study of Feeney et al. but lower then found in other studies.[36] Since the method used to classify PROP taster status in this study was a simple-screening procedure which lacked the sensitivity of standard threshold techniques, part of the non-explained phenotypic variation in PROP tasting may be due to phenotypic misclassification. Furthermore, environmental factors, modifying genes or unknown genes that have yet to be identified may also play a role.[7, 38-40]

Prior findings regarding the association of PROP phenotype and body weight have been inconsistent.[3, 6, 8, 9, 12, 15] Some studies showed a higher BMI among tasters.[8, 12] Other studies found non-taster females to have higher body weights as compared to taster females,[5, 6, 15, 36] while other studies have not found any relationship between PROP status and body weight.[9, 11] Furthermore, our findings were in contrast with the findings of Keller et al.,[3, 4] which showed non-taster boys to have higher body weights than taster boys. The reports of association between BMI and PROP phenotype may be due to chance associations, since there is no replication of consistent findings. Furthermore, the discrepancies could be due to failure to adjust fully for confounding factors, such as socioeconomic position, lifestyle and family dietary habits, which are associated with body weight and are difficult to assess comprehensively. Another explanation might be that the impact of PROP taster status on body composition depends on the typical diet of a population.

Thus, perhaps there is true heterogeneity of the effect of PROP taster status on body composition, depending on dietary 'environment'.

We build importantly on prior work by strengthening evidence for a causal effect of PROP phenotype on body composition measures among females. We found a similar magnitude and direction of both effect estimates derived from 2 complementary study designs which were not significantly different when confirmed by the Hausman test. However, the effect estimates of the 2 SLS regression among girls were imprecise and not statistically significant at conventional thresholds. MR models have advantages with respect to reducing bias but are nearly always less efficient, i.e., require larger sample sizes, than conventional models because the genotype does not *perfectly* predict the phenotype. This loss of efficiency was exacerbated here because our MR analyses excluded over 1000 individuals who were not genotyped. For this reason, conventional estimates are generally preferred if they are unbiased. We therefore used the MR effect estimates to evaluate the plausibility of substantial bias in the conventional effect estimates and found no evidence for bias in the Hausman tests comparing the MR to the OLS effect estimates. However, this should be interpreted cautiously because the Hausman test also may be underpowered to detect bias. This means that replication in diverse samples, along with meta-analyses to maximize effective sample sizes, will be valuable to confirm these findings.

The reason PROP phenotype is only associated with BMI and body fat mass among girls is unknown. An interaction between PROP phenotype, gender and diet could possibly explain the different influence of PROP phenotype on BMI in females since studies show differences in diets between boys and girls.[41, 42] It has been suggested that non-taster phenotype and female gender contribute to higher fat intake and ultimately to greater weight gain as compared to boys.[13, 15] Another study found that more than 20% of the variation in food preferences could be contributed to taster status and this influence was found to vary with gender.[36] Also, a recent study demonstrated that girls have a greater sensitivity to disliked foods [43] suggesting that PROP status may be better captured in girls than in boys since girls may be more sensitive to taste.

Study limitations

A potential limitation in our study is the method that we used to classify PROP phenotype could only distinguish tasters from non-tasters. Previous studies showed that tasters consist of two subgroups; medium tasters, who show moderate taste sensitivity to PROP, and supertasters, who are highly sensitive.[37, 44] The association with BMI and fat mass could be different for these two groups. The method, however, used to make this PROP classification is probably too complicated for 6-year-old children and may have given

unreliable results. Also, no information on parental feeding factors was available which might act as a confounder in the association between PROP status and body composition. Furthermore, we did not have comprehensive data on dietary intake and dietary behaviour such as restraint and disinhibition at the age of 6 years. One of the underlying causal pathways in the relationship between PROP status and body composition that has been hypothesized is related to dietary behaviour.[33] Therefore, further studies on the underlying mechanisms including dietary behaviour are necessary.

Conclusion

This study adds to the small body of literature that showed non-taster females to have higher body weights and fat mass than taster females. We provide novel evidence from a Mendelian Randomization design on the validity of the effect estimates; using *TAS2R38* genotype as a natural experiment, we find no evidence of confounding in the observational estimates of the effect of PROP status on adiposity measures. PROP status may play a role in the aetiology of obesity in girls and may be useful for prevention and intervention strategies in the future. Further research should focus on possible underlying pathways, such as dietary behaviour. Also, larger MR studies are necessary to confirm our findings.

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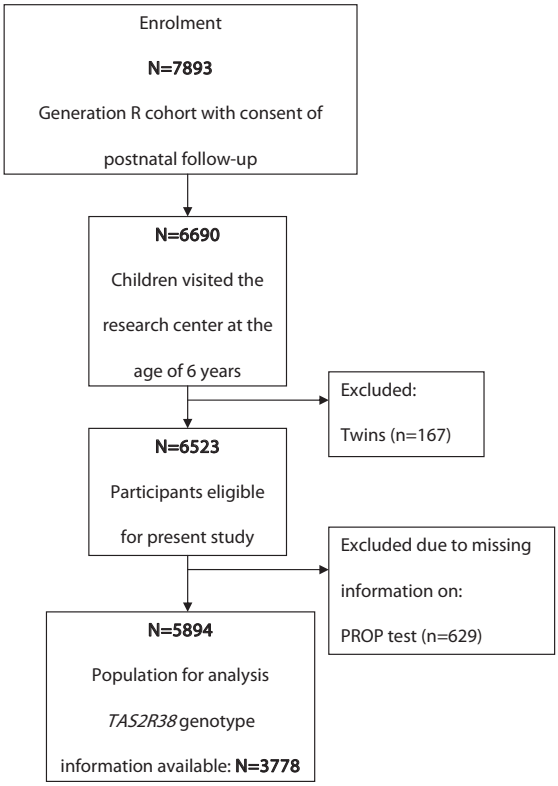
Supplemental Material



Supplemental Figure S1. Flow chart of the study population

10

Maternal smoking during pregnancy and the development of higher body mass index and blood pressure during childhood

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Submitted

Abstract

Objective: To investigate the extent to which maternal prenatal smoking is associated with change in child BMI and blood pressure from birth to mid-childhood, and with BMI z-scores, fat mass index (FMI) and SBP in mid-childhood .

Methods: We studied 1755 mother-child pairs in Project Viva, a prospective cohort study. Maternal smoking status was self-reported; we categorized it into never smoked, formerly smoked and smoked during pregnancy. Main outcome measures were BMI z-score, FMI and systolic blood pressure. We analyzed mid-childhood data with multivariable linear regression models and longitudinal data with linear mixed models.

Results: 209 (11.9%) of the pregnant women smoked during pregnancy and 344 (19.6%) were former smokers; their mean age was 31.9 (5.2) years. Compared to never smokers, BMI z-score in the offspring of mothers who smoked during pregnancy was similar at birth (-0.01 units, 95% CI:-0.18,0.16) but increased by 0.07 units per year (95% CI: 0.03, 0.10) and in mid-childhood BMI z-score was 0.11 units higher (95% CI:-0.09, 0.31). Offspring SBP of both former smoking mothers (0.18 mm Hg; 95% CI: -0.07, 0.43 per year) and smoking mothers (0.18 mm Hg; 95% CI: -0.16, 0.52 per year) tended to increase faster with age than that of never smokers. In mid-childhood SBP was 1.45 mm Hg (95% CI: 0.19, 2.72) and 1.82 mm Hg (95% CI: 0.02, 3.61) higher in these two groups, respectively. Mid-childhood FMI also tended to be higher in offspring of pregnancy smokers (0.27 kg/m^2; 95% CI: -0.15, 0.69).

Conclusions: Women who smoked during pregnancy had children who developed a higher BMI from infancy onwards and a higher blood pressure from birth onwards. Offspring of former smokers also had higher blood pressure levels than never smokers. Public health strategies to reduce smoking among fertile women are imperative.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in most parts of the world. Obesity and hypertension are major CVD risk factors, and the prevalence of these CVD risk factors increases with age from childhood to adulthood.[1, 2] Consequently, a major public health goal is preventing these modifiable risk factors to reduce the burden of CVD. To identify targets for prevention, understanding the nature and onset of obesity and hypertension is crucial.

In recent years, evidence emerged that CVD has its origins in pre and postnatal periods through the phenomenon of 'programming',[3] wherein environmental exposures occurring at a critical period of development may have lasting effects.[4] One such exposure may be maternal smoking during pregnancy, which is associated, seemingly paradoxically, with intrauterine growth retardation,[5, 6] but also with obesity in later childhood and adulthood.[7-9] Also, some studies have shown that maternal smoking during pregnancy may be related to higher offspring blood pressure,[10-13] but other studies did not find a relation.[14, 15]

While confounding by social patterning could explain associations of maternal smoking with offspring obesity and higher blood pressure, biological mechanisms are also possible. Animal studies suggest that nicotine exposure during pregnancy may cause childhood obesity by acting on neurotransmitter systems in the brain that may increase appetite and reduce physical activity.[16-18] The effect of smoking during pregnancy on blood pressure might be explained by its effect on obesity or by separate pathways such as by restricting the number of nephrons in the developing fetus.[19]

Most studies on prenatal smoking and childhood obesity lack a longitudinal perspective, and, to our knowledge, no studies have yet examined whether prenatal smoking is associated with longitudinal blood pressure patterns in childhood. Also, more research is necessary to confirm previous findings that showed a relation of prenatal smoking with body mass index (BMI) and blood pressure after adjusting for diverse confounding factors. Therefore, in a prospective prenatal cohort study, we investigated the extent to which maternal smoking during pregnancy is associated with longitudinally measured child body mass index (BMI) and blood pressure from birth to mid-childhood. We also investigated the association of maternal smoking during pregnancy with BMI z-scores, fat mass index (FMI) and SBP in mid-childhood itself.

Methods

Study design and population

Study subjects were participants in Project Viva, a prospective cohort study of pregnant women and their offspring, which is described in detail elsewhere.[20] We recruited women who were attending their initial prenatal visit at one of 8 urban and suburban obstetrical offices of a multi-specialty group practice located in eastern Massachusetts between April 1999 and July 2002. All participating women provided written informed consent. Institutional review boards of participating institutions approved the study, and all procedures were in accordance with the ethical standards established by the Declaration of Helsinki.[21]

Of the 2128 mothers with a live birth, we excluded those with missing information on all outcomes (n=364) or maternal smoking (n=9). We followed children with in-person visits just after delivery, in infancy (6-10 months), early childhood (3-5 years), and mid-childhood (7-10 years) in a research office or at home, and with annual mailed questionnaires. We augmented our in-person research measures of weight and length/height by obtaining clinical measures from well-child visits. Our final sample included children with data on maternal smoking and at least one measurement of weight and length/height, or blood pressure measured at one of the in-person visits, leaving a study population of 1755 participants.

Measurements

Exposure

At the first study visit in early pregnancy, women reported on self-completed questionnaires whether they had ever smoked and the quantity. We categorized women who had smoked > 100 cigarettes in their lifetime as ever smokers. Secondly, ever smokers reported whether they had smoked in the 3 months before learning they were pregnant. We classified women who did not smoke during this period as former smokers (quit before pregnancy). Thus, we assigned each woman to 1 of 3 smoking categories: 1. Never smoked 2. Formerly smoked 3. Smoked during pregnancy.

Outcomes

We abstracted birth weights from medical records. At each of the in-person visits at birth, in infancy, in early childhood, and in mid-childhood, trained research assistants measured length at birth and infancy; and height at early and mid-childhood using a calibrated stadiometer (Shorr Productions, Olney, Maryland). We measured weight using a calibrated scale (early childhood: Seca model 881, Seca Corp, Hanover, Maryland;

mid-childhood: Tanita model TBF-300A, Tanita Corporation of America, Inc., Arlington Heights, IL). At interim time points, we also used height and weight measures retrieved from medical records from birth to 7 years of age from the children's various pediatric practices. We calculated age- and sex-specific BMI z-scores using W.H.O. reference data for children aged < 24 months and U.S. national reference data for children aged ≥ 24 months, as recommended by CDC.[22, 23]

Trained research assistants performed whole body DXA scans on the children at the in-person visit in mid-childhood using a Hologic model Discovery A (Hologic, Bedford, MA) checked for quality control daily by scanning a standard synthetic spine to check for machine drift. We used Hologic software QDR version 12.6 for scan analysis. A single trained investigator checked all scans for positioning, movement, and artefacts, and defined body regions for analysis. We calculated fat mass index using the following formula: (mass in kg)/(height in meters)^2.

Using a Dinamap 8100 or Pro-100 (since 2001) oscillometric automated monitors (GE Medical Services, Tampa, FL) trained research assistants recorded child blood pressure after delivery, in infancy, in early childhood and in mid-childhood up to five times at 1-minute intervals. We recorded conditions of measurement including order of readings, cuff size, limb, and child position and activity. Our primary outcome for blood pressure analyses was systolic blood pressure (SBP) because of the validity of its measurement and because SBP is a better predictor of later blood pressure.[24] Research assistants performing the measurements followed standardized techniques and participated in biannual in-service training to ensure measurement validity.

Covariates

Using a combination of questionnaires and interviews, we collected information about maternal race/ethnicity, age, education, parity, household income, environmental smoke exposure during pregnancy (hours/day), pre-pregnancy weight and height, paternal weight and height from which we calculated BMI (kg/m^2), and breastfeeding duration in infancy (months). From the medical records we obtained pregnancy weights and child's sex. We calculated gestational weight gain as the difference between the last clinically recorded weight before delivery and pre-pregnancy weight. In SBP models, we additionally included child height, which was measured using a calibrated stadiometer (Shorr Productions, Olney, MD).

Mediators

We considered birth weight-for-gestational age and gestational age as mediators in the pathway between maternal smoking, BMI and SBP. We calculated sex-specific birth

weight for gestational age z-values based on U.S. natality data.[25] We determined gestational age from the last menstrual period or from the second trimester ultrasound if the two estimates differed by > 10 days. For SBP as an outcome, we additionally considered child BMI, and maternal third trimester SBP, which we obtained from medical records, to be mediators.

Statistical analyses

We explored bivariate associations of maternal smoking with all covariates and outcomes using Chi-square tests and ANOVAs. Using multivariable linear regression models we assessed the association of maternal smoking with BMI z-scores and SBP in mid-childhood. In the multivariable models, we adjusted for potential confounders and in additional models we also adjusted for potential mediators. In the models with SBP as an outcome, we also adjusted for measurement conditions and height. To assess associations between maternal smoking and mid-childhood SBP, we used mixed models that incorporated each of the blood pressure measurements from each child as repeated outcome measures.[26] Mixed models weight subjects based on the number of measurements and their variability. We examined interactions between maternal prenatal smoking and child sex, and between maternal smoking and environmental smoke exposure on both outcomes. All p-values for interactions were > 0.5; therefore we did not do stratified analyses.

We used linear mixed models to assess associations of maternal smoking with longitudinally measured z-scores of BMI and SBP from birth to mid-childhood. The potential confounders included in the linear mixed models were maternal age, education, parity, race/ethnicity, pre-pregnancy BMI, gestational weight gain, paternal BMI, household income, exposure to environmental smoking during pregnancy and breastfeeding duration in infancy. SBP model is additionally adjusted for child sex. In the model with BMI as outcome, the p-value for interaction between maternal smoking and child age was <0.01. For SBP, the corresponding p-value was 0.26.

The proportions of missing values in the covariates ranged from 0 to 17%. Assuming missing at random, we employed multiple imputation to account for the missing data in the covariates. To generate imputation data sets, we used a set of variables chosen from the thousands available in Project Viva to reflect demographic and other factors that we deemed plausibly related to potential missingness mechanisms and to the exposures and outcome measures. We generated 50 imputed data sets. Pooled estimates from these 50 imputed datasets were used to report beta's, and their 95% confidence intervals (CIs). All statistical analyses were performed using SAS version 9.3(Cary, NC).

Results

Among the 1755 mothers, 209 (11.9%) smoked during pregnancy, 344 (19.6%) were former smokers and 1202 (68.5%) never smoked (Table 1). Only 52 of the 209 pregnancy smokers reported smoking throughout pregnancy; the others quit between the initial study visit and delivery. Compared with never smokers mothers who smoked during pregnancy were on average 3.0 years younger, less educated, had a lower household income, were more frequently nulliparous and breastfed for fewer months (Table 1). Also pre-pregnancy BMI was 1.3 kg/m^2 higher, they experienced 1.3 kg more gestational weight gain, and were exposed more frequently to environmental tobacco smoke during pregnancy. In mid-childhood, mean BMI z-scores in the offspring of smoking women was 0.33 units higher than among children of never smoking mothers, their FMI was 0.70 kg/m^2 higher, and their SBP was 2.3 mmHg higher (Table 1).

Table 1. Characteristics of 1755 participating mother-child pairs from Project Viva

Characteristics[b]	Total (N=1755)	Never smoked (N=1202)	Former smoker, quit before pregnancy (N=344)	Smoked in pregnancy (N=209)	P-value[a]
Maternal socio-demographics	Mean (SD) or N (%)				
Age (years)	31.9 (5.2)	32.0 (5.2)	33.4 (4.3)	29.0 (5.8)	<0.001
Education (% college degree)	1164 (66.3)	836 (69.5)	250 (72.7)	78 (37.1)	<0.001
Household income < $70.000/yr	733 (41.8)	495 (41.2)	109 (31.8)	129 (61.8)	<0.001
Parity (% nulliparous)	839 (47.8)	583 (48.5)	144 (41.9)	112 (53.6)	0.02
Race/ethnicity (%)					<0.001
Black	281 (16.0)	229 (19.0)	20 (5.8)	33 (15.6)	
Hispanic	122 (7.0)	84 (7.0)	12 (3.5)	26 (12.6)	
Asian	95 (5.4)	77 (6.4)	9 (2.6)	9 (4.3)	
White	1186 (67.6)	763 (63.5)	294 (85.5)	129 (61.7)	
Other	70 (4.0)	49 (4.1)	9 (2.6)	12 (5.8)	
Infant/child characteristics					
Sex (% female)	848 (48.3)	584 (48.6)	168 (48.8)	96 (45.9)	0.76
Gestational age at birth (weeks)	39.5 (1.8)	39.5 (1.8)	39.7 (1.7)	39.5 (1.8)	0.33
Breastfeeding duration (months)	5.9 (4.6)	6.3 (4.6)	6.0 (4.6)	3.2 (3.7)	<0.001
Parental characteristics					
Pre-pregnancy BMI (kg/m^2)	24.9 (5.6)	24.8 (5.6)	24.5 (4.8)	26.1 (6.4)	0.003
Paternal BMI (kg/m^2)	26.4 (4.1)	26.4 (4.0)	26.5 (3.7)	26.7 (4.9)	0.51
Gestational weight gain (kg)	15.5 (5.7)	15.2 (5.5)	16.0 (5.4)	16.5 (6.7)	0.001
Exposure to environmental smoking during pregnancy (hours/day)	1.09 (1.76)	0.91 (1.40)	0.79 (1.01)	2.66 (3.22)	<0.001
Third trimester systolic blood pressure (mm Hg)	111.3 (8.3)	111.1 (8.3)	111.4 (8.0)	112.3 (8.3)	0.16

Table 1. Characteristics of 1755 participating mother-child pairs from Project Viva (continued)

Characteristics[b]	Total (N=1755)	Never smoked (N=1202)	Former smoker, quit before pregnancy (N=344)	Smoked in pregnancy (N=209)	P-value[a]
Outcomes at birth					
Birth weight gestational age and sex adjusted z-score	0.18 (0.96)	0.17 (0.95)	0.28 (0.95)	0.05 (1.03)	0.15
Length at birth (cm)	49.8 (2.1)	49.7 (2.1)	50.3 (2.1)	49.3 (2.3)	<0.001
BMI (kg/m^2)	14.2 (1.3)	14.2 (1.3)	14.2 (1.3)	14.0 (1.2)	0.14
BMI z-score	0.60 (0.93)	0.63 (0.95)	0.59 (0.92)	0.47 (0.86)	0.22
Systolic blood pressure (mm Hg)	72.5 (9.0)	72.5 (9.2)	72.6 (9.1)	72.0 (7.5)	0.82
Outcomes in infancy					
Height (cm)	66.8 (2.7)	66.7 (2.7)	66.9 (2.7)	67.1 (2.6)	0.24
Weight (kg)	8.1 (1.0)	8.1 (1.0)	8.2 (1.0)	8.3 (1.1)	0.21
BMI (kg/m^2)	18.2 (1.6)	18.2 (1.6)	18.2 (1.7)	18.3 (1.8)	0.76
BMI z-score	0.66 (1.03)	0.65 (1.01)	0.68 (1.03)	0.70 (1.11)	0.81
Systolic blood pressure (mm Hg)	90.4 (13.3)	89.8 (13.0)	91.1 (14.0)	92.8 (13.9)	0.06
Early childhood outcomes					
Height (cm)	97.6 (4.7)	97.5 (4.8)	97.5 (4.7)	98.3 (4.6)	0.16
Weight (kg)	15.8 (2.3)	15.6 (2.2)	15.8 (2.2)	16.4 (2.9)	0.001
BMI (kg/m^2)	16.5 (1.5)	16.4 (1.4)	16.6 (1.5)	17.0 (2.2)	<0.001
BMI z-score	0.46 (1.03)	0.40 (0.98)	0.52 (1.06)	0.71 (1.23)	0.003
Systolic blood pressure (mm Hg)	92.2 (10.7)	91.8 (10.4)	93.2 (11.7)	93.0 (10.9)	0.15
Mid-childhood outcomes					
Height (cm)	128.8 (7.8)	128.6 (7.7)	128.9 (7.6)	130.0 (8.8)	0.24
Weight (kg)	29.0 (7.9)	28.7 (7.7)	28.7 (7.0)	31.7 (10.2)	0.001
BMI (kg/m^2)	17.3 (3.1)	17.1 (3.0)	17.1 (2.8)	18.4 (3.9)	<0.001
BMI z-score	0.39 (1.00)	0.36 (0.98)	0.38 (1.02)	0.69 (1.10)	0.01
Systolic blood pressure (mm Hg)	94.6 (8.8)	94.0 (8.7)	95.7 (8.5)	96.3 (9.0)	0.004
Fat mass index (kg/m^2)	4.44 (1.96)	4.38 (1.95)	4.32 (1.64)	5.08 (2.43)	0.01

[a]P-values from Chi-square test for categorical variables and ANOVA for continuous variables.

[b] Estimates are from multiple imputation to account for missing data for household income (10.6%), breast-feeding duration (16.8%), paternal BMI (5.1%), gestational weight gain (2.2%), exposure to environmental smoking during pregnancy (8.6%), and maternal third trimester SBP (1.8%).

Table 2 shows the association of maternal smoking during pregnancy with BMI and FMI in mid-childhood adjusted for confounders and mediators. For BMI z-score, the effect estimate was 0.11 (95% CI: -0.09, 0.31) after adjustment for all confounders, and for FMI the effect estimate was 0.27 kg/m^2 (95% CI: -0.15, 0.69). For both BMI and FMI, parental BMI and gestational weight gain were responsible for the largest attenuation. Inclusion of the mediators birth weight and gestational age did not further attenuate the effect

Table 2. Associations of maternal smoking during pregnancy with offspring BMI z-score and fat mass index at 7-10 years of age. Data from 1106 (BMI) and 872 (FMI) mother-child pairs participating in Project Viva[a]

	Never smoked	Former smoker	P-value	Smoked during pregnancy	P-value
Models[b]		**Difference in BMI z-score (95% CI)**		**Difference in BMI z-score (95% CI)**	
1	0.0 (ref)	0.02 (-0.13, 0.17)	0.79	0.33 (0.13, 0.53)	<0.001
2	0	0.06 (-0.09, 0.23)	0.43	0.21 (0.00, 0.42)	0.04
3	0	0.03 (-0.11, 0.17)	0.69	0.17 (-0.03, 0.36)	0.09
4	0	0.02 (-0.12, 0.16)	0.75	0.14 (-0.06, 0.33)	0.16
5	0	0.03 (-0.11, 0.16)	0.72	0.11 (-0.09, 0.31)	0.28
6	0	0.03 (-0.11, 0.17)	0.68	0.11 (-0.09, 0.31)	0.27
		Difference in FMI[c] (kg/m^2) (95% CI)		**Difference in FMI[c] (kg/m^2) (95% CI)**	
1	0	-0.02 (-0.34, 0.31)	0.92	0.59 (0.17, 1.01)	0.01
2	0	0.05 (-0.28,0.37)	0.77	0.48 (0.04,0.91)	0.03
3	0	0.00 (-0.30,0.30)	0.99	0.34 (-0.06,0.75)	0.10
4	0	0.00 (-0.31,0.30)	0.98	0.29(-0.12,0.70)	0.16
5	0	0.00 (-0.30,0.30)	0.98	0.27 (-0.15,0.69)	0.21
6	0	-0.01 (-0.31,0.29)	0.97	0.27 (-0.15,0.69)	0.21

CI= confidence interval

[a]Estimates from multivariable linear regression incorporating multiple imputation for missing covariate values.

[b]Model 1: Unadjusted Model 2: Model 1 + additionally adjusted for maternal age, education, household income, parity, and race/ethnicity Model 3: Model 2 + additionally adjusted for maternal pre-pregnancy BMI, gestational weight gain, and paternal BMI Model 4: Model 3 + additionally adjusted for breastfeeding Model 5: Model 4+ additionally adjusted for environmental smoking during pregnancy Model 6: Model 5+ additionally adjusted for the mediators sex-specific birth weight for gestational age z-score and gestational age at birth.

[c] All models for FMI are additionally adjusted for child age at outcome and sex

estimates. Effect estimates of children born to former smoking mothers were more attenuated than the effect estimates of children born to mothers who never smoked.

Compared with children of never smoking mothers, the fully confounder adjusted mean difference in SBP in the offspring of smoking mothers was 1.82 mm Hg (95% CI: 0.02, 3.61), and it was 1.45 mm Hg (95% CI: 0.19, 2.72) among the offspring of mothers who had quit smoking before they learned they were pregnant. After inclusion of the mediating factors maternal third trimester SBP, birth weight for gestational age, gestational age, and mid-childhood BMI z-score the effect estimate for pregnancy smoking was attenuated to 1.35 mm Hg (95% CI: -0.35, 3.06) with the largest reduction by child BMI z-score (Table 3).

Table 3. Associations of maternal smoking during pregnancy and systolic blood pressure at 7-10 years of age. Data form 1102 mother-child pairs participating in Project Viva[a]

	Never smoked	Former smoker	P-value	Smoked during pregnancy	P-value
Models[b]		Difference in SBP (mm Hg) (95% CI)		Difference in SBP (mm Hg) (95% CI)	
1	0.0 (ref)	1.63 (0.41, 2.84)	0.01	1.76 (0.15, 3.37)	0.03
2	0	1.49 (0.22, 2.75)	0.02	1.93 (0.19, 3.66)	0.03
3	0	1.44 (0.18, 2.70)	0.03	1.84 (0.11, 3.58)	0.04
4	0	1.45 (0.19, 2.71)	0.02	1.88 (0.12, 3.63)	0.04
5	0	1.45 (0.19, 2.72)	0.02	1.82 (0.02, 3.61)	0.05
6	0	1.40 (0.15, 2.66)	0.03	1.76 (-0.03, 3.54)	0.05
7	0	1.35 (0.14, 2.56)	0.03	1.43 (-0.29, 3.15)	0.10
8	0	1.28 (0.08, 2.48)	0.04	1.35 (-0.35, 3.06)	0.12

CI= confidence interval

[a]Estimates from multivariable linear regression incorporating multiple imputation for missing covariate values.

[b]Model 1: Adjusted for child age, sex, height and conditions of measurement Model 2: Model 1 + additionally adjusted for maternal age, education, household income, parity, maternal race/ethnicity Model 3: Model 2 + additionally adjusted for maternal pre-pregnancy BMI, gestational weight gain and paternal BMI Model 4: Model 3 + additionally adjusted for breastfeeding Model 5: Model 4+ additionally adjusted for environmental smoking exposure during pregnancy Model 6: Model 5 + additionally adjusted for the mediators sex-specific birth weight for gestational age z-score, gestational age at birth and maternal third trimester SBP Model 7: Model 5 + additionally adjusted for the mediator BMI z-score at age 7 Model 8: fully adjusted model (includes all potential confounders and mediators)

Patterns derived from linear mixed models showed that BMI z-score in the offspring of smoking mothers was similar to that of never smokers directly after birth (0.01 units; 95% CI 0.18,0.16), but increased over time by 0.07 units per year (95% CI 0.03, 0.10). As a result, BMI-z of children of smoking mothers and never smoking mothers crossed just before 2 months of age and widened with increasing age (Figure 1A). Despite interaction terms that did not meet traditional levels of statistical significance, SBP in the offspring of both former smoking mothers and smoking mothers appeared to increase with age faster than that of never smokers (children of smoking mothers: 0.18 mm Hg (95% CI -0.16, 0.52) increase per year; children of former smoking mothers; 0.18 mm Hg (95% CI -0.07, 0.43) increase per year) (Figure 1B). For both the BMI-z and SBP outcomes, we present regression coefficients for age-independent (intercept) and age-dependent differences (interaction maternal smoking * age) in Supplemental Table S1.

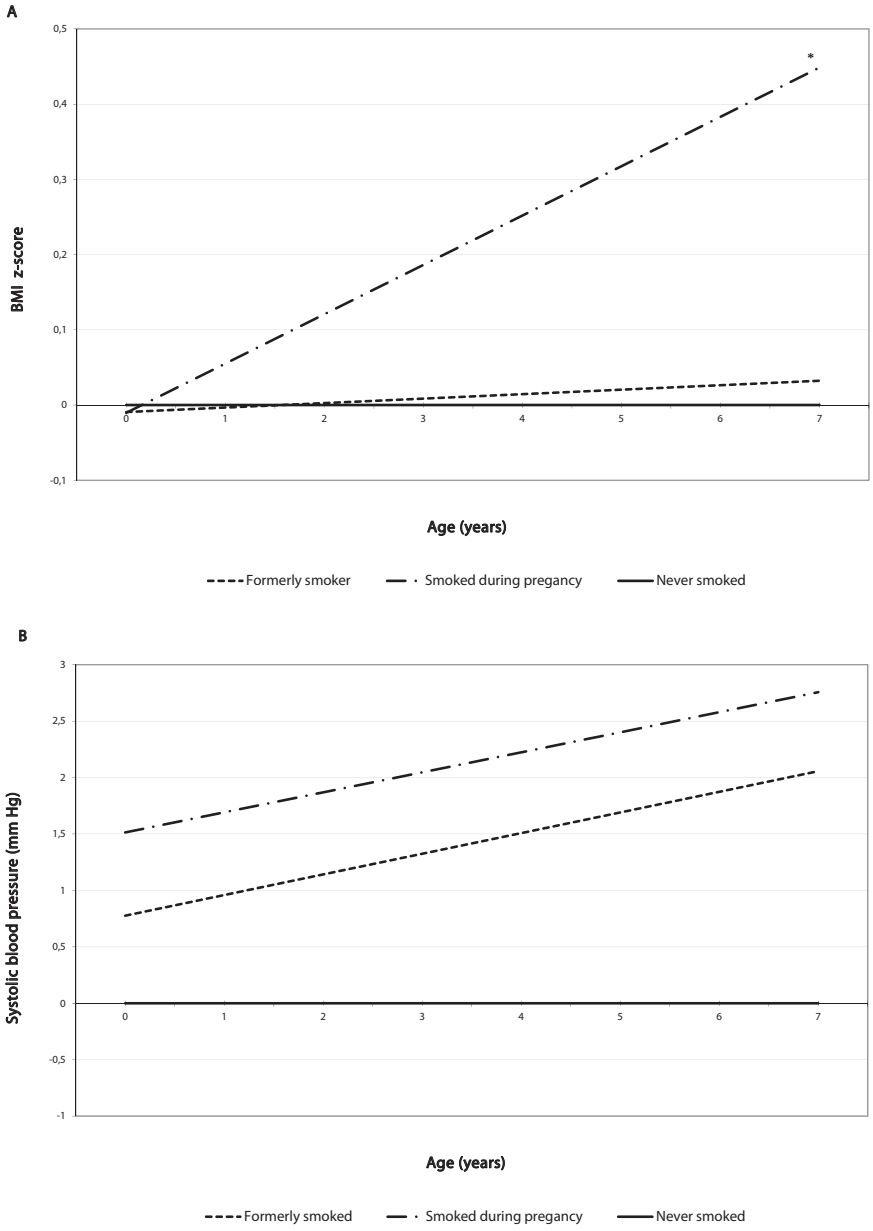

Figure 1. Association between maternal smoking during pregnancy and longitudinally measured offspring BMI z-s-core (A) and systolic blood pressure (B).
Estimates from linear mixed models adjusted for maternal age, education, household income, parity, race/ethnicity, maternal pre-pregnancy BMI, paternal BMI, gestational weight gain, exposure to environmental smoking, breastfeeding and paternal BMI. SBP model additionally adjusted for child sex and height. Based on 3624 measurements for BMI z-score and 3588 measurements for SBP. * P<0.001

Discussion

In this pre-birth cohort study, we showed that children of mothers who smoked during pregnancy had a higher increase in BMI from birth to 7-10 years of age than children of mothers who never smoked. While they started at a similar BMI at birth, the children of smoking mothers developed a higher BMI in early infancy that continued to widen with age. Like BMI and FMI, SBP in 7-10-year-old children of mothers who smoked during pregnancy was higher than SBP in the offspring of mothers who never smoked. In contrast to BMI, children of smokers and former smokers also had somewhat higher SBP starting from birth.

Maternal smoking during pregnancy and childhood BMI and blood pressure

Our finding that maternal smoking was associated with the development of higher BMI in the offspring is in line with previous studies.[8, 27-29] We found that a higher BMI among children of smoking mothers started to develop in early infancy. 'Fetal programming' may explain the association between prenatal smoking and the development of a higher BMI.[4] Prenatal smoking creates an adverse intrauterine environment for the fetus; increased carbon monoxide levels in maternal blood supply may reduce oxygen unloading to the fetus, and nicotine may have vasoconstrictive effects on maternal and uteroplacental vasculature affecting fetal growth [30]. Subsequently, this may lead to structural, physiological and metabolic fetal adaptations which are beneficial in the short term, but may enhance later obesity.[4] According to this hypothesis fetal growth retardation might be an intermediate in the pathway from prenatal smoking to childhood obesity, but in contrast to other studies, in our study it did not seem that prenatal smoking influences later obesity by an effect on fetal growth.[31, 32] Adjustment for birth weight did not attenuate either the observed association in mid-childhood or the increase in BMI from birth to mid-childhood (data not shown). An explanation could be that maternal smoking might exert an effect on childhood obesity independent of its effect on fetal growth retardation. In line with our findings, a previous study found a small intermediate effect of birth weight, and another study found an increased risk of obesity in 4-year-old children exposed to prenatal smoking but born normal weight, supporting that the effect of prenatal smoking on offspring BMI might act, at least partly, independent from fetal growth retardation.[27, 33] Alternative biological pathways of prenatal smoking on obesity, supported by animal studies, may operate through effects on neural regulation, causing increased appetite and reduced physical activity, or through effects on adipose tissue, causing an increase and hypertrophy of fat cells, and prenatal smoking may alter pancreatic development.[16-18] These pathways do not necessarily have to affect fetal growth. One of the issues in the literature on maternal smoking and offspring obesity is whether

the association is causal; confounding by sociodemographic and environmental factors is a leading alternative that cannot be eliminated.

Because few women smoked after the first trimester, our results reflect mainly the effect of early pregnancy smoking. A previous study with trimester-specific smoking data showed that the effect of maternal prenatal smoking on offspring weight depended largely on cigarette smoking during the first trimester, whereas the additional impact of smoking throughout pregnancy was small.[34] Also, other previous research showed that smoking in first trimester was associated with childhood obesity.[29, 32] However, Durmus et al. showed that 4-year old children of mothers who continued smoking during pregnancy, but not mothers who only smoked in the first trimester, had an increased risk of obesity.[27] The discrepancies in the studies can possibly be explained by differences in study populations, as they are conducted in different countries with different ethnic groups. Our findings add to the evidence that the fetus is particularly sensitive to the adverse effects of prenatal smoking in the first trimester of pregnancy and that it is very important to support women to stop smoking already before conception.

Our findings showed that both children of smoking mothers and children of mothers who quit smoking before pregnancy had a higher SBP than children of mothers who never smoked, consistent with our previous analysis in early childhood.[9] Our observation of a higher SBP at birth corroborates previous research,[35] but our study extends the previous literature by showing that SBP appears to diverge as the children grow older. Prenatal smoking exposure may cause a smaller number of nephrons by its adverse effects on fetal growth which may increase blood pressure, but this is unlikely in our study as we did not found an intermediate effect of birth weight.[19, 36] However, there are also studies which suggest that prenatal smoking is associated with higher offspring blood pressure, independent of birth weight, possibly by an adverse effect on kidney development without decreasing kidney weight.[37, 38] Other suggested pathways are that smoking exposure increases the resistance of fetal and uteroplacental vasculature which then may cause elevated blood pressure levels after birth.[39] Future research is necessary to unravel the exact pathways how prenatal smoking may lead to an adverse cardiovascular risk profile in childhood.

It is somewhat surprising that women who quit smoking even before pregnancy had children with higher SBP, but no differences in BMI. A possible explanation might be that periconceptional smoking causes vascular damage leading to altered placentation during conception and subsequently adverse effects on the development of organs such as kidneys. Another possible explanation is that misclassification existed; that is some women who endorsed stopping before pregnancy actually continued smoking

during pregnancy. However, in that case one might have expected similar associations with BMI. A third explanation might be inadequate adjustment for sociodemographic confounding factors. The associations shown are then more likely to be the result of shared familial and environmental factors, rather than having an causal biological mechanism. This is in line with a previous study which found maternal and paternal smoking to have similar effects on offspring blood pressure, and concluded that confounding is the most likely explanation rather than biological effects of cigarette exposure.[15] In our study, however, the effect estimate of maternal prenatal cigarette exposure on offspring SBP did not materially change after adjustment for environmental smoking, which is in part from paternal smoking, suggesting an independent effect of maternal prenatal smoking.

Methodological considerations

The major strength of this study was our use of repeated measurements of height, weight and SBP from birth to 7-10 years of age, which allowed us to investigate development of higher BMI and SBP in a crucial time periods of infancy onwards. Also, we had information about fat mass measured by DXA in mid-childhood, a more sensitive measure than BMI to discriminate between fat mass and lean mass.

Several limitations need to be addressed. We assessed smoking status by questionnaires, which may have resulted in underreporting, particularly in the maternal smoking group. This misclassification would probably have biased our results towards an underestimation of the contribution of smoking to the outcomes reported. In our study population, few mothers continued smoking after the first trimester. Thus, our results mainly reflect the influence of maternal early pregnancy smoking on childhood SBP and BMI, and we could not address trimester-specific effects. Also, we had no repeated measurements of fat mass measured by DXA, which made it impossible to investigate the development of fat mass from birth to mid-childhood. Despite the fact that we incorporated information about a large number of potential confounders, residual confounding due to unmeasured factors might still be an issue. Study participants were relatively highly educated and all resided in eastern Massachusetts, which could limit generalizability to other populations. The effect estimates of BMI and SBP derived from the linear mixed models were somewhat different for 7-year-old children of former and smoking mothers as compared to the effect estimates derived from the linear regression models at this age. This is because linear mixed models are based on multiple measurements and take the correlation between repeated measurements of the same subject into account, while a linear regression only takes into account one measurement of a subject.

Conclusion

Children who were prenatally exposed to maternal cigarette smoking had a higher development of BMI than children who were not prenatally exposed to smoking. Furthermore, they had higher SBP, starting at birth and lasting at least through mid-childhood. Given that overweight/obesity and high blood pressure are main determinants of CVD and track into adulthood, a public health strategy is imperative and should be aimed at women of reproductive age. As smoking rates are particularly high among women from socially disadvantaged subgroups and in low and middle income countries, this strategy should be especially aimed at women from low social classes and among women in low and middle income countries. Randomized controlled trials before and during pregnancy are necessary to further investigate and develop interventions on smoking cessation, as the effects of current interventions are small. Also, evidence-based interventions such as brief cessation counselling should be implemented as standard care to help these women to stop smoking before pregnancy.[40, 41]

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Supplemental Material

Supplemental Table S1. Longitudinal associations between maternal smoking status and BMI (z-score) and SBP (mm Hg) in their offspringa

	Difference in slope of BMI (z-score) with age			
Maternal smoking status	**Intercept (at birth)**	**P-value**	**Slope (increase in z-score/year) (SDS (95% CI))**	**P-value**
Never smoker	-1.22	<0.001	0.0 (ref)	
Former smoker	-0.01	0.87	0.01 (-0.02, 0.03)	0.61
Smoked during pregnancy	-0.01	0.90	0.07 (0.03, 0.10)	<0.001
	Difference in the slope of SBP (mm Hg) with age			
	Intercept (at birth)	**P-value[b]**	**Slope (increase in SBP/year) (mm Hg (95% CI))**	**P-value[b]**
Never smoker	73.28	<0.001	0.0 (ref)	
Former smoker	0.78	0.28	0.18 (-0.07, 0.43)	0.15
Smoked during pregnancy	1.51	0.12	0.18 (-0.16, 0.52)	0.31

[a]Values are based on linear mixed models (based on 15697 measurements for BMI and 3588 measurements for SBP) and reflect the difference in BMI (z-score) and SBP (mm Hg) in children by maternal smoking status compared to the never smoking group, which is the reference group. Models are adjusted for the following confounders: maternal age, education, household income, parity, race/ethnicity, maternal pre-pregnancy BMI, paternal BMI, gestational weight gain, exposure to environmental smoking during pregnancy and breastfeeding. SBP model is additionally adjusted for child sex and height.

11 General discussion

General Discussion

The aim of this thesis was to investigate social inequalities in cardiovascular health among mothers and their offspring, particularly in pregnant women and in early childhood. Furthermore, we examined underlying pathways of these social inequalities. This chapter will first summarize and interpret the main findings of the studies presented in this thesis. Subsequently, relevant methodological considerations for the interpretation of the results will be described, as well as the implications of the findings for public health policy and future research.

Summary and interpretation of main findings

Inequalities in maternal cardiovascular health during pregnancy

Ethnic differences in blood pressure and hypertensive disorders during pregnancy

Several studies showed that a non-native as compared to a native ethnic background is associated with higher blood pressure levels and a higher prevalence of hypertension.[1, 2] These studies, however, were mostly conducted among non-pregnant, older people or in countries with other cultures, life styles and health care systems than in the Netherlands. According to another Dutch study, we found that Dutch women had higher systolic blood pressure (SBP) levels during pregnancy as compared to non-Dutch pregnant women, despite more favorable characteristics such as a higher educational level and a lower BMI.[3] Turkish and Moroccan women had the lowest diastolic blood pressure (DBP) levels and the lowest risk of pregnancy induced hypertension, which was also in line with a previous Dutch study.[3] We also found that Cape Verdean women had the highest risk of developing preeclampsia, which is in line with several previous studies showing that black descent is a risk factor of preeclampsia.[3-5] Thus, although Dutch women had the highest SBP levels, they did not have an increased risk of gestational hypertensive disorders. The ethnic inequalities in blood pressure and gestational hypertensive disorders could not be explained by known risk factors, such as socioeconomic position (SEP) or lifestyle factors. Thus, substantial ethnic differences were shown in blood pressure levels during pregnancy and gestational hypertensive disorders, but in contrast to our expectations, we demonstrated that non-native ethnicity was not a risk factor for higher blood pressure levels during pregnancy, whereas native ethnicity was. Ethnic differences in blood pressure and gestational hypertensive disorders might be explained by a different adaptation of the cardiovascular system during pregnancy across various ethnic groups due to genetic differences, different environmental factors such as dietary patterns or a different biological response to risk factors.[2, 6-8]

Socioeconomic inequalities in cardiovascular health during pregnancy

Socioeconomic inequalities in gestational hypertensive disorders and birth outcomes, as a consequence of poor maternal health during pregnancy, are reported.[9-11] These adverse pregnancy and birth outcomes may lead to a higher risk of developing cardiovascular disease (CVD) in the long term for both mother and child, as it has been found that gestational hypertensive disorders and small for gestational age births are associated with CVD later in life.[12-15] Consequently, socioeconomic inequalities in CVD may originate in prenatal life, and understanding the prenatal mechanisms causing these disparities is necessary in order to develop effective interventions.

Higher placental resistance indices, reflecting impaired placental blood flow, has been associated with preeclampsia, small for gestational age births and preterm births.[16, 17] The exact pathways leading from impaired placental blood flow to adverse maternal and childhood outcomes are largely unknown, but it has been suggested that oxidative stress may play a role.[18] Also, oxidative stress may be involved in long-term childhood cardiovascular outcomes due to impaired fetal-placental blood flow in third trimester.[19] Impaired placental blood flow may underlie the association between SEP and adverse pregnancy and birth outcomes. Indeed, we found that women from low socioeconomic subgroups had higher placental resistance indices, which was mainly explained by smoking during pregnancy. As far as we know, our study was the first to demonstrate socioeconomic inequalities in placental blood flow and its underlying pathways. The results, as many others, emphasize the imperative need to reduce smoking rates among pregnant women. Furthermore, it is well known that gestational diabetes is associated with adverse outcomes in both mother and child. Women who suffered from gestational diabetes are more likely to develop type 2 diabetes later in life.[20, 21] Also, children who are exposed to gestational diabetes in utero have an increased risk to develop childhood obesity, childhood metabolic syndrome and type 2 diabetes in later life.[22-24] Several studies have shown that low SEP is one of the major risk factors of type 2 diabetes,[25] but less is known about the role of SEP in the development of gestational diabetes.[26] We showed that a low SEP is associated with a higher risk of gestational diabetes. Additionally, the role of several life style related factors in the association between SEP and gestational diabetes was assessed. A higher pre-pregnancy BMI among women in lower socioeconomic groups appeared to be the most important contributor to the association between SEP and gestational diabetes.

Pre-pregnancy overweight and obesity have not only been associated with gestational diabetes, but also with gestational hypertensive disorders and delivering large size for gestational age infants.[27, 28] In developed countries, the prevalence of pre-pregnancy overweight and obesity is much higher among women with a low SEP.[29] Less is known

about a socioeconomic gradient in the amount of weight gained during pregnancy. Gestational weight gain is another important aspect of a healthy pregnancy, and may, independently from pre-pregnancy BMI, affect short and long-term maternal and childhood outcomes.[30] Excessive gestational weight gain is associated with large for gestational age births, maternal adiposity after pregnancy and childhood obesity, whereas inadequate gestational weight gain has been linked to small for gestational age births.[31-33] Low SEP was found to be associated with excessive gestational weight gain, as well as with inadequate gestational weight gain. Strikingly, there was no socioeconomic gradient in excessive gestational weight gain among women who were already overweight/obese before pregnancy, suggesting that pre-pregnancy BMI is a stronger determinant of excessive gestational weight gain than SEP. Future research is necessary to unravel the exact pathways leading from a low SEP to excessive and inadequate gestational weight gain. So, in addition to placental vascular dysfunction and gestational diabetes, inadequate and excessive gestational weight gain may contribute to socioeconomic inequalities in maternal and childhood health outcomes. We assume that these adverse pregnancy conditions have independent effects on maternal and childhood outcomes; however, because they may be correlated part of the effects may overlap.

Taken together, a low SEP is associated with an adverse cardiovascular health profile among pregnant women, including a higher prevalence of impaired placental blood flow, gestational diabetes, and excessive gestational weight gain and inadequate gestational weight gain. The most important factors underlying these associations were maternal smoking during pregnancy and a high pre-pregnancy BMI. Based on our findings, it is likely that socioeconomic inequalities in cardiovascular morbidity and mortality in adult life originate in fetal life. Thus, it should be a top priority to reduce overweight/obesity and smoking rates among women of reproductive age prior to conception, especially among women from low socioeconomic groups. Also, more attention should be given to weight control during pregnancy, especially among socially disadvantaged women, in order to reduce both inadequate and excessive gestational weight gain. This will not only have short-term benefits by improving birth and pregnancy outcomes, but based on the concept of in utero programming might also have long-term benefits by reducing the socioeconomic inequalities in adult CVD among either mothers and their offspring.

Inequalities in growth and cardiovascular health in childhood

Socioeconomic inequalities in head circumference

Several aspects of growth are related to a low SEP. Children from families with a low as compared to a high SEP are found to be smaller, both pre- and postnatal.[10, 34] Growth

is an excellent marker of the general health status in childhood as it captures cumulative nutritional status,[35] and has also been associated with adult health outcomes.[36] An important aspect of growth is brain growth. Head circumference correlates highly with brain volume, especially in early childhood.[37] A small head circumference at birth might reflect serious fetal growth restriction in early pregnancy, and has been related to CVD in adult life.[38, 39] Smaller head circumference in early childhood is associated with a lower intelligence quotient, already for values within the normal range.[40, 41] The relationship between SEP and brain size in childhood has been evaluated previously, but underlying pathways are unknown.[40] In line with former studies we showed that a low SEP is associated with a smaller head circumference in early childhood.[40, 42] This effect was most pronounced in the first 6 months of life; no significant effect was found at 11 months of age anymore. The latter findings are remarkable given that socioeconomic inequalities in head circumference in adult life have been recognized.[43] A considerable part of inequalities in head circumference in early childhood could be explained by a lower birth weight and a shorter gestational age among children from families with a low SEP. This adds to the conjecture that improving pregnancy and birth outcomes among low socioeconomic subgroups, including reducing low birth weights and preterm births, may contribute to the reduction of socioeconomic inequalities in multiple aspects of health.

Inequalities in body composition, blood pressure and cardiac structures

Overweight and obesity are strong determinants of CVD and have multiple causes.[44] A disturbance of the energy-balance, with a high caloric intake and low physical activity, is the most important cause. It has been suggested that the inability to taste the bitter substance 6-n-propulthiouracil (PROP) is associated with a greater energy intake, resulting in higher body weights as compared to tasters, but results were inconsistent.[45-48] Inconsistent findings in these observational studies may be related to confounding. We conducted a Mendelian randomization study on PROP taster status and body weight, since there is a strong genetic variant associated with taster status. Our results showed that it is likely that PROP taster status exerts a causal role in the development of overweight/obesity in girls, but not in boys. The reason that PROP taster status was not associated with body composition in boys is unknown. Possibly, there is an interaction between PROP phenotype, gender and diet, whereby the relationship between non-taster status and dietary intake is stronger for girls; i.e. non-taster status and female gender contribute to higher fat intake and ultimately to greater weight gain as compared to boys.[49] Future research is highly recommended to investigate underlying pathways, such as dietary behaviors.

Maternal prenatal smoking has also been associated with higher childhood obesity and higher childhood SBP levels, possibly through the phenomenon of 'programming', wherein environmental exposures occurring at a critical period of development may have lasting effects.[50, 51] However, most studies on prenatal smoking and childhood obesity lack a longitudinal perspective. Furthermore, only few studies investigated the association between prenatal smoking and offspring SBP, and no studies have examined whether prenatal smoking is associated with longitudinal blood pressure patterns in childhood. We showed that children of mothers who smoked during pregnancy as compared to mothers who never smoked had a higher increase in BMI from birth to 7-10 years of age. While they started at a similar BMI at birth, the children of smoking mothers developed a higher BMI in early infancy that continued to widen with age. In addition, we also found that SBP in 7-10-year-old children of mothers who smoked during pregnancy was higher than SBP in the offspring of mothers who never smoked, starting directly after birth.

Overweight and obesity are unequally distributed among the socially disadvantaged.[52, 53] However, it is unknown when the inverse socioeconomic gradient in overweight and obesity arises. In a previous study in the same study cohort there was no socioeconomic gradient observed in overweight among 2-3 year old children.[54] Our study extended the latter study and investigated socioeconomic inequalities in overweight and obesity until school age (6 years of age), where socioeconomic inequalities in overweight are consistently reported.[52] It was found that the inverse socioeconomic gradient in overweight and obesity emerged around the age of 3 to 4.5 years. Furthermore, substantial socioeconomic inequalities in body composition were observed in 6-year old children, which was mainly explained by parental BMI and prenatal smoking. A public health strategy aimed at low socioeconomic families should already start during the preconception period, which is in line with the recommendations based on the other results in this thesis, and should include the prevention of prenatal smoking and promoting healthy life styles among parents-to-be, such as increasing physical activity.

A large part of the effect of overweight and obesity on CVD is mediated through blood pressure, cholesterol and glucose.[55] Blood pressure was found to be the most important mediator, especially for stroke.[55] The effect of adiposity on blood pressure might act through increased peripheral vascular resistance and renal salt retention, the latter due to higher activity of sympathetic nervous system, leptin concentrations and angiotensin-aldosterone activity.[56] Since marked socioeconomic inequalities in overweight and obesity were identified at the age of 6, it was not completely unexpected that we also found socioeconomic inequalities in blood pressure at 6 years of age, with higher childhood BMI being the main contributor, followed by lower physical activity. Although the

majority of studies is consistent with our findings, including a recent Dutch study, not all studies have supported our findings.[57-59] A different study population with children having a different ethnic background may explain the different findings. It is alarming, though, that blood pressure inequalities already exist at this young age, because it is known to track into adulthood where it is likely to contribute to the CVD burden in the lower socioeconomic subgroups.[59-61] Also, a higher fractional shortening was found among children from low socioeconomic families, but no consistent association with other cardiac structures was shown. Although a change finding cannot be excluded, it is also possible that this is a first cardiac adaptation to a higher blood pressure and a higher BMI, but conclusions must be drawn carefully because clinical implications are not yet fully understood.

Methodological considerations

In each study presented in this thesis, specific limitations have been described. This paragraph will describe more general methodological considerations with regard to study design, internal validity of the results, mediation and socioeconomic indicators which should be taken into account when interpreting the results in this thesis.

Study design

Studies described in this thesis were embedded within the Generation R Study, a population-based prospective birth cohort study.[62] Cohort studies have an observational design and compare in a pre-defined population outcomes across groups with and without certain exposures after these are followed-up over time. Many determinants, covariates and outcomes can be studied during follow-up. Disadvantages are that it may require long duration for follow-up before outcomes occur, and different types of bias may threaten the validity of results, including selection bias, information bias and confounding. Perhaps most problematic is inferring causality from observational studies. Since there is no random assignment of the exposure of interest, participants with and without the exposure may differ in many ways (e.g. have different lifestyle behaviors), and therefore residual confounding cannot be excluded.

Internal validity

Internal validity refers to the degree to which a study measures what it is supposed to measure. In general three types of biases can be identified in epidemiological studies: selection bias, information bias and confounding.

Selection bias

Selection biases may arise in cohort studies if the association between the exposure and outcome is different in those who participate in the study and those who were

eligible, but do not participate, either as a result of selective participation at baseline or selective losses to follow-up. Of all eligible children at birth, the initial participation rate in the Generation R Study was 61%. There was some selection towards a relatively higher educated, more native-Dutch and healthier study population. This selection towards a more healthy study population may have led to reduced prevalence rates in the studied outcomes, and consequently reduced statistical power. Indeed, participating women suffered less frequently from pregnancy complications, including gestational hypertensive disorders and gestational diabetes, as what was expected from the general population. However, the interpretation of our findings would only be altered when the association that is studied would be different between non-participants and those who participated in the study. Several studies showed that associations in cohort studies are not strongly influenced by selective non-participation at baseline,[63, 64] and we therefore assume that selection bias due to selective participation at baseline may not be a major threat to the validity of our results. Selective loss to follow-up refers to selective non-response to questionnaires and visits to the research centers. Loss to follow up during pregnancy was low, minimizing selection bias in the studies presented in chapter 2. Loss to follow-up during the first postnatal phase (0-4 years) and the school age period was also low, with general follow-up rates until the age of 6 years exceeding 80%.[62] Mothers from children who did not participate in the postnatal phase and the school age period were less well educated, more often had a non-native ethnic background and had more frequently unhealthy lifestyle habits. Similar to non-participation at baseline, selection bias will only occur when the association between exposure and outcome is different for participants and non-participants. This is difficult to ascertain since we don't have information on the association between exposure and outcome of non-participants. One can argue that selection bias will be small, because the outcome under study is unknown at the start of the study, but this is not always true, because the outcome may be associated with social, educational and health related characteristics of those not participating in the study. However, a recent study showed that even a considerable loss to follow-up did not change the qualitative conclusions about the direction and magnitude of socioeconomic inequalities.[65]

Information bias

Information bias may arise in a study because of misclassification of determinant or outcome measurements. Misclassification can be either differential or non-differential. In non-differential misclassification the exposure does not depend on the outcome, and vice versa. Examples of non-differential misclassification are typing errors during measurement and data entry, which cannot completely be avoided. In general, non-differential misclassification leads to an underestimation of effect measures. Differential misclassification occurs when the misclassification of the exposure is related to the

outcome, and vice versa. Unhealthy life style behaviors, such as weight and smoking, are more likely to be underreported.[66] These unhealthy behaviors are more frequently observed among low educated women, and it has been found that these women are more likely to underreport their unhealthy behavior.[67] This results in an underestimation of the true association. However, self-reported weight before pregnancy and weight measured at intake were strongly correlated ($r > 0.80$). Therefore, we assume that our results might be somewhat attenuated, but that differential misclassification did not occur to a large extent.

Confounding

A confounder is an extraneous variable that is associated with both the exposure and the outcome, which is not on the causal pathway. A confounder may cause a spurious relationship between exposure and outcome if it is not taken into account. Therefore, adjustment for confounding factors is needed to obtain an unbiased estimate of the association of interest. In most studies presented in this thesis, we selected potential confounders on the basis of existing literature. However, residual confounding by unmeasured or poorly measured variables (e.g. due to misclassification) cannot be ruled out completely, as is the case in every observational study. A solution to overcome the problems of residual confounding in observational designs is to use Mendelian Randomization, as we performed in chapter 3.2. Mendelian Randomization is based on Mendel's second law that inheritance of one trait is independent of inheritance of other traits.[68] It uses a genetic variant, which is randomly allocated at conception, as proxy for the exposure, the so-called 'instrumental variable'.[69] Associations between genetic variants and outcome are generally not confounded by behavioral or environmental exposures. This means that Mendelian Randomizaton studies have similar properties to intention to treat analyses in randomized controlled trials.[68] However, three key consumptions need to be fulfilled to obtain valid result from a Mendelian Randomization design. First, there must be no unmeasured common causes of the genetic variant and the outcome. Second, the genetic variant needs to be associated with the exposure. Third, there must be no direct association between the genetic variant and the outcome and every directed pathway from the genetic variant to the outcome passes through the exposure.[69] Not all of these assumptions can be tested statistically and a Mendelian Randomization is nearly always less efficient, i.e., require larger sample sizes, than observational studies because the genotype does not perfectly predict the phenotype. Therefore, it might be useful to conduct a Mendelian Randomization study in addition to an observational design.

Mediation

In the studies conducted on social inequalities, we assumed that the effect on health by ethnic background and SEP act (partly) through more proximal determinants of health, the so called 'mediators'. Rather than hypothesizing a direct causal relationship between the exposure and outcome, mediation hypothesizes that the exposure causes the mediator, which in turn causes the outcome. Identifying mediators is necessary in order to disentangle the different pathways that could explain the effect of the exposure, such as ethnic background and SEP, on the outcomes. By doing this it is possible to define the relationship between an exposure on an outcome (direct effect) and the effect of the exposure that is explained by a given set of mediators (indirect effect). In this thesis we used a traditional approach of mediation analysis by comparing the percentage change between two effect estimates. The use of regression adjustment to assess mediation has been criticized, since the required assumptions on causality cannot be verified and the percentage change can be different for similar absolute changes in effect estimates.[70] In most studies conducted in this thesis we applied bootstrap analyses to assess the significance of the change in effect estimate. However, this does not solve the problem that different percentage changes reflect similar absolute changes in effect estimates. Other methods used for mediation analyses, such as structural equation models, have similar problems with inferring causality.[71] More causal inference of mediation analysis is important in future research, and there are now methods in development trying to overcome the problems of traditional mediation analyses.[72] Since no other method have been shown to be superior yet, it seems justified to use the traditional method of regression adjustment.

Socioeconomic indicators

SEP refers to the 'social and economic factors that influence what positions individuals or groups hold within the structure of society'.[73] SEP is a complex and multifactorial construct, and most frequently used socioeconomic indicators are educational level, income and occupation. These indicators are correlated, but also reflect different dimensions of the broad concept of SEP. In this thesis we mainly used maternal educational level as indicator of SEP. Education is not only an important determinant of employment and economic circumstances which partly captures material resources, but it mainly reflects non-economical social characteristics, such as general and health-related knowledge which influences health behavior.[74] Level of education has been shown to be a good predictor of health, particularly of pregnancy outcomes and cardiovascular health.[75, 76] Also, educational level is relatively easy to measure with questionnaires and response rates tend to be high.[74] Furthermore, education is relatively stable over time. Although educational level is a useful indicator of SEP, it may not entirely capture the material and financial aspects of SEP. Therefore, in this thesis we repeated most of our analyses using

household income level as determinant, and we found comparable results, but regularly somewhat attenuated.

Implications

Public health policy

Socioeconomic inequalities in cardiovascular morbidity and mortality are a major public health concern. Probably the best way of reducing socioeconomic inequalities in CVD is to improve someone's SEP, but this is very difficult to achieve. There is however, some evidence showing that this is not completely impossible. A randomized control trial among three- and four-year old African-American children living in poverty showed that children who received extra daily classes and weekly home visits had better academic performance, higher SEP, and lower lifetime rates of crime and welfare dependence at age 40 than children who did not.[77] Another study from the U.S. among low-income minority children showed that those who received preschool education had higher rates of educational attainment and health insurance, lower rates of severe criminal behaviors and lower rates of depressive symptom at age 24 than children who did not receive preschool education.[78] In Rotterdam, intensive preschool education programs are now developed and implemented for 2-4 year old toddlers with a developmental delay. These programs are particularly focused on language and social development, and include education focused on language and social behavior, education given by higher educated teachers, and improving the involvement of parents in the preschool education.[79] Whether these programs actually improve children's SEP need to be evaluated in the future.

It remains very challenging, though, to change one's SEP, and therefore it is important to additionally focus intervention strategies on more proximal, modifiable risk factors underlying socioeconomic inequalities in CVD. This requires knowledge of the underlying pathways leading to these inequalities. The findings from the studies presented in this thesis clearly show that reducing socioeconomic inequalities in CVD requires a life course approach, and should already start in the preconception period; the two major contributors to the socioeconomic inequalities investigated in this thesis were pre-pregnancy BMI and prenatal smoking. Promoting healthy lifestyles prior to conception will not only improve the health of parents-to-be, but most importantly will also positively affect their offspring's health in different periods in life; this results in a healthy intrauterine environment, and creates a healthy eating and activity environment for their future children. Consequently, this will give them a similar prospect of a healthy life from the start of their lives onwards.

To reduce socioeconomic inequalities in cardiovascular health it is important to implement structured preconception care, to increase public awareness, and particularly to reach the most disadvantaged population groups. In 2009, a pilot study on preconception care was initiated in Rotterdam, the Netherlands, with special attention for vulnerable population groups.[80] The study is now being implemented nationwide to improve perinatal health outcomes especially among the high risk population. During preconception care evidence-based interventions in relation to weight loss and smoking cessation should be applied to ensure the most optimal and effective interventions. For pregnant smokers, behavioral counseling is recommended, increasing quit rates by 6%-10% over usual care.[81] Nicotine-replacement therapy has also been found to increase quit rates among non-pregnant smokers. However, nicotine-replacement therapy was not efficacious for smoking cessation during pregnancy, but adherence to therapy among pregnant women was low.[82] Furthermore, with respect to pre-pregnancy BMI, reducing overweight and obesity will not only improve pregnancy outcomes, but it will also increase a woman's chance of conception.[83] It is shown that life style interventions targeting both dietary intake and physical activity are preferred to achieve weight reduction and to support maintenance of weight loss, but effects are small.[84] Also, interventions promoting diet and exercise will help women lose weight after childbirth, since the postpartum period might be related to an increase in food intake and a decrease in physical activity which makes a woman prone for developing overweight.[85] It is important to recognize that prevention and intervention strategies should not stop after the preconception period but need to continue along the life course, since risk factors for CVDs accumulate during life. Consequently, social inequalities in CVD need to be tackled along the life course, starting in the preconception period. Also, midwives, obstetricians, general practitioners and pediatricians should be aware of the fact that low SEP should be recognized as a major risk factor for poor maternal and childhood health outcomes, partly because a lot of risk factors are likely to cluster in these patients, and therefore socioeconomic factors should be assessed during individual consultations.[86]

Furthermore, appropriate health policies can also help people make more healthy choices in relation to behavioral risk factors, including smoking and diet, through for example action on tobacco advertising and pricing, and agreements with industry to reduce trans fats and salt in processed food.[87] However, research is conflicting about the effectivity of such policies in reducing socioeconomic inequalities, because they may be less effective in lower socioeconomic groups. The type of policy may determine its effectivity among low socioeconomic groups.[88, 89] Tobacco control policies have shown that increasing the price of cigarettes resulted in a larger decrease in smoking prevalence among lower SEP groups, while mass media campaigns were more effective among higher SEP groups.[90-92] More research is needed to identify and develop health policies

that are effective in lower socioeconomic groups, specifically among pregnant women in lower SEP groups, to close the social gap in cardiovascular health.

Directions for future research

To strengthen conclusion in this thesis, some of our findings need confirmation in future research. These include the finding that Dutch women have higher systolic blood pressure levels during pregnancy than non-Dutch women, that low educated women have higher placental resistance indices, and that babies born from low educated women have a smaller head circumference in early childhood. Furthermore, we were unable to investigate whether the investigated inequalities actually contribute to social inequalities in CVD in adult life. It is therefore important to follow-up this cohort into adulthood. Additionally, more explanatory factors in the association between social disadvantage and cardiovascular health need to be investigated, as the associations in the studies presented in this thesis could not entirely be explained by the included factors. In particular it is interesting to investigate the contribution of extensive psychosocial factors, such as low self-esteem, lack of social support and social exclusion, since these have been mentioned to play a crucial role in the emergence of social inequalities in CVD.[93, 94] In addition, objective measures of stress, such as cortisol levels in hair, can also be used in future research.[95] Physical activity and sedentary behaviors are other important explanatory factors that require further investigation, as these were measured with questionnaires only in children. Future studies are necessary to investigate the role of physical activity and sedentary behaviors in the association between low SEP and maternal cardiovascular health during pregnancy. Accelerometers, instead of questionnaires, can be used to objectively measure physical activity among both mothers and children. Furthermore, epigenetic mechanisms are being suggested as underlying biological mechanism leading from low SEP to CVDs, but research is lacking.[96] Epigenetics refer to chemical modifications to the genome that occur in response to adverse early life exposures. Epigenetic mechanisms regulate gene activity but do not involve a change in DNA nucleotide sequence; DNA methylation is the most studied epigenetic mechanism.[97] Currently it is unknown whether epigenetic mechanisms contribute to the explanation of low SEP and CVD, although there is some recent evidence that childhood SEP is linked to DNA methylation profiles in adult blood.[96] SEP is a multidimensional construct comprising diverse socioeconomic indicators, including income, education, occupation, neighborhood socioeconomic conditions and economic resources. Different socioeconomic indicators could affect health at different times in the life course, and influence health through different pathways.[98] Therefore, it is important to measure and include a large array of different socioeconomic indicators in future studies.

Causality is mainly inferred from randomized control trials, but this is not feasible for the topics studied in this thesis. As previously discussed Mendelian Randomization is a method to establish causality in observational studies, but it is probably impossible to find a valid genetic instrument as proxy for the complex construct of SEP. However, most of the effects of SEP act through more proximal determinants of SEP which makes it interesting, though, to perform Mendelian Randomization studies to examine the causal role of these more proximal determinants such as smoking and maternal BMI on cardiovascular outcomes in both mother and child, including placental resistance indices and childhood BMI.[99, 100] Since Mendelian Randomization studies need very large sample sizes, multiple cohort studies need to collaborate. Finally, findings in this thesis emphasize the need to perform intervention trials in the preconception period focused on smoking cessation and weight reduction, as previous trials have shown only small effects of their interventions.

General conclusions

Several conclusions can be drawn from the findings in this thesis. Substantial ethnic differences in blood pressure levels and gestational hypertensive disorders were found. Also, women with a low SEP had a more adverse cardiovascular risk profile during pregnancy than their high socioeconomic counterparts, including impaired placental blood flow, gestational diabetes and inadequate and excessive gestational weight gain. Pregnancy might serve as a stress test and uncover women with an increased risk of CVD in future, which makes it essential to follow-up these mothers for several years. Furthermore, an adverse cardiovascular risk profile during pregnancy may affect the developing fetus and increase the child's cardiovascular risk later in life. Hence, pregnancy might be the period in which socioeconomic inequalities in CVD originate. Socioeconomic inequalities in growth and cardiovascular risk factors were also found to exist already in early childhood, including a smaller head circumference, a higher BMI and higher blood pressure levels among children from low socioeconomic families. Main modifiable contributors to the socioeconomic inequalities in either pregnancy complications and childhood outcomes were prenatal smoking and pre-pregnancy BMI. Our studies highlight the importance of tackling social inequalities during the life course which should already start in the preconception period. Future studies are necessary to confirm our findings, to unravel the yet unidentified underlying mechanisms and to follow-up the children into adulthood to investigate the actual contribution of investigated inequalities in cardiovascular risk factors to CVDs in adulthood.

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12 Summary/Samenvatting

Summary

Chapter 1 Cardiovascular disease (CVD) is the leading cause of disability and mortality worldwide and is a major public health concern. Risk of CVD is not equally distributed among populations, but strongly associated with social disadvantage, as indicated by a low socioeconomic position (SEP) or non-native ethnicity. Women who suffered from pregnancy complications, such as gestational hypertensive disorders, obesity and gestational diabetes, are more likely to develop CVD on the long term. Children born from these mothers also have an increased risk of adverse health outcomes both on the short and long term, including small for gestational age birth, preterm birth, macrosomia, childhood obesity and CVD in adulthood. Based on these findings, it has been hypothesized that exposures to adverse circumstances in fetal and early postnatal life may lead to persistent metabolic, physiological and structural adaptations, enhancing short-term survival, and as such the 'programming' of adult CVD. The latter implies that social inequalities in CVD may originate in prenatal life and emphasizes the importance of studying social inequalities in this particular period. Given the evidence of CVD as disease that develops over the life course it is also important to continue the study on social inequalities in CVD and its risk factors in postnatal life. The main purpose of this thesis was to improve the understanding of the pre-, peri- and postnatal development of socioeconomic and ethnic inequalities in cardiovascular risk factors. The following specific research questions were formulated:

1. Is there an association between ethnic background and blood pressure levels in pregnancy and in gestational hypertensive disorders, and which factors explain this association?
2. Is there an association between socioeconomic position and maternal cardiovascular health during pregnancy, and which factors explain this association?
3. Is maternal socioeconomic position associated with the child's head growth in the first year of life, and which factors explain this association?

4a. Are there socioeconomic inequalities in child's body composition at the age of 6, and which factors explain this association?

b. At what age does the inverse socioeconomic gradient in body mass index emerge?

5. Is there an association of socioeconomic position with childhood blood pressure and cardiac structures at the age of 6, and which factors explain this relation?
6. Is there a causal relation between the ability to taste the bitter substance 6-n-propylthiouracil (PROP) and child's body composition at the age of 6?

7a. Is maternal smoking during pregnancy associated with the development of child's body mass index and systolic blood pressure in the first 7-10 years of life?

b. Is there an association of maternal smoking during pregnancy with body mass index, fat mass index and systolic blood pressure at the age of 7-10?

The studies presented in this thesis were embedded in the Generation R Study, Rotterdam, The Netherlands and in Project Viva, Massachusetts, U.S.A., two prospective cohort studies from early pregnancy onwards. The Generation R Study has been designed to identify early, environmental and genetic causes of normal and abnormal growth, development and health during fetal life, childhood and adulthood. The goal of Project Viva is to find ways to improve health of mothers and their children by looking at the effects of mother's diet as well as other factors during pregnancy and after birth.

Chapter 2 shows that Dutch pregnant women had a higher systolic blood pressure than non-Dutch pregnant women despite more favorable characteristics such as a higher educational level and a lower body mass index. Turkish and Moroccan women had the lowest diastolic blood pressure and the lowest risk of gestational hypertension. Cape Verdean women had the highest risk of developing preeclampsia. The ethnic inequalities in blood pressure and gestational hypertensive disorders could not be explained by known risk factors such as

SEP or lifestyle factors.

Chapter 3 describes that women with a low as compared to a high educational level had higher placental resistance indices, reflecting impaired placental blood flow in both the second and third trimester of pregnancy. This was mainly explained by higher smoking rates among low educated women. They also had an increased risk of tracking of placental resistance indices from the second to third trimester which has been associated with the risk of adverse pregnancy outcomes.

In **Chapter 4** the association between maternal educational level and gestational diabetes mellitus was investigated. Mothers with a low educational level had a three times higher risk of developing gestational diabetes mellitus than mothers with a high educational level. The mediating effects of alcohol use and BMI explained a substantial part of the increased risk, whereas smoking, diet and stress did not contribute to the increased risk.

Chapter 5 shows that low educational level is a risk factor for excessive gestational weight gain among women with a normal pre-pregnancy body mass index. No association was observed between educational level and excessive gestational weight gain among overweight/obese women. High educated overweight/obese women had a higher risk of excessive gestational weight gain than low educated normal weight women. Furthermore, low as compared to high education was associated with an increased risk for inadequate gestational weight gain. More detailed analyses showed that women in the lowest educational subgroup had a higher gestational weight gain in early pregnancy, but not in later pregnancy.

In **Chapter 6** the relation of the mother's educational level with growth of the child's head in the first year of life was examined. It was shown that children of mothers with a low educational level had smaller head circumferences in the first 6 months than children

of high educated mothers. The factors that predominantly explained the socioeconomic inequalities in head circumference were found to be birth weight, gestational age and the child's length and weight. Our result were further evidence that SEP affects different aspects of growth, as it is already known that SEP influences height and birth weight.

Chapter 7 provides insight in the emergence of the inverse SEP gradient in overweight and obesity. We found that the inverse SEP gradient in body composition emerged in the preschool period around 3 to 4.5 years of age. At 6 years of age, marked socioeconomic inequalities were observed in children's body mass index and total body fat mass. Parental body mass index and prenatal smoking were the most important explanatory factors, which highlights the importance of public health strategies to focus on promoting healthy life styles and preventing smoking among parents-to be.

Chapter 8 shows that 6-year-old children born to mothers with a low educational level had already higher systolic and diastolic blood pressure levels than children born to high educated mothers, which was mainly explained by socioeconomic inequalities in the child's body mass index and physical activity. Furthermore, a positive association was found between maternal education and fractional shortening, which was explained by higher blood pressure levels and a higher BMI among children of lower educated mothers. No evidence was found for socioeconomic inequalities in carotid- femoral pulse wave velocity, aortic root diameter, left atrial diameter, and left ventricular mass at the age of 6 years.

In **Chapter 9** the causal relation between the ability to taste the bitter substance 6-propylthiouracil was assessed by using a Mendelian Randomization design. This study suggests that PROP taster status is causally related to body composition measures in girls. Non-taster girls had a higher BMI and a higher body fat mass as compared to taster girls. For boys, no differences were observed in BMI and body fat mass between PROP tasters and non-tasters.

Chapter 10 shows that children of mothers who smoked during pregnancy had a higher increase in BMI from birth to 7-10 years of age than children of mothers who never smoked. SBP in 7-10-year-old children of mothers who smoked during pregnancy was higher than SBP in the offspring of mothers who never smoked. Additionally, children of smokers and former smokers had somewhat higher SBP levels starting from birth.

Finally, **Chapter 11** provides a general discussion in which the studies presented in this thesis are described in a broader context, and implications for public health policy and future research are discussed.

Several conclusions can be drawn from the findings in this thesis. Substantial ethnic differences in blood pressure levels and gestational hypertensive disorders were found. Also, women with a low SEP had a more adverse cardiovascular risk profile during pregnancy than their high socioeconomic counterparts, including impaired placental blood flow, gestational diabetes and inadequate and excessive gestational weight gain. An

adverse cardiovascular risk profile during pregnancy may affect the developing fetus and increase its cardiovascular risk later in life. Hence, pregnancy might be the period in which socioeconomic inequalities in cardiovascular diseases originate. Socioeconomic inequalities in growth and cardiovascular risk factors were also found to exist in early childhood, including a smaller head circumference, a higher BMI and higher blood pressure levels among children from low socioeconomic families. The main modifiable contributors to the socioeconomic inequalities in either pregnancy complications and childhood outcomes were prenatal smoking and pre-pregnancy BMI. Our studies highlight the importance of tackling social inequalities during the life course which should already start in the preconception period.

Samenvatting

Hoofdstuk 1 geeft een algemene introductie en achtergrondinformatie en beschrijft de doelstellingen en opzet van dit proefschrift. Cardiovasculaire ziekten (CVZ) zijn wereldwijd de meest voorkomende oorzaak van morbiditeit en mortaliteit en dus een groot probleem voor de volksgezondheid. Het risico op CVZ is niet gelijk verdeeld onder de bevolking, maar sterk gerelateerd aan sociale achterstand, hetgeen verwijst naar een lage sociaaleconomische positie (SEP) of een niet-Nederlandse etniciteit. Vrouwen met zwangerschapscomplicaties, zoals hypertensieve aandoeningen, obesitas en zwangerschapsdiabetes hebben een grote kans op het ontwikkelen van CVZ op de lange termijn. Kinderen van deze moeders hebben ook een verhoogd risico op ongunstige gezondheidsuitkomsten, zowel op de korte als op lange termijn, waaronder een te laag geboortegewicht voor de zwangerschapsduur, vroeggeboorte, macrosomie, obesitas en CVZ op de volwassen leeftijd. Gebaseerd op deze bevindingen is de hypothese ontstaan dat blootstelling aan ongunstige factoren in het foetale en het vroege postnatale leven kan leiden tot blijvende metabole, fysiologische en structurele aanpassingen, deze aanpassingen zijn gunstig voor overleving op de korte termijn, maar vergroten het risico op cardiovasculaire aandoeningen op latere leeftijd. Dit impliceert dat sociale ongelijkheden in CVZ al kunnen ontstaan in de prenatale fase, hetgeen het belang benadrukt om sociale ongelijkheden al in deze periode te bestuderen. Daarnaast is het eveneens belangrijk om sociale ongelijkheden in cardiovasculaire aandoeningen en cardiovasculaire risicofactoren te bestuderen in het postnatale leven, gezien het bewijs dat cardiovasculaire aandoeningen zich ontwikkelen in de loop van het gehele leven. Het doel van dit proefschrift was om bij te dragen aan de kennis over de pre-, peri- en postnatale ontwikkeling van sociaaleconomische en etnische ongelijkheden in cardiovasculaire risicofactoren. De volgende specifieke onderzoeksvragen werden geformuleerd:

1. Is er een associatie tussen etniciteit, bloeddruk tijdens de zwangerschap en hypertensieve zwangerschapscomplicaties en welke factoren verklaren deze associatie?
2. Is er een associatie tussen sociaaleconomische positie en cardiovasculaire gezondheid van de moeder tijdens de zwangerschap en welke factoren verklaren deze associatie?
3. Is sociaaleconomische positie van de moeder geassocieerd met de groei van het hoofd van haar kind in het eerste levensjaar en welke factoren verklaren deze associatie?

4a. Zijn er sociaaleconomische verschillen in de lichaamssamenstelling van een 6 jarig kind en welke factoren verklaren deze associatie?

b. Op welke leeftijd ontstaat de negatieve sociaaleconomische gradiënt in body mass index?

5. Is er een associatie tussen sociaaleconomische positie en bloeddruk en cardiovasculaire structuren op 6 jarige leeftijd en welke factoren verklaren deze associatie?
6. Is er een causaal verband tussen het vermogen om de bittere stof 6-n-propylthiouracil (PROP) te proeven en lichaamssamenstelling op 6 jarige leeftijd?

7a. Is roken van de moeder tijdens de zwangerschap geassocieerd met de ontwikkeling van body mass index en bloeddruk van haar kind in de eerste 7-10 jaar?

b. Is roken van de moeder tijdens de zwangerschap geassocieerd met body mass index en bloeddruk van haar 7-10 jarige kind?

De studies die worden gepresenteerd in dit proefschrift waren ingebed in het Generation R onderzoek te Rotterdam, Nederland en Project Viva, Massachusetts, U.S.A., twee prospectieve cohort onderzoeken. Het Generation R onderzoek heeft tot doel factoren te onderzoeken die van invloed zijn op groei, ontwikkeling en gezondheid in het foetale leven, op de kinderleeftijd en op de volwassen leeftijd. Het doel van Project Viva is de gezondheid van moeders en hun kinderen te verbeteren door het effect van dieet van de moeder te onderzoeken en door andere factoren te bestuderen tijdens de zwangerschap en na de geboorte.

Hoofdstuk 2 laat zien dat Nederlandse zwangere vrouwen een hogere systolische bloeddruk hadden dan niet-Nederlandse vrouwen, ondanks gunstigere karakteristieken zoals een hoger opleidingsniveau en een lagere body mass index. Turkse en Marokkaanse vrouwen hadden de laagste diastolische bloeddruk en het laagste risico op het ontwikkelen van zwangerschapshypertensie. Kaapverdiaanse vrouwen hadden het grootste risico op de ontwikkeling van preeclampsie. De etnische ongelijkheden in bloeddruk en hypertensieve zwangerschapscomplicaties kon niet worden verklaard door bekende risicofactoren zoals SEP en leefstijl factoren.

Hoofdstuk 3 beschrijft dat vrouwen met een laag opleidingsniveau in het tweede en derde trimester van de zwangerschap hogere placentaire resistance indices hadden dan vrouwen met een hoog opleidingsniveau, duidend op een abnormale placentaire doorbloeding. Dit kon grotendeels worden verklaard doordat laag opgeleide vrouwen vaker rookten. Ook hadden deze vrouwen een groter risico op tracking van de placentaire resistance indices in het derde tertiel van het tweede naar derde trimester, hetgeen is geassocieerd met het risico op ongunstige zwangerschapsuitkomsten.

In **hoofdstuk 4** werd de associatie onderzocht tussen opleidingsniveau van de moeder en zwangerschapsdiabetes. Een laag opleidingsniveau was geassocieerd met een driemaal hoger risico op zwangerschapsdiabetes. De mediërende effecten van alcohol gebruik en body mass index verklaarden een groot deel van het verhoogde risico, terwijl roken, dieet en stress niet bijdroegen aan de verklaring.

Hoofdstuk 5 laat zien dat een laag opleidingsniveau van de moeder een risicofactor was voor excessieve gewichtstoename tijdens de zwangerschap bij vrouwen die een normale body mass index hebben voor de zwangerschap. Geen associatie werd gevon-

den tussen opleidingsniveau van de moeder en excessieve gewichtstoename tijdens de zwangerschap bij vrouwen die overgewicht/obesitas hadden voor de zwangerschap. Hoog opgeleide vrouwen met overgewicht/obesitas hadden een hoger risico op een excessieve gewichtstoename tijdens de zwangerschap dan laag opgeleide vrouwen met een normaal gewicht voor de zwangerschap. Ook werd gevonden dat een laag opleidingsniveau een risico was voor het ontwikkelen van inadequate gewichtstoename tijdens de zwangerschap. Bij het nader bestuderen van deze associaties werd gevonden dat vrouwen met het laagste opleidingsniveau een grotere gewichtstoename hadden tijdens de vroege zwangerschap, maar niet later in de zwangerschap.

In **hoofdstuk 6** werd de relatie tussen opleidingsniveau van de moeder en de groei van het hoofd van haar kind in het eerste levensjaar onderzocht. Gevonden werd dat kinderen van moeders met een laag opleidingsniveau een kleinere hoofdomtrek hadden in de eerste 6 maanden van hun leven dan kinderen van hoog opgeleide moeders. De factoren die deze sociaaleconomische ongelijkheden in hoofdomtrek het meest verklaarden waren geboortegewicht, zwangerschapsduur en lengte en gewicht van het kind. Onze resultaten zijn aanvullend bewijs dat SEP meerdere aspecten van groei beïnvloedt, aangezien het al bekend is dat SEP lengte en geboortegewicht beïnvloedt.

Hoofdstuk 7 geeft inzicht in het ontstaan van de negatieve SEP gradiënt in overgewicht en obesitas. We vonden dat de negatieve SEP gradiënt in lichaamssamenstelling ontstond op de voorschoolse leeftijd rond de leeftijd van 3 tot 4.5 jaar. Op de leeftijd van 6 jaar werden er aanzienlijke sociaaleconomische ongelijkheden gevonden in body mass index en totale vetmassa. Body mass index van de ouders en roken tijdens de zwangerschap waren de belangrijkste verklarende factoren. Dit benadrukt het belang om volksgezondheidsstrategieën te richten op het bevorderen van een gezonde leefstijl en de preventie van roken bij aanstaande ouders.

In **hoofdstuk 8** werd gevonden dat al op de leeftijd van 6 jaar kinderen van moeders met een laag opleidingsniveau een hogere systolische en diastolische bloeddruk hadden, hetgeen met name werd verklaard door sociaaleconomische ongelijkheden in body mass index en fysieke activiteit. Ook werd een positieve associatie gevonden tussen opleidingsniveau van de moeder en fractional shortening, wat werd verklaard door een hogere bloeddruk en een hogere body mass index bij kinderen van laag opgeleide moeders. Er waren geen aanwijzing voor sociaaleconomische ongelijkheden in arteriële vaatwandstijfheid, diameter van de aortabasis, linker atrium diameter en linker ventrikel massa.

In **hoofdstuk 9** werd de causale relatie tussen het vermogen om de bittere stof PROP te proeven en de lichaamssamenstelling op 6 jarige leeftijd onderzocht door het gebruik van een Mendelian Randomization design. Onze studie suggereert dat het vermogen om PROP te proeven causaal gerelateerd is aan de lichaamssamenstelling bij meisjes. Meisjes die PROP niet konden proeven hadden een hogere body mass index en

totale vetmassa dan meisjes die PROP wel konden proeven. Bij jongens werden er geen verschillen gevonden tussen proevers en niet proevers van PROP in body mass index en totale vetmassa.

Hoofdstuk 10 laat zien dat kinderen van moeders die hadden gerookt tijdens de zwangerschap een grotere toename van body mass index hadden vanaf de geboorte tot de leeftijd van 7-10 jaar dan kinderen van moeders die niet hadden gerookt. De systolische bloeddruk van 7-10 jarige kinderen van rokende moeders was hoger dan de systolische bloeddruk van kinderen van niet-rokende moeders. Ook hadden de kinderen van moeders die gerookt hadden tot hun zwangerschap bekend was een iets hogere systolische bloeddruk.

Hoofdstuk 11 bestaat uit een algemene discussie waarin de studies in dit proefschrift in een bredere context worden geplaatst, implicaties voor volksgezondheidsbeleid worden besproken en suggesties voor toekomstig onderzoek worden gedaan.

Verschillende conclusies kunnen worden getrokken uit de bevindingen gepresenteerd in dit proefschrift. Substantiële etnische verschillen in bloeddruk tijdens de zwangerschap en hypertensieve zwangerschapscomplicaties werden gevonden. Ook werd gevonden dat vrouwen met een lage SEP een ongunstiger cardiovasculair risicoprofiel hebben tijdens de zwangerschap dan vrouwen met een hoge SEP, waaronder een abnormale placentaire doorbloeding, zwangerschapsdiabetes en een inadequate en excessieve gewichtstoename tijdens de zwangerschap. Een ongunstig cardiovasculair risicoprofiel tijdens de zwangerschap kan de ontwikkelende foetus ongunstig beïnvloeden, evenals zijn/haar cardiovasculaire risico later in het leven. Dus de zwangerschap is mogelijk de periode waarin sociaaleconomische ongelijkheden in CVZ ontstaan. Sociaaleconomische verschillen in groei en cardiovasculaire risicofactoren werden ook gevonden op de vroege kinderleeftijd, waaronder een kleinere hoofdomtrek, een hogere body mass index en een hogere bloeddruk bij kinderen uit lage sociaaleconomische families. Belangrijke bijdragende en modificeerbare factoren van sociaaleconomische ongelijkheden in zowel zwangerschapscomplicaties als kinduitkomsten waren roken tijdens de zwangerschap en body mass index. De studies in dit proefschrift benadrukken het belang om sociale ongelijkheden te bestrijden gedurende de levensloop en hiermee zo vroeg mogelijk te beginnen, bij voorkeur al in de preconceptionele periode.

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Authors' affiliations
Publication list
About the author
PhD portfolio
Dankwoord

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Gisthi O, Gaillard R, Felix JF, **Bouthoorn SH**, Steegers EAP, Raat H, Hofman A, Duijts L, Franco OH, Jaddoe VWV. Early origins of ethnic disparities in cardiovascular risk factors. *Submitted.*

About the author

Selma Bouthoorn was born on the 15th of October 1983 in Utrecht, the Netherlands. She graduated from secondary school at the Kalsbeek College in Woerden in 2002. In 2003, she started her medical training at the Leiden University, Leiden, the Netherlands. She obtained her medical degree in 2009 (cum laude). Subsequently, she started working as a resident in pediatrics (ANIOS) at the Reinier de Graaf Gasthuis in Delft (dr. N. van der Lely). She combined this work with research on 'alcohol intoxication among adolescents', which resulted in her first scientific publications. In 2011, she started her PhD project at the Generation R Study Group and the department of Public Health at Erasmus Medical Center, Rotterdam, the Netherlands under supervision of prof. dr. Hein Raat and dr. Frank J. van Lenthe. The results of this research are presented in this thesis. In 2013, she obtained a Master of Science degree in Health Sciences, specialization Clinical Epidemiology, from the Netherlands Institute of Health Sciences. In the fall of 2013, she spent time working with another birth cohort (Project Viva) at Harvard Medical School in Boston, USA. From December 2014 onwards, Selma works as a resident in psychiatry (ANIOS) at SymforaMeander in Amersfoort.

PhD portfolio

Summary of PhD training and teaching activities

Name PhD student:	Selma H. Bouthoorn
Erasmus MC Department:	Public Health
Research School:	Netherlands Institute for Health Sciences
PhD period:	2011 - 2015
Promotor:	Prof. dr. H. Raat
Copromotor:	Dr. F.J. van Lenthe

	Year	Workload (ECTS)
1. PhD training		
Master's degree Health Sciences, specialization Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2011-2013	
General courses		
Principles of Research in Medicine and Epidemiology		0.7
Clinical Decision Analysis		0.7
Methods of Public Health Research		0.7
Pharmaco-epidemiology		0.7
Health Economics		0.7
Case-control Studies		0.7
Introduction to Global Public Health		0.7
Primary and Secondary Prevention Research		0.7
Causal Inference		0.7
History of Epidemiologic Ideas		0.7
Markers and Prognostic Research		0.7
The Practice of Epidemiologic Analyses		0.7
Core courses		
Study Design		4.3
Biostatistical Methods I: Basic Principles		5.7
Clinical Epidemiology		5.7
Methodologic Topics in Epidemiologic Research		1.4
Biostatistical Methods II: Popular Regression Models		4.3
Advanced courses		
Planning and Evaluation of Screening		1.4
Maternal and Child Health		0.9

	Year	Workload (ECTS)
Quality of Life Measurement		0.9
Ethnicity, Health and Health Care		1.1
General academic skills		
English Language		1.4
Introduction to Medical Writing		1.1
Seminars and workshops		
Seminars at the department of Public Health, Erasmus MC, the Netherlands	2011-2014	1.0
Generation R Research meetings, Erasmus MC, The Netherlands	2011-2014	1.0
INRICH 4th Workshop, Rotterdam, the Netherlands	2012	0.5
(Inter)national congresses and presentations		
EUPHA congress, Copenhagen, Denmark. *Poster presentation*	2011	1.4
Gemeente Rotterdam, Sport en Recreatie, Rotterdam, the Netherlands. *Oral presentation*	2012	0.7
XVIII ISSHP World Congress, Geneva, Switzerland. *Oral presentation*	2012	1.4
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands. *Oral presentation*	2012	1.4
Generation R Research Meeting, Erasmus MC, the Netherlands. *Oral presentation*	2013	0.7
Working group 'sociale ongelijkheden in leefstijl en cardiovasculaire risicofactoren', Rotterdam, the Netherlands. *Oral presentation*	2013	0.7
Research seminar Public Health, Erasmus MC, the Netherlands. *Oral presentation*	2014	0.7
Haagsche Praat, GGD, Den Haag, the Netherlands. *Oral presentation*	2014	0.7
CEPHIR seminar, Erasmus MC, the Netherlands. *Oral presentation*	2014	0.7
International research projects		
International research project at Harvard School of Public Health, Boston, USA	2013	
Other		
Reviewed articles for *Plos One, Pediatrics, European Journal of Epidemiology, International Journal of Behavioral Nutrition and Physical Activity*	2011-2014	0.3
Organising working group 'sociale ongelijkheden in leefstijl en cardiovasculaire risicofactoren'	2013-2014	1.0
2. Teaching activities		
'Instrumental variable analysis', Hot topic as part of the course Public Health Research: Analysis of Determinants, NIHES, Rotterdam, the Netherlands	2012,2014	0.7

Dankwoord

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