Early growth and childhood adiposity

The Generation R Study

Büşra Durmuş

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MANUSCRIPTS BASED ON THIS THESIS

1. Mook-Kanamori DO, Holzhauer S, Hollestein LM, **Durmuş B**, Manniesing R, Koek M, Boehm G, van der Beek EM, Hofman A, Witteman JC, Lequin MH, Jaddoe VWV. Abdominal fat in children measured by ultrasound and computed tomography. *Ultrasound Med Biol* 2009;35(12):1938-46.

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4. **Durmuş B**, Kruithof CJ, de Jongste JC, Manniesing R, Raat H, Steegers EAP, Hofman A, Jaddoe VWV. Fetal and infant growth and body fat distribution in school-age children. The Generation R Study. *Submitted*.

5. **Durmuş B**, Arends LR, Ay L, Hokken-Koelega AC, Raat H, Hofman A, Steegers EAP, Jaddoe VWV. Parental anthropometrics, early growth and the risk of obesity in early childhood: The Generation R Study. *Pediatr Obes* 2012*:* DOI: 10.1111/j.2047-6310.2012.00114.x.

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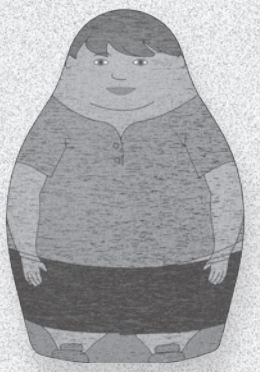


CHAPTER 1

General introduction

The World Health Organization defines overweight and obesity as abnormal or excessive accumulation of adipose tissue, which is an established risk factor for harmful health.[1] Common health consequences of overweight and obesity include cardiometabolic diseases – mainly diabetes, stroke and heart diseases – orthopedical disorders and some cancers such as breast- and colon cancer.[1-3] Currently, overweight and obesity are the fifth leading cause of global deaths.[1] The burden of diabetes and ischemic heart disease are for 44% and 23% attributable to overweight and obesity, respectively. Overall, in 2008 more than 1.4 billion adults in the world were overweight.[1] The dramatic increase in the worldwide prevalence of overweight and obesity might be designated as a 'global epidemic'.

Also, children with overweight or obesity experience more often inhalation difficulties, non-alcoholic fatty liver disease, adverse lipid profile, hypertension, insulin resistance, and depression and other psychological effects.[1,4] In 2010, more than 40 million children worldwide under the age of 5 years were estimated as overweight.[1] In the Netherlands, the prevalence of childhood overweight and obesity is fluctuating, but has an overall increasing trend.[5] In 2010, 13.7% and 13.0% of the boys and girls in the Netherlands, respectively, were overweight. On average, the percentage of overweight in young persons has been increased with 40% in the last 30 years.[5,6] Also, the prevalence of cardiometabolic risk factors associated with overweight and obesity is increasing in children.[7,8]

Childhood overweight and obesity are important risk factors for overweight and obesity in adulthood. The concept of persistence or relative stability of overweight over time is often referred to as 'tracking'.[9,10] Tracking is the phenomenon that children keep their body mass index (BMI) position in the population distribution from childhood into adulthood.[11,12] Several studies have demonstrated that overweight in childhood tracks into adolescence and adulthood.[9] It has also been suggested that clustered cardiometabolic risk factors associated with overweight and obesity track from childhood into adolescence.[13]

A growing body of research suggests that overweight and cardiometabolic diseases at least partly originate in intrauterine life. The retrospective cohort studies of David Barker and colleagues during the late 1980s established the principle of the 'Barker' hypothesis, later termed as the 'DOHaD' hypothesis.[14] This *Developmental Origins of Health and Disease* hypothesis proposes that the developing fetus responds to suboptimal conditions during critical periods of cellular proliferation and differentiation by enabling structural and functional adaptations in cells, tissues and organ systems.[15] These changes may have long-term consequences for body composition, and cardiovascular and metabolic dysfunction and disease in later life.[15]

Several environmental and behavioral factors during the intrauterine period have been identified that might lead to overweight, obesity and cardiometabolic dysfunc-

Table 1 Body mass index classification in adults (*source: WHO*)

Underweight	<18.5
Normal range	18.5 – 24.9
Overweight	>= 25
Pre-obese	25.0 – 29.9
Obese	>= 30.0
Obese class I	30.0 – 34.9
Obese class II	35.0 – 39.9
Obese class III	>= 40

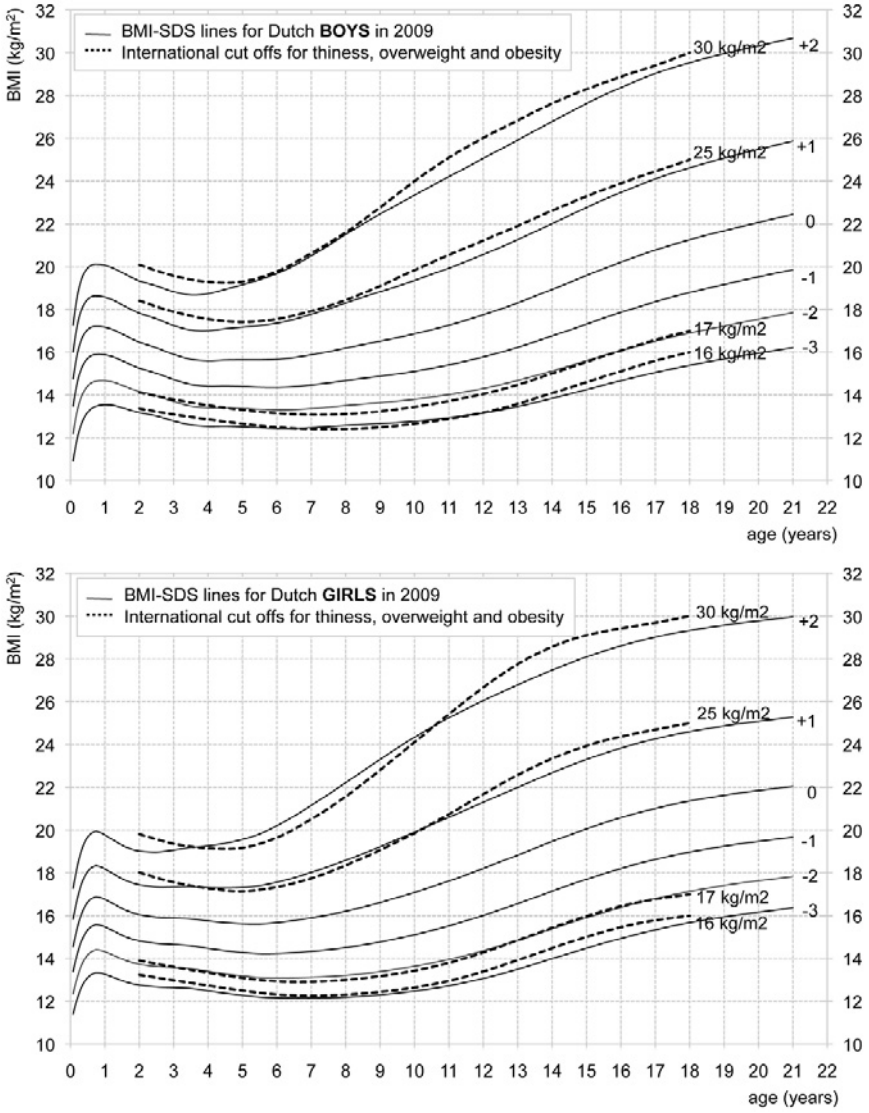
tion.[14,15] Fetal and early infant growth patterns seem to be strongly associated with later risk of obesity and cardiometabolic diseases.[16] However, the specific fetal and infant growth patterns that might contribute to an adverse body fat distribution in children are not well-known.

The majority of studies assessing childhood adiposity used BMI as outcome measure. BMI is a very simple index of weight-for-height that is internationally used to classify overweight and obesity in adult populations (**Table 1**).[1] BMI is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). BMI is safe, inexpensive and an easy to obtain population-level diagnostic tool for defining general adiposity, but lacks good accuracy and reproducibility.[17,18] A high BMI is difficult to interpret when the relative proportions of fat, muscle, bone and organ mass are changing, especially during childhood and adolescence.[19]

The classification for adults is also used to calculate the cut-off values for overweight and obesity in children by Cole *et al.*[20] Cole defined overweight and obesity in children based on age- and sex-adjusted BMI distributions from six international studies. **Figure 1** shows the sex-adjusted BMI distributions in the Netherlands in 2009 for boys and girls. The curves also include the international cut-off values for overweight and obesity.[21]

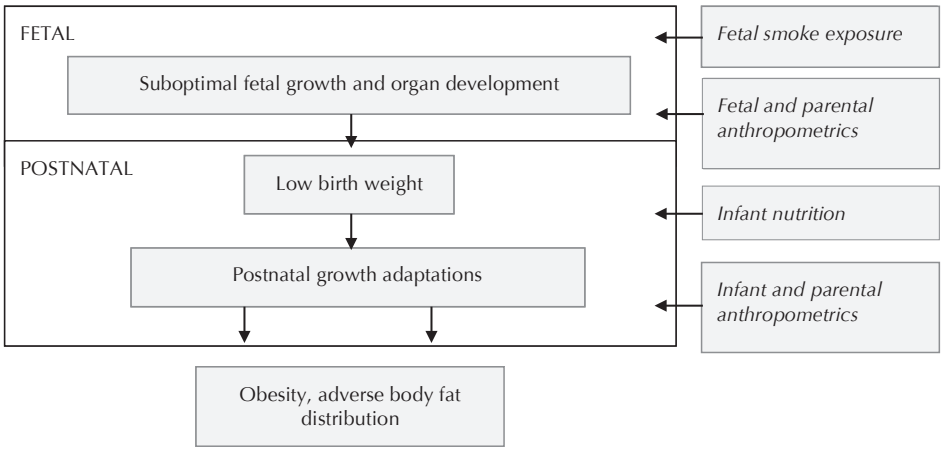
Previous studies suggested that body fat distribution rather than BMI is related to the risks of cardiovascular and metabolic diseases.[22,23] Additional measures such as skinfold thicknesses, waist circumference and waist-to-hip ratio are used in clinical practice to derive estimates of total and abdominal fat distribution. These measures may be imprecise and do not give any insight into the amount or differential effects of visceral and subcutaneous fat compartments.[24] These two compartments are distinct in their endocrine and paracrine secretion profiles of hormones and cytokines.[17,24] Especially visceral fat is associated with a cluster of pro-inflammatory and thrombotic abnormalities.[25-27] Visceral fat has a direct circulatory connection with the liver via the portal circulation and might therefore directly lead to insulin resistance, inflammation processes in the liver and non-alcoholic fatty liver disease.[25-27]

Figure 1 Body mass index distributions for Dutch boys and girls in 2009 (Schönbeck *et al*; PLoS One 2011)



Visceral fat increases with age throughout childhood into adulthood.[28] Two recent studies demonstrated that tracking for particularly abdominal fat was moderate for girls and high in boys after a follow-up period of 7 to 8 years in children aged 7 to 16 years.[29,30] Moreover, recent studies suggest that, already in childhood, abdominal visceral fat appears to be associated with an unfavorable lipid profile, including decreased HDL- and elevated LDL-cholesterol, and high concentrations of triglycerides.[17]

Figure 2 Overview of the assessed associations in this thesis



Overall, the cardiometabolic risks associated with adipose tissue seem to be mainly related to the visceral fat tissue compartments, while subcutaneous fat tissue plays a controversial role.[17-24] Most studies focused on total body and abdominal fat distribution have been performed in adults. Not much is known about adipose tissue development and fat distribution in children.

From these perspectives, we can conclude that it is important to gain more knowledge on the associations of early life factors with detailed measures of body fat distribution and cardiometabolic risk factors in childhood.

The key objectives for this thesis are:

- To examine the associations of repeatedly measured fetal and infant **growth** patterns with childhood body fat distribution (**chapter 3**);
- To examine the associations of **parental factors** such as anthropometrics before and during pregnancy, and smoking during pregnancy with childhood body fat distribution and cardiometabolic outcomes (**chapter 4**);
- Finally, to examine the associations of **infant nutrition** with childhood body fat distribution and cardiometabolic outcomes (**chapter 5**).

An overview of the assessed associations in this thesis is given in **Figure 2**. The general design of the study is presented in **chapter 2**. In **chapter 6**, the results of the observed associations in this thesis are discussed and recommendations for future research and policy are presented. **Chapter 7** includes an English and Dutch summary of the thesis.

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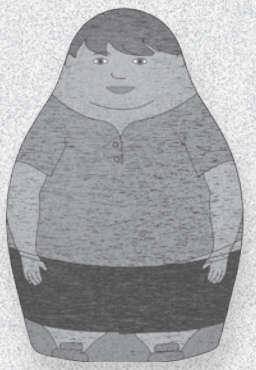


CHAPTER 2

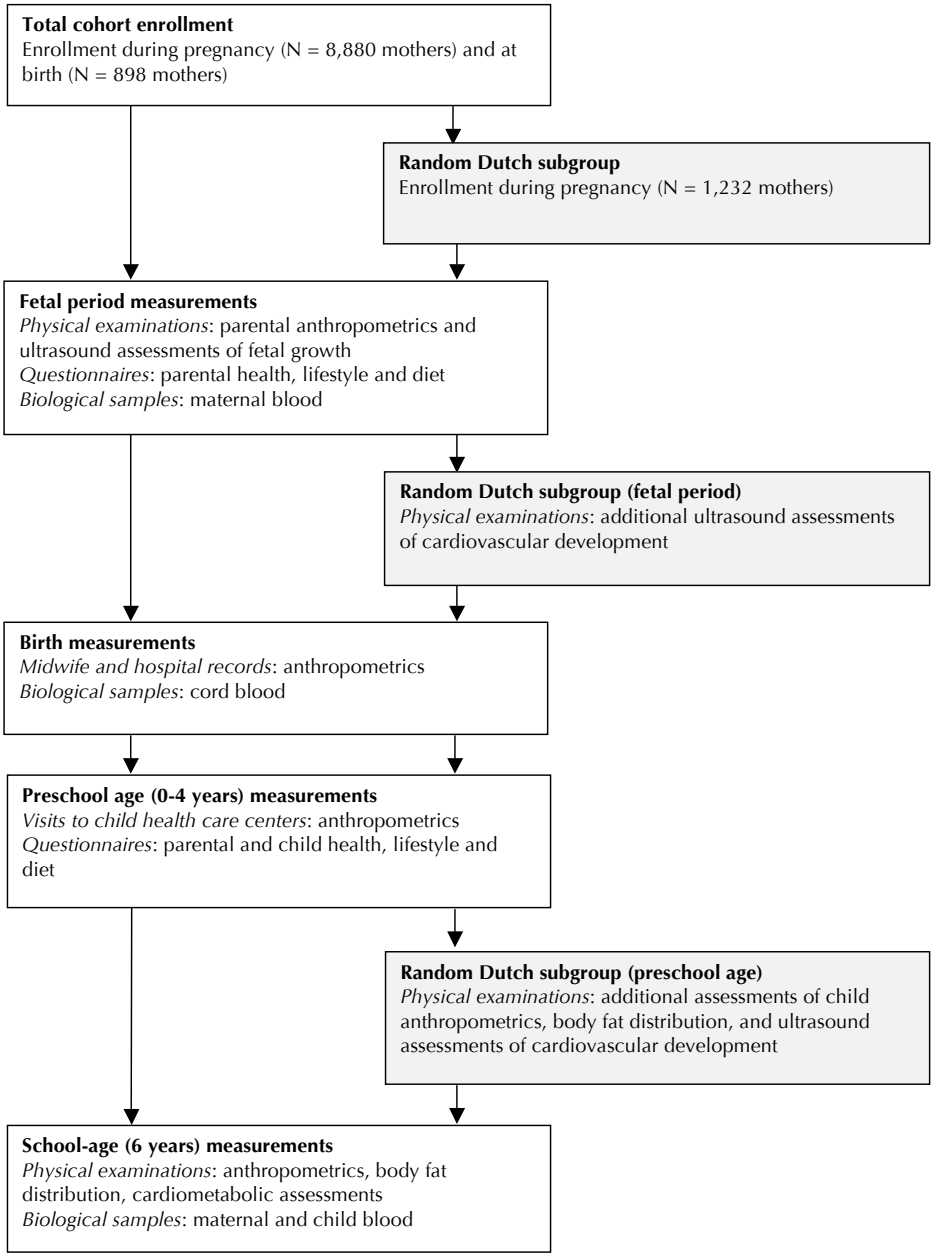
Study design

All studies described in this thesis were embedded in the Generation R Study, an ongoing population-based prospective cohort study from early fetal life onwards. This study is designed to identify early environmental, biological and social determinants of growth, development and health.[1,2] The Generation R Study is conducted in Rotterdam, the second largest city of the Netherlands. The total population consists of about 600,000 inhabitants and almost 170 different ethnicities.[3] All pregnant women living in the study area and with a delivery date from April 2002 until January 2006 were eligible for enrollment in the study. Enrollment was aimed in early pregnancy but was possible until birth of the child. In total, 9,778 mothers were enrolled in the study. Of these mothers, 91% (N =8,880) was enrolled in pregnancy. In total, 71% of all partners was enrolled and only partners from mothers enrolled in pregnancy were invited to participate (**Figure 1**).[1]

DATA COLLECTION

Assessments were planned in early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age >25 weeks). Assessments in the mother included physical examinations, questionnaires, fetal ultrasound examinations and blood and urine samples.[1,2] Their partners were assessed once in pregnancy by a physical examination and blood sample in early pregnancy, and a questionnaire in mid-pregnancy. Physical examinations of the parents included height, weight and blood pressure measurements. Topics of the questionnaires were mainly based on lifestyle habits. The ultrasound examinations were used for both establishing gestational age and assessing fetal growth patterns.[4] Additional detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch pregnant women and their children from late pregnancy.[1] This subgroup is ethnically homogeneous to exclude confounding or effect modification by ethnicity. Assessments in the child at birth were performed by a physical examination including weight, length, head circumference, and cord blood tests. Postnatal information on growth, development and health of the participating children was obtained from hands-on measurements at the routine child health centers and by questionnaires at the ages of 2, 6, 12, 18, 24, 36 and 48 months. At the age of 6 years, all participating children were invited to visit the dedicated research center in the Sophia Children's Hospital in Rotterdam for detailed measurements.[1]

Figure 1 Overview of the study population



Body fat distribution and cardiometabolic outcome assessments

The studies presented in this thesis were specifically focused on detailed measurements of growth, body fat distribution and cardiometabolic outcomes. In this paragraph, these measurements are described.

Infant and childhood anthropometrics

Information on birth characteristics (length and weight) was obtained from community midwife and hospital registries. We created gestational age- and sex-adjusted birth length and weight standard deviation scores (SDS) within our study population using Growth Analyser 3.5 (Dutch Growth Research Foundation, Rotterdam, The Netherlands) based on the Swedish reference growth charts. Postnatal growth at preschool ages was measured at the Community Health Centers according to a standard schedule and procedures by a well-trained staff at the ages of 3, 6, 12, 24, 36 and 48 months. Head circumference was measured to the nearest millimeter (mm) using a standardized tape (SECA, Hamburg, Germany) until the age of 12 months. Length was determined in a supine position to the nearest mm until the age of 12 months using a neonatometer. From the age of 24 months, height was measured in a standing position by a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight was measured using a mechanical personal scale (SECA, Almere, The Netherlands). BMI (kg/m^2) was calculated. Overweight (+1.1 SDS) and obesity (+2.3 SDS) were defined based on the national age- and sex- adjusted BMI distributions of the 1997 Dutch Growth Study for children. These definitions correspond to the international adult cut-off points of overweight (25 kg/m^2) and obesity (30 kg/m^2), and the definition of Cole *et al*.[5,6]

Child anthropometrics at the age of 6 years were measured in a dedicated research center by a well-trained staff. Height, weight and BMI were determined or calculated using the same methods as in preschool ages. Age- and sex-adjusted SDS for postnatal growth characteristics were obtained using Dutch reference growth charts (Growth Analyser 3.5, Dutch Growth Research Foundation, Rotterdam, The Netherlands). Childhood overweight and obesity were based on the definition of Cole *et al*.[5]

Skinfold thicknesses

The thickness of subcutaneous fat is specific to adipose tissue and can be measured noninvasively, easily and quickly. Therefore, skinfold thickness (SFT) is assumed to be an important, valid and informative anthropometric indicator of regional and total body fatness in most age groups, especially in research settings.[7,8] In general, intra- and inter-

observer error are low compared to between-subject variability, but in obese children accuracy and precision are poorer.[8,9] The accuracy of SFT in predicting body fatness may also vary according to the selected sites and prediction equation. It has been suggested that one SFT equation is inaccurate (R^2 = 0.51) compared to a 4-compartment model (R^2 = 0.85).[10] Furthermore, SFT has a limitation in assessing lean and fat mass of the whole body, especially in the differentiation of visceral and subcutaneous fat mass.[8]

Methods

At the ages of 1.5, 6 and 24 months, SFT were measured in mm within a subgroup. These measurements were performed on the left side of the body at four different sites (biceps, triceps, suprailiacal and subscapular) according to standard procedures using a skinfold caliper (Slim Guide, Creative Health Products).[11] Four well-trained research nurses performed all measurements (intra-observer intraclass correlation coefficient (ICC) 0.88; inter-observer ICC 0.77).[12-14] Total subcutaneous fat mass was calculated from the sum of biceps + triceps + suprailiacal + subscapular SFT. Central subcutaneous fat mass was calculated from the sum of suprailiacal + subscapular SFT. Peripheral subcutaneous fat mass was calculated from the sum of triceps + biceps SFT.[15,16]

Dual-energy- X-ray absorptiometry (DXA)

The main measurement technique of DXA is based on the differential attenuation by bone, fat, and lean tissue of transmitted photons at two energy levels.[17] The DXA devices

Figure 2 Example of total body composition measurements by a DXA device

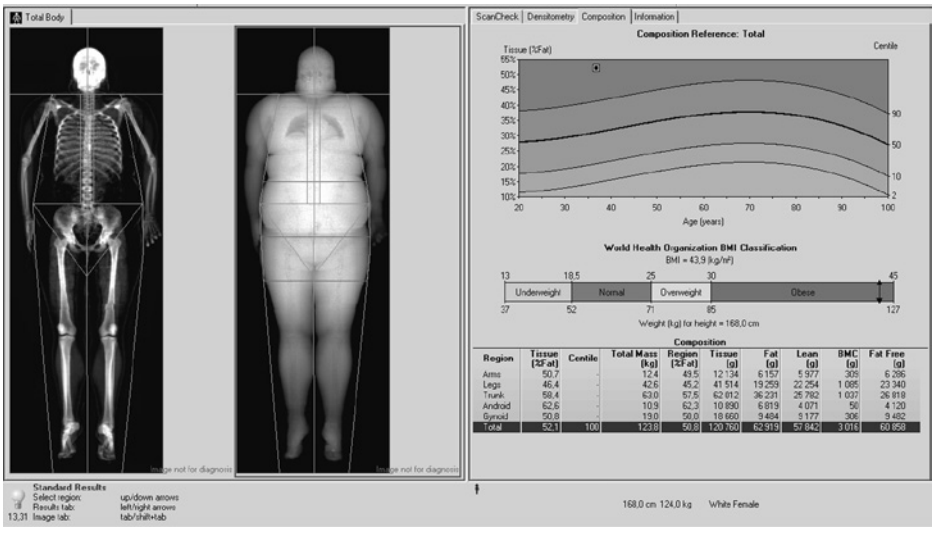


Table 1 DXA-derived total and regional body fat measures used in this thesis

Total fat mass%	= total fat mass (kg) / total body weight (kg)
Android / gynoid fat ratio	= android fat (kg) / gynoid fat (kg)

used nowadays quantify body fat content with high precision (within 2% coefficient of variation), low X-ray exposure (5-10 micro Sv) and short scanning time (7 minutes).[18] The DXA output provides information about the following masses (in grams): fat mass, lean mass, and bone mineral mass of the whole body and specific regions (**Figure 2**). The total fat content is given in %. The specific body fat measures and their definitions that are used in this thesis are provided in **Table 1**. Although DXA is not capable to differentiate between visceral and subcutaneous fat, it has been validated against Computed Tomography (CT) that is considered as the reference standard in establishing the amount of body fat (distribution).[18] Also, in children the use of DXA has been demonstrated to be valid for the assessment of body fat.[19,20]

Methods

All children that attended the research center at the 6-year visit were invited for a DXA scan. Well-trained research assistants obtained the DXA scans following standard manufacturer and positioning protocols and performed adjustments when necessary. Quality assurance tests were run every day using a standard calibration block of tissue-equivalent material supplied by the manufacturer. Repeated measurements on the calibration block had coefficients of variation <0.25%.

Abdominal ultrasound

Adipose tissue is loose connective tissue replete with adipocytes. It is composed of about 80% fat but also contains protein, minerals and water. Adipose tissue accumulates as a child ages and varies considerably during infancy, but after the first year of life, total body fat declines or stabilizes until the age of 6 years.[21] Young children have in general little intra-abdominal fat proportional to total body fat compared to adults and more of the fat deposition in children than in adults is subcutaneous fat.[22] Between 12 to 14 years it has been observed that children accumulate <10% of their adipose tissue in the intra-abdominal fat deposit. Visceral fat in particular is already present at birth and increases with age throughout childhood and adulthood, independent of an increase in total body fat. Subcutaneous fat also increases as children age.[21,22] As abdominal fat has been shown to track moderately for girls and highly for boys after a 7 to 8 year long follow-up period in 7 to 15 old children[22], it might probably be concluded that there is an early predisposition to the development of diseases related to abdominal fat.

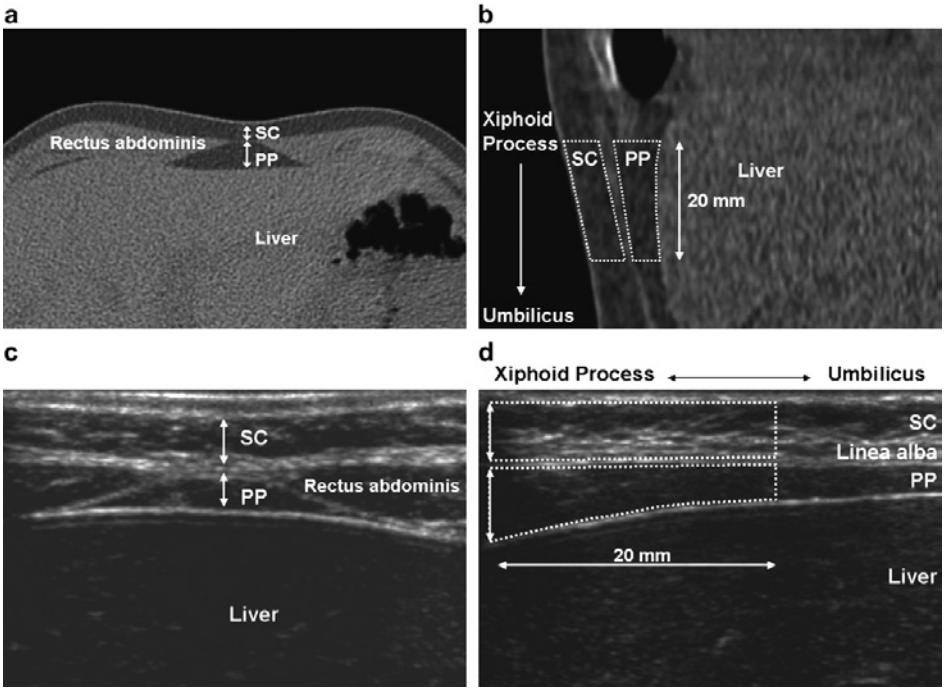
Ultrasound has been proposed as a suitable technique to estimate abdominal fat tissue in a research setting as described in the next paragraph.[23] The time needed for single measurement is very short. More importantly, ultrasound offers a noninvasive and reliable method to differentiate between visceral and subcutaneous fat thickness compartments.[23,24] Ultrasound can also be used to estimate the preperitoneal to subcutaneous fat thickness ratio, which is termed the abdominal wall fat index.[20,25,26] The evaluation of the preperitoneal fat thickness – which is a proxy for visceral abdominal fat – by ultrasound may be the most sensitive and reliable method for predicting insulin resistance-associated metabolic derangements in children.[26]

Methods

All children that attended the research center at the 6-year visit were invited for an abdominal ultrasound in order to measure abdominal fat tissue compartments. Preperitoneal and subcutaneous fat thickness and area were measured with a Linear Array probe L12-5 (38 mm, 5-12 MHz), according to the method of Suzuki.[25] The linear probe was carefully positioned perpendicular to the skin of the median upper abdomen and touched as lightly as possible to prevent compression of the fat layers. Scanning was performed while moving the probe longitudinally from the xiphoid process to the umbilicus along the midline (linea alba) to obtain an image containing the maximum preperitoneal fat thickness, represented by the maximum height of a triangular shaped area (**Figure 3c**). In this transversal image, only the *maximum subcutaneous fat thickness* was measured directly above this triangular area (Figure 3c). To obtain a sagittal image, the probe was kept parallel to the linea alba (**Figure 3d**). In this image, first the *maximum preperitoneal fat thickness* was determined, which is, as in the CT images, located at the upper part of the ventral side of the liver. At this same level the *minimum subcutaneous fat thickness* was determined. Subsequently, the *maximum preperitoneal and subcutaneous fat areas* were measured starting from the position of the maximum preperitoneal fat thickness over a distance of 20 mm in the caudal direction (Figure 3d). The maximum subcutaneous fat area was measured in parallel along the same 20 mm distance. Finally, preperitoneal to subcutaneous fat thickness ratio was calculated. All measurements were performed offline using a 2D measuring tool on the personal computer developed by the Biomedical Imaging Group Rotterdam at the Erasmus MC. This tool has been calibrated to the nearest mm according to the ultrasound machine.

Previously, we demonstrated within the Generation R Study good correlations between preperitoneal fat and visceral fat, both measured by CT (r ranged from 0.58 to 0.76) (*substudy 1*). In *substudy 2* strong correlations of abdominal ultrasound measures with corresponding measures obtained by CT (r ranged from 0.75 to 0.97) were found **(Figure 3a, b)**.[27] To measure the exact agreement between ultrasound and CT a Bland and

Figure 3 Measurements of abdominal fat with Computed Tomography (CT) and ultrasound: transversal images from a CT-scan **(a)** and ultrasound **(c)**, at the location where the maximum preperitoneal (PP) and subcutaneous (SC) fat thicknesses were measured. Sagittal images from a CT-scan **(b)** and ultrasound **(d)**, where the PP and SC areas were measured starting at the location of the maximum PP fat thickness to 20 mm in caudal direction.



Altman analysis was performed.[13,28] We estimated the maximum acceptable difference between CT and ultrasound to be 2 mm (+/- 1 standard deviation (SD)) for fat thickness measures. For the areas this resulted in an acceptable difference of 40 mm^2 (2 mm over the length of 20 mm). Systematic differences were observed for all preperitoneal fat measurements and for subcutaneous fat thickness in the transversal image.

Measuring preperitoneal fat by ultrasound systematically resulted in smaller maximum fat thickness and area than measured by CT. Similarly, in the transversal image, the subcutaneous fat thickness was slightly larger measured by ultrasound than by means of CT. There was a good inter- and intra-observer reproducibility (ICC ranged from 0.93 to 0.97), from which we can conclude that the measurements were highly reproducible.[29]

Cardiometabolic risk factors

Blood pressure in childhood increases with age and children who are either heavier or taller or both have a higher blood pressure than smaller children of the same age.[30] The

relationship between body weight and blood pressure in children is stronger than in adults and children with high blood pressure who are taller and heavier are more likely to become hypertensive as adults.[31] Tracking of high blood pressure over time is a widely demonstrated phenomenon in children.

Insulin is secreted by the B-cells of the pancreas and passes into circulation via the portal vein and the liver.[32] There are simple methods that act as surrogate marker for insulin resistance. In children and adults, the use of fasting insulin in presence of normoglycemia, could be an estimate of insulin resistance as good as the most common tests.[33,34] We had insulin levels available in children at the age of 6 years. Insulin resistance is a good predictor for the development of impaired glucose tolerance and type 2 diabetes, especially in obese children and adolescents.[33] Increased adiposity is a major risk factor for the development of insulin resistance in children and adults.[33]

Cholesterol is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Thereafter, it is transported by Low Density Lipoprotein (LDL) in the circulation. High Density Lipoprotein (HDL) allows excess cholesterol in the periphery to be transported to the liver and excreted from the body as bile salts. It also inhibits lipid oxidation and prevent endothelial cell death and damage.[35] Hypercholesterolemia and increased LDL/HDL ratios are well-known risk factors for cardiovascular diseases in later life.[36] Increased adiposity is strongly related to cholesterol, HDL- and LDL-cholesterol concentrations.[37] It has been suggested that clustered cardiometabolic risk factors as described in this paragraph track from childhood into adolescence.[38]

Methods

Measurements of cardiometabolic risk factors were conducted at the 6-year visit at the same time as the body fat distribution measurements. Systolic and diastolic blood pressure was measured at the right brachial artery, four times with one-minute intervals, using a validated automatic sphygmanometer.[39] Thirty-minutes fasting blood samples were collected in participants by well-trained research nurses. These samples were drawn by antecubital venipuncture and transported to and processed in a dedicated laboratory facility of the regional laboratory in Rotterdam (STAR-MDC). Finally, measurements were determined enzymatically using Cobas 8000 analyser (Roche, Almere, The Netherlands) in the Erasmus MC.

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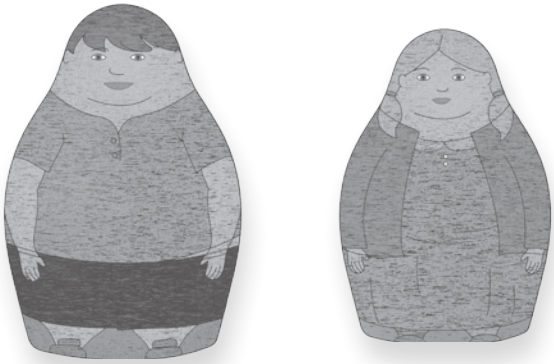

CHAPTER 3

Early growth

CHAPTER 3.1

Fetal and infant growth and the risk of obesity in preschool children

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ABSTRACT

Objective: To examine whether infant growth rates are influenced by fetal growth characteristics and are associated with the risks of overweight and obesity in early childhood.
Methods: This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onward. Fetal growth characteristics (femur length and estimated fetal weight) were assessed in second and third trimesters and at birth (length and weight). Infant peak weight velocity (PWV), peak height velocity (PHV) and body mass index at adiposity peak (BMIAP) were derived for 6,267 infants with multiple height and weight measurements.
Results: Estimated fetal weight measured during the second trimester was positively associated with PWV and BMIAP during infancy. Subjects with a smaller weight gain between the third trimester and birth had a higher PWV. Femur length measured during the second trimester was positively associated with PHV. Gradual length gain between the second and third trimesters and between the third trimester and birth were associated with higher PHV. Compared to infants in the lowest quintile, the infants in the highest quintile of PWV had strongly increased risks of overweight/obesity at the age of 4 years (odds ratio (OR) (95% confidence interval (CI)) 15.01 (9.63, 23.38)).
Conclusions: Fetal growth characteristics strongly influence infant growth rates. A higher PWV, which generally occurs in the first month after birth, is associated with an increased risk of overweight and obesity at 4 years of age. Longer follow-up studies are necessary to determine how fetal and infant growth patterns affect the risk of disease in later life.

INTRODUCTION

The inverse relationship between birth weight and adverse metabolic phenotypes in adulthood has been well established.[1-3] Increasing evidence suggests that infant growth patterns, such as rapid postnatal weight gain, are also risk factors for diseases in later life.[4,5] Recent data from the Northern Finnish Birth Cohort 1966 Study suggest that infant growth characteristics such as the peak weight velocity (PWV) and peak height velocity (PHV) are predictors of increased blood pressure, waist circumference, and body mass index (BMI) at the age of 31 years.[6] Also, BMI at the adiposity peak (BMIAP), which occurs at around 9 months of age, was positively associated with BMI at the age of 31 years.[7] Growth rate in early postnatal life is highly dependent of birth weight, since smaller babies tend to catch-up and heavier babies tend to catch-down during the first months of postnatal life.[8] Birth weight is a crude measure of fetal growth as different fetal growth patterns may lead to the same birth weight.[9] Growth restriction during different critical periods of fetal growth can have different metabolic consequences in adult life.[10] An adverse environment has been demonstrated to influence fetal growth as early as the 10th week of pregnancy.[11] Infant growth rates and patterns might be intermediates in the association between impaired fetal growth and the increased risks of obesity and metabolic diseases in later life. However, the associations between fetal growth characteristics and early postnatal growth rates are not known.

Therefore, we examined in a prenatally recruited prospective cohort study in 6,267 children whether infant growth rates are influenced by fetal growth characteristics and are associated with the risks of overweight and obesity in early childhood.

METHODS

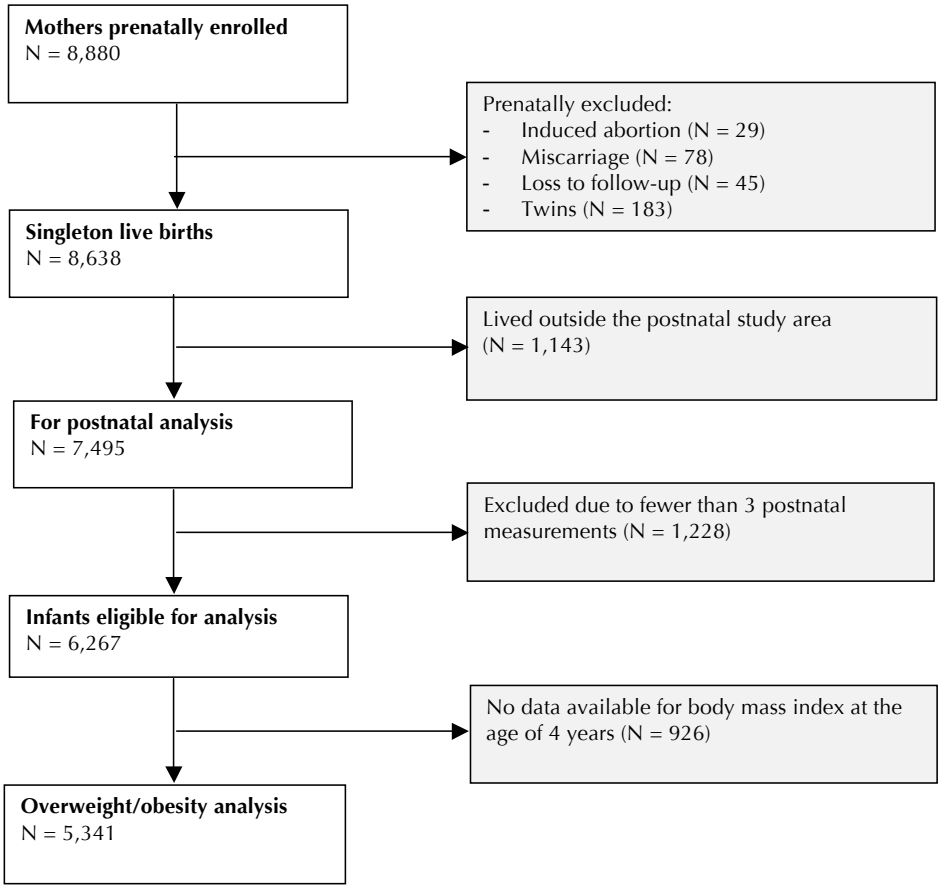
Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study of 9,897 children and their parents from early fetal life onward. This study is designed to identify early determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail.[12] Pregnant women were asked to enroll between 2001 and 2005, and enrollment was aimed to be in the first trimester but was allowed until birth. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. All parents gave written informed consent.

Population for analysis

In total, 9,897 children and their parents were enrolled in the study. Of those, 8,880 mothers were enrolled during pregnancy. These mothers gave birth to 8,638 singleton live births (**Figure 1**). Of these children, 13% (N = 1,143) lived outside the study area for postnatal follow-up, and 14% (N = 1,228) children had fewer than 3 postnatal measurements, which is necessary for the infant growth modeling, leaving N = 6,267 subjects for the analyses. Of these children, 85% (N = 5,341) were available for the analyses regarding overweight and obesity at the age of 4 years.

Figure 1 Population for analysis



Fetal growth measurements and birth outcomes

In a dedicated research facility, we measured fetal crown-rump length (CRL) in the first trimester and fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) in the second and third trimesters to the nearest millimeter (mm) using standardized ultrasound procedures.[13] Estimated fetal weight (EFW) was calculated using the formula by Hadlock *et al.*[14] ($\log_{10}$ EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 (HC)^2 – 0.003685 (AC * FL)). Standard deviation scores (SDS) for all fetal growth characteristics were constructed on data from the study group.[13] Ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). For first trimester CRL, gestational age was based on the first day of the last menstrual period. Analyses were limited to women who had a CRL measurement between 10 weeks 0 days and 13 days 6 days, with a known and reliable first day of last menstrual period and a menstrual cycle between 24 and 32 days (N = 1,377).[11] Fetal growth measurements in the second and third trimesters were available in 6,004 and 6,181 children, respectively. For second trimester, third trimester, and birth, gestational age was based on first trimester CRL according to standard obstetric practice. Date of birth, birth weight and length, and infant sex were obtained from community midwives and hospital registries. Birth length was only available for 4,164 individuals (66.4%), since birth length is not routinely measured in obstetric practices in the Netherlands. Gestational age-adjusted SDS for birth weight and length were constructed using growth standards from Niklasson *et al*.[15]

Postnatal growth measurements and derived infant growth parameters

Well-trained staff in the Community Health Centers obtained postnatal growth characteristics (weight and length) using standardized procedures and BMI (kg/m^2) was calculated.[12] The ages at which the children were measured were based on the national health care program in the Netherlands: 1 month; 2 months; 3 months; 4 months; 6 months; 11 months; 14 months; 18 months; 24 months; 36 months; and 48 months. The median number of postnatal growth measurements was 5 (90% range: 3 – 8). Overweight and obesity were defined as described by Cole *et al*.[16]

Peak weight velocity and peak height velocity

PWV and PHV were derived from the postnatal data using the Reed1 model for boys and girls separately using the previously described procedure.[6,17] The Reed1 model[18] was chosen since it showed a better fit to the early growth data than the Kouchi, Carlberg,

and Count models, and it showed an equally good fit to the Reed2 model which has one more parameter than the Reed1 model. The difference compared with the simpler models, for example, the Count model, is that the Reed1 model allows the velocity to peak after birth, whereas other models force it to peak at birth. In the first couple of weeks after birth, weight may drop up to 10% in normal individuals. The PWV is thus usually not in the first weeks after birth, but slightly later. Therefore, the Reed1 model is more realistic (especially for weight) and more flexible. The Reed1 model was fitted by sex on all weight and height measurements taken at 0 to 3 years of age, including birth weight and length. We assumed both a fixed and a random component for all four parameters in the model. For each person, the first derivative of the fitted distance curve was taken to get the weight or height velocity curve. Subsequently, the maximum of this curve was taken to obtain the PWV or PHV in infancy. The Reed1 model is a 4-parameter extension of the 3-parameter Count model[19] and its functional form is[18]:

$Y = A + Bt + C\ln(t) + D/t$

Since this model is not defined at birth (t=0), it was modified for this study in the same way as in Simondon *et al*[20]:

$Y = A + Bt + C\ln(t+1) + D/(t+1)$, where

t = postnatal age

Y = weight or height reached at age t

and A, B, C and D the function parameters.

Of the function parameters, A is related to the baseline weight or height at birth, B to the linear component of the growth velocity, C to the decrease in the growth velocity over time, and D to the inflection point that allows growth velocity to peak after birth rather than exactly at birth. The Reed1 model is linear in its constants.[19] Having 2 measurements was inadequate to capture the shape of the growth curve, and therefore, we restricted all association analyses to those with a minimum of 3 measurements per person.

Adiposity peak

For BMIAP, a cubic mixed effects model was fitted on log(BMI) from 14 days to 1.5 years, using sex as a covariate.[6] Modelization of BMI growth was performed from the age of 14 days onwards, since children may lose up to 10% of their body weight in the first 2 weeks of life. When fitting the model, age was centralized to 0.75 years. In addition to fixed effects, we included random effects for the constant and the slope in the model. We assumed autoregressive AR(1) within-person correlation structure between the measurements. Then, BMI was derived for each individual at the point where the curve reaches its maximum, i.e. at infant adiposity peak.

Covariates

At enrollment, data regarding maternal age, pre-pregnancy weight, parity, smoking, and paternal height and weight were obtained by questionnaires.[12] Both parents were asked to provide details regarding the country of birth of their parents. This information was used to classify ethnic background of the child according to Statistics Netherlands, as previously described in detail.[21] Maternal height was measured at our research center and BMI was calculated (weight (kg) / height $(m)^2$). We obtained information regarding breastfeeding duration by postnatal questionnaires at the ages of 2, 6 and 12 months. Mothers were asked whether they ever breastfed their child and, if so, at what age they stopped breastfeeding.

Statistical analysis

First, using multivariate linear regression models and adjusting for covariates, we assessed the associations of CRL in the first trimester, estimated fetal weight in the second and third trimesters and birth weight with infant PWV and BMIAP. The covariates in the model were fetal ethnicity, maternal age, maternal educational level, maternal pre-pregnancy BMI, maternal smoking, paternal BMI, parity, duration of breastfeeding, and number of postnatal measurements. The covariates were based on whether they were associated with the postnatal growth parameters. The interaction parameters 'fetal growth-sex' and 'fetal growth-smoking' were not associated with postnatal growth and were therefore not included in the models. Using similar models, we then examined whether weight change (in SDS) between the second trimester and third trimesters (second trimester weight gain), and between the third trimester and birth (third trimester weight gain), was associated with infant PWV and BMIAP. Subsequently, similar analyses were repeated for the associations of (femur) length with PHV and BMIAP. Since fetal body length cannot be measured, femur length in the second and third trimesters was used as a proxy for body length.[22] Finally, using multivariate logistic regression models, we assessed whether PWV, PHV and BMIAP were associated with the risks of overweight and obesity during infancy at the age of 4 years.[16] To distinguish between antenatal and postnatal determinants, this model was subsequently additionally adjusted for birth weight. For this purpose, PWV, PHV, and BMIAP were stratified into quintiles and the lowest quintile was used as the reference category. Analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc, Chicago, IL, USA) and R version 2.10.1 (The R Foundation for Statistical Computing).

RESULTS

Subject characteristics are shown in **Table 1**. Of all the children, 67% were of Caucasian ethnicity. The mean maternal age was 30.3 years, the median maternal weight was 67.0 kg, and the mean maternal height was 167.7 cm.

There were no significant associations between first trimester CRL and PWV, PHV, and BMIAP (**Table 2**). Estimated fetal weight measured during the second trimester was positively associated with PWV and BMIAP (both P-value for linear trend <0.05; **Table 3**). Also, we found a positive association between birth weight and BMIAP (P-value for linear trend <0.0001), while the association between birth weight and PWV was inverse (P-value for

Table 1 Parental and child characteristics (N = 6,267)[1]

Maternal characteristics	
Age (years)	30.3 (5.1)
Weight (kg)	67.0 (52.0 – 94.0)
Height (cm)	167.7 (7.4)
Body mass index (kg/m^2)	23.7 (19.4 – 33.3)
Educational level	
Primary (%)	9.2%
Secondary (%)	42.6%
Higher (%)	48.2%
Smoked during pregnancy (% yes)	23.9%
Parity (% primiparous)	56.3%
Paternal characteristics	
Age (years)	33.1 (5.4)
Weight (kg)	83.0 (65.0 – 106.0)
Height (cm)	182.2 (7.8)
Body mass index (kg/m^2)	24.9 (20.2 – 31.1)
Fetal and child characteristics	
Sex (% males)	50.6%
Ethnicity	
Caucasian (%)	66.5%
Turkish (%)	7.6%
Surinamese (%)	7.0%
Moroccan (%)	6.1%
Other / mixed (%)	13.8%
First trimester	
Gestational age (weeks)	12.4 (10.0 – 13.9)
Crown-rump length (mm)	60.9 (11.4)

Table 1 Parental and child characteristics (N = 6,267)[1] (continued)

Second trimester	
Gestational age (weeks)	20.5 (18.9 – 22.7)
Estimated fetal weight (grams)	380 (91)
Femur length (mm)	33.4 (3.5)
Third trimester	
Gestational age (weeks)	30.4 (28.9 – 32.2)
Estimated fetal weight (grams)	1,623 (254)
Femur length (mm)	57.4 (3.0)
Birth	
Gestational age (weeks)	40.1 (37.1 – 42.1)
Weight (grams)	3,442 (543)
Length (cm)	50.2 (2.4)
Infancy	
Number of postnatal measurements	5 (3 – 8)
Peak weight velocity (PWV) (kg/year)	12.3 (9.1 – 16.1)
Age at peak weight velocity (PWV) (months)	0.8 (0.6 – 1.0)
Peak height velocity (PHV) (cm/year)	48.5 (38.7 – 64.9)
Age at peak height velocity (PHV) (months)	0.6 (0.2 – 1.0)
Adiposity peak, (body mass index) (kg/m^2)	17.6 (0.8)
Breastfeeding duration (months)	3.5 (0.5 – 12.0)

[1]Values are means (standard deviation), percentages or medians (90% range).

Table 2 The association of first trimester crown-rump length (CRL) with peak weight velocity (PWV), peak height velocity (PHV), and body mass index at adiposity peak (BMIAP)[1,2]

Crown-rump length 1st trimester (SDS)	Peak weight velocity (PWV) (kg/year) (N = 1,376)	Peak height velocity (PHV) (cm/year) (N = 1,349)	Adiposity peak (body mass index) (kg/m^2) (N = 1,282)
1st quintile	11.79 (1.18)	48.79 (1.16)	17.45 (0.78)
2nd quintile	11.95 (1.19)	48.30 (1.17)	17.59 (0.79)
3rd quintile	12.08 (1.19)	48.94 (1.17)	17.58 (0.82)
4th quintile	12.02 (1.20)	49.00 (1.17)	17.57 (0.84)
5th quintile	11.95 (1.19)	48.35 (1.17)	17.56 (0.75)
P-value for linear trend	0.32	0.85	0.65

[1]Values represent geometric means (standard deviation).
[2]Model is adjusted for sex, age, fetal ethnicity, age of mother, menstrual cycle duration, maternal pre-pregnancy body mass index, maternal educational level, maternal smoking, paternal body mass index, parity, duration of breastfeeding, and number of postnatal measurements. Median age at measurement in first trimester (in weeks): 12.4 (90% range: 10.0 – 13.9).
SDS: Standard Deviation Score

Table 3 The association of (estimated fetal) weight with peak weight velocity (PWV) and body mass index at adiposity peak (BMIAP)[1,2]

Estimated fetal weight 2nd trimester (SDS)	**Peak weight velocity (PWV) (kg/year)**	**Adiposity peak (body mass index) (kg/m²)**
	(N = 5,943)	**(N = 5,421)**
1st quintile	12.02 (1.19)	17.52 (0.80)
2nd quintile	12.01 (1.18)	17.56 (0.80)
3rd quintile	12.12 (1.18)	17.60 (0.80)
4th quintile	12.22 (1.19)	17.64 (0.82)
5th quintile	12.16 (1.16)	17.68 (0.77)
P-value for linear trend	< 0.05	<0.05
Estimated fetal weight 3rd trimester (SDS)	**Peak weight velocity (PWV) (kg/year)**	**Adiposity peak (body mass index) (kg/m²)**
	(N = 6,114)	**(N = 5,598)**
1st quintile	12.00 (1.18)	17.41 (0.80)
2nd quintile	12.09 (1.19)	17.53 (0.80)
3rd quintile	12.18 (1.18)	17.64 (0.77)
4th quintile	12.12 (1.20)	17.67 (0.81)
5th quintile	12.16 (1.19)	17.80 (0.79)
P-value for linear trend	0.47	< 0.0001
Birth weight (SDS)	**Peak weight velocity (PWV) (kg/year)**	**Adiposity peak (body mass index) (kg/m²)**
	(N = 6,265)	**(N = 5,705)**
1st quintile	12.16 (1.18)	17.25 (0.78)
2nd quintile	12.23 (1.19)	17.46 (0.76)
3rd quintile	12.18 (1.19)	17.62 (0.76)
4th quintile	12.08 (1.19)	17.74 (0.76)
5th quintile	11.86 (1.20)	17.95 (0.79)
P-value for linear trend	< 0.05	< 0.0001
Weight change from 2nd to 3rd trimester (SDS)	**Peak weight velocity (PWV) (kg/year)**	**Adiposity peak (body mass index) (kg/m²)**
	(N = 5,829)	**(N = 5,332)**
1st quintile	12.16 (1.18)	17.50 (0.78)
2nd quintile	12.09 (1.19)	17.55 (0.79)
3rd quintile	12.08 (1.19)	17.59 (0.82)
4th quintile	12.11 (1.19)	17.63 (0.80)
5th quintile	12.05 (1.19)	17.75 (0.81)
P-value for linear trend	0.09	< 0.0001

Table 3 The association of (estimated fetal) weight with peak weight velocity (PWV) and body mass index at adiposity peak (BMIAP)[1,2] (continued)

Weight change from 3rd trimester to birth (SDS)	Peak weight velocity (PWV) (kg/year)	Adiposity peak (body mass index) (kg/m²)
	(N = 6,141)	(N = 5,596)
1st quintile	12.39 (1.18)	17.43 (0.82)
2nd quintile	12.15 (1.19)	17.54 (0.78)
3rd quintile	12.14 (1.19)	17.64 (0.78)
4th quintile	12.09 (1.18)	17.71 (0.79)
5th quintile	11.78 (1.19)	17.78 (0.80)
P-value for linear trend	< 0.0001	< 0.0001

[1]Values represent geometric means (standard deviation).
[2]Model is adjusted for sex, age, fetal ethnicity, age of mother, maternal pre-pregnancy body mass index, maternal educational level, maternal smoking, paternal body mass index, parity, duration of breastfeeding, and number of postnatal measurements. Median age at measurement in second trimester (in weeks): 20.5 (90% range: 18.9 – 22.7). Median age at measurement in third trimester (in weeks): 30.4 (90% range: 28.9 – 32.2). Median age at measurement at birth (in weeks): 40.1 (90% range: 37.1 – 42.1).
SDS: Standard Deviation Score

linear trend <0.05). Weight gain between both the second and third trimesters and the third trimester and birth was positively associated with BMIAP (both P-values for linear trends <0.0001). Infants with a smaller weight gain between the third trimester and birth had a higher PWV (P-value for linear trend <0.0001). Prenatal growth parameters were not associated with the ages of PWV and PHV (data not shown).

Femur length measured during the second trimester was positively associated with PHV and negatively associated with BMIAP (both P-value for linear trend <0.05; **Table 4**). At birth, these associations were both reversed where length was negatively associated with PHV and positively associated with BMIAP (P-values for linear trends <0.0001). Gradual length gain between both the second and third trimesters and between the third trimester and birth was associated with higher PHV after birth (P-values for linear trends <0.05). Length gain between the third trimester and birth was positively associated with BMIAP (P-value for linear trend <0.0001)

Table 5 shows the associations between PWV, PHV, and BMIAP with the risks of overweight and obesity at the age of 4 years. Subjects in the highest quintile of PWV had an increased risk of being overweight/obese at the age of 4 years (odds ratio (OR) (95% confidence interval (CI) 15.01 (9.63, 23.38)). There was no association between PHV and the risk of overweight or obesity at the age of 4 years. These results did not materially change after additional adjustment for birth weight. The ages at PWV and PHV were not associated with the risk of obesity at the age of 4 years (data not shown).

Table 4 The association of (femur) length with peak height velocity (PHV) and body mass index at adiposity peak (BMIAP)[1,2]

Femur length 2nd trimester (SDS)	**Peak height velocity (PHV) (cm/year) (N = 5,802)**	**Adiposity peak (body mass index) (kg/m²) (N = 5,448)**
1st quintile	48.89 (1.16)	17.67 (0.82)
2nd quintile	49.45 (1.18)	17.63 (0.76)
3rd quintile	48.73 (1.17)	17.62 (0.80)
4th quintile	49.48 (1.18)	17.55 (0.84)
5th quintile	49.28 (1.17)	17.54 (0.79)
P-value for linear trend	< 0.05	< 0.05
Femur length 3rd trimester (SDS)	**Peak height velocity (PHV) (cm/year) (N = 5,993)**	**Adiposity peak (body mass index) (kg/m²) (N = 5,619)**
1st quintile	49.53 (1.18)	17.64 (0.82)
2nd quintile	49.21 (1.18)	17.66 (0.81)
3rd quintile	49.41 (1.17)	17.60 (0.79)
4th quintile	49.18 (1.17)	17.61 (0.79)
5th quintile	48.45 (1.16)	17.54 (0.80)
P-value for linear trend	0.47	< 0.01
Birth length (SDS)	**Peak height velocity (PHV) (cm/year) (N = 4,125)**	**Adiposity peak (body mass index) (kg/m²) (N = 3,833)**
1st quintile	56.26 (1.20)	17.46 (0.78)
2nd quintile	50.52 (1.16)	17.51 (0.80)
3rd quintile	48.51 (1.14)	17.62 (0.78)
4th quintile	46.76 (1.14)	17.66 (0.79)
5th quintile	43.22 (1.14)	17.77 (0.79)
P-value for linear trend	< 0.0001	< 0.0001
Length change from 2nd to 3rd trimester (SDS)	**Peak height velocity (PHV) (cm/year) (N = 5,717)**	**Adiposity peak (body mass index) (kg/m²) (N = 5,369)**
1st quintile	49.82 (1.19)	17.57 (0.83)
2nd quintile	49.56 (1.17)	17.60 (0.79)
3rd quintile	49.01 (1.17)	17.61 (0.81)
4th quintile	48.71 (1.16)	17.62 (0.77)
5th quintile	48.53 (1.16)	17.62 (0.81)
P-value for linear trend	< 0.05	0.66
Length change from 3rd trimester to birth (SDS)	**Peak height velocity (PHV) (cm/year) (N = 4,007)**	**Adiposity peak (body mass index) (kg/m²) (N = 3,789)**
1st quintile	55.47 (1.20)	17.42 (0.77)
2nd quintile	50.39 (1.16)	17.56 (0.79)
3rd quintile	48.40 (1.15)	17.56 (0.79)
4th quintile	47.04 (1.15)	17.70 (0.80)
5th quintile	44.10 (1.15)	17.81 (0.74)
P-value for linear trend	< 0.001	< 0.0001

[1]Values represent geometric means (standard deviation).
[2]Model is adjusted for sex, age, fetal ethnicity, age of mother, maternal pre-pregnancy body mass index, maternal educational level, maternal smoking, paternal body mass index, parity, duration of breastfeeding, and number of postnatal measurements. Median age at measurement in second trimester (in weeks): 20.5 (90% range: 18.9 – 22.7). Median age at measurement in third trimester (in weeks): 30.4 (90% range: 28.9 – 32.2). Median age at measurement at birth (in weeks): 40.1 (90% range: 37.1 – 42.1).
SDS: Standard Deviation Score

Table 5 The association of peak weight velocity (PWV), peak height velocity (PHV), and body mass index at adiposity peak (BMIAP) with the risk of overweight/obesity[16] at the age of 4 years[1]

Peak weight velocity (PWV) (kg/year)	**Model 1[2]: Overweight/Obesity**	**Model 2[3]: Overweight/Obesity**
1st quintile	Reference	Reference
2nd quintile	2.70 (1.74, 4.19)***	2.79 (1.79, 4.34)***
3rd quintile	3.77 (2.43, 5.84)***	4.06 (2.61, 6.31)***
4th quintile	6.00 (3.88, 9.29)***	6.49 (4.18, 10.09)***
5th quintile	15.01 (9.63, 23.38)***	16.33 (10.43, 25.55)***
P for linear trend	< 0.0001	< 0.0001
Peak height velocity (PHV) (cm/year)	**Model 1: Overweight/Obesity**	**Model 2: Overweight/Obesity**
1st quintile	Reference	Reference
2nd quintile	1.14 (0.83, 1.56)	1.25 (0.91, 1.71)
3rd quintile	1.01 (0.73, 1.40)	1.18 (0.84, 1.64)
4th quintile	0.82 (0.58, 1.16)	0.96 (0.67, 1.37)
5th quintile	1.00 (0.70, 1.41)	1.26 (0.88, 1.82)
P for linear trend	0.57	0.35
Body mass index at adiposity peak (BMIAP) (kg/m²)	**Model 1: Overweight/Obesity**	**Model 2: Overweight/Obesity**
1st quintile	Reference	Reference
2nd quintile	3.46 (1.68, 7.14)***	3.49 (1.69, 7.12)***
3rd quintile	7.66 (3.86, 15.21)***	7.75 (3.84, 15.42)***
4th quintile	16.65 (8.54, 32.48)***	16.96 (8.64, 33.28)***
5th quintile	47.28 (24.26, 92.12)***	48.38 (24.57, 95.27)***
P for linear trend	< 0.0001	< 0.0001

[1]Overweight/obesity are based on standard definitions established by Cole *et al*.[16] Values represent odds ratios (95% confidence interval) based on multivariate logistic regression.
[2]Model 1 is adjusted for sex, age, fetal ethnicity, age of mother, maternal pre-pregnancy body mass index, maternal educational level, maternal smoking, paternal body mass index, parity, duration of breastfeeding, and number of postnatal measurements.
[3]Model 2 is additionally adjusted for birth weight (SDS).
*** P < 0.001

DISCUSSION

We demonstrated strong associations between fetal growth characteristics and infant growth rates. The direction and size of the associations were dependent on the timing of the fetal growth variation. Estimated fetal weight measured during the second trimester was positively associated with both PWV and BMIAP during infancy. Gradual weight and height gain between the third trimester and birth were associated with higher PWV and PHV, respectively. Both higher PWV and BMIAP during infancy were strongly positively associated with increased risks of overweight and obesity at the age of 4 years.

Methodological considerations

To our knowledge, this is the first study that has examined the associations of infant growth rates with both fetal growth characteristics and the risks of overweight and obesity in childhood. Analyses were performed in a large sample that made our study well powered. Furthermore, data were available for a large number of covariates. A limitation might be that 16.4% of the children had fewer than 3 postnatal measurements and were therefore not included in the analyses. A minimum of 3 measurements was set for the postnatal growth modeling. Birth weight and birth length were lower in children without postnatal data available for analyses (70.6 (95% CI 42.8, 98.4) grams and 0.26 (95% CI 0.06, 0.46) cm, respectively). Also, birth length was missing in 33.6% of our sample, since this measurement is not a part of the routine obstetric practice in the Netherlands. Subjects without birth length measurements had a slightly smaller femur length in the second and third trimesters (P = 0.07 and P = 0.04, respectively) and a lower PHV (-0.60 (95% CI -1.05, -0.16) cm/year). Smaller babies at birth are more likely to show lower growth rates in the third trimester and increased growth rates during early infancy than normal size newborns. Therefore, we expect that this selection most likely will lead to an underestimation of inverse associations between growth rates in the third trimester and peak growth velocity during infancy.

Comparison of main findings with other studies

Recently, it was demonstrated in a population-based study from Finland that both PWV and PHV in the first months after life were associated with increased risks of higher blood pressure and BMI in adulthood.[6] Previously, catch-up growth or upward growth realignment in the first 2 years of postnatal life was shown to be associated with an adverse adult metabolic phenotype.[5,23] Moreover, it has been shown that children who were born small for gestational age and had a rapid weight gain in the first 3 months of life were at

increased risk of development of risk factors for cardiovascular disease and type 2 diabetes.[24] It seems that rapid weight gain in the first months immediately after birth may be of greater importance than catch-up growth during the first 2 years.[25] Adaptations in early postnatal growth rates are influenced by a drive to compensate for prenatal fetal growth restriction or growth acceleration caused by the maternal-uterine environment.[26] In our study, we indeed found that there was a strong negative association between weight or height gain from the third trimester until birth and PWV and PHV during infancy. In contrast, growth in weight and height measured in the second trimester was positively associated with PWV and PHV, respectively. Body stature and size are known to be a highly heritable trait, with a large genetic component.[27,28] It could be hypothesized that the fetus grows along its growth curve during the first half of pregnancy but that this curve is more susceptible to maternal-uterine factors during late pregnancy. After birth, however, the child may continue along its original genetically determined growth curve or may deviate from this due to compensatory accelerated or decelerated growth as a response to decreased or increased fetal third trimester growth, respectively. Finally, the first trimester CRL was not associated with any of the derived postnatal growth parameters. We have previously described that first trimester CRL is associated with prenatal and early postnatal growth but that these associations are much stronger before birth than after birth.[11] Thus, though the first trimester analyses were not nearly as well powered as the analyses of later pregnancy, this lack of associations is most likely due to the fact that there is no relationship between first trimester growth and PWV, PHV, or BMIAP.

The relationship between obesity during infancy and during later life (both childhood and adulthood) is complex. In the study of Rolland-Cachera *et al.*,[29] the authors found a twofold increased risk of being obese at the age of 21 years if the individual was also obese at the age of 1 year. This would be similar with our current study, where we find a strong association between BMIAP (which occurs at around 0.75 years) and obesity at the age of 4 years. Also, in the Northern Finnish Birth Cohort Study 1966, it was found that BMIAP was associated with higher BMI at 31 years of age.[7] The phenomenon where children tend to stay more or less in the same percentile of growth is also called tracking. In contrast, the study of Eriksson *et al.*[30] shows an inverse relationship between BMI at the age of 1 year and obesity in adulthood. These findings are in line with our previous study regarding the association between obesity gene FTO and BMI during early life.[31] Here, we found that the obesity risk allele was associated with lower BMIAP (at the age of about 0.75 years).[31] This finding may reflect rapid early weight gain or sometimes called catch-up growth. The most plausible explanation for this apparent contradiction is that there are actually two phenomena occurring simultaneously, namely tracking and early rapid weight gain. The most convincing evidence for this theory is the study of Parsons *et al.*[32] using data from the 1958 Birth Cohort. In this study, they found the association between

birth weight and BMI in adulthood to be J-shaped. Children in the lower ranges of (birth) weight in early life tend to show rapid weight gain in early life, which ultimately may lead to obesity in adulthood. On the other side of the spectrum, children in the upper ranges tend to track and continue to have a high BMI in adulthood. In our study, estimated fetal weight measured during the second trimester was positively associated with BMIAP. Also, birth weight itself was strongly positively associated with BMI at the age of 4 years (data not shown). Based on the data from the current study, it could be hypothesized that fetuses that show third trimester growth restriction in late pregnancy, which might lead to a lower birth weight, show rapid weight gain postnatally and thus are at increased risk of developing obesity in later life. In contrast, fetuses that grow in the highest percentiles for weight, from second trimester onwards, are more likely to continue following this curve during postnatal life, which could ultimately lead to a higher BMI as adults.

Conclusion

We demonstrated strong associations between fetal growth characteristics and infant growth rates. Estimated fetal weight measured during the second trimester is positively associated with a higher PWV during infancy. Both gradual weight gain and height gain between the third trimester and birth are strongly associated with higher postnatal PWV and PHV, respectively. Higher PWV, which generally occurs in the first month after birth, is a strong predictor of childhood overweight and obesity. Results from our study suggest that studies relating birth size with outcomes in later life should take the longitudinal fetal and infant growth measures into account. Longer follow-up studies are necessary to determine how infant growth patterns affect the risk of disease in later life.

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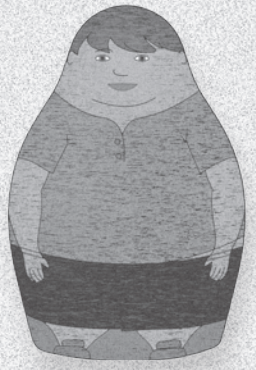

CHAPTER 3.2

Growth in fetal life and infancy and abdominal adiposity at the age of 2 years

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ABSTRACT

Objective: Early weight gain is associated with an increased risk of obesity. It is not known whether rapid weight gain in fetal life and infancy is also associated with increased abdominal adiposity. We examined the associations of fetal and postnatal growth characteristics with abdominal fat mass at the age of 2 years.

Methods: This study was performed in 481 children participating in a prospective cohort study from early fetal life onward. Fetal and postnatal growth characteristics in second and third trimester, at birth and at the age of 2 years were related to abdominal fat mass (subcutaneous distance and area, preperitoneal distance and area) measured by ultrasound at the age of 2 years.

Results: Fetal and birth weight were not associated with abdominal subcutaneous fat mass. Estimated fetal weight in second trimester of pregnancy was inversely associated with preperitoneal fat area (-3.73% (95% confidence interval (CI) -7.23, -0.10) per standard deviation score (SDS) increase in weight. Weight gain from birth to the age of 2 years was positively associated with preperitoneal fat mass measures. These associations remained significant after adjustment for age, sex, breastfeeding and body mass index. Positive associations were found between catch-up growth in weight and abdominal fat mass measures.

Conclusions: Our results suggest that rapid growth rates during fetal life and infancy are associated with increased abdominal subcutaneous and preperitoneal fat mass in healthy children. Further studies need to explore whether these associations persist in later life and are related to metabolic syndrome outcomes.

INTRODUCTION

The prevalence of childhood overweight and obesity has dramatically increased over the past two decades.[1,2] Childhood obesity is an important risk factor for various adverse health outcomes in childhood and adulthood, including type 2 diabetes and cardiovascular diseases.[3,4] Rather than high body mass index (BMI), increased central and visceral fat seem to lead to higher risks of development of obesity and metabolic and cardiovascular diseases in later life.[5,6] Studies in adults showed that increased abdominal fat mass, a measure of central and visceral fat, is associated with an increased risk of insulin resistance, dyslipidemia, hypertension and coronary heart disease and overall mortality rates.[7-9] Risk factors for childhood obesity have been studied extensively. Increased growth rates in early postnatal life are strongly associated with obesity in childhood and adulthood.[10,11] It has been suggested that also low birth weight children with an increased postnatal growth rate are at increased risk for developing obesity.[12] Not much is known specifically about development of abdominal visceral fat in childhood and its growth-related determinants.

To test the hypothesis that high growth rates in early life are associated with an increase in abdominal visceral fat mass, which may be related to later health outcomes, we examined the associations of fetal and early postnatal growth characteristics with abdominal subcutaneous and preperitoneal fat mass at the age of 2 years. We also examined whether catch-down and catch-up growth from birth to the age of 2 years are associated with an increase in abdominal fat mass development. We used a recently developed noninvasive ultrasound method to measure abdominal fat in 481 children participating in a prospective cohort study from early fetal life onwards.[13,14]

METHODS

Study design

The present study was embedded in the Generation R Study, a prospective cohort study from early fetal life onwards in Rotterdam, the Netherlands. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail.[13,15] Assessments during pregnancy, including physical and fetal ultrasound examinations and questionnaires, were performed in first trimester (gestational age 14.5 (range 11.2-16.4) weeks), second trimester (gestational age 20.5 (range 19.0 - 22.0) weeks) and third

trimester (gestational age 30.3 (range 28.7 - 32.2) weeks). The individual time scheme of these assessments depended on the specific gestational age at enrollment. At the age of 2 years, abdominal fat measurements were performed in a subgroup of 481 infants. This was a randomly selected subgroup of Dutch children from the total cohort population. No specific selection criteria were used. Eighty percent of all mothers approached, participated in this study. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

Fetal growth

Fetal ultrasound examinations were carried out at the research centers in each trimester of pregnancy.[13,16] These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics. Crown-rump length was used for pregnancy dating in early pregnancy (gestational age until 12 weeks and 5 days, crown-rump length smaller than 65 mm) and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter larger than 20 mm). Fetal growth measurements used in the present study included head circumference (HC), abdominal circumference (AC) and femur length (FL), measured in second and third trimester of pregnancy and measured to the nearest millimeter (mm) using standardized ultrasound procedures. Estimated fetal weight (EFW) was calculated using the formula by Hadlock *et al.*[17]: (log10 EFW=1.5662-0.0108 (HC)+0.0468 (AC)+0.171 (FL)+0.00034 $(HC)^2$ – 0.003685 (AC x FL)). Fetal measurements in early pregnancy were not included as growth characteristics because these ultrasound examinations were primarily performed to establish gestational age.

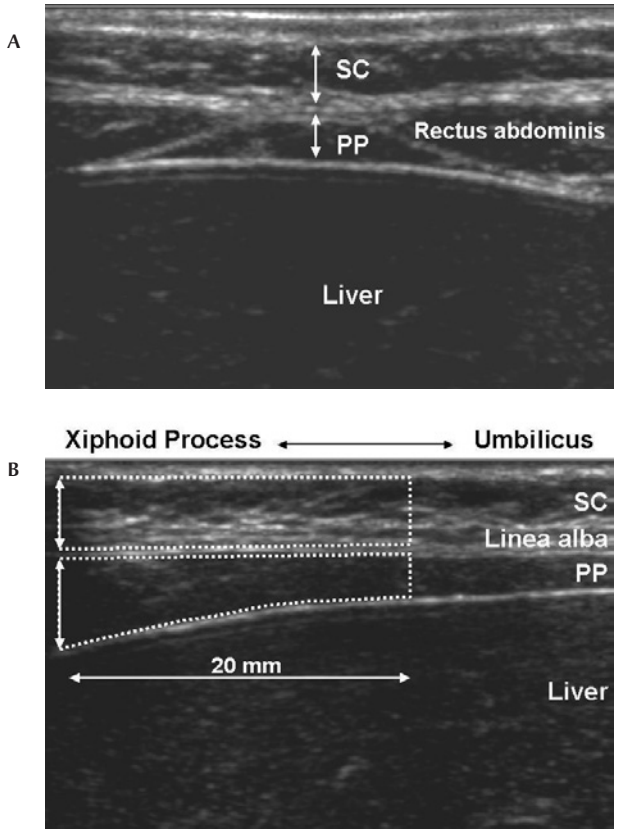
Birth characteristics

Date of birth, birth weight (standard deviation score, SDS) and sex were obtained from midwife and hospital registries. A decrease and an increase in SDS for weight between second trimester of pregnancy and the age of 2 years and between birth and the age of 2 years greater than 0.67 SDS were considered as catch-down and catch-up growth, respectively. A change of 0.67 SDS represents the width of each percentile band on standard growth charts.[18]

Abdominal fat mass

Abdominal fat mass measures were measured at the age of 2 years with ultrasound (Philips / ATL HDI 5000, Seattle, Washington, USA) in the supine position. A Linear Array probe L12-5 (38 mm, 5-12 MHz) was placed at the median upper abdomen, according to the method described by Suzuki *et al*.[14] Scanning was performed between the xiphoid process and the umbilicus. A transversal image was obtained by placing the probe perpendicularly to the linea alba. Only the maximum thickness of the subcutaneous fat layer was measured in this image by scanning the direction of the xiphoid process (**Figure 1A**). To obtain a sagittal image, the probe was kept parallel to the linea alba (**Figure 1B**). In this image the maximum preperitoneal fat thickness and the minimum subcutaneous fat

Figure 1 Measurements of abdominal fat with ultrasound



Transversal images from an ultrasound (**A**) at the location where the maximum subcutaneous (SC) fat thickness was measured. Sagittal images from an ultrasound (**B**) where the maximum preperitoneal (PP) fat thickness and the PP and SC areas were measured starting at the location of the maximum PP thickness to 20 mm in caudal direction.

thickness were measured. In the same image, preperitoneal and subcutaneous fat areas were measured by starting from the position where the maximum preperitoneal fat thickness is seen, to 20 mm in the caudal direction (Figure 1B). Subcutaneous fat thickness reflects subcutaneous central fat mass and preperitoneal fat is found to be a proxy of visceral abdominal fat.[19] Both preperitoneal and subcutaneous fat are associated with metabolic syndrome outcomes. The ratio between this preperitoneal and subcutaneous fat thickness, based on ultrasound measures, is a useful method to estimate the abdominal fat distribution. A higher ratio reflects a more adverse abdominal fat distribution. This method has been used in several studies.[14]

We performed a validation study in 34 children (aged 1-18 years) and showed that abdominal fat measurements by ultrasound were strongly correlated with measurements obtained by Computer Tomography (CT). Of the total group, 9 children were between the ages of 1 and 4 years.[20] Overall correlation coefficients ranged from 0.75 to 0.97 for the whole group and from 0.56 to 0.94 for children younger than 4 years. To assess the intra-observer repeatability of our measurements, we calculated intra-class correlation coefficients (ICC). For the ultrasound measurements, the ICC's ranged from 0.93 to 0.97 from which we can conclude that our measurements for ultrasound were highly reproducible. Results were similar for children younger and older than 4 years.[21] Agreement between ultrasound and CT measurements was assessed using Bland and Altman plots and showed small systematic differences between the measurements obtained by ultrasound and CT. Thus, measuring abdominal fat distribution using ultrasound is a useful method for epidemiological research in children, but should be used carefully for obtaining absolute measurements in individual children.

Anthropometrics of the child

Weight was measured at the age of 2 years by a mechanical personal scale (SECA, Almere, The Netherlands). Height was measured by a Harpenden stadiometer (Holtain Limited, Dyfed, UK) in standing position. BMI (kg/m^2) and body surface area (BSA) (m^2) were calculated.

Covariates

Information on maternal age and weight was obtained by the first questionnaire at enrollment in the study. Maternal weight gain during pregnancy was calculated as pre-pregnancy to third trimester weight change. As enrollment in our study was in early pregnancy, we were not able to measure maternal weight before pregnancy. We obtained information on pre-pregnancy weight by questionnaire. Correlation of pre-pregnancy weight obtained

by questionnaire and weight measured at enrollment was 0.97 ($P < 0.001$). Maternal height was measured without shoes at our research center and pre-pregnancy BMI (kg/m^2) was calculated. In total, 4 of the 481 mothers did have gestational diabetes and 45 of the 481 subjects did have a family history of diabetes. This information was obtained from midwife and hospital registries and questionnaires, respectively. Information on duration of breastfeeding was collected by questionnaires at 2, 6 and 12 months.

Statistical analysis

Differences between boys and girls were examined with Student's t-tests and Chi- square tests. The associations of fetal weight in the second and third trimester of pregnancy and birth weight with abdominal fat mass were assessed using linear regression models. For this purpose, we calculated relative fat mass (%) at the age of 2 years as percentage of abdominal fat mass. The interpretation of the differences in abdominal fat mass is easier by using relative differences expressed as percentages. To compare the effects of weight at different ages, the effects are presented per change in SDS. As preperitoneal area and the preperitoneal/subcutaneous ratio were not normally distributed, natural log transformation was applied and effect estimates are presented as geometric means. These regression models were adjusted for age at visit (months), sex, breastfeeding and current BMI. As, no associations were found for maternal pre-pregnancy weight and pregnancy weight gain, age, smoking and paternal anthropometrics with abdominal fat mass measures and adding these variables to the regression models did not materially change the effect estimates, they were not included in the final models. Similarly, we additionally adjusted our regression models for gestational diabetes, family history of diabetes and birth weight using a multiple regression analysis. As our effect estimates did not change, these covariates were not included in the final models. Similar regression models were used to assess the effect of fetal and postnatal weight change (standard deviation (SD)) on abdominal fat mass measures. Next, we constructed tertiles of birth weight and used linear regression models to examine the effect of catch-down and catch-up growth on abdominal fat mass compared with nonchangers. These models were adjusted for age at visit (months), sex and breastfeeding. Tests for trends within strata were performed by using the continuous variables in the fully adjusted linear regression model. To test whether the associations of birth weight and postnatal growth tertiles with abdominal fat mass measures were modified by sex, we added interaction terms of sex with birth weight and postnatal growth to our regression models. These were not significant (all P-value > 0.1) and therefore not further used in the models. We calculated that, with $\alpha = 0.05$ and 80% power, we were able to detect differences of 0.13 SD (SD 0.2 for both independent and dependent variables). We are not aware of previous studies that studied the associations

of growth characteristics in early life with abdominal fat mass measures in childhood. However, previous studies focused on BMI as outcomes showed much larger effect estimates.[22] All measures of association are presented with their 95% confidence interval (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 presents the clinical characteristics of the children and their mothers. Of all participating children, 51% were male. Birth weight was higher in boys than in girls. No differences were found between boys and girls in breastfeeding. At the age of 2 years, girls had a higher subcutaneous transversal distance and area (differences 10.27% (95% CI 3.82, 16.72) and 11.13% (95% CI 4.49, 17.78)), respectively. **Table 2** shows that weight, BSA and BMI were all strongly positively associated with all abdominal fat measures. The smallest effect estimates were found for height.

Table 3 shows weak tendencies towards positive associations of fetal weight with both subcutaneous fat distance and area. These were all not significant. Tendencies towards inverse associations of fetal weight with preperitoneal fat were found. The strongest effect was found for second trimester fetal weight, which was inversely associated with preperitoneal fat area (-3.73% (95% CI -7.23, -0.10) per SDS increase in weight. Fetal weight in second and third trimester were inversely associated with the preperitoneal/subcutaneous ratio. The associations were of borderline significance. No significant associations between birth weight and abdominal fat measures were found. Weight gain from birth to the age of 2 years was positively associated with preperitoneal fat mass measures ($P < 0.01$). No associations were found between postnatal weight gain and subcutaneous fat mass measures.

Table 4 shows that within each tertile of birth weight catch-up growth was positively associated with subcutaneous and preperitoneal fat mass measures. Tests for trend for the ratio preperitoneal/ subcutaneous distance were not significant. Except for subcutaneous area, the lowest and highest fat mass percentages, were found in children in the lowest birth weight tertile with catch-down growth and in children in the highest birth weight tertile with catch-up growth, respectively. Differences up to 22% were observed.

Table 1 Maternal, fetal and child characteristics (N = 481)[1,2]

Maternal characteristics		
Age (years)		31.9 (3.8)
Weight (kg)		69.0 (13.3)
Height (cm)		170.9 (6.2)
Body mass index (kg/m^2)		23.6 (4.3)
Smoking habits (%)	Never smoked	83%
	Smoked until pregnancy was known	7%
	Throughout pregnancy	10%
Fetal and child characteristics		
Second trimester	Gestational age (weeks)	20.5 (19.0 – 22.0)
	Estimated fetal weight (g)	366 (69)
Third trimester	Gestational age (weeks)	30.3 (28.7 – 32.2)
	Estimated fetal weight (g)	1,614 (254)
Birth	Gestational age (weeks)	40.1 (37.4 – 42.1)
	Weight (grams)	3,501 (529)
	Low birth weight (<2500 grams) %	3.5
	Preterm birth (<37 weeks) (%)	3.3
	Males (%)	51
2 years	Age at visit (months)	25.3 (23.4- 27.9)
	Weight (grams)	12,550 (1 358)
	Height (cm)	88.9 (3.3)
	Body surface area (m^2)	0.56 (0.04)
	Body mass index (kg/m^2)	15.8 (1.3)
Breastfeeding	Ever (%)	91
	Duration (months)	4.9 (0.5 – 12.0)
Abdominal measures at 2 years	Subcutaneous transversal distance (mm)	2.88 (1.37 – 4.80)
	Subcutaneous area (mm^2)	45.24 (23.0 – 79.0)
	Preperitoneal distance (mm)	2.26 (1.33 – 3.47)
	Preperitoneal area (mm^2)	28.76 (17.0 – 19.45)
	Ratio preperitoneal/ subcutaneous transversal distance	1.07 (0.58 – 1.79)

[1]Values are means (standard deviation), percentages or medians (90% range).
[2]Of the total group, data were missing on weight before pregnancy (N = 61), maternal height at intake (N = 3), body mass index before pregnancy (N = 62), gestational age second trimester (N = 15), estimated fetal weight second trimester (N = 20), gestational age third trimester (N = 15), estimated fetal weight third trimester (N = 21), age at visit at 2 years (n = 7), current weight (N = 9), current height (N = 22), body mass index at 2 years (N = 22), ever breastfeeding (N = 4), duration of breastfeeding (N = 57), SC trans (N = 3), SC area (N = 12), PP distance (N = 8), PP area (N = 11) and ratio PP/SC (N = 8).

Table 2 Associations between anthropometrics and abdominal fat measures (%) at the age of 2 years[1]

	Abdominal fat measures (%)				
(N = 481)	**Subcutaneous transversal distance**	**Subcutaneous area**	**Preperitoneal distance**	**Preperitoneal area[2]**	**Ratio preperitoneal/ subcutaneous distance[2]**
Height (1 SD = 3.3 cm)	1.94 (-1.39, 5.31) P = 0.25	1.49 (-2.00, 4.98) P = 0.40	3.19 (0.35, 6.03) P = 0.03	4.60 (1.51, 7.79) P < 0.01	1.11 (-2.18, 4.50) P = 0.51
Weight (1 SD = 1.4 kg)	12.49 (9.37, 15.65) P < 0.01	11.88 (8.63, 15.13) P < 0.01	6.34 (3.59, 9.09) P < 0.01	8.33 (5.23, 11.52) P < 0.01	-5.54 (-8.52, -2.57) P < 0.01
BSA (1 SD = 0.04 m^2)	10.44 (7.18, 13.67) P < 0.01	9.88 (6.52, 13.25) P < 0.01	5.85 (3.06, 8.64) P < 0.01	7.68 (4.60, 10.96) P < 0.01	-4.30 (-7.41, -1.19) P < 0.01
BMI (1 SD = 1.3 kg/m^2)	15.30 (12.21, 18.39) P < 0.01	14.75 (11.56, 17.94) P < 0.01	5.63 (2.84, 8.42) P < 0.01	6.82 (3.77, 9.97) P < 0.01	-8.61 (-11.40, -5.64) P < 0.01

[1]Values are regression coefficients (95% confidence interval) and reflect the difference (%) in abdominal fat mass measures for each SD change.
[2]Preperitoneal area and ratio values are geometric means.
BSA: body surface area; BMI: body mass index.

Table 3 Associations of fetal and postnatal weight and weight gain with abdominal fat mass measures (%) at the age of 2 years[1,2]

	Abdominal fat measures (%)				
(N = 481)	**Subcutaneous transversal distance**	**Subcutaneous area**	**Preperitoneal distance**	**Preperitoneal area[3]**	**Ratio preperitoneal/ subcutaneous distance[3]**
Weight (SDS)					
Second trimester	1.46 (-2.50, 5.38) P =0.47	0.68 (-3.43, 4.79) P = 0.75	-3.24 (-6.69, 0.22) P = 0.07	-3.73 (-7.23, -0.10) P = 0.05	-3.34 (-7.13, 0.50) P =0.09
Third trimester	1.46 (-2.08, 5.00) P =0.42	1.49 (-2.24, 5.21) P = 0.43	-1.99 (-5.14, 1.15) P = 0.21	-0.60 (-4.02, 2.84) P = 0.72	-3.54 (-6.95, 0) P = 0.05
Birth	0.28 (-3.23, 3.75) P = 0.88	-1.05 (-4.70, 2.60) P = 0.57	-1.91 (-5.01, 1.20) P = 0.23	-1.00 (-4.21, 2.43) P = 0.58	-0.70 (-4.21, 2.84) P = 0.68
Weight change (SDS)					
3rd trimester - birth	-1.01 (-4.58, 2.57) P = 0.58	-2.59 (-6.35, 1.16) P = 0.18	0.09 (-3.10, 3.28) P = 0.95	-0.30 (-3.63, 3.25) P = 0.88	2.84 (-0.80, 6.61) P = 0.13
Birth – 2 years	1.08 (-2.50, 4.61) P = 0.56	2.13 (-1.59, 5.84) P = 0.26	3.50 (0.31, 6.65) P = 0.03	3.77 (0.30, 7.36) P = 0.04	0.60 (-2.96, 4.29) P = 0.74

[1]Values are regression coefficients (95% confidence interval) and reflect the difference (%) in abdominal fat mass measures for change in SD in weight.
[2]Models are adjusted for age at visit (months), sex, breastfeeding and current body mass index.
[3]Preperitoneal area and ratio values are geometric means.

Table 4 The associations between catch-down and catch-up growth with abdominal fat mass (%)[1,2]

	Postnatal growth			
	Catch-down growth (N = 163)	**Nonchangers (N = 215)**	**Catch-up growth (N = 76)**	
Subcutaneous transversal distance				
Birth weight				
1st tertile (N = 160)	-17.14 (-34.42, 0.14)	-3.96 (-16.07, 8.12)	5.27 (-7.95, 18.49)	P trend = 0.02
2nd tertile (N = 152)	-15.16 (-29.01, -1.32)	Reference	18.56 (1.63, 35.50)	P trend < 0.01
3rd tertile (N = 157)	-4.86 (-16.07, 6.32)	11.73 (-0.80, 24.29)	-15.27 (-65.68, 35.15)	P trend = 0.02
	P trend < 0.01	P trend < 0.01	P trend = 0.08	
Subcutaneous area				
Birth weight				
1st tertile (N = 160)	-17.96 (-35.28, -0.63)	-7.44 (-19.78, 4.90)	6.01 (-7.36, 19.38)	P trend < 0.01
2nd tertile (N = 152)	-10.81 (-24.82, 3.19)	Reference	16.13 (-0.82, 33.07)	P trend < 0.01
3rd tertile (N = 157)	-10.91 (-22.21, 0.39)	14.29 (1.63, 26.94)	2.25 (-48.12, 52.63)	P trend < 0.01
	P trend < 0.01	P trend < 0.01	P trend = 0.07	
Preperitoneal distance				
Birth weight				
1st tertile (N = 160)	-21.99 (-33.73, -7.76)	-8.55 (-18.71, 1.55)	-3.24 (-14.14, 7.62)	P trend = 0.04
2nd tertile (N = 152)	-16.45 (-27.93, -4.96)	Reference	2.62 (-11.30, 16.49)	P trend = 0.02
3rd tertile (N = 157)	-11.04 (-20.30, -1.82)	-2.53 (-12.90, 7.85)	14.45 (-19.46, 48.36)	P trend = 0.07
	P trend = 0.02	P trend = 0.33	P trend = 0.95	
Preperitoneal area[3]				
Birth weight				
1st tertile (N = 160)	-24.87 (-35.40, -12.54)	-6.95 (-16.47, 3.67)	1.21 (-9.97, 13.77)	P trend < 0.01
2nd tertile (N = 152)	-15.38 (-25.10, -4.30)	Reference	5.76 (-8.88, 22.63)	P trend < 0.01
3rd tertile (N = 157)	-7.41 (-16.14, 2.22)	3.15 (-7.69, 15.26)	16.77 (-18.70, 67.70)	P trend = 0.10
	P trend < 0.01	P trend = 0.12	P trend = 0.80	
Ratio preperitoneal/subcutaneous distance[3]				
Birth weight				
1st tertile (N = 160)	-1.09 (-16.31, 17.00)	0.30 (-10.95, 13.09)	-4.40 (-15.89, 8.76)	P trend = 0.34
2nd tertile (N = 152)	-3.54 (-15.72, 10.52)	Reference	-12.10 (-25.40, 3.56)	P trend = 0.28
3rd tertile (N = 157)	2.94 (-7.69, 14.80)	-11.57 (-21.81, -0.10)	-4.78 (-36.17, 42.05)	P trend = 0.05
	P trend = 0.28	P trend < 0.01	P trend = 0.02	

[1]Values are regression coefficients (95% confidence interval) and reflect the difference in abdominal fat measures (%) compared with children in the 2nd tertile of birth weight and without growth realignment.
[2]All models are adjusted for age at visit (months), sex and breastfeeding. Tests for trends within each stratum of birth weight and catch-up growth are performed by using the continuous variables in the fully adjusted linear regression model.
[3]Preperitoneal area and ratio values are geometric means.

DISCUSSION

This population-based prospective cohort study showed that second trimester estimated fetal weight was not associated with abdominal subcutaneous fat mass measures but showed tendencies towards inverse associations with preperitoneal fat measures at the age of 2 years, which are related to abdominal visceral fat mass. Birth weight was not associated with abdominal fat mass measures. Weight gain from birth to the age of 2 years was positively associated with the preperitoneal abdominal fat mass measures, but not with subcutaneous abdominal fat mass measures. Similarly, we found tendencies towards positive associations between postnatal catch-up growth and subcutaneous and preperitoneal fat mass measures in each tertile of birth weight.

Methodological considerations

To our knowledge, this is the first prospective cohort study examining the associations of fetal and postnatal growth characteristics with abdominal fat mass measured by ultrasound. Thus far, most studies focused on adiposity in early childhood used BMI or waist-to-hip ratio as outcome.[9,23] However, abdominal visceral fat has been suggested to be stronger related to adverse metabolic syndrome outcomes and is therefore of greater interest. In this study, abdominal fat mass was measured by ultrasound, a valid method for measuring abdominal fat distribution in children in which preperitoneal fat mass is related to abdominal visceral fat mass.[14] The strength of this study is the population-based cohort with a relative large number of subjects studied with ultrasound. The study group consisted of healthy children participating in an ongoing prospective cohort study. Potential limitations of this study are the small numbers of preterm births and children born with low birth weight. Preterm born infants may be at risk of development of metabolic syndrome outcomes in later life through increased and aberrant adiposity.[24,25] These small numbers make it difficult to study the effect of preterm birth and low birth weight on abdominal fat mass and limits extrapolation of our results to this specific group of children. However, our results suggest similar effects of early growth characteristics on abdominal fat mass development in healthy children.

Comparison of main findings with other studies

Previous studies have shown that accelerated postnatal growth is related to development of obesity and type 2 diabetes. Stettler *et al*.[26] showed in the National Collaborative Perinatal Project in 19,397 participants that a rapid weight gain during the first 4 months of life leads to an increased risk of childhood overweight at the age of 7 years. A similar

association of weight gain in infancy with obesity in adulthood was found in a cohort of 300 African Americans. Rapid weight gain seems not only to be associated with obesity but also with metabolic syndrome outcomes. In a prospective birth cohort study in 851 children, associations were found between early postnatal weight gain and decreased insulin sensitivity at the age of 8 years.[27] Recently, it was shown that postnatal catch-up growth in the first 6 weeks of life led to higher total fat mass measured as skinfold thickness.[28] Highest growth rates in infancy seem to occur particularly in children born with low birth weight.[28] It is known that a majority of children born with low birth weight have a postnatal catch-up growth during the first 2 postnatal years.[29] These findings suggest that especially children with low birth weight and high growth rates in infancy are at increased risk for developing metabolic syndrome outcomes. Our results are in line with these findings. Fetal and birth weight were inversely associated with preperitoneal fat mass. The largest effect was found for fetal weight in second trimester of pregnancy. In addition, growth from birth to the age of 2 years was associated with increased abdominal fat mass. Our results suggest that growth patterns that have previously been related to increased BMI and development of metabolic syndrome outcomes in adults are also associated with an increased abdominal fat mass in early childhood. Catch-up growth during the first 2 years was associated with increased levels of both subcutaneous and preperitoneal fat measures. However, the increase in subcutaneous fat mass measures was larger, leading to lower preperitoneal and subcutaneous fat area ratios. Thus, although catch-up growth leads to an increase in abdominal adiposity, the distribution is not adversely affected.

Postnatal catch-up growth is associated with both increased BMI and abdominal fat mass. BMI could be an intermediate in the associations between postnatal catch-up growth and abdominal fat mass in childhood. The models in Table 4 were therefore not additionally adjusted for BMI. As previous studies have demonstrated adverse effects of increased abdominal fat mass in children and adults with the same BMI, further studies in larger cohorts are needed to assess development of BMI and abdominal adiposity, separately. To date no studies have assessed whether abdominal fat mass tracks from early childhood into adulthood. However, tracking of BMI has been extensively studied and described.[30-32] The overall tracking coefficient from childhood to adulthood is about 0.6, but is strongly dependent on age and age interval.[33,34] Studies focused on tracking of abdominal fat mass from childhood to adulthood are important, as they may reveal insight in the early origins of obesity and metabolic syndrome. Consequences of abdominal fat mass in young children for metabolic readouts have not been studied yet. Studies in adults showed that abdominal visceral obesity leads to cardiovascular diseases and type 2 diabetes.[35] Additionally, it has been suggested that abdominal adiposity is associated with an increased risk of mortality.[9]

Several studies have shown positive associations between maternal and offspring anthropometrics, like height, weight and BMI.[36,37] We did not find significant associations of maternal and paternal anthropometrics with abdominal fat mass measures in the offspring. However, no measures of abdominal adiposity or waist-hip circumference were available in parents. Further studies are needed to assess whether measures of abdominal fat mass in parents are related to similar measures in the offspring.

Conclusion

This study suggests that growth characteristics and patterns in late fetal and in early postnatal life are associated with increased abdominal fat mass in early childhood. Catch-up growth after birth, even in the normal range of birth weight, is associated with higher abdominal fat mass accumulation. Abdominal fat measured by ultrasound may be an interesting biomarker for predicting later adverse outcomes. Although high abdominal fat mass is an important risk factor for cardiovascular disease, metabolic syndrome and mortality in adulthood, not much is known about the consequences of increased abdominal fat mass in childhood. Further studies are needed to examine whether the associations persist in later life and are associated with development of metabolic syndrome outcomes.

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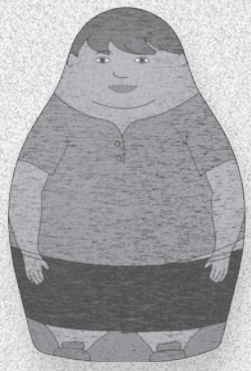

CHAPTER 3.3

FETAL AND INFANT GROWTH AND BODY FAT DISTRIBUTION IN CHILDHOOD

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Submitted

CHAPTER 4

Parental factors

CHAPTER 4.1

Parental anthropometrics, growth and the risk of overweight in early childhood

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ABSTRACT

Objective: There are limited data regarding the associations of both maternal and paternal anthropometrics with longitudinally measured postnatal growth measures in early childhood. We assessed the associations of maternal and paternal anthropometrics with growth characteristics and the risk of overweight in preschool children.

Methods: Population-based prospective cohort study from early fetal life onwards in the Netherlands. Maternal pre-pregnancy anthropometrics and gestational weight gain, and paternal anthropometrics were related to fetal and postnatal growth measures and the risk of overweight until the age of 4 years. Analyses were based on 5,674 mothers, fathers and their children.

Results: Both pre-pregnancy maternal and paternal height, weight and body mass index (BMI) were associated with corresponding fetal and postnatal anthropometric measures. Maternal BMI had a significantly stronger effect on childhood BMI than paternal BMI. As compared to children from parents with normal BMI, children from two obese parents had an increased risk of overweight at the age of 4 years (odds ratio (OR) 6.52 (95% confidence interval (CI) 3.44, 12.38)). Maternal gestational weight gain was only in mothers with normal BMI associated with BMI and the risk of overweight in the children.

Conclusions: Maternal and paternal anthropometrics affect early growth in preschool children differently. Gestational weight gain in mothers without overweight and obesity is related to the risk of overweight in early childhood.

INTRODUCTION

Prevalences of childhood overweight and obesity are increasing in Western countries.[1,2] One of the strongest risk factors of childhood obesity is parental body mass index (BMI).[3] Having both a mother and father with obesity more than doubles the risk of childhood obesity under the age of 10 years.[4] Previously, it has also been suggested that higher maternal gestational weight gain is related to an increased risk of overweight in children aged 2 to 14 years.[5-8] Direct exposures during fetal life might be critical for the development of obesity across the life course.[9-11] Both low and high birth weight have been suggested to be associated with high BMI or obesity in postnatal life.[12,13] Intrauterine under and overnutrition have both been proposed as underlying mechanisms for these associations.[14-16] Maternal anthropometrics and gestational weight gain may be markers of maternal and fetal metabolism and tissue expansion.[17,18] To our knowledge, there are limited data regarding the associations of both maternal and paternal anthropometrics with repeated measures of postnatal growth in early childhood. There are also inconsistencies in whether these associations are explained by direct intrauterine programming effects, other environmental exposures or genetic influences. Observing stronger effect estimates for the associations of maternal obesity, than paternal obesity with offspring BMI, might suggest direct intrauterine effects of maternal nutritional status.[15,19] However, if the associations are of similar magnitude for both maternal and paternal BMI, genetic or postnatal environmental and behavioral exposures might be involved in the underlying pathways.

We examined in a population-based prospective cohort study in 5,674 mothers, fathers and their children followed from early fetal life onwards, the associations of both maternal anthropometrics before and during pregnancy and paternal anthropometrics with early growth characteristics and the risk of overweight until the age of 4 years.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands.[20,21] Enrollment in the study was aimed in first trimester of pregnancy but was possible 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 followed until

young adulthood. This cohort is representative of the regional population. Of all eligible children in the study area, 61% were participating in the study at birth.[20] The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

Parental anthropometrics

Parental anthropometrics were measured in first, second and third trimester of pregnancy. Maternal height was measured in standing position without shoes and heavy clothing at enrollment. Information on maternal weight and BMI just before pregnancy was obtained by questionnaires. We used questionnaire data as enrollment in our study was in early pregnancy. Paternal height and weight were measured lightly clothed without shoes and BMI was calculated. Maternal and paternal BMI were both used as continuous and categorical variables (< 25 kg/m^2; 25 to 29.99 kg/m^2; ≥ 30 kg/m^2) in the analyses. Information on maternal maximum weight during the whole pregnancy was collected postnatally by questionnaire and was missing in 33% (N = 1,740). Therefore, for the current study, we defined maximum weight gain during pregnancy as the difference between weight before pregnancy and weight at gestational age of 30 weeks because this corresponds to the third trimester ultrasound and was available in 97%. The correlation between weight measured at the age of 30 weeks and maximum weight from questionnaire was r = 0.87 (P < 0.001).

Fetal growth characteristics

Fetal ultrasound examinations were carried out at the research centers in first trimester (median 13.4 weeks (90% range 10.4 to 16.9)), second trimester (median 20.5 weeks (90% range 19.0 to 22.7)), and third trimester (median 30.4 weeks (90% range 28.9 to 32.2)).[22-24] Second and third trimester ultrasounds were used to assess fetal growth. We measured fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) to the nearest millimeter (mm) using standardized ultrasound procedures.[25] Estimated fetal weight (EFW) was calculated using the formula by Hadlock *et al.*[26] Standard deviation scores (SDS) for all fetal growth characteristics were constructed based on data from the study group. For the present study we used SDS for femur length and estimated fetal weight as measures for length and weight, respectively. Birth anthropometrics (head circumference, length and weight) were available from medical records.

Postnatal growth characteristics

Postnatal growth was measured at the Community Child Health Centers according to standard schedules and procedures by a well-trained staff at the ages of 3 (median 3.2 months (90% range 1.3 to 4.2)), 6 (median 6.2 months (90% range 5.5 to 10.2)), 12 (median 12.7 months (90% range 11.1 to 15.3)), 24 (median 24.5 months (90% range 18.6 to 27.5)), 36 (median 36.6 months (90% range 31.0 to 39.2)) and 48 months (median 45.8 months (90% range 44.7 to 48.1)). Length was measured in supine position to the nearest mm until the age of 14 months using a neonatometer, after which height was measured in standing position without shoes by a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight was measured without clothing and shoes using a mechanical personal scale (SECA, Almere, The Netherlands), and BMI (kg/m^2) was calculated. SDS for postnatal growth characteristics were obtained using Dutch reference growth charts (Growth Analyser 3.0, Dutch Growth Research Foundation). Overweight (+1.1 SDS) and obesity (+2.3 SDS) were defined based on the national age- and sex- adjusted BMI distributions of the 1997 Dutch Growth Study for children, which correspond to the international adult cut-off points of overweight (25 kg/m^2) and obesity (30 kg/m^2), and the definition of Cole *et al*.[27-29]

Covariates

Gestational age at birth and sex were obtained from midwife and hospital records at birth. Information on maternal educational level (primary, secondary, higher), ethnicity (European, Non-European) and parity (nulliparity, multiparity) were obtained from the first questionnaire at enrollment in the study. Information on maternal smoking was obtained by self-reported questionnaires sent in first, second and third trimester of pregnancy. Response rates for these questionnaires were 91%, 80% and 77%, respectively.[30] Active maternal smoking at enrollment was assessed in the first questionnaire by asking each mother whether she smoked during pregnancy thus far (no, first trimester only and continued smoking). In the second and third questionnaires, the mothers were asked whether they had smoked during the past 2 months (yes, no).[31] Thereafter, we classified the mothers into 2 groups: never smoked and ever smoked (first trimester only and continued smoking). Ethnicity and educational level of the mothers were defined according to the classification of Statistics Netherlands.[32,33] Child's ethnicity was based on the country of birth of the parents and grandparents. Information on breastfeeding initiation and continuation was obtained from delivery reports and postal questionnaires at the ages of 2, 6 and 12 months after birth. Mothers were asked whether they ever breastfed their child (yes, no) and also at what age they quitted breastfeeding.

Population for analysis

In total, 6,969 children and their mothers had been included prenatally and fully participated in the postnatal phase of the study (flow chart is given in **Supplemental Figure 1**). Mothers without information on their pre-pregnancy weight in the first questionnaire were excluded from the present analyses (18.6%, N = 1,295), leaving 5,674 mothers. First, for the analyses focused on the associations of both maternal and paternal anthropometrics with corresponding outcomes in children, we excluded those with missing data on paternal anthropometrics (N = 1,363). Also, twin pregnancies (N = 90) were excluded to prevent bias due to correlation. Of the remaining 4,221 singleton live births with complete data, information about at least one postnatal growth characteristic measure was available in 4,116 children. Second, for analyses focused on the associations of gestational weight gain with outcomes in the children, we excluded from the total of 5,674 mothers those with missing data on gestational weight gain (N = 188), and those with twin pregnancies (N = 95). Of the remaining 5,381 singleton live births with complete data, information about at least one postnatal growth characteristic measure was available in 5,227 children.

Statistical analysis

We assessed the associations between pre-pregnancy maternal and paternal anthropometric measures per 1 standard deviation (SD) and corresponding fetal and postnatal growth characteristics (height, weight and BMI), using linear regression models. These models were adjusted for child's age at visit and sex. The explained variance (R^2) was estimated for each outcome. Subsequently, we examined the associations of maternal and paternal BMI and their interaction with longitudinally measured childhood BMI at the ages of 1, 2, 3 and 4 years. We observed a significant interaction between maternal and paternal BMI at all ages ($P < 0.05$). We used repeated measures three-way Mixed ANOVA, taking into account correlations within-subjects and assessing both time-dependent and -independent associations. The F-test was used to assess the overall main effects of maternal and paternal BMI categories and the interaction between different categories of BMI. The models were adjusted for child's age at visit, sex, maternal ethnicity and education, parity, maternal smoking and breastfeeding. Similarly, we used multiple logistic regression models to analyze the associations of parental BMI with the risk of overweight in children at the age of 4 years. Finally, we explored the associations of pre-pregnancy maternal BMI and gestational weight gain with childhood BMI and the risk of overweight at the age of 4 years, using general linear mixed models and multiple logistic regression models, respectively (interaction between weight gain and maternal BMI $P < 0.001$).

These models were additionally adjusted for gestational age at the third trimester weight measurement. Tests for trends were performed by treating each categorized variable as a continuous term and entering the variable into the fully adjusted model. We did not perform multiple imputations for the general linear mixed models as multiple imputations has no added value in standard linear mixed models.[34] We did perform multiple imputations for the logistic regression models by generating 5 independent datasets.[35] Imputations were based on the relationships between covariates included in this study using the Markov Chain Monte Carlo (MCMC) method. All measures of associations are presented with their 95% confidence interval (CI). Statistical analyses including general linear mixed models were performed using the Statistical Package of Social Sciences version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Of all mothers and fathers included in the analyses, 18.1% (N = 946) and 31.6% (N = 1,652) were overweight, and 7.3% (N = 382) and 6.0% (N = 314) were obese, respectively (**Table 1**). In total, 47.9% of the mothers completed higher educational level and had a total gestational weight gain of 10.6 kg (range 3.0 - 18.0 kg). **Supplemental Table 1** gives the fetal and postnatal growth characteristics at different ages. Furthermore, we observed moderate correlations for height (r = 0.33, P < 0.01), and weak correlations for weight (r = 0.20, P < 0.01) and BMI (r = 0.21, P < 0.01) between mothers and fathers (**Supplemental Figure 2A-C**). Also, **Supplemental Figure 3A,B** shows a weak negative correlation between maternal BMI and gestational weight gain (r = -0.21, P < 0.01).

Pre-pregnancy maternal, paternal and childhood anthropometrics

Table 2 shows the associations of pre-pregnancy maternal and paternal anthropometrics (height, weight and BMI) with corresponding fetal, birth and postnatal growth characteristics until the age of 4 years. The effect estimates are given in SD scores to enable comparison of effect estimates. Pre-pregnancy maternal and paternal anthropometrics were highly correlated with all corresponding fetal and postnatal growth characteristics (all P < 0.01; data not shown). The largest effect estimates and explained variances were observed for the associations between maternal and paternal height and childhood height measurements (combined explained variance 16.7% at the age of 4 years). The

Table 1 Characteristics of mothers, fathers and their children (N = 5,227)[1]

Maternal characteristics	
Age (years)	30.9 (21.3 – 38.1)
Height (cm)	167.9 (7.4)
Pre-pregnancy weight (kg)	65.9 (11.9)
Pre-pregnancy body mass index (kg/m^2)	23.4 (4.0)
Overweight (body mass index > 25 -29.9 kg/m^2) (%)	18.1
Obesity (body mass index ≥ 30 kg/m^2) (%)	7.3
Weight gain during pregnancy (kg)	10.6 (3.0 – 18.0)
Parity (%)	
0	57.0
>=1	43.0
Highest completed education (%)	
Primary school	9.4
Secondary school	42.7
Higher education	47.9
Ethnicity (%)	
European	62.4
Non-European[2]	37.6
Smoked during pregnancy (%)	
Yes	24.9
No	75.1
Alcohol consumption during pregnancy (%)	
Yes	55.7
No	44.3
Paternal characteristics	
Age (years)	33.0 (23.7 – 43.0)
Height (cm)	182.4 (7.8)
Weight (kg)	84.0 (12.9)
Body mass index (kg/m^2)	25.2 (3.4)
Overweight (body mass index > 25 -29.9 kg/m^2) (%)	31.6
Obesity (body mass index ≥ 30 kg/m^2)(%)	6.0
Child characteristics	
Males (%)	50
Gestational age at birth (weeks)	40.1 (37.3 – 42.1)
Birth weight (grams)	3,460 (526)
Ever breast fed (%)	92.2
Breastfeeding duration (months)	4.9 (0.5 - 12.0)

[1]Values are means (standard deviation), percentages (%), or medians (90% range) for variables with skewed distribution.

[2]Non-European: Indonesian, Moroccan, Turkish, Antillean, Surinamese, Cape Verdean, African, American, Asian and Australian.

Table 2 Associations of pre-pregnancy maternal and paternal anthropometrics (in SD) with fetal and postnatal growth characteristics[1]

(N = 4,116)	Estimates based on maternal anthropometrics[2]		Estimates based on paternal anthropometrics[2]		Estimates based on both parents
	Standard deviation score	Explained variance R2 (%)	Standard deviation score	Explained variance R2 (%)	Combined explained variance (%)
Outcome offspring length / height	SD		SD		
2nd trimester[3]	0.08 (0.05, 0.11)**	3.9	0.05 (0.02, 0.08)**	3.4	3.9
3rd trimester[3]	0.17 (0.14, 0.20)**	4.0	0.14 (0.11, 0.17)**	3.1	4.9
Birth	0.24 (0.20, 0.29)**	4.5	0.21 (0.16, 0.25)**	3.4	5.9
3 months	0.31 (0.28, 0.34)**	9.1	0.25 (0.22, 0.28)**	6.2	11.7
6 months	0.27 (0.24, 0.30)**	8.2	0.26 (0.23, 0.28)**	7.6	11.9
12 months	0.24 (0.21, 0.27)**	6.4	0.24 (0.21, 0.27)**	6.6	9.8
24 months	0.29 (0.25, 0.32)**	7.6	0.28 (0.25, 0.32)**	7.8	11.6
36 months	0.34 (0.30, 0.38)**	10.4	0.32 (0.29, 0.36)**	10.0	15.3
48 months	0.36 (0.32, 0.40)**	11.9	0.33 (0.29, 0.36)**	10.2	16.7
Outcome offspring weight	SD		SD		
2nd trimester[3]	0.13 (0.10, 0.16)**	5.4	0.06 (0.03, 0.09)**	4.1	5.5
3rd trimester[3]	0.22 (0.19, 0.26)**	5.3	0.13 (0.10, 0.16)**	2.2	6.0
Birth	0.26 (0.23, 0.29)**	6.8	0.14 (0.11, 0.17)**	2.7	7.7
3 months	0.15 (0.11, 0.18)**	2.0	0.15 (0.12, 0.19)**	2.5	3.5
6 months	0.14 (0.11, 0.17)**	2.1	0.14 (0.11, 0.17)**	2.3	3.6
12 months	0.17 (0.14, 0.20)**	3.2	0.18 (0.15, 0.21)**	3.8	5.8
24 months	0.22 (0.19, 0.26)**	5.1	0.22 (0.19, 0.25)**	5.2	8.4
36 months	0.23 (0.19, 0.27)**	5.0	0.23 (0.19, 0.27)**	5.2	8.7
48 months	0.25 (0.21, 0.29)**	6.0	0.26 (0.22, 0.29)**	6.7	10.5
Outcome offspring body mass index	SD		SD		
3 months	0.08 (0.04, 0.11)**	1.5	0.11 (0.08, 0.15)**	2.3	2.5
6 months	0.11 (0.07, 0.14)**	1.1	0.10 (0.06, 0.13)**	1.1	1.7
12 months	0.09 (0.06, 0.13)**	0.9	0.10 (0.07, 0.13)**	1.2	1.6
24 months	0.14 (0.11, 0.18)**	1.7	0.11 (0.08, 0.15)**	1.2	2.4
36 months	0.15 (0.11, 0.19)**	1.9	0.14 (0.11, 0.18)**	1.9	3.2
48 months	0.17 (0.13, 0.21)**	3.0	0.18 (0.14, 0.22)**	3.6	5.5

[1]Values are regression coefficients (95% confidence interval) and reflect the association of childhood growth characteristic per 1 standard deviation (SD) change in corresponding parental anthropometric characteristic. To enable comparison of effect estimates, results are given in SD scores with the explained variance (R2) for the childhood outcomes. Models are adjusted for child's age at visit and sex.

[2]1 SD in height = 22.79 cm; 1 SD in weight = 5.34 kg; 1 SD in body mass index = 5.54 kg/m^2.

[3]Femur length and estimated fetal weight are used as measures of length and weight in fetal life, respectively.

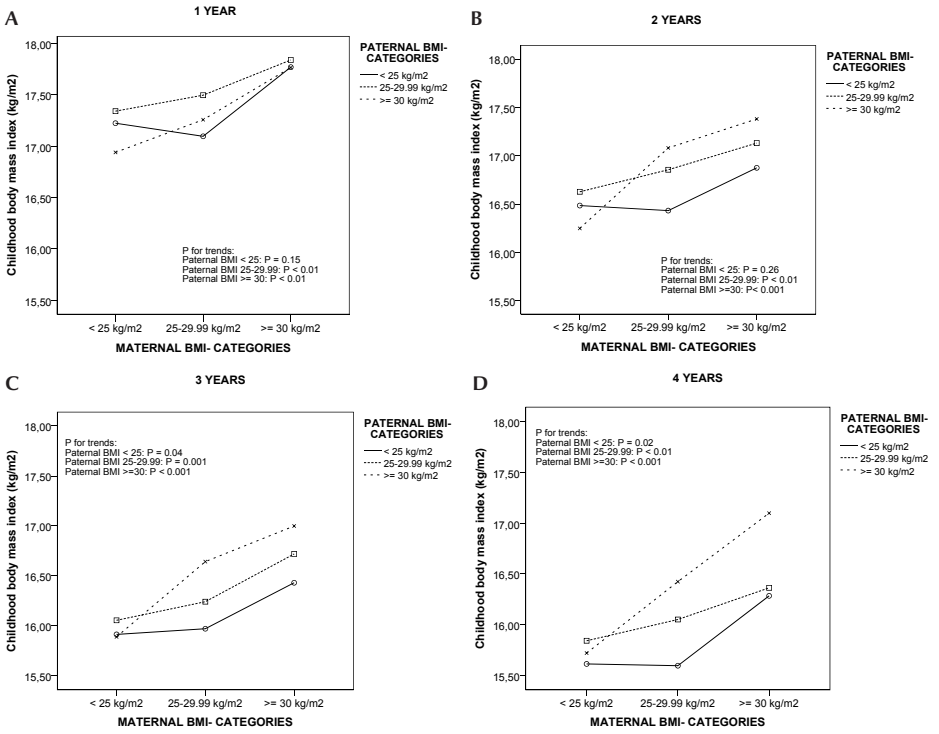
**P < 0.01

effect estimates increased with age and the effect estimates for associations of maternal height with fetal length were slightly stronger than for paternal height. Also, the effect estimates for the associations of maternal and paternal weight with weight during fetal life were stronger for mothers than for fathers. Also, these effect estimates slightly increased postnatally. The effect estimates and explained variances for the associations of maternal and paternal BMI with childhood BMI at the ages of 1, 2, 3 and 4 years were of similar magnitude (combined explained variance 5.5% at the age of 4 years).

Parental anthropometrics, childhood BMI and the risk of overweight

Figure 1A-1D shows the associations of maternal and paternal BMI categories with longitudinally measured childhood BMI (kg/m²) at the ages of 1, 2, 3 and 4 years. At all ages, children from both mothers and fathers with obesity have the highest BMI. Also, both

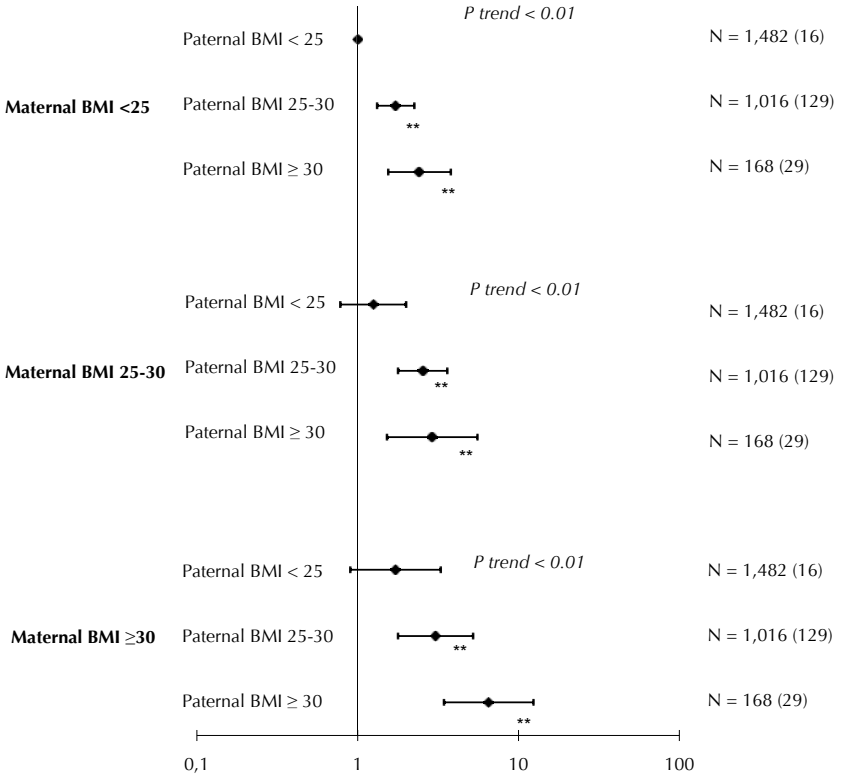
Figure 1 Associations of parental body mass index and their interaction with childhood body mass index (N = 4,116)[1]



[1]Values are estimated marginal means (body mass index in kg/m²) based on repeated measurements using general linear mixed models. Models are adjusted for child's age at visit, sex, maternal ethnicity and education, parity, smoking (yes, no), and breastfeeding (yes, no).
BMI = body mass index

maternal and paternal BMI were positively associated with childhood BMI (P-values for trend are given in Figure 1). In fathers with normal BMI, maternal overweight was not strongly associated with childhood BMI, but maternal obesity led to a strong increase of childhood BMI. At the age of 1 year, maternal obesity had a strong effect on childhood BMI, without an effect of paternal BMI. After the age of 2 years, paternal BMI was strongly associated with childhood BMI in both overweight and obese mothers, whereas paternal BMI had a limited influence on childhood BMI in mothers with normal BMI.

Figure 2 Associations of parental body mass index with the risk of childhood overweight at the age of 4 years (N = 4,116)[1,2]



[1]Values are odds ratios (95% confidence interval) based on logistic regression models. Models are adjusted for child's age at visit, sex, maternal ethnicity and education, parity, smoking (yes, no), and breastfeeding (yes, no).

[2]Overweight is defined as age- and sex- specific body mass index > 1.1 - 2.3 SDS. Values in parentheses represent the cases of childhood overweight.

** P < 0.01

BMI = body mass index

At the age of 4 years, both maternal and paternal obesity was associated with a strong increase in childhood BMI compared to the effect of mothers and fathers with normal BMI. The main effects of maternal BMI on childhood BMI were significantly stronger than the main effects of paternal BMI (F-value 18.8; P < 0.001; and F-value 4.3; P = 0.013, respectively).

Figure 2 shows the associations of paternal BMI with the risk of childhood overweight at the age of 4 years, within strata of maternal BMI categories. Within each pre-pregnancy maternal BMI category, children of both overweight and obese fathers had an increased risk of overweight at the age of 4 years, compared to the reference group (P < 0.01). Compared to children from parents with normal BMI, children from two obese parents had a strongly increased risk of overweight at the age of 4 years ((OR 6.52 (95% CI 3.44, 12.38)).

Gestational weight gain, childhood BMI and the risk of overweight

Table 3 shows the associations of gestational weight gain (per 1 SD) and childhood BMI in SDS according to maternal BMI category. Overall weight gain during gestation was associated with childhood BMI at all ages (P < 0.05). The strongest effect estimate was seen at the age of 4 years ((difference 0.08 (95% CI 0.03, 0.12) SD per 1 SD change in gestational weight gain)). The associations of higher gestational weight gain with childhood BMI was observed at all ages in mothers with normal BMI. In overweight mothers, higher gestational weight gain was only associated with childhood BMI at the age of 4 years

Table 3 Associations of gestational weight gain with childhood body mass index according to maternal body mass index categories[1,2]

	Body mass index (Standard Deviation Score)			
(N = 5,227)	**Age 1 year**	**Age 2 years**	**Age 3 years**	**Age 4 years**
Overall weight gain (1 SD = 4.7 kg)	0.07 (0.03, 0.10)** N = 3,875	0.04 (0.01, 0.08)* N = 3,691	0.06 (0.02, 0.10)** N = 2,990	0.08 (0.03, 0.12)** N = 2,657
Maternal body mass index < 25 kg/m^2	0.12 (0.08, 0.15)** N = 2,921	0.08 (0.04, 0.12)** N = 2,761	0.10 (0.06, 0.14)** N = 2,221	0.11 (0.07, 0.15)** N = 1,988
Maternal body mass index 25-29.99 kg/m^2	0.02 (-0.04, 0.09) N = 684	0.03 (-0.04, 0.09) N = 661	0.06 (-0.004, 0.13) N = 553	0.08 (0.02, 0.15)* N = 480
Maternal body mass index ≥ 30 kg/m^2	-0.01 (-0.10, 0.07) N = 270	-0.02 (-0.10, 0.07) N = 269	0.01 (-0.08, 0.10) N = 216	0.05 (-0.03, 0.14) N = 189

[1]Values are obtained by general linear mixed models and reflect the association of childhood body mass index per 1 standard deviation (SD) change in gestational weight gain.
[2]Models are adjusted for child's age at visit, sex, maternal ethnicity and education, parity, smoking (yes, no), breastfeeding (yes, no) and gestational age in 3rd trimester of pregnancy.
* P < 0.05 and ** P < 0.001

(P < 0.05). Similarly, at the age of 4 years, gestational weight gain was only associated with an increased risk of childhood overweight in mothers with a BMI of < 25 kg/m² ((OR 1.23 (95% CI 1.10, 1.37; P < 0.01)) (data not shown).

DISCUSSION

This population-based prospective cohort study showed that pre-pregnancy maternal and paternal anthropometrics were strongly associated with corresponding fetal and postnatal growth characteristics until the age of 4 years. Both maternal and paternal BMI affect BMI in early childhood, but the overall effect of maternal BMI was stronger than paternal BMI. In children of mothers without pre-pregnancy overweight and obesity, higher gestational weight gain was associated with childhood BMI and the risk of overweight.

Methodological considerations

An important strength of this study was the population-based cohort, with a large number of subjects being studied from early pregnancy onwards and information about a large number of potential confounders being available. Another strength is the possibility of studying the longitudinal effects of parental anthropometrics on childhood BMI. However, some methodological issues need to be considered. Information on pre-pregnancy weight and BMI was missing in 19% of all mothers. This nonresponse at baseline would lead to biased effect estimates if the associations of maternal anthropometrics before pregnancy with fetal and postnatal growth characteristics would be different between those included and not included in the analyses. This seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline.[36] In the present analysis, overall loss to follow-up was limited (< 10%). However, the number of follow-up measurements was smaller with increasing age. We observed no differences in parental anthropometrics or birth characteristics between children with and without follow-up data at the age of 4 years. Weight gain was partly based on self-reported weights. These self-reported pre-pregnancy weights were highly correlated with weight measured in the first visit at the research center (r > 0.95; P < 0.01). Furthermore, mothers in this age group may systematically underestimate their weights.[37] As we were interested in differences between subjects and the effect on postnatal growth characteristics and the risk of overweight in early childhood, systematic underestimation of pre-pregnancy weight and BMI does not bias our results. Moreover, we did not examine possible effects of non-paternity in the parental-offspring associa-

tions. Maternal gestational weight gain was defined as the difference between weight at gestational age of 30 weeks and weight just before pregnancy. Ideally, gestational weight gain is defined as the difference between the highest weight in pregnancy and the weight just before pregnancy, which was only available in 67% of the participants. Therefore, we could not assess specific effects of third trimester weight gain. Ideally, instead of BMI, outcomes assessing body composition like fat mass or fat distribution should be used, as fat distribution is a much more accurate predictor of risk on adulthood disease. Finally, we should be careful with childhood overweight definitions, as at this young age there is no clear cut- off point to define overweight or obesity.[38]

Comparison of main findings with other studies

The associations of pre-pregnancy maternal anthropometrics associated with postnatal BMI in childhood seem to be well established.[39-42] In our study, anthropometrics of mother and father were strongly related with corresponding fetal and postnatal anthropometrics. The influence of effect estimates of maternal and paternal anthropometrics on the corresponding anthropometrics of the child mainly increased after birth with age and was highest for length or height measurements. Similar effect estimates for both mothers and fathers suggest that same genetic and/or shared environmental risk factors might be important.[15] However, several studies showed inconsistent results for the associations between parental and offspring anthropometrics. Few studies conducted in Europe and Asia reported stronger effect estimates for maternal than paternal anthropometrics,[43,44] while others found similar effect estimates for maternal and paternal anthropometrics.[19,45,46] Also, some studies suggested that the maternal-offspring associations for BMI at birth are stronger than the paternal-offspring associations.[47,48] Furthermore, previous studies suggested that early childhood BMI include paternal but not maternal BMI as independent contributing factor and that the association of paternal with childhood BMI gets stronger with increasing age of the children, whereas the association of maternal with childhood BMI seems to stay more stable.[19,49]

In our study the effect estimates for the associations of maternal height and weight with the corresponding measures in the offspring in fetal life were higher than for paternal height and weight, which suggest stronger influences of maternal characteristics during fetal life. Another important observation was that maternal BMI had a stronger effect on childhood BMI than paternal BMI, and that this effect was already present in early postnatal life. These effects are in line with other studies demonstrating stronger effects for maternal than paternal anthropometrics on childhood growth.[43,44] In contrast, the Cardiovascular Risk in Young Finns Study observed no differences in the strength of the associations of maternal and paternal BMI with childhood BMI at the ages of 3 to 39

years.[46] Similar results were observed in population-based cohort studies in the UK and Norway.[19,50] We found that both maternal and paternal overweight and obesity were associated with an increased risk of childhood overweight until the age of 4 years, especially when having both parents with obesity.

The fetal overnutrition hypothesis is referring to some causes that may act during pregnancy and increase the fetus' risk of adiposity in later life.[19] Included mechanisms may be permanent changes in neuro-endocrine functioning or energy metabolism. Maternal BMI is also associated with diet and glucose levels during pregnancy, both of which may lead to fetal overnutrition and thus childhood obesity.[39] Some support for an effect of the intrauterine environment would provide a larger maternal-offspring association as in our study.[50] Nevertheless, the associations between parental and offspring anthropometrics might not only explained by intrauterine or genetic factors but also partly by familial and non-familial shared characteristics in the environment such as socioeconomic status, food and sedentary habits, and physical activity.[51]

Recently, it has been shown that high gestational weight gain is associated with an increased risk of overweight in children at the ages of 2 to 14 years, after adjustment for maternal BMI.[5-8] The most recent and largest studies on gestational weight gain and offspring obesity revealed that the effects of gestational weight gain on the offspring risks of overweight and obesity is strongest in underweight or normal-weight mothers.[5,7,8] We found similar tendencies by showing that gestational weight gain was associated with BMI in the offspring of mothers with a normal BMI. It is already known that increased fat mass predisposes mother and fetus to elevated concentrations of leptin and insulin which can affect neurodevelopment and energy balance.[5] However, it seems explicable that the effect of gestational weight gain in normal weight mothers is based on shared environmental characteristics and that the effect in overweight mothers is partly explained by intrauterine mechanisms.[5]

Conclusion

Pre-pregnancy maternal and paternal BMI are differently associated with fetal and postnatal growth characteristics. Having both parents with obesity is associated with an increased risk of childhood overweight at the age of 4 years. Gestational weight gain in mothers with normal BMI is related to childhood BMI. Further studies are needed to identify genetic and environmental underlying mechanisms for these associations. Also, further follow-up studies are needed to identify associations of parental anthropometrics with body composition and risk factors for type 2 diabetes and cardiovascular disease in children at older ages.

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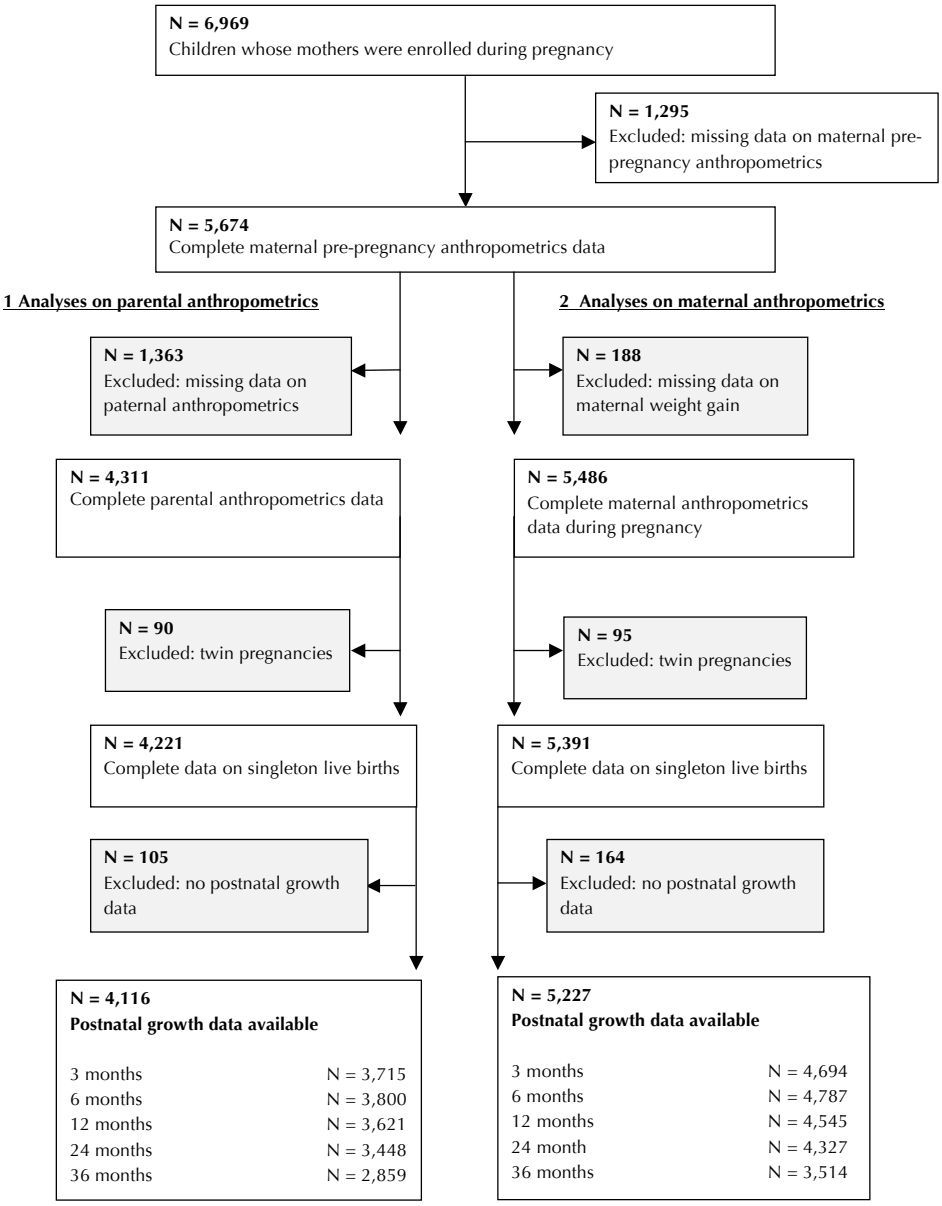
Supplemental Table 1 Fetal and postnatal growth characteristics of all children[1]

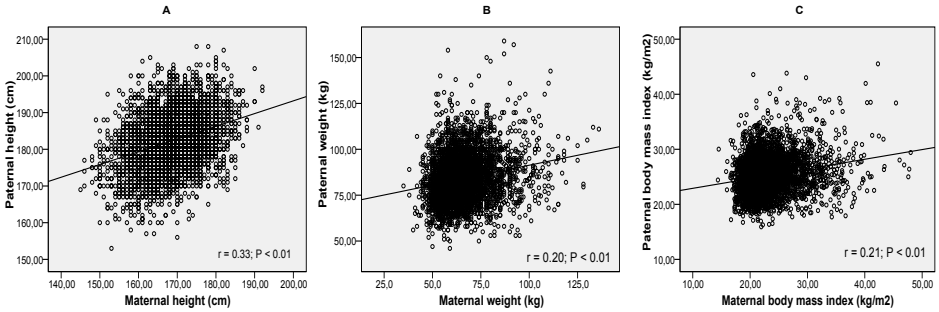
Second trimester fetal growth (N = 4,938)	
Gestational age (weeks)	20.5 (19.0 – 22.7)
Femur length (cm)	3.3 (0.3)
Estimated fetal weight (g)	381 (91)
Third trimester fetal growth (N = 5,128)	
Gestational age (weeks)	30.4 (28.9 – 32.2)
Femur length (cm)	5.7 (0.3)
Estimated fetal weight (g)	1,626 (254)
Birth (N = 5,227)	
Gestational age (weeks)	40.1 (37.3 – 42.1)
Length (cm)	50.3 (2.4)
Weight (grams)	3,460 (526)
3 months (N = 4,694)	
Age (months)	3.2 (1.3 – 4.2)
Length (cm)	60.5 (3.2)
Weight (kg)	6.0 (0.9)
6 months (N = 4,787)	
Age (months)	6.2 (5.5 – 10.2)
Length (cm)	68.2 (3.1)
Weight (kg)	8.0 (1.0)
Body mass index (kg/m^2)	17.3 (1.4)
12 months (N = 4,545)	
Age (months)	12.7 (11.1 – 15.3)
Height (cm)	76.6 (3.3)
Weight (kg)	10.2 (1.2)
Body mass index (kg/m^2)	17.3 (1.4)
24 months (N = 4,327)	
Age (months)	24.5 (18.6 – 27.5)
Height (cm)	87.7 (3.9)
Weight (kg)	12.8 (1.6)
36 months (N = 3,514)	
Age (months)	36.6 (31.0 – 39.2)
Height (cm)	96.9 (4.1)
Weight (kg)	15.2 (1.9)
48 months (N = 3,055)	
Age (months)	45.8 (44.7 – 48.1)
Height (cm)	103.2 (4.2)
Weight (kg)	17.0 (2.2)
Body mass index (kg/m^2)	15.9 (1.4)
Overweight %	13.2
Obesity %	2.8

[1]Values are means (standard deviation) or medians (90% range) for variables with skewed distribution.

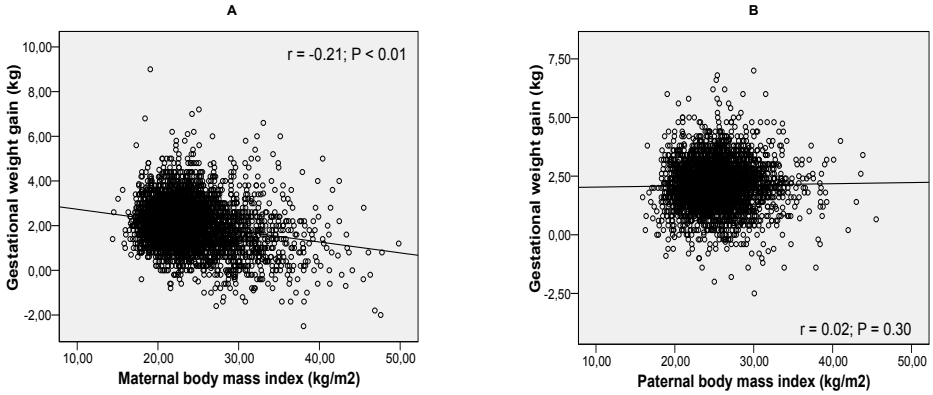
Supplemental Figure 1 Flow chart of participants in study



Supplemental Figure 2 Correlations between pre-pregnancy maternal and paternal anthropometrics



Supplemental Figure 3 Correlations between maternal and paternal body mass index and maternal weight gain during pregnancy



CHAPTER 4.2

Consequences of maternal obesity and excessive weight gain during pregnancy

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ABSTRACT

Objective: The prevalence of overweight and obesity in women of reproductive age is increasing. We aimed to determine risk factors and maternal, fetal and childhood consequences of maternal obesity and excessive gestational weight gain.

Methods: The study was embedded in a population-based prospective cohort study in 6,959 mothers and their children. The study was based in Rotterdam, the Netherlands (2001-2005).

Results: Maternal lower educational level, lower household income, multiparity, and FTO risk allele were associated with an increased risk of maternal obesity, whereas maternal European ethnicity, nulliparity, higher total energy intake, and smoking during pregnancy were associated with an increased risk of excessive gestational weight gain (all P-values <0.05). As compared to normal weight, maternal obesity was associated with increased risks of gestational hypertension (odds ratio (OR) 6.31 (95% confidence interval (CI) 4.30, 9.26)), preeclampsia (OR (3.61, (95% CI 2.04, 6.39)), gestational diabetes (OR 6.28 (95% CI 3.01, 13.06)), Caesarean delivery (OR 1.91 (95% CI 1.46, 2.50)), delivering large size for gestational age infants (OR 2.97 (95% CI 2.16, 4.08)), and childhood obesity (OR 5.02 (95% CI 2.97, 8.45)). Weaker associations of excessive gestational weight gain with maternal, fetal and childhood outcomes were observed, with the strongest effects for first-trimester weight gain.

Conclusions: In conclusion, our study shows that maternal obesity and excessive weight gain during pregnancy are associated with sociodemographic, lifestyle, and genetic factors and with increased risks of adverse maternal, fetal and childhood outcomes. As compared to pre-pregnancy overweight and obesity, excessive gestational weight gain has a limited influence on adverse pregnancy outcomes.

INTRODUCTION

Maternal obesity seems to be associated with short-term adverse maternal and fetal outcomes.[1-5] It has also been suggested that maternal obesity is associated with long-term maternal and offspring consequences, such as postpartum weight retention, metabolic syndrome and obesity in the offspring.[1,3,6] Excessive gestational weight gain might also influence the risk of adverse maternal and fetal outcomes.[4-5,7] The mechanisms of these associations remain unclear, as gestational weight gain reflects both maternal nutritional status, as well as tissue expansion during pregnancy, because of fat storage and fluids.[4] Not much is known about specific risk factors for maternal obesity and excessive weight gain during pregnancy. Identification of these risk factors and critical periods of gestational weight gain might be useful for the development of preventive strategies.

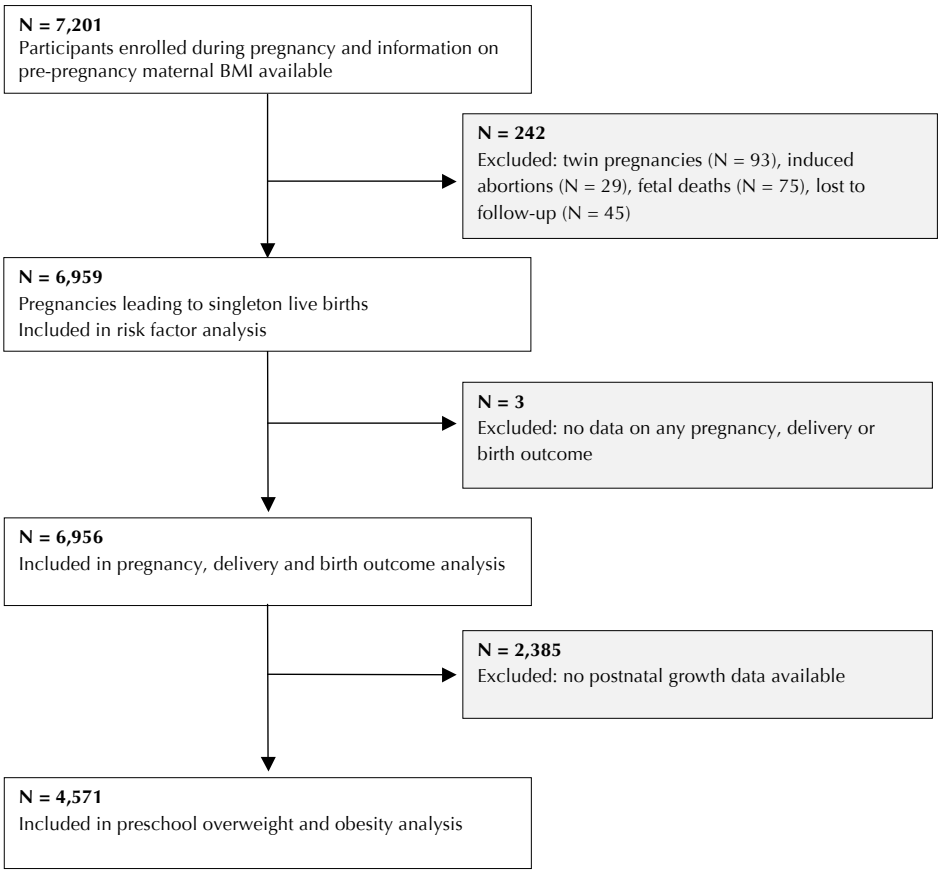
In a population-based prospective cohort study in 6,959 mothers and their children, we examined the associations of several sociodemographic, lifestyle, and genetic factors with the risks of maternal obesity and excessive gestational weight gain. Next, we examined the associations of maternal obesity, excessive gestational weight gain and trimester-specific weight gain with the risks of adverse maternal, fetal and childhood outcomes.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, the Netherlands.[8] Pregnant women were enrolled between 2001 and 2005. Of all the eligible children in the study area, 61% participated at birth in the study. The Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, approved the study (MEC 198.782/2001/31). Written informed consent was obtained from all mothers.[8] In total, 8,880 mothers were enrolled during pregnancy, of whom information on pre-pregnancy body mass index (BMI) was available in 7,201 subjects. We excluded pregnancies not leading to singleton live births (N = 242). The population for analysis was 6,959 mothers and their children (**Figure 1**).

Figure 1 Selection of study participants



Data collection and measurements

Maternal anthropometrics, obesity, and weight gain during pregnancy

Maternal anthropometrics were measured in first, second, and third trimester of pregnancy. Height (cm) and weight (kg) were measured without shoes and heavy clothing and BMI (kg/m^2) was calculated. Information on maternal weight just before pregnancy was obtained by questionnaire. In our population for analysis, 46.2% of all women were enrolled before a gestational age of 14 weeks. Correlation of pre-pregnancy weight, obtained by questionnaire, and weight measured at enrollment was 0.95 ($P < 0.001$) (regression coefficient for this correlation: 0.93 (95% confidence interval (CI) 0.93, 0.94). Pre-pregnancy BMI was categorized into 4 categories (underweight (<20 kg/m^2, normal weight (20-24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obesity (≥30 kg/m^2)). Weight

gain until a gestational age of 30 weeks was measured and available for 6,623 mothers. Information on maximum weight during pregnancy was available in a subgroup of 3,314 mothers and was assessed by questionnaire 2 months after delivery. Maximum weight from questionnaire and weight measured at 30 weeks were strongly correlated ($r = 0.87$ ($P < 0.001$)). According to Institute of Medicine guidelines, we defined excessive gestational weight gain in relation to maternal pre-pregnancy BMI (for underweight and normal weight mothers: total weight gain > 16 kg; for overweight mothers: total weight gain > 11.5 kg; for obese mothers: total weight gain > 9 kg.[9] Weight gain was further analyzed in each trimester of pregnancy.

Risk factors

Sociodemographic exposures

Maternal age was assessed at intake. Highest completed maternal educational level (primary school, secondary school, higher education) and maternal ethnicity (European, Surinamese, Turkish, Moroccan, Cape Verdean and Dutch Antilles) were available from questionnaire.[8]

Diet and lifestyle-related exposures

First trimester nutritional information (total energy intake (kCal), carbohydrates (energy %), fat (energy %), protein (energy %)) was obtained by a food frequency questionnaire at enrollment.[10] Mothers who were enrolled after first trimester of pregnancy did not receive this food frequency questionnaire. Information on folic acid supplementation use was obtained at enrollment. Information on smoking and alcohol consumption was assessed by questionnaire in each trimester.[8] Maternal smoking and alcohol consumption were categorized in smoking during pregnancy (yes, no) and alcohol consumption during pregnancy (yes, no).

Maternal FTO polymorphism

Maternal genotyping of the FTO polymorphism (rs8050136) was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg Germany). The genotyping reaction was amplified using the GeneAmp® PCR system 9600 [95 C (15 min), then 40 cycles of 94 C (15 sec) and 60 C (1 min)]. The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems).

Paternal exposures

Information on paternal age was obtained at enrollment in the study.[8] At enrollment, paternal height (cm) and weight (kg) were measured and BMI (kg/m^2) was calculated.[8]

Gestational hypertension, preeclampsia, and gestational diabetes

Information on pregnancy complications was obtained from medical records. Details of these procedures have been described elsewhere.[11] Briefly, the following criteria were used to identify women with gestational hypertension: development of systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg after 20 weeks of gestation in previously normotensive women. These criteria plus the presence of proteinuria (defined as two or more dipstick readings of 2+ or greater, one catheter sample reading of 1+ or greater, or a 24–hour urine collection containing at least 300 mg of protein) were used to identify women with preeclampsia.[11] Information on gestational diabetes was obtained from medical records. Gestational diabetes 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, a 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.[12] 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.

Delivery and birth complications

Information on assisted delivery, including prelabour rupture of membranes (PROM), Caesarian delivery, ventouse extraction, and postpartum hemorrhage, was obtained from midwife registries and hospital registries at birth. Gestational age was established by fetal ultrasound examination during the first ultrasound visit. Dating of the pregnancy was performed using the first ultrasound measurement of crown-rump length (CRL) or biparietal diameter (BPD), using dating curves derived from this cohort.[13] Gestational age at birth, birth weight and sex were obtained from midwife and hospital registries at birth.[8] Preterm birth was defined as a gestational age of < 37 weeks at birth. Small size for gestational age at birth (SGA) and large size for gestational age at birth (LGA) were defined as a gestational age-adjusted birth weight below the 10th percentile and above the 90th percentile in the study cohort.

Childhood overweight and obesity

In children aged 4 years, growth was measured at the Community Child Health Centers.[8] Height and weight were measured in standing position and BMI (kg/m^2) was calculated. Childhood overweight and obesity were defined by the International Obesity Task Force cut-offs.[14]

Statistical analysis

We examined the associations of risk factors with maternal underweight, overweight, obesity and excessive gestational weight gain using multivariate logistic regression models. Using similar models, we explored the associations of maternal underweight, overweight, obesity and excessive gestational weight gain with the risks of pregnancy complications in mothers and children. These models were adjusted for maternal age, educational level, ethnicity, parity, folic acid supplementation use, smoking habits and alcohol consumption. The models in which we examined maternal overweight and obesity as exposure were also adjusted for maximum gestational weight gain. We tested potential interactions between maternal BMI and gestational weight gain for these models, but after adjustment for multiple testing, we found no significant interactions. Furthermore, we used stepwise regression analyses to compare the strength of the associations of pre-pregnancy overweight and obesity and excessive gestational weight gain with the risks of adverse pregnancy outcomes (data not shown). We performed a sensitivity analysis to examine whether the associations of pre-pregnancy BMI with the risk of adverse pregnancy outcomes differed between women enrolled in first trimester (before 14 weeks of gestation) and women enrolled later in pregnancy. Sensitivity analyses using weight gain until third trimester instead of maximum weight gain were performed for the analyses focused on excessive gestational weight gain and the risk of adverse outcomes. Finally, we examined the associations of trimester-specific weight gain with pregnancy, delivery, fetal and childhood outcomes using multivariate logistic regression models. Missing data of the covariates were imputed using multiple imputation. The percentages of missing values within the population for analysis were lower than 10%, except for information on maternal nutrition (23.7%) and folic acid supplementation use (17.3%). All analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics

Characteristics of the included mothers, fathers and children are given in **Table 1**. Of all mothers, 16.2%, 55.8%, 19.2% and 8.8% were underweight, normal weight, overweight and obese, respectively, and 44.5% had excessive weight gain. Subject characteristics according to maternal BMI category are given in **Supplemental Table 1**.

Table 1 Characteristics of mothers, fathers and their children (N = 6,959)

Characteristics	Value
Maternal Characteristics	
Age, median (90% range), years	30.3 (20.4 – 37.9)
Height, mean (SD) (cm)	167.4 (7.4)
Weight, mean (SD) (kg)	69.3 (13.1)
Body mass index, mean (SD) (kg/m^2)	23.6 (4.4)
Maximum weight gain (SD) (kg)	13.6 (8.0)
First-trimester weight gain (SD) (kg)	2.32 (3.6)
Second-trimester weight gain (SD) (kg)	3.25 (2.4)
Third-trimester weight gain (SD) (kg)	5.01 (2.7)
Education, no. (%)	
Primary	744 (11.1)
Secondary	3,135 (46.6)
Higher	2,852 (42.4)
Household income per month, no. (%)	
< € 1,600	1,606 (29.3)
> € 1,600 - € 2,200	834 (15.3)
> € 2,200	3,035 (55.4)
Race / Ethnicity, no. (%)	
Dutch or European	3,958 (57.8)
Surinamese	618 (9.0)
Turkish	640 (9.3)
Moroccan	444 (6.5)
Cape Verdean or Dutch Antilles	496 (7.2)
Others	689 (10.1)
Parity, no. nulliparous (%)	3,959 (56.9)
Folic acid supplement use, no. (%)	4,085 (71.0)
Diet	
Total energy intake, mean (SD) (KCal)	2,044 (563)
Carbohydrates, mean (SD) (energy%)	48.7 (5.9)
Proteins, mean (SD) (energy%)	14.8 (2.5)
Fat, mean (SD) (energy%)	36.3 (5.2)
Smoking, no. (%)	1,713 (25.9)
Alcohol consumption, no. (%)	3,353 (50.5)
FTO rs8050136, no. (%)	
CC	2,235 (38.3)
AC	2,737 (46.8)
AA	869 (14.9)

Table 1 Characteristics of mothers, fathers and their children (N = 6,959) (continued)

Characteristics	Value
Maternal pregnancy complications	
Gestational hypertension, no. (%)	264 (4.0)
Preeclampsia, no. (%)	133 (2.1)
Gestational diabetes, no. (%)	70 (1.0)
Prelabour rupture of membranes, no, (%)	260 (3.9)
Postpartum hemorrhage, no. (%)	342 (5.1)
Paternal Characteristics	
Age, median (90% range), years	33.1 (22.0 – 44.9)
Height, mean (SD) (cm)	181.2 (7.7)
Weight, mean (SD) (kg)	83.5 (11.6)
Body mass index, mean (SD) (kg/m^2)	25.4 (3.2)
Delivery and child characteristics	
Caesarian section, no. (%)	778 (12.3)
Ventouse extraction, no. (%)	858 (13.6)
Males, no. (%)	3,518 (51%)
Gestational age, median (90% range), weeks	40.1 (36.9 – 42.0)
Preterm birth[1], no. (%)	354 (5.1)
Birth weight, mean (SD) grams	3,419 (557)
Small size for gestational age[1] (<10 birth centile), no. (%)	680 (9.9)
Large size for gestational age[1] (>90 birth centile), no. (%)	692 (10.0)
Preschool overweight and obesity, no. (%)	708 (15.5)

[1]SGA is defined as < 10th percentile of age-and sex-adjusted birth weight; LGA is defined as > 90th percentile of age-and sex-adjusted birth weight; preterm birth is defined as < 37 weeks.

Risk factors of maternal overweight and obesity and excessive gestational weight gain

In the multivariate analyses, maternal low educational level, multiparity, no alcohol consumption during pregnancy, FTO risk allele and higher paternal BMI were all associated with the risk of maternal overweight and obesity (all P-values <0.05) (**Table 2**). Maternal European ethnicity, nulliparity, higher total energy, carbohydrate, protein and fat intake, no alcohol consumption during pregnancy, smoking during pregnancy and higher paternal BMI were associated with a higher risk of excessive gestational weight gain (all P-values < 0.05).

Table 2 Risk factors of maternal overweight, obesity and excessive weight gain during pregnancy using multivariate analyses[1]

(N = 6,959)	Maternal underweight (OR, (95% CI)) N = 1,123	Maternal overweight (OR, (95% CI)) N = 1,334	Maternal obesity (OR, (95% CI)) N = 611	Excessive weight gain (OR, (95% CI)) N = 1,474
Maternal risk factors				
Age (1 SD = 5.3y)	0.83 (0.75, 0.93)**	1.03 (0.94, 1.14)	1.04 (0.90,1.19)	0.97 (0.85, 1.07)
Education				
Primary	0.91 (0.68, 1.22)	1.64 (1.26,2.12)**	2.48 (1.71, 3.59)**	0.92 (0.62,1.34)
Secondary	1.00 (0.84, 1.19)	1.39 (1.18,1.65)**	2.75 (2.12, 3.56)**	1.13 (0.96, 1.36)
Higher	Reference	Reference	Reference	Reference
Household income per month				
< €1,600	1.10 (0.86, 1.41)	1.05 (0.84, 1.31)	1.36 (1.03, 1.79)*	0.91 (0.69, 1.14)
> €1,600 – 2,200	1.03 (0.79, 1.34)	1.09 (0.89,1.35)	1.20 (0.84, 1.72)	0.90 (0.72, 1.12)
> €2,200	Reference	Reference	Reference	Reference
Ethnicity				
Dutch or European	Reference	Reference	Reference	Reference
Non-European	0.94 (0.79, 1.12)	1.23 (1.03, 1.44)*	1.06 (0.87, 1.36)	0.78 (0.65, 0.94)**
Parity				
Nulliparous	Reference	Reference	Reference	Reference
Multiparous	0.98 (0.84, 1.15)	1.51 (1.31, 1.75)**	1.68 (1.37, 2.06)**	0.71 (0.61, 0.83)**
Folic acid supplement use				
No	Reference	Reference	Reference	Reference
Yes	1.07 (0.86, 1.31)	0.94 (0.78, 1.15)	0.81 (0.61, 1.07)	1.25 (1.00, 1.56)
Total Energy intake (1 SD = 563 kcal)	1.01 (0.93, 1.10)	0.95 (0.88, 1.04)	0.88 (0.79, 0.98)*	1.13 (1.03, 1.23)**
Carbohydrates (1 SD = 6.5% energy)	1.21 (0.62, 2.36)	1.21 (0.54, 2.70)	5.38 (1.42, 20.21)*	4.49 (1.61, 12.46) **
Proteins (1 SD = 2.6% Energy)	0.94 (0.71, 1.24)	1.18 (0.87, 1.59)	2.23 (1.32, 3.75)**	1.91 (1.26, 2.88)**
Fat (1 SD = 5.6% Energy)	1.19 (0.66, 2.13)	1.12 (0.55, 2.27)	4.51 (1.40, 14.39)*	4.00 (1.62, 9.83)**
Smoking				
No	Reference	Reference	Reference	Reference
Yes	1.09 (0.93, 1.29)	0.96 (0.82, 1.13)	1.01 (0.81, 1.25)	2.08 (1.74, 2.48)**
Alcohol				
No	Reference	Reference	Reference	Reference
Yes	1.08 (0.92, 1.26)	0.76 (0.65, 0.89)**	0.73 (0.59, 0.90)**	0.83 (0.71, 0.98)*
FTO rs8050136				
CC	Reference	Reference	Reference	Reference
AC	1.00 (0.94, 1.16)	1.11 (0.96, 1.28)	1.25 (0.99, 1.58)	1.10 (0.86, 1.41)
AA	0.99 (0.80, 1.25)	1.30 (1.06, 1.58)*	1.64 (1.21, 2.23)**	1.14 (0.95, 1.36)
Paternal risk factors				
Age at intake (1 SD = 5.8 y)	1.07 (0.97, 1.17)	1.02 (0.93, 1.11)	1.05 (0.91, 1.18)	0.98 (0.88, 1.08)
Body mass index (1 SD = 3.49 units)	0.80 (0.73, 0.87)**	1.32 (1.21, 1.44)**	1.53 (1.35, 1.73)**	1.12 (1.02, 1.22)*

OR: odds ratio; CI: confidence interval; SD: standard deviation
[1]Values are multivariate logistic regression coefficients (95% CI). For continuous variables, estimates reflect the risk of maternal underweight, overweight and obesity and excessive gestational weight gain per standard deviation of the risk factor. For categorical variables or dichotomous variables, the effect estimates represent the risk of maternal underweight, overweight and maternal obesity and excessive gestational weight gain, compared to reference group. Estimates are based on multiple imputed data. * P < 0.05 and **P < 0.01

Maternal underweight, overweight, obesity, and excessive gestational weight gain and risks of pregnancy, delivery, birth and childhood outcomes

As compared to normal weight, maternal underweight was associated with a higher risk of PROM (odds ratio (OR) 1.61 (95% CI 1.17, 2.22) and a higher risk of delivering a small size for gestational age infant (OR 1.66 (95% CI 1.36, 2.07)), but with a lower risk of delivering a large size for gestational age infant (OR 0.42 (95% CI 0.30, 0.57)) and a lower risk of childhood overweight of the offspring (OR 0.62 (95% CI 0.44, 0.87).

As compared to normal weight mothers, mothers with overweight had increased risks of gestational hypertension (OR 2.15 (95% CI 1.55, 2.97)), preeclampsia (OR 1.91 (95% CI 1.21, 3.00)), gestational diabetes (OR 4.25 (95% CI 2.32, 7.76)), Caesarean delivery (OR 1.52 (95% CI 1.24, 1.85)), postpartum hemorrhage (OR 1.34 (95% CI 1.01, 1.78)), large size for gestational age infants (OR 1.69 (95% CI 1.35, 2.12)), and childhood overweight (OR 1.48 (95% CI 1.15, 1.91)) (**Table 3**). We observed stronger effect estimates for the associations of maternal obesity with these outcomes. Repeating these analyses in women who were enrolled during first trimester and in women enrolled later in pregnancy showed that effect estimates differed only slightly between first trimester enrolled and later enrolled women (**Supplemental Table 2**).

As compared to low or recommended weight gain, excessive gestational weight gain was associated with a higher risk of gestational hypertension (OR 2.07 (95% CI 1.43, 2.99)), Caesarean delivery (OR 1.26 (95% CI 1.00, 1.57)), and large size for gestational age infants (OR 2.17 (95% CI 1.72, 2.74)), and a lower risk of preterm delivery (OR 0.67 (95% CI 0.46, 0.98)) and small size for gestational age infants (OR 0.34 (95% CI 0.26, 0.46)). Excessive gestational weight gain was associated with the risk of childhood overweight (OR 1.51 (95% CI 1.16, 1.97)). Associations of excessive gestational weight gain with these adverse pregnancy outcomes attenuated when pre-pregnancy overweight and obesity were included in the model (data not shown). Similar results for the associations with excessive gestational weight gain were found when we used weight in third trimester instead of maximum weight (**Supplemental Table 3**).

Table 3 Associations of maternal underweight, overweight, obesity and excessive gestational weight gain with maternal, delivery, birth and childhood complications

	Underweight[1,2] (OR, (95% CI))	Overweight[1,2] (OR, (95% CI))	Obesity[1,2] (OR, (95% CI))	Excessive gestational weight gain[1,3] (OR, (95% CI))
Maternal complications N = 6,956	N = 1,123	N = 1,334	N = 611	N = 1,474
Gestational hypertension	0.65 (0.41, 1.02)	2.15 (1.55, 2.97)**	6.31 (4.30, 9.26)**	2.07 (1.43, 2.99)**
Preeclampsia	1.25 (0.76, 2.06)	1.91 (1.21, 3.00)**	3.61 (2.04, 6.39)**	1.12 (0.67, 1.89)
Gestational diabetes	0.61 (0.18, 2.06)	4.25 (2.32, 7.76)**	6.28 (3.01, 13.06)**	1.54 (0.66, 3.56)
Delivery complications N = 6,956	N = 1,123	N = 1,334	N = 611	N = 1,474
PROM	1.61 (1.17, 2.22)**	0.95 (0.65, 1.37)	1.66 (1.08, 2.55)*	0.69 (0.47, 1.03)
Ventouse extraction	0.98 (0.79, 1.20)	1.00 (0.81, 1.23)	1.12 (0.82, 1.52)	1.21 (0.98, 1.48)
Caesarean section	0.94 (0.74, 1.18)	1.52 (1.24, 1.85)**	1.91 (1.46, 2.50)**	1.26 (1.00, 1.57)*
Postpartum hemorrhage	0.92 (0.66, 1.27)	1.34 (1.01, 1.78)*	1.44 (0.96, 2.16)	1.04 (0.76, 1.42)
Birth complications N = 6,956	N = 1,123	N = 1,334	N = 611	N = 1,474
Preterm birth[4]	1.29 (0.96, 1.72)	1.04 (0.77, 1.42)	1.53 (1.05, 2.20)*	0.67 (0.46, 0.98)*
Large size for gestational age	0.42 (0.30, 0.57)**	1.69 (1.35, 2.12)**	2.97 (2.16, 4.08)**	2.17 (1.72, 2.74)**
Small size for gestational age	1.66 (1.36, 2.07)**	0.81 (0.64, 1.03)	0.54 (0.38, 0.78)**	0.34 (0.26, 0.46)**
5 minute APGAR <7	0.65 (0.30, 1.39)	1.56 (0.90, 2.71)	2.05 (1.04, 4.01)*	1.09 (0.50, 2.39)
Childhood complications N = 4,571	N = 736	N = 844	N = 372	N = 1,263
Overweight[5]	0.62 (0.44, 0.87)**	1.48 (1.15, 1.91)**	2.41 (1.75, 3.33)**	1.51 (1.16, 1.97)**
Obesity[5]	0.61 (0.29, 1.28)	1.61 (0.94, 2.74))	5.02 (2.97, 8.45)**	0.93 (0.51, 1.68)

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risks of complications for underweight, overweight and obese women as compared to women with a normal body mass index, 20-24.9 kg/m^2, and for women with excessive gestational weight gain as compared to women with a recommended or less than recommended gestational weight gain. Estimates are from multiple imputed data.
[2]Models for underweight, overweight and obesity are adjusted for educational level, maternal age, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and gestational weight gain.
[3]Models for excessive gestational weight gain are adjusted for educational level, maternal age, ethnicity, parity, folic acid supplement use, smoking habits and alcohol consumption.
[4]Models are adjusted for sex as well.
[5]Models are also adjusted for breastfeeding (yes, no).
* P < 0.05 and **P < 0.01
OR: odds ratio; CI: confidence interval; PROM: prelabour rupture of membranes

Trimester-specific weight gain and risks of pregnancy, delivery, birth, and childhood outcomes

Table 4 shows that first-trimester weight gain was associated with the risk of gestational hypertension, gestational diabetes, and Caesarean delivery (OR 1.24 (95% CI 1.12, 1.39),

Table 4 Associations of trimester-specific weight gain with maternal, delivery, birth and childhood complications[1,2]

	First trimester	Second trimester	Third trimester
Complication	**OR (95% CI) per SD change in gestational weight per week**	**OR (95% CI) per SD change in gestational weight per week**	**OR (95% CI) per SD change in gestational weight per week**
Maternal complications N = 6,956	N = 5,695	N = 5,469	N = 3,313
Gestational hypertension	1.24 (1.12, 1.39)**	1.18 (1.03, 1.34)*	1.27 (1.06, 1.51)**
Preeclampsia	1.10 (0.92, 1.32)	1.14 (0.96, 1.37)	1.35 (1.08, 1.69)**
Gestational diabetes	1.29 (1.10, 1.51)**	1.31 (1.04, 1.64)*	1.03 (0.72, 1.46)
Delivery complications N = 6,956	N = 5,695	N = 5,469	N = 3,313
Caesarean delivery	1.19 (1.10, 1.29)**	1.05 (0.96, 1.15)	1.00 (0.90, 1.20)
Birth complications N = 6,956	N = 5,695	N = 5,469	N = 3,313
Preterm delivery[3]	1.04 (0.93, 1.17)	1.00 (0.88, 1.14)	0.96 (0.80, 1.14)
Large size for gestational age infants	1.24 (1.14, 1.34)**	1.41 (1.29, 1.53)**	1.42 (1.26, 1.60)**
Small size for gestational age infants	0.91 (0.82, 0.99)*	0.72 (0.66, 0.80)**	0.74 (0.66, 0.84)**
Childhood complications N = 4,571	N = 3,812	N = 3,712	N = 2,777
Overweight[4]	1.20 (1.08, 1.34)**	1.17 (1.04, 1.30)**	0.94 (0.83, 1.07)
Obesity[4]	1.44 (1.21,1.70)**	0.93 (0.75, 1.16)	0.94 (0.73, 1.20)

[1]Values are odds ratios (95% CI) for the risk of complications per SD change in gestational weight gain per week. Estimates based on multiple imputed data.
[2]Models are adjusted for educational level, maternal age, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and maternal pre-pregnancy body mass index.
[3]Models are adjusted for sex as well.
[4]Models are also adjusted for breastfeeding (yes, no).
* P < 0.05 and **P < 0.01
OR: odds ratio; CI: confidence interval; SD: standard deviation

OR 1.29 (95% CI 1.10, 1.51) and OR 1.19 (95% CI 1.10, 1.29) per standard deviation (SD) of change in gestational weight gain per week, respectively). First-trimester weight gain was also associated with the risk of childhood overweight and obesity (OR 1.20 (95% CI 1.08, 1.34) and OR 1.44 (95% CI 1.21, 1.70) per SD of change in gestational weight gain per week, respectively). Weight gain in third trimester was associated with the risk of gestational hypertension and preeclampsia (OR 1.27 (95% CI 1.06, 1.51), OR 1.35 (95% CI 1.08, 1.69), per SD of change in gestational weight gain per week, respectively). The risks of delivering a large size for gestational age infant and a small size for gestational age infant were influenced by first-, second- and third-trimester weight gain.

DISCUSSION

Results from this prospective cohort study showed that the risks of maternal overweight and obesity were higher in lower educated, non-European origin, and multiparous mothers and mothers with an obese partner. The risk of excessive gestational weight gain was increased by maternal European ethnicity, nulliparity, higher dietary intake, smoking during pregnancy, and having an obese partner. Maternal overweight and obesity were strongly associated with increased risks of gestational hypertensive disorders, gestational diabetes, Caesarean delivery, large size for gestational age infants, and overweight and obesity in the offspring. Excessive gestational weight gain was associated with increased risks of gestational hypertension, Caesarean delivery, large size for gestational age infants and overweight in the offspring. However, the risk of delivering a small size for gestational age infant and the risk of delivering preterm were decreased in women who gained excessively. As compared to pre-pregnancy overweight and obesity, excessive gestational weight gain tended to have a limited influence on adverse pregnancy outcomes. Pre-pregnancy overweight and obesity were associated with more adverse pregnancy outcomes compared with excessive gestational weight gain. Furthermore, stepwise regression analysis showed that the effect estimates for the associations of excessive gestational weight gain with pregnancy complications attenuated when pre-pregnancy overweight and obesity were taken into account.

Methodological considerations

Some methodological issues need to be considered. One of the strengths of this study was the prospective data collection from early pregnancy onwards. We had a large sample size of 6,959 participants. The response rate at baseline for participation in the Generation R Study cohort was 61%. The nonresponse would lead to biased effect estimates if the associations were different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline.[15] Furthermore, not all women were already enrolled in the study in first trimester. Therefore, we did not have first-trimester weight measurements in approximately 53% of the participating women. It seemed unlikely that late enrollment has biased our results. We observed small differences in the effect estimates for the associations of pre-pregnancy BMI with the risk of adverse pregnancy outcomes between women who were enrolled during first trimester or later in pregnancy. For all associations, effect estimates were in similar direction in women enrolled during first trimester or later in pregnancy. Detailed information about a large number of potential risk factors and confounding factors was available in this study.

However, because of the observational design, residual confounding because of other sociodemographic and lifestyle-related determinants might still be an issue. In addition, information about many covariates in this study was self-reported, which may have resulted in underreporting of certain adverse lifestyle-related determinants. Some data of these covariates were missing. It is unlikely that these data were missing completely at random, so a complete case analysis might lead to biased results. To avoid bias and to maintain statistical power, we used multiple imputations for missing information of the covariates. As compared to the complete case analysis, effect estimates only changed marginally after using multiple imputations to deal with the missing values. Information on maternal pre-pregnancy weight was self-reported. Self-reported weight tends to be underestimated, so some misclassification might have occurred. Also, maximum weight during pregnancy was self-reported 2 months after delivery. Weight assessment by questionnaire might have led to an underestimation of maximum pregnancy weight. This might have led to an underestimation of the observed effects. However, self-reported pre-pregnancy weight and weight measured at intake, and self-reported maximum weight and weight measured at 30 weeks of gestation, were highly correlated in our study. Furthermore, for the analyses focused on the associations between trimester-specific weight gain and the risk of adverse pregnancy outcomes, we performed a sensitivity analyses in normal weight women only, as overweight and obese women are more likely to underestimate self-reported weight (results not shown). The effect estimates changed slightly, when overweight and obese women were excluded from the analyses, but were in similar direction. The observed smaller effect sizes might be explained by smaller numbers of subjects and less power to detect differences because of the exclusion of extremes.

Comparison of main findings with other studies

The risk of maternal obesity and excessive gestational weight gain varied among different ethnic groups and socioeconomic groups, which is in line with previous studies.[16-18] We observed that multiparous women were more frequently obese and had a lower risk of excessive gestational weight gain, as compared to nulliparous women. Accordingly, a study in 57,700 Danish women showed that women with low gestational weight gain were more often multiparous.[17] The risks of overweight and obesity were higher in women who carry the risk variants of the FTO gene. Many studies have already shown an association of FTO polymorphism with the risk of obesity in children and adults.[19-20] In pregnant women, the FTO gene has been suggested to influence pre-pregnancy weight as well.[21] We also showed an association of the FTO gene with the risk of pre-pregnancy overweight and obesity in pregnant women. However, we did not replicate our findings. Therefore, our results should be considered as hypothesis generating and need replication in further

studies. Furthermore, we observed that excessive gestational weight gain was more likely in women who smoked during pregnancy and in women who did not consume alcohol during pregnancy, which is in agreement with the study in Danish women.[17] Higher total energy intake was also associated with an increased risk of excessive gestational weight gain, which has been reported by a previous study.[22]

Previous studies suggested associations between maternal overweight and obesity and the risks of gestational hypertensive disorders and gestational diabetes.[16,23-26] A large review among 13 cohort studies showed that there was a strong positive association between pre-pregnancy BMI and preeclampsia.[24] Another review suggested that the risk of developing gestational diabetes was two times higher for overweight women and four times higher for obese women compared with normal weight women.[26] We observed similar results as maternal overweight and obesity were strongly associated with the risks of gestational hypertensive disorders and gestational diabetes. For the associations with gestational diabetes, it needs to be noted that accurate diagnosis of gestational diabetes is difficult. A fasting glucose greater that 7.0 mmol/L might also represent preexisting diabetes, and a fasting glucose between 6.1 and 6.9 mmol/L might also represent impaired glucose tolerance, instead of gestational diabetes. Unfortunately, in our study, no data were available on glucose tolerance before pregnancy. Excessive gestational weight gain was associated with the risk of gestational hypertension, but not associated with the risks of gestational diabetes and preeclampsia. This might be because of the small number of cases of gestational diabetes and preeclampsia in our study population. Overweight and obese mothers, and mothers with excessive weight gain, were at increased risk of Caesarean delivery. This is in line with observations in other studies that examined the association of maternal obesity and antenatal complications.[16-18,27] These associations might be influenced by the effect of obesity and excessive gestational weight gain on birth weight. However, after additional adjustment for birth weight, the associations only changed slightly and remained highly significant (results not shown). The association between maternal obesity and the risk of instrumental delivery remains more controversial. A study in 18,643 women reported that maternal obesity was not associated with the risk of instrumental delivery.[28] Accordingly, we observed no association of maternal BMI and excessive gestational weight gain with ventouse extraction. We observed a positive association between pre-pregnancy obesity and the risk of preterm delivery, which might partly be explained by the association of pre-pregnancy obesity with the risk of PROM. In our study, we do not have further data available about the specific causes of preterm birth. Further research to assess whether maternal obesity is associated with the risk of idiopathic or indicated preterm birth is necessary. We also observed that the risk of preterm delivery was lower in women who gained excessive weight. Thus far, published studies focused on the associations of maternal anthropometrics with the risk of preterm

delivery seem to be inconsistent. Some studies found no association between maternal obesity and preterm delivery, whereas other studies suggested that the risk of preterm birth is higher in obese women.[16,28-30] A study in 76,682 adolescent women reported that the risk of preterm delivery was lower in women who gained excessively, independently of pre-pregnancy BMI.[31] It has also been suggested that the association between gestational weight gain and preterm delivery is a modest U-shape.[32] A study in 33,872 women reported that compared with a gestational weight gain of 10-14 kg, women who gained less than 10 kg and women who gained more than 20 kg were at increased risk of preterm delivery.[32] In our study population, approximately 65% of the women who gained excessive weight, gained below 20 kg. Modest excessive weight gain might have a protective effect for preterm delivery. We observed that maternal obesity and excessive gestational weight gain were associated with an increased risk of large size for gestational age infants and a lower risk of small size for gestational age infants. Similar findings have been reported by other studies.[4,17,18] Previously, we have shown that maternal pre-pregnancy BMI is positively associated with birth weight of the offspring.[4] The associations between maternal obesity and excessive gestational weight gain with the risk of delivering a small size for gestational age infant or large size for gestational age infant attenuated after adjustment for gestational hypertensive disorders and gestational diabetes, but remained highly significant (results not shown). Furthermore, multiple studies have suggested that pre-pregnancy overweight and obesity are associated with an increased risk of longer length of hospital stay, an increased risk of having a neonate with a low Apgar score, and a higher risk of referral to neonatal intensive care unit.[33-35] We also observed that maternal obesity was associated with a higher risk of having a neonate with a low Apgar score. Other information about the neonate's health is not available within our study.

The fetal overnutrition hypothesis suggests that higher maternal plasma concentrations of glucose and free fatty acids because of maternal obesity during pregnancy might increase placental transfer of nutrients during embryonic and fetal development. This might cause permanent changes in appetite, energy metabolism and neuro-endocrine function of offspring, predisposing an individual to a greater risk of obesity in later life.[6] In line with this suggested pathway, we observed that maternal overweight and obesity are associated with overweight and obesity in the offspring.

Maternal underweight has also been suggested to be associated with adverse pregnancy outcomes. A large review among 78 studies showed that underweight women had a higher risk of both spontaneous and induced preterm birth and a higher risk of delivering a low birth weight infant.[36] In line with these findings, we observed that maternal underweight was associated with an increased risk of PROM, and an increased risk of delivering a small size for gestational age infant. We did not observe a significant effect on overall preterm birth.

Weight gain during pregnancy may vary greatly, and the effect of gestational weight in first, second and third trimester on maternal and fetal outcomes might be different. We observed that maternal weight gain was slow in first trimester and increased in second and third trimester. Few studies have examined the influence of trimester-specific weight gain on adverse outcomes.[37-39] We observed that weight gain in first trimester was associated with the risks of gestational diabetes and gestational hypertension and weight gain in third trimester was associated with the risks of preeclampsia and gestational hypertension. When examining the associations between third-trimester gestational weight gain and the risk of these disorders, it is difficult to differentiate between cause and consequence. The occurring edema might partly explain the excessive gestational weight gain. Further research is necessary to explore reversed causation and to examine underlying mechanisms of these associations. Studies examining the effect of gestational weight gain per trimester have mainly focused on the association of low weight gain and the risk of low birth weight infants.[37-39] A study in 10,696 women showed that low weight gain in second and third trimester, but not in first trimester, was associated with the risk of intrauterine growth retardation.[39] Accordingly, we observed that higher maternal weight gain in second and third trimester was more strongly associated with a lower risk of delivering a small size for gestational age infant, as compared to first-trimester weight gain. Furthermore, higher weight gain in each trimester was associated with a higher risk of delivering a large size for gestational age infant, but the strongest effects of weight gain were during second and third trimester. After additional adjustment for total weight gain and weight gain in the other trimesters, results only changed marginally (results not shown). These associations suggest that the effect of weight gain in early pregnancy might be different from the effect of weight gain later in pregnancy.

Current preventive strategies have mainly focused on restricting gestational weight gain during pregnancy. A meta-analysis of randomized controlled trials, focused on diet and physical activity during pregnancy as intervention, showed that interventions may be effective to control weight gain during pregnancy.[40] However, as maternal overweight and obesity are strongly associated with short-term and long-term adverse consequences, future preventive strategies should also focus on pre-pregnancy overweight and obesity.

Conclusion

Maternal sociodemographic characteristics and lifestyle habits are associated with increased risks of maternal obesity and excessive weight gain during pregnancy. Both maternal underweight, overweight, obesity and excessive gestational weight gain are associated with increased risks of maternal, fetal and childhood health outcomes. Future

preventive strategies, focused on especially pre-pregnancy BMI, are needed to improve maternal pregnancy outcomes and health of offspring.

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Supplemental Table 1 Characteristics by maternal body mass index (N = 6,959)[1,2]

	Underweight (20-24.9 kg/m²) N = 1,123	Normal (<25 kg/m²) N = 3,888	Overweight (25-29.9 kg/m²) N = 1,334	Obesity (>=30 kg/m²) N = 611	P-value
Height (cm), mean (SD)	168.4 (7.1)	167.8 (7.3)	166.3 (7.5)	165.7 (7.5)	P < 0.01
Pre-pregnancy weight (kg), mean (SD)	53.6 (5.3)	62.7 (6.4)	74.7 (7.5)	93.4 (13.2)	P < 0.01
Body mass index, mean (SD)	18.9 (0.9)	22.6 (1.4)	27.0 (1.4)	34.0 (3.7)	P < 0.01
Maximum weight gain (kg), mean (SD)	15.0 (5.3)	15.4 (5.4)	14.0 (6.6)	11.3 (8.6)	P < 0.01
Age (yrs), mean (SD)	29.1 (5.4)	30.0 (5.3)	29.8 (5.2)	29.3 (5.1)	P < 0.01
Parity, nulliparous (%)	63.4	60.4	47.3	43.5	P < 0.01
Gestational age at intake (wks), median (95% range)	14.5 (10.2, 28.9)	14.2 (10.4, 25.5)	14.4 (10.2, 24.9)	14.6 (10.5, 28.2)	P = 0.13
Highest completed education (%)					
Primary school	9.1	9.0	15.8	17.5	P < 0.01
Secondary school	45.7	42.9	50.2	64.3	
Higher education	45.1	46.1	34.0	18.1	
Ethnicity (%)					
European	61.3	62.2	48.9	42.5	P < 0.01
Non-European	38.7	37.8	51.1	57.5	
Alcohol consumption (%)					
None	45.0	45.2	58.8	66.3	P < 0.01
Yes	55.0	54.8	41.2	33.7	
Smoking habits (%)					
None	70.8	73.7	75.8	73.9	P = 0.04
Yes	29.2	26.3	24.2	26.1	
Folic acid supplement use (%)					
None	26.5	25.5	34.8	43.7	P < 0.01
Yes	73.5	74.5	65.2	56.3	

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

Supplemental Table 2 Associations between pre-pregnancy overweight and obesity and risk of adverse pregnancy outcomes according to gestational age at enrollment[1]

	Overweight[2]		Obesity[2]	
	First trimester enrolled (N = 601)	Second or third trimester enrolled (N = 733)	First trimester enrolled (N = 254)	Second or third trimester enrolled (N = 357)
Maternal complications				
Gestational hypertension	1.78 (1.13, 2.80)*	2.66 (1.66, 4.25)**	6.01 (3.59, 10.01)*	7.18 (4.02, 12.78)**
Preeclampsia	1.69 (0.83, 3.44)	2.07 (1.15, 3.73)*	4.06 (1.71, 9.57)**	3.38 (1.57, 7.28)**
Gestational diabetes	3.94 (163, 9.49)**	4.42 (1.91, 10.20)**	7.96 (3.13, 20.19)**	4.26 (1.14, 15.19)*
Delivery complications				
PROM	0.84 (0.45, 1.56)	1.03 (0.65, 1.64)	1.99 (1.01, 3.93)*	1.47 (0.84, 2.58)
Ventouse extraction	1.05 (0.78, 1.41)	0.93 (0.69, 1.26)	1.53 (1.00, 2.32)	0.82 (0.52, 1.29)
Caesarean section	1.62 (1.21, 2.15)**	1.46 (.09, 1.91)*	1.59 (1.05, 2.40)*	2.24 (1.56, 3.21)**
Postpartum hemorrhage	1.09 (0.71, 1.65)	1.64 (1.11, 2.43)*	1.15 (0.62, 2.12)	1.73 (1.00, 2.99)
Birth complications				
Preterm birth[3]	1.08 (0.67, 1.74)	1.02 (0.68, 1.51)	1.60 (0.86, 2.97)	1.50 (0.92, 2.44)
Large size for gestational age	1.42 (1.02, 1.96)*	2.01 (1.49, 2.70)**	2.53 (1.59, 4.01)**	3.33 (2.27, 4.88)**
Small size for gestational age	0.88 (0.62, 1.25)	0.77 (0.55, 1.05)	0.53 (0.30, 0.93)*	0.55 (0.35, 0.89)*
Childhood complications				
Overweight[4]	1.69 (1.18, 2.43)	1.32 (0.92, 1.87)	2.85 (1.75, 4.63)**	2.15 (1.38, 3.33)**
Obesity[4]	1.72 (0.67, 4.41)	1.56 (0.80, 3.02)	6.37 (2.65, 15.22)**	4.07 (2.10, 7.89)**

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risks of complications for overweight and obese women as compared to women with a normal body mass index, 20-24.9 kg/m^2. Estimates are from multiple imputed data.

[2]Models for overweight and obesity are adjusted for educational level, maternal age, ethnicity, parity, folic acid supplement use, smoking habits, alcohol consumption and gestational weight gain.

[3]Models are adjusted for sex as well.

[4]Models are also adjusted for breastfeeding (yes, no).

* P < 0.05 and **P < 0.01

OR: odds ratios; CI: confidence interval; PROM: prelabour rupture of membranes

Supplemental Table 3 Associations of excessive gestational weight gain with maternal, delivery, birth and childhood complications using weight gain until third trimester[1]

(N = 6,956)	Excessive gestational weight gain[2] (OR, (95% CI))
Maternal complications	N = 2,996
Gestational hypertension	1.57 (1.21, 2.04)**
Preeclampsia	1.20 (0.83, 1.72)
Gestational diabetes	1.90 (1.16, 3.08)*
Delivery complications	N = 2,996
Caesarean section	1.34 (1.14, 1.58)**
Birth complications	N = 2,996
Preterm birth[3]	1.06 (0.84, 1.35)
Large size for gestational age	2.08 (1.76, 2.45)**
Small size for gestational age	0.56 (0.47, 0.66)**
Childhood complications	N = 1,949
Childhood overweight[4]	1.44 (1.21, 1.72)**
Childhood obesity[4]	1.23 (0.82, 1.85)

[1]Values are odds ratios (95% confidence interval) that reflect the difference in risks of complications for women with excessive gestational weight gain as compared to women with a recommended or less than recommended gestational weight gain. Estimates are from multiple imputed data.
[2]Models for excessive gestational weight gain are adjusted for educational level, maternal age, ethnicity, parity, folic acid supplement use, smoking habits and alcohol consumption.
[3]Models are adjusted for sex as well.
[4]Models are also adjusted for breastfeeding (yes, no).

CHAPTER 4.3

Parental smoking during pregnancy, growth and the risk of obesity in preschool children

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ABSTRACT

Objective: Maternal smoking during pregnancy seems to be associated with obesity in offspring. Not much is known about the specific critical exposure periods or underlying mechanisms for this association. We assessed the associations of active maternal and paternal smoking during pregnancy with early growth characteristics and risks of overweight and obesity in preschool children.

Methods: This study was a population-based, prospective cohort study from early fetal life until the age of 4 years in 5,342 mothers and fathers and their children. Growth characteristics (head circumference, length, weight and body mass index (BMI)), and overweight and obesity were repeatedly measured at the ages of 1, 2, 3 and 4 years.

Results: In comparison with children from nonsmoking mothers, children from mothers who continued smoking during pregnancy had persistently smaller head circumferences and heights until the age of 4 years, whereas their weights were lower only until the age of 3 months. This smaller length and normal to higher weight led to an increased BMI (standard deviation (SD) score difference: 0.11 (95% confidence interval (CI) 0.02, 0.20; $P < 0.05$)), and an increased risk of obesity (odds ratio (OR): 1.61 (95% CI 1.03, 2.53; $P < 0.05$)) at the age of 4 years. In nonsmoking mothers, paternal smoking was not associated with postnatal growth characteristics or risk of obesity in offspring. Maternal smoking during pregnancy was associated with a higher BMI at the age of 4 years in children with a normal birth weight and in those who were small for gestational age at birth.

Conclusions: Our findings suggest that direct intrauterine exposure to smoke until late pregnancy leads to different height and weight growth adaptations and increased risks of overweight and obesity in preschool children.

INTRODUCTION

The hypothesis of developmental origins proposes that fetal adaptations in organ function and metabolism in response to adverse intrauterine conditions lead to fetal growth retardation and predispose the individual to increased risks of obesity and type 2 diabetes in adult life.[1,2] Not much is known about the influence of specific adverse exposures. In Western countries, active maternal smoking during pregnancy is a common and preventable specific adverse environmental exposure.[3,4] Maternal smoking during pregnancy is associated with fetal growth retardation and increased risks of preterm birth and low birth weight.[5-7] It has been suggested that maternal smoking during pregnancy also increases risk of obesity in offspring.[8,9] A recent systematic review suggested that prenatal smoke exposure led to a 50% increased risk of overweight in childhood.[10] Most previous studies were not able to assess the effect of maternal smoking exposure in different periods of pregnancy. This information is important because it might identify specific critical time windows. It is also not known whether the associations between maternal smoking during pregnancy and risk of childhood obesity are explained by intrauterine effects or just reflect various unmeasured environmental confounders. Stronger effect estimates for maternal smoking than for paternal smoking with childhood obesity may suggest direct intrauterine effects, whereas similar effect estimates may suggest that the associations are explained by unmeasured environmental exposures.[11,12]

Therefore, in a population-based prospective cohort study in 5,342 mothers and fathers and their children, who were followed from early fetal life onwards, we examined associations of exposure to maternal and paternal smoking during pregnancy with early growth characteristics and risks of overweight and obesity until the age of 4 years.

METHODS

Study design

This study was embedded in the Generation R Study, which is a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands.[13,14] Enrollment in the study was aimed at early pregnancy (gestational age <18 weeks) but was possible until the birth of the child. Assessments during pregnancy, including physical examinations, fetal ultrasound examinations, and questionnaires, were planned in each trimester.[14] All children were born between April 2002 and January 2006, and form a prenatally enrolled birth-cohort that is currently fol-

lowed until young adulthood. Postnatal growth data for the current study were available until the age of 4 years. Of all eligible children in the study area, 61% of children were participating in the study at birth.[14] The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center (Rotterdam, Netherlands). Written informed consent was obtained from all parents.

Data collection and measurements

Maternal and paternal smoking during pregnancy

Information on maternal smoking was obtained by postal questionnaires sent in first, second and third trimester of pregnancy. Response rates for these questionnaires were 91%, 80%, 77%, respectively.[14] Active maternal smoking at enrollment was assessed in the first questionnaire by asking whether she smoked during her pregnancy. We grouped mothers into 3 categories as follows: 1) never smoked during pregnancy; 2) only smoked until their pregnancy was acknowledged (first trimester only); and 3) continued to smoke during pregnancy. This questionnaire was sent to all mothers independent of the gestational age at enrollment. In the second and third questionnaires, mothers were asked whether they had smoked during the past 2 months (yes, no). Mothers who reported in the first questionnaire not to have smoked or to have smoked until their pregnancy was acknowledged but reported to have smoked in the second or third questionnaire were reclassified as ''continued smoking''. Active paternal smoking was assessed in the first questionnaire by asking the mother whether the father smoked during pregnancy (yes, no, or do not know). Similar information completed by the father was available in a subset of participants (N = 3,558). Agreement between these assessments was good (sensitivity 91%, specificity 95%). We used data collected from the mother's questionnaire because this information was available for all children. No difference in effect estimates were observed when we used information completed by the father himself. In smokers, the numbers of cigarettes smoked daily was available in the following categories: no smoking; <5 cigarettes/day; and >=5 cigarettes/day. All mothers included in these analyses were selected on the basis of complete information on the duration of smoking during pregnancy. Because we used 2 different questions (i.e., did you smoke; what is the number of smoked cigarettes), the number of cigarettes smoked per day was not known for all mothers.

Fetal growth characteristics

Fetal ultrasound examinations were carried out at the research centers in first trimester (median 13.5 weeks (95% range 11.0, 17.0)), second trimester (median 20.7 weeks (95% range 18.9, 22.8)), and third trimester (median 30.5 weeks (95% range 28.9, 32.4)). The first ultrasound was used for establishing gestational age because these methods were superior than the use of the last menstrual period because of its limitations, including the large number of women who did not know the exact date of their last menstrual period or had irregular menstrual cycles.[15-17] Second and third trimester ultrasounds were used to assess fetal growth. We measured fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) to the nearest millimeter (mm) by using standardized ultrasound procedures[18] and the estimated fetal weight (EFW) was calculated by using the following formula of Hadlock *et al.*:[19]

$(\log_{10} EFW = 1.5662 - 0.0108\,(HC) + 0.0468\,(AC) + 0.171\,(FL) + 0.00034\,(HC)^2 - 0.003685\,(AC * FL))$.

Standard deviation scores (SDS) for all fetal growth characteristics were constructed by using data from the study group. Ultrasound examinations were performed with an Aloka® model SSD-1700 (Aloka Co Ltd, Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Philips, Seattle, WA).

Postnatal growth characteristics

Information on weight at birth was obtained from community midwife and hospital registries. Because head circumferences and lengths were not routinely measured at birth, these measurements were only available in a subset. Postnatal growth was measured by a well-trained staff at Community Health Centers according to a standard schedule and procedures at the ages of 3 months (median 3.1 (95% range 1.3, 4.2)), 6 months (median 6.7 (95% range 5.5, 10.3)), 12 months (median 13.0 (95% range 11.1, 15.3)), 24 months (median 24.4 (95% range 18.6, 27.5)), 36 months (median 36.4 (95% range 31.1, 39.2)) months and 48 months (median 45.3 (95% range 25.7, 47.8)). Head circumferences were measured to the nearest millimeter (mm) with a standardized tape (SECA, Hamburg, Germany) until the age of 12 months. Lengths were measured in a supine position to the nearest mm until the age of 12 months with a neonatometer. From the age of 24 months, heights were measured in a standing position with a Harpenden stadiometer (Holtain Ltd, Dyfed, UK). Weights were measured with a mechanical personal scale (SECA, Almere, The Netherlands). Body mass index (BMI) (kg/m^2) was calculated. SDS for postnatal growth characteristics were obtained with Dutch reference growth charts (Growth Analyser 3.0, Dutch Growth Research Foundation, Rotterdam, Netherlands). Definitions of overweight (BMI

>1.1 to 2.3 SDS) and obesity (BMI >2.3 SDS) were based on the age- and sex-adjusted BMI distributions on the basis of the definition of Cole *et al*.[20] Fredriks *et al*.[21] transformed the international criteria for overweight and obesity to SD's to identify the pediatric centiles at younger ages and showed that an adult BMI of 25 kg/m^2 (overweight) corresponded to a +1.1 SDS and that an adult BMI of 30 kg/m^2 (obesity) corresponded to a +2.3 SDS in the reference growth diagrams on the basis of the 1997 Dutch Growth Study. Therefore, the +1.1- and +2.3- SDS lines in the 1997 BMI charts correspond to the recommended limits for overweight and obesity, respectively, that Cole *et al*.[20] also used.

Covariates

Gestational age at birth and sex were obtained from midwife and hospital registries at birth. Information on parental educational level and ethnicity were obtained from the first questionnaire at enrollment in the study. Ethnicity and educational level of parents were defined according to the classification of Statistics Netherlands.[22,23] Parental anthropometric measurements were assessed at enrollment. Height and weight were measured while the parent stood without shoes and heavy clothing, and BMI (kg/m^2) was calculated. Information on breastfeeding was obtained by postnatal questionnaires at the ages of 2, 6, and 12 months.

Population for analysis

In total 6,969 children and their mothers had been included prenatally and fully participated in the postnatal phase of the study (**Supplemental Figure 1**). Subjects without information on smoking during pregnancy in the three questionnaires were excluded from the current analyses (13%, N = 936). Of the remaining mothers, those with twin pregnancies (N = 125) and those with second or third participating infants of the same mother in the study (N = 382) were excluded from the current analyses to prevent a bias because of correlation. Of the remaining 5,526 singleton live births with complete data on maternal smoking during pregnancy, information about at least one postnatal growth characteristics measure was available in 5,342 children. There were no differences in the categories of active smoking between participants compared with those of lost to follow-up subjects (P = 0.14).

Statistical analysis

Differences in baseline characteristics between maternal smoking categories were compared by using the t-tests and analysis of variance with Bonferroni correction in Table 1.

Table 1 Characteristics of mothers and their children according to category of maternal smoking during pregnancy[1,2]

	Smoking during pregnancy (N = 5,342)			
	No (N = 4,028, 75.4%)	First trimester only (N = 481, 9.0%)	Continued (N = 833, 15.6%)	ANOVA
Maternal characteristics				
Age (years)	30.4 (21.4, 38.2)	29.7 (20.4, 37.5)*	29.0 (19.9, 37.8)**	< 0.01
Height (cm)	167.6 (7.5)	168.7 (7.1)**	167.1 (7.2)	< 0.01
Weight (kg)	69.0 (12.9)	69.2 (12.5)	70.1 (14.0)	0.10
Body mass index (kg/m^2)	24.6 (4.4)	24.3 (4.3)	25.1 (4.7)**	< 0.01
Education (%)				< 0.01
Primary	9.0	7.9	16.6**	
Secondary	40.4	45.1	62.2**	
Higher	50.6	47.0	21.2**	
Ethnicity (%)				0.04
Dutch or European	60.4	65.1	58.0**	
Non-European	39.6	34.9	42.0**	
Paternal characteristics				
Age (years)	33.4 (24.5, 43.5)	32.2 (22.5, 41.9)**	31.7 (21.4, 42.3)**	< 0.01
Weight (kg)	83.5 (12.7)	83.7 (12.7)	82.2 (13.3)	0.25
Height (cm)	181.4 (7.7)	182.5 (7.8)	179.9 (8.0)**	< 0.01
Body mass index (kg/m^2)	25.4 (3.4)	25.1 (3.3)	25.4 (3.6)	0.33
Smoking (%)				< 0.01
Yes	34.7	65.1**	74.1**	
Birth				
Males (%)	50	48	52	0.30
Gestational age (weeks)	40.0 (37.1, 42.1)	39.9 (37.1, 42.0)	39.8 (36.4, 42.1)**	< 0.01
Weight (grams)	3,463 (540)	3,462 (532)	3,265 (540)**	< 0.01
Small size for gestational age (<10%) (%)	9.0	8.1	15.5**	< 0.01
Low birth weight (<2500 g) %	3.8	3.3	6.6**	< 0.01
Preterm birth (%)	4.0	4.0	6.1*	0.02
Breastfeeding				
Ever (%)	93.7	92.7	84.3**	< 0.01
Duration (months)	5.1 (0.5, 12.0)	4.0 (0.5, 12.0)**	3.4 (0.5, 12.0)**	< 0.01

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.

[2]Differences in parental and child characteristics (compared with the maternal nonsmoking category) were evaluated by using the t-tests and ANOVA with Bonferroni correction. Values were missing for maternal height (N = 6), maternal weight (N = 16), maternal education (N = 93), maternal ethnicity (N = 20), paternal age (N = 482), paternal height (N = 1,227), paternal weight (N = 1,232), paternal smoking (N = 83), birth weight (N = 2), ever breastfeeding (N = 553), and duration of breastfeeding (N = 1,932).

*P < 0.05 and **P < 0.01

Associations of the period of maternal smoking during pregnancy (no, first trimester only, or continued) with growth characteristics (SDS of head circumference, height, weight, and BMI) were assessed by using linear mixed models. These models take the correlation between repeated measurements of the same participant into account and allow for incomplete outcome data.[24] To account for the within-child correlation, we included a random intercept in the model. The models were adjusted for potential confounders including the visit (second trimester, third trimester, birth, and 3, 6, 12, 24, 36, or 48 months), because the intercept might not have been the same at every visit, child's age at the visit relative to the mean per visit, sex, maternal ethnicity and education, maternal height and weight at enrollment, and breastfeeding (yes, no). All interactions between the visit and the other confounders where also included in the model because of the possible variability of confounder effects. Confounders were included in the models on the basis of their associations with postnatal BMI in previous studies or a change in effect estimates of interest >10% because this criterion took into account the covariate-outcome association and the change in the estimate upon removal of the covariate.[25] Similar linear mixed models were used for the assessment of associations of reported numbers of cigarettes smoked by the mother during pregnancy, smoking of the father, and the number of cigarettes smoked by the father with growth characteristics in offspring. Postnatal smoking, parity, and maternal alcohol consumption were not included in models because they did not materially change effect estimates. Multiple logistic regression models were used for the analysis of associations of the period of maternal and paternal smoking during pregnancy with risks of overweight and obesity at the age of 4 years. Analyses that focused on associations of maternal and paternal smoking with anthropometrics in offspring were not adjusted for multiple testing because these were closely correlated outcomes. Finally, to assess whether associations of maternal smoking during pregnancy with postnatal BMI and risks of overweight and obesity were modified by gestational age-adjusted birth weight, we repeated these analyses with overweight and obesity as outcomes in strata of small size for gestational age defined as the lowest 10% of gestational age-adjusted birth weight in the cohort. Tests for trends were performed by treating each categorized variable as a continuous term and entering the variable into the fully adjusted regression model. To handle missing values in covariates (<23% missing values), we performed multiple imputations for linear mixed models in Table 2 and Supplemental Table 2 by using the chained equations approach in the R program (version 2.12.1; The R Foundation for Statistical Computing, Vienna, Austria)[26] and for Table 3 by generating 5 independent datasets using the Markov chain Monte Carlo (MCMC) method in the Statistical Package of Social Sciences program (version 17.0 for Windows; SPSS Inc, Chicago, IL). According to both methods, SEs from each of the 5 imputation sets were combined to an overall SE on the basis of the within-imputation variance and the between-imputation variance. All

Table 2 Associations of maternal smoking during pregnancy with repeatedly measured postnatal growth characteristics[1,2]

	Difference (95% confidence interval) in head circumference (SDS)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Maternal smoking category							
No (N = 4,028)	Reference	Reference	Reference	Reference	-	-	-
First trimester only (N = 481)	-0.07 (-0.18, 0.05)	0.03 (-0.06, 0.12)	0.01 (-0.08, 0.10)	-0.03 (-0.13, 0.07)	-	-	-
Continued (N = 833)	-0.26 (-0.35, -0.17)**	-0.19 (-0.27, -0.11)**	-0.11 (-0.18, -0.03)**	-0.10 (-0.18, -0.01)*	-	-	-
0-4 cigarettes/day (N = 313)	-0.22 (-0.35, -0.08)**	-0.08 (-0.19, 0.02)	-0.04 (-0.14, 0.07)	-0.08 (-0.20, 0.03)	-	-	-
>=5 cigarettes/day (N = 296)	-0.31 (-0.45, -0.17)**	-0.31 (-0.43, -0.20)**	-0.20 (-0.31, -0.08)**	-0.13 (-0.25, -0.01)*	-	-	-
P for trend	P < 0.01	P < 0.01	P < 0.01	P < 0.01	-	-	-
	Difference (95% confidence interval) in length (SDS)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Maternal smoking category							
No (N = 4,028)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
First trimester only (N = 481)	-0.05 (-0.16, 0.05)	0 (-0.09, 0.10)	-0 (-0.10, 0.09)	-0.02 (-0.12, 0.07)	0.04 (-0.06, 0.13)	0.01 (-0.10, 0.11)	0.02 (-0.09, 0.13)
Continued (N = 833)	-0.40 (-0.49, -0.31)**	-0.30 (-0.38, -0.23)**	-0.14 (-0.21, -0.06)**	-0.14 (-0.21,-0.06)**	-0.13 (-0.21,-0.05)**	-0.11 (-0.20,-0.03)**	-0.10 (-0.19, -0.01)*
0-4 cigarettes/day (N = 313)	-0.36 (-0.49, -0.23)**	-0.15 (-0.26, -0.04)**	-0.04 (-0.15, 0.07)	-0.04 (-0.14, 0.07)	-0.03 (-0.14, 0.07)	-0.04 (-0.15, 0.07)	0 (-0.12, 0.12)
>=5 cigarettes/day (N = 296)	-0.45 (-0.59, -0.31)**	-0.48 (-0.59, -0.37)**	-0.26 (-0.38,-0.14)**	-0.25 (-0.36, -0.14)**	-0.25 (-0.36, -0.13) **	-0.20 (-0.32,-0.08)**	-0.23 (-0.35, -0.10)**
P for trend	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01	P < 0.01
	Difference (95% confidence interval) in weight (SDS)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Maternal smoking category							
No (N = 4,028)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
First trimester only (N = 481)	-0.01 (-0.10, 0.08)	0.04 (-0.05, 0.14)	0 (-0.09, 0.09)	-0.03 (-0.12, 0.07)	-0.04 (-0.13, 0.06)	-0 (-0.10, 0.10)	0.03 (-0.08, 0.13)
Continued (N = 833)	-0.35 (-0.43, -0.28) **	-0.17 (-0.24, -0.09)**	-0.05 (-0.13, 0.03)	-0.04 (-0.11, 0.04)	-0.07 (-0.15, 0.01)	-0 (-0.08, 0.08)	0.02 (-0.07, 0.11)
0-4 cigarettes/day (N = 313)	-0.32 (-0.42, -0.22)**	-0.08 (-0.19, 0.03)	0.02 (-0.09, 0.13)	0.01 (-0.10, 0.12)	-0.03 (-0.14, 0.08)	0.01 (-0.10, 0.12)	0.05 (-0.07, 0.17)
>=5 cigarettes/day (N = 296)	-0.39 (-0.50, -0.28)**	-0.26 (-0.38, -0.14)**	-0.13 (-0.25, -0.01)*	-0.10 (-0.21, 0.02)	-0.13 (-0.24,-0.02)*	-0.02 (-0.14, 0.10)	-0.02 (-0.14, 0.11)
P for trend	P < 0.01	P < 0.01	P = 0.25	P = 0.06	P = 0.01	P = 0.24	P = 0.39

Table 2 Associations of maternal smoking during pregnancy with repeatedly measured postnatal growth characteristics[1,2] (continued)

	Difference (95% confidence interval) in body mass index (SDS)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Maternal smoking category							
No (N = 4,028)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
First trimester only (N = 481)	-	0.04 (-0.06, 0.14)	0.01 (-0.08, 0.11)	-0.01 (-0.11, 0.09)	-0.07 (-0.18, 0.03)	0 (-0.10, 0.11)	0.01 (-0.10, 0.12)
Continued (N = 833)	-	0.04 (-0.04, 0.12)	0.05 (-0.03, 0.13)	0.06 (-0.02, 0.14)	0.03 (-0.05, 0.11)	0.10 (0.02, 0.19)*	0.11 (0.02, 0.20)*
0-4 cigarettes/day (N = 313)	-	0.02 (-0.09, 0.13)	0.05 (-0.05, 0.16)	0.04 (-0.07, 0.14)	0.01 (-0.10, 0.12)	0.05 (-0.06, 0.16)	0.07 (-0.06, 0.19)
>=5 cigarettes/day (N = 296)	-	0.08 (-0.04, 0.20)	0.06 (-0.06, 0.17)	0.07 (-0.04, 0.19)	0.05 (-0.07, 0.16)	0.16 (0.04, 0.28)*	0.15 (0.03, 0.28)*
P for trend	-	P = 0.02	P = 0.27	P = 0.24	P = 0.24	P = 0.02	P = 0.03

[1]Values are standardized regression coefficients (95% confidence interval) assessed by using linear mixed models. Trend tests for the number of cigarettes smoked per day were performed by using fully adjusted linear regression models and by treating the categorized dose variables as continuous variables in these models.
[2]Models are adjusted for child's age at visit, sex, maternal ethnicity and education, maternal height and weight, and breastfeeding (yes, no).
* P < 0.05 and ** P < 0.01

Table 3 Associations of maternal and paternal smoking with overweight and obesity at the age of 4 years compared with nonsmokers[1,2]

	Risk of overweight[3] (odds ratio (95% confidence interval))	**Risk of obesity[3] (odds ratio (95% confidence interval))**	**Risk of overweight or obesity[3] (odds ratio (95% confidence interval))**
Maternal smoking category	N = 4,540 (590)[4]	N = 4,540 (106)[4]	N = 4,540 (696)[4]
No (N = 4,028)	Reference	Reference	Reference
First trimester only (N = 481)	1.39 (1.04, 1.85)*	0.76 (0.32, 1.79)	1.32 (0.99, 1.73)
Continues (N = 833)	1.00 (0.78, 1.28)	1.61 (1.03, 2.53)*	1.11 (0.89, 1.39)
P for trend	P = 0.57	P = 0.07	P = 0.19
Paternal smoking category	N = 3,394 (420)	N = 3,394 (69)	N = 3,394 (489)
No (N = 2,527)	Reference	Reference	Reference
Yes (N = 1,397)	1.17 (0.95, 1.45)	1.09 (0.66, 1.76)	1.16 (0.95, 1.42)
P for trend	P = 0.15	P = 0.75	P = 0.16

[1]Values are odds ratios (95% confidence interval) by using multivariate logistic regression models.
[2]Models are adjusted for child's age at visit, sex, parental ethnicity and education, parental height and weight, and breastfeeding (yes, no).
[3]Overweight was defined as age-and sex-adjusted body mass index >1.1 – 2.3 SDS; obesity was defined as age-and sex-adjusted body mass index > 2.3 SDS; and overweight or obesity was defined as age-and sex-adjusted body mass index > 1.1 SDS.
[4]Values in parentheses represent the cases of overweight, obesity, and overweight or obesity, respectively.
* P < 0.05

measures of associations are presented with their 95% confidence interval (CI). Statistical analyses were performed with the Statistical Package of Social Sciences (version 17.0 for Windows; SPSS Inc) and R (version 2.12.1; The R Foundation for Statistical Computing) programs.

RESULTS

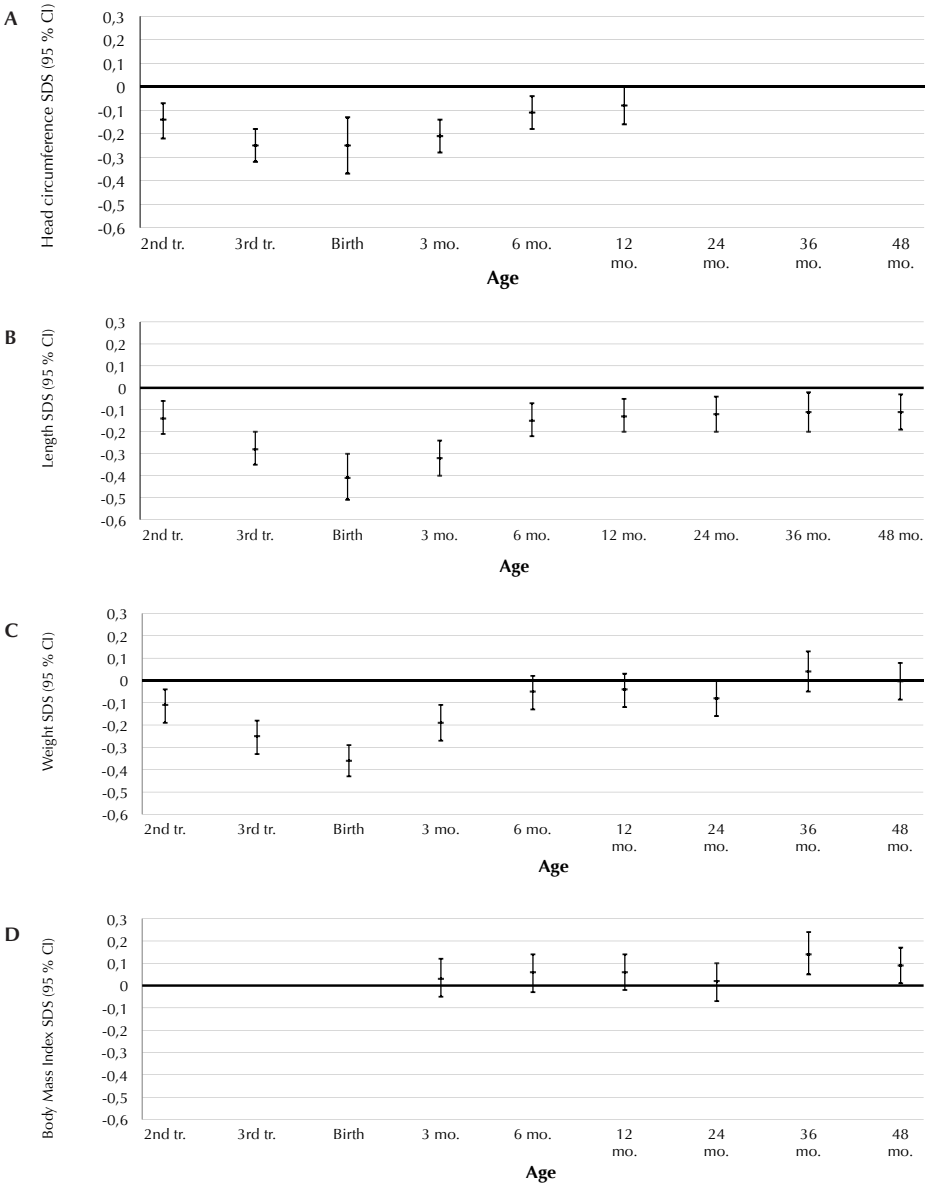
Subject characteristics

Of all mothers included in the analyses, 9.0% (N = 418) of them reported only smoking in the first trimester, and 15.6% (N = 833) of them continued smoking during pregnancy (**Table 1**). Mothers who continued smoking were younger and less educated than mothers who never smoked during pregnancy. The largest ethnic group was Dutch or other European (60.4%). Mean birth weights of children from mothers who never smoked during pregnancy and who continued smoking were 3,463 grams (standard deviation (SD) 540) and 3,265 grams (SD 540), respectively. The unadjusted growth characteristics per maternal smoking category are given in **Supplemental Table 1**.

Parental smoking during pregnancy, growth, and obesity in offspring

Compared with no maternal smoking, maternal smoking in the first trimester only was not associated with growth differences in head circumferences, lengths, weights, and BMI of offspring (**Table 2**). Children from mothers who continued smoking had smaller head circumferences until the age of 12 months and smaller heights until the age of 4 years, whereas their weights were only lower until the age of 3 months (P for trend < 0.01). The persistently smaller heights and normal to higher weights led to a higher BMI at the age of 4 years (difference: 0.11 SDS (95% CI 0.02, 0.20 SDS; P < 0.05). In mothers who continued smoking, we observed the largest effect estimates for mothers who smoked 5 cigarettes or more per day (at 4 years: height difference of -0.23 SDS (95% CI -0.35, -0.10 SDS; P < 0.01); weight difference of -0.02 SDS (95% CI -0.14, 0.11 SDS; P = 0.97); and BMI difference of 0.15 SDS (95% CI 0.03, 0.28 SDS; P < 0.05)). No dose-response associations between maternal smoking during the first trimester only and postnatal childhood growth characteristics were observed (data not shown). In mothers who did not smoke during pregnancy, we did not observe associations of paternal smoking with postnatal growth characteristics (**Supplemental Table 2**). Estimated differences in age- and sex-adjusted SDS for fetal and childhood head circumferences, femur and body lengths,

Figure 1 Associations of continued maternal smoking during pregnancy with repeatedly measured fetal and postnatal growth characteristics (SDS) compared with no maternal smoking (N = 5,342)[1]



[1]Values are standardized coefficients (95% confidence interval) on the basis of repeated measurements using linear mixed models. Models are adjusted for child's age at visit, sex, maternal ethnicity and education, maternal height and weight, and breastfeeding (yes, no).
tr. = trimester; mo. = months.

estimated fetal weights and body weights and BMI between children from mothers who did not smoke and mothers who continued smoking during pregnancy are presented in **Figure 1A-D.** The largest effect estimates for head circumferences, lengths and weights in mothers who continued smoking during pregnancy were observed in the third trimester of pregnancy and at birth.

As shown in **Table 3**, continued maternal smoking during pregnancy was not associated with risk of overweight at the age of 4 years. Children of mothers who continued smoking during pregnancy had an increased risk of obesity at the age of 4 years (odds ratio (OR) 1.61 (95% CI 1.03, 2.53; P < 0.05)). Paternal smoking during pregnancy of the partner was not associated with risks of overweight or obesity in offspring.

Smoking during pregnancy, small size for gestational age, and obesity

The additional adjustment of the logistic regression models focused on associations between maternal smoking during pregnancy and risks of overweight and obesity for gestational age-adjusted birth weight resulted in stronger effect estimates in terms of the odds ratio (OR at the age of 4 years 1.10 (95% CI 0.86, 1.41; P = 0.45) for overweight, 1.73 (95% CI 1.09, 2.74; P = 0.02) for obesity, and 1.23 (95% CI 0.98, 1.56; P = 0.08)) for overweight or obesity. Maternal smoking during pregnancy was associated with a higher BMI at the age of 4 years in children with normal birth weight and in those who were small for gestational age at birth (interaction between smoking and SDS birth weight was P < 0.001). Compared with children from nonsmoking mothers who were normal size for gestational age, children from mothers who did not smoke during pregnancy and who were born small for gestational age had a lower BMI at the age of 4 years (difference -0.56 kg/m^2 (95% CI -0.72, -0.41; P < 0.01)), whereas no difference in BMI at the age of 4 years was observed in children from mothers who smoked during pregnancy and who were born small for gestational age (data not shown).

DISCUSSION

This population-based prospective cohort study showed that continued maternal smoking during pregnancy, and not maternal smoking in the first trimester only, was associated with persistent smaller head and length growths and increased weights and BMI in offspring at the age of 4 years. Children of mothers who continued smoking during pregnancy also showed an increased risk of obesity at the age of 4 years. No association between paternal smoking during pregnancy and postnatal growth characteristics were observed.

Methodological considerations

An important strength of this study was the population-based cohort with a large number of subjects who were studied from early pregnancy onwards, and information about a large number of potential confounders was available. To our knowledge, this was the largest population-based prospective cohort study that has examined the associations of maternal and paternal smoking habits during specific periods in pregnancy with postnatal growth characteristics. Some methodologies need to be considered. Information on smoking during pregnancy at enrollment was missing for 13% of all mothers. This nonresponse would lead to biased effect estimates if associations of maternal smoking in pregnancy with postnatal growth characteristics would be different between those mothers included and not included in the analyses. However, this bias seemed unlikely because biased estimates in large cohort studies mainly arise from a loss to follow-up rather than from nonresponse at baseline.[27] The percentage of mothers who smoked during pregnancy may have been higher in those who were not included in the current analyses than in those who were included. This might have led to loss of statistical power and some underestimation of estimated effects. In the current analysis, the loss to follow-up was limited (< 5%). Because active smoking categories were similarly distributed at baseline in women who participated and in women who did not participate, we did not expect that the results were biased because of the loss to follow-up. Information on maternal and paternal smoking during pregnancy was collected by questionnaires without reference to postnatal growth characteristics. Although the assessment of smoking during pregnancy by questionnaire seems to be a valid method, misclassifications may occur.[28] Underreporting of maternal smoking across the various smoking categories may have been present and led to misclassification. In general, underreporting would lead to underestimation of differences between children from smoking and nonsmoking mothers. To overcome these limitations, some smaller previous studies used biomarkers such as cotinine in maternal urine samples.[29,30] However, this method does not seem to be superior to the use of self-report data of smoke exposure because of the low correlations between cotinine amounts and self-reported smoking habits.[31,32]

Comparison of main findings with other studies

The associations of maternal smoking during pregnancy associated with fetal growth retardation and increased risks of preterm birth and low birth weight are well established.[3,4,33-35] Various studies have suggested that exposure to smoke during fetal life led to overweight and obesity in childhood.[9,36,37] A systematic review by Oken *et al.*[10] suggested that prenatal smoke exposure led to a 50% increased risk of overweight in the offspring

aged 3 to 33 years. Also, a recent meta-analysis that used 17 studies showed that maternal smoking was consistently associated with obesity in children with a mean age of 9 years.[9] Our results are in line with this recent meta-analysis by showing that children of mothers who continued smoking during pregnancy had an increased risk of obesity (OR 1.61) at the age of 4 years. It is likely that this high risk of obesity at this young age is part of a trajectory, and risk of obesity tracks into late childhood and adolescently. Our results also showed that there was a dose-response relation between the number of cigarettes and postnatal growth characteristics and risk of obesity. Only a few studies assessed associations of exposure to maternal smoke in different periods of pregnancy with postnatal growth characteristics.[10,38,39] However, this assessment might identify critical time periods that are important from a developmental and preventive perspective. In addition to Adams *et al*.[38] and Mendez *et al*.[39], we observed that smoking in only the first trimester was not associated with postnatal growth and childhood obesity, whereas continued smoking until the third trimester of pregnancy was associated with these outcomes. Similarly, it has been shown that smoking in only the first trimester did not adversely affects risks of spontaneous preterm birth and small size for gestational age compared with risks for nonsmoking mothers.[40] Therefore, advising pregnant women and offering them help to quit smoking during pregnancy, by using proven methods is important.[41] Encouraging reproductive-age women to quit smoking before pregnancy is also important.

Previous studies suggested that the observed associations between maternal smoking during pregnancy and childhood obesity were not affected by the adjustment for potential confounders such as sociodemographic factors.[10] However, residual confounding might still be an issue because of unmeasured social- and lifestyle-related factors. To overcome this limitation, we also examined whether paternal smoking during pregnancy in nonsmoking mothers is associated with postnatal growth and risks of childhood overweight and obesity. This approach was previously used for other outcomes.[11,12] We did not observe any associations between paternal smoking during pregnancy and these outcomes. This result was in line with results from a cross-sectional study in 5,899 children in Bavaria that showed that paternal smoking could only partially explain the association of maternal smoking before or in pregnancy with childhood obesity.[42] Our findings suggested that underlying mechanisms might include direct intrauterine processes. Smoking during pregnancy might permanently lead to impaired skeletal growth, a shorter stature, and a normal or higher weight. Maternal smoking may also lead to impaired embryogenic growth and fetal growth retardation, which was associated with a more rapid postnatal weight gain.[43,44]

We showed that maternal smoking during pregnancy is associated with a higher BMI in children with and without small size for gestational age at birth. Thus, the small size for gestational age did not explain the associations shown. The mechanisms by which mater-

nal smoking during pregnancy may program postnatal child height and weight growths need to be studied further. We observed that continued maternal smoking, but not first trimester smoking, led to a persistent smaller length and higher BMI. Our results suggested that exposure to active maternal smoking during fetal life led to impaired skeletal growth and persistently a shorter height in postnatal life. The mechanisms of nicotine on skeletal growth might include programming effects on growth and adiposity hormones such as growth hormone, leptin, and ghrelin responsive pathways and a direct stimulation of the fetal hypothalamic-pituitary axis leading to increased adrenocorticotropic hormone (ACTH) and chronic changes in the proportion of body fat.[45] It has also been shown that maternal smoking during pregnancy is related to changes in DNA-methylation.[46] However, whether these changes in methylation underlie the associations between fetal smoke exposure and postnatal obesity remains to be studied.

Conclusion

Our results underlined the importance of health care interventions to reduce the smoking of mothers during pregnancy for the prevention of short-term outcomes during pregnancy and long-term outcomes in their children. Additional follow-up studies are needed in children at older ages and to identify associations of maternal smoking during pregnancy with more refined metabolic syndrome measures such as concentrations of glucose, triglycerides and total cholesterol and detailed measures of body composition.

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Supplemental Table 1 Fetal and postnatal growth characteristics according to category of maternal smoking during pregnancy[1,2]

	Smoking during pregnancy (N = 5,342)		
	No (N = 4,028, 75.4%)	**First trimester only (N = 481, 9.0%)**	**Continued (N = 833, 15.6%)**
Second trimester fetal growth (N = 5,110)			
Gestational age (weeks)	20.7 (18.9 – 22.8)	20.5 (18.9 – 22.7)**	20.6 (19.0 – 22.7)*
Head circumference (cm)	18.0 (1.4)	17.7 (1.4)**	17.8 (1.3)**
Femur length (cm)	3.35 (0.35)	3.31 (0.34)*	3.30 (0.33)**
Estimated fetal weight (g)	383 (93)	372 (86)*	372 (86)**
Third trimester fetal growth (N = 5,256)			
Gestational age (weeks)	30.5 (28.9 – 32.4)	30.4 (29.0 – 32.0)	30.3 (28.6 – 32.0)**
Head circumference (cm)	28.6 (1.2)	28.5 (1.2)	28.3 (1.2)**
Femur length (cm)	5.76 (0.30)	5.74 (0.28)	5.67 (0.28)**
Estimated fetal weight (g)	1,633 (261)	1,618 (234)	1,566 (239)**
Birth (N = 5,342)			
Gestational age (weeks)	40.0 (37.1 – 42.1)	39.9 (37.1 – 42.0)	39.8 (36.4 – 42.1)**
Head circumference (cm)	33.9 (1.7)	33.8 (1.7)	33.4 (1.7)**
Length (cm)	50.4 (2.4)	50.2 (2.4)	49.5 (2.4)**
Weight (grams)	3,463 (540)	3,462 (532)	3,265 (540)**
Body mass index (kg/m^2)	13.8 (1.5)	13.8 (1.3)	13.6 (1.4)**
3 months (N = 4,758)			
Age (months)	3.1 (1.3 – 4.2)	3.0 (1.3 – 4.1)	3.1 (1.2 – 4.3)
Head circumference (cm)	40.5 (1.6)	40.5 (1.6)	40.2 (1.7)**
Length (cm)	60.5 (3.2)	60.4 (3.2)	59.8 (3.5)**
Weight (kg)	6.1 (0.9)	6.0 (0.9)	6.0 (1.0)*
Body mass index (kg/m^2)	16.5 (1.5)	16.5 (1.5)	16.6 (1.6)
6 months (N = 4,883)			
Age (months)	6.7 (5.5 – 10.3)	6.6 (5.5 – 9.3)	6.6 (5.4 – 9.4)
Head circumference (cm)	43.8 (1.6)	43.8 (1.5)	43.7 (1.6)*
Length (cm)	68.2 (3.2)	68.1 (3.0)	67.8 (3.2)**
Weight (kg)	8.1 (1.0)	8.0 (1.0)	8.0 (1.0)
Body mass index (kg/m^2)	17.3 (1.4)	17.3 (1.4)	17.4 (1.4)*
12 months (N = 4,670)			
Age (months)	13.0 (11.1 – 15.3)	13.0 (11.1 – 15.3)	13.0 (11.1 – 15.6)
Head circumference (cm)	46.5 (1.5)	46.5 (1.4)	46.4 (1.4)
Height (cm)	76.6 (3.3)	76.6 (3.3)	76.4 (3.4)
Weight (kg)	10.2 (1.2)	10.1 (1.2)	10.2 (1.2)
Body mass index (kg/m^2)	17.3 (1.4)	17.3 (1.4)	17.4 (1.4)*
24 months (N = 4,437)			
Age (months)	24.4 (18.6 – 27.5)	24.3 (18.7 – 27.7)	24.3 (18.5 – 27.6)

Supplemental Table 1 Fetal and postnatal growth characteristics according to category of maternal smoking during pregnancy[1,2] (continued)

	Smoking during pregnancy (N = 5,342)		
	No (N = 4,028, 75.4%)	**First trimester only (N = 481, 9.0%)**	**Continued (N = 833, 15.6%)**
Height (cm)	87.7 (3.9)	87.9 (3.8)	87.3 (3.9)*
Weight (kg)	12.8 (1.6)	12.8 (1.6)	12.7 (1.6)
Body mass index (kg/m^2)	16.7 (1.5)	16.5 (1.3)	16.7 (1.5)
36 months (N = 3,686)			
Age (months)	36.4 (31.1 – 39.2)	36.4 (30.9 – 39.0)	36.3 (30.7 – 39.3)
Height (cm)	96.9 (4.0)	97.1 (4.3)	96.4 (4.3)*
Weight (kg)	15.1 (1.9)	15.2 (2.0)	15.2 (2.0)
Body mass index (kg/m^2)	16.1 (1.4)	16.1 (1.5)	16.3 (1.6)**
48 months (N = 4,540)			
Age (months)	45.3 (25.7 – 47.8)	45.4 (25.2 – 47.9)	45.3 (25.7 – 48.2)
Height (cm)	100.7 (6.0)	100.9 (6.2)	100.2 (6.2)
Weight (kg)	16.2 (2.4)	16.4 (2.6)	16.3 (2.6)
Body mass index (kg/m^2)	16.0 (1.4)	16.0 (1.5)	16.2 (1.6)**

[1]Values are means (standard deviation) or medians (90% range) for variables with skewed distribution.
[2]Differences in child characteristics (compared with the maternal nonsmoking category) were evaluated using t-tests. Gestational age was log-transformed for the t-tests.
*P < 0.05 and **P < 0.01

Supplemental Table 2 Associations of the number of cigarettes smoked by father with postnatal growth characteristics in the children[1,2]

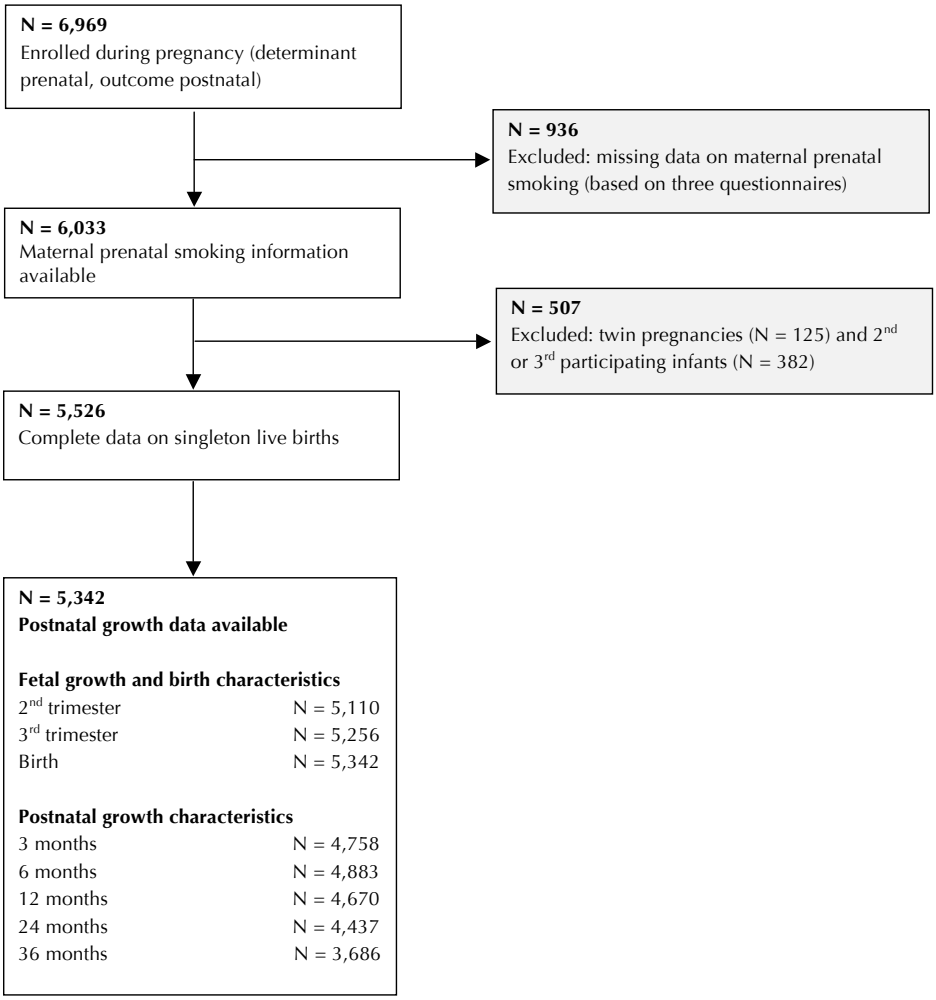
	Difference (95% confidence interval) in head circumference (cm)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Paternal smoking	N = 2,239	N = 3,489	N = 3,555	N = 2,608			
No (N = 2,572)	Reference	Reference	Reference	Reference	-	-	-
0-4 cigarettes/day (N = 607)	-0.10 (-0.29, 0.09)	0.02 (-0.11, 0.15)	-0.02 (-0.14,0.11)	0.11 (-0.03, 0.25)	-	-	-
> 5 cigarettes/day (N = 753)	-0 (-0.17, 0.17)	-0.01 (-0.13, 0.10)	0.01 (-0.11, 0.13)	0.13 (-0, 0.26)	-	-	-
P for trend	P = 0.76	P = 0.89	P = 0.96	P = 0.03	-	-	-
	Difference (95% confidence interval) in length/height (cm)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Paternal smoking	N = 2,604	N = 3,361	N = 3,482	N = 3,411	N = 3,242	N = 2,671	N = 3,362
No (N = 2,572)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
0-4 cigarettes/day (N = 607)	-0.27 (-0.49, -0.04)*	-0.10 (-0.36, 0.16)	-0.06 (-0.32, 0.19)	-0.22 (-0.47, 0.03)	-0.30 (-0.64, 0.04)	-0.21 (-0.62, 0.19)	-0.23 (-0.59, 0.13)
> 5 cigarettes/day (N = 753)	0.08 (-0.13, 0.29)	0.18 (-0.07, 0.42)	0.14 (-0.10, 0.38)	0.25 (0.02, 0.48)*	0.24 (-0.07, 0.55)	0.16 (-0.23, 0.55)	0.22 (-0.13, 0.55)
P for trend	P = 0.94	P = 0.28	P = 0.35	P = 0.15	P = 0.35	P = 0.65	P = 0.43
	Difference (95% confidence interval) in weight (grams)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Paternal smoking	N = 3,932	N = 3,513	N = 3,579	N = 3,406	N = 3,262	N = 2,685	N = 3,362
No (N = 2,572)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
0-4 cigarettes/day (N = 607)	-25 (-63, 13)	-14 (-89, 60)	-17 (-104, 70)	-44 (-146, 57)	-96 (-242, 50)	-21 (-215, 172)	-45 (-235, 146)
> 5 cigarettes/day (N = 753)	-8 (-39, 32)*	56 (-14, 126)	90 (8, 172)*	139 (43, 235)**	91 (-44, 227)	106 (-78, 290)	142 (-36, 321)
P for trend	P = 0.60	P = 0.19	P = 0.06	P = 0.02	P = 0.38	P = 0.33	P = 0.20

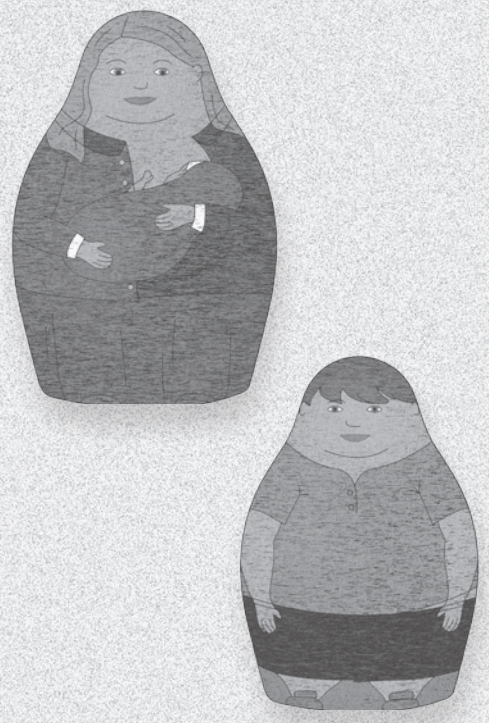
Supplemental Table 2 Associations of the number of cigarettes smoked by father with postnatal growth characteristics in the children[1,2] (continued)

	Difference (95% confidence interval) in body mass index (kg/m²)						
	Birth	**3 months**	**6 months**	**12 months**	**24 months**	**36 months**	**48 months**
Paternal smoking	N = 2,602	N = 3,359	N = 3,471	N = 3,398	N = 3,241	N = 2,667	N = 3,362
No (N = 2,572)	Reference	Reference	Reference	Reference	Reference	Reference	Reference
0-4 cigarettes/day (N = 607)	-0.03 (-0.19, 0.12)	-0.02 (-0.16, 0.12)	0.02 (-0.12, 0.15)	0.03 (-0.10, 0.16)	-0.01 (-0.15, 0.14)	0.03 (-0.12, 0.17)	0.03 (-0.10, 0.16)
> 5 cigarettes/day (N = 753)	-0.06 (-0.20, 0.08)	0.05 (-0.09, 0.18)	0.13 (0.01, 0.26)*	0.13 (0.01, 0.26)*	0.02 (-0.12, 0.15)	0.08 (-0.06, 0.22)	0.09 (-0.03, 0.21)
P for trend	P = 0.39	P = 0.58	P = 0.06	P = 0.04	P = 0.84	P = 0.27	P = 0.15

[1]Values are regression coefficients (95% confidence interval) and odds ratios (95% confidence interval), by using multiple linear and logistic models.
[2]Models are adjusted for child's age at visit, sex, paternal ethnicity and education, paternal height and weight, and breastfeeding (yes, no).
* P < 0.05 and ** P < 0.01

Supplemental Figure 1 Flow chart of participants in study





CHAPTER 4.4

Maternal smoking during pregnancy and subcutaneous fat mass in early childhood

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ABSTRACT

Objective: Maternal smoking during pregnancy increases the risk of obesity in the offspring. Not much is known about the associations with other measures of body composition. We assessed the associations of maternal smoking during pregnancy with the development of subcutaneous fat mass measured as peripheral and central skinfold thickness measurements in early childhood, in a population-based prospective cohort study from early fetal life onward in the city of Rotterdam, the Netherlands.

Methods: The study was performed in 907 mothers and their children at the ages of 1.5, 6 and 24 months.

Results: As compared to nonsmoking mothers, mothers who continued smoking during pregnancy were more likely to have a younger age and a lower educational level. Their children had a lower birth weight, higher risk of small size for gestational age and were breastfed for a shorter duration (P- values < 0.01). We did not observe differences in peripheral, central and total subcutaneous fat mass between the offspring of nonsmoking mothers, mothers who smoked in first trimester only and mothers who continued smoking during pregnancy (P > 0.05). Also, the reported number of cigarettes smoked by mothers in both first and third trimester of pregnancy was not associated with peripheral, central and total subcutaneous fat mass in the offspring at the ages of 1.5, 6 and 24 months.

Conclusions: Our findings suggest that fetal exposure to cigarette smoke during pregnancy does not influence subcutaneous fat mass in early childhood. Follow-up studies are needed in children at older ages and to identify associations of maternal smoking during pregnancy with other measures of body composition.

INTRODUCTION

Active maternal smoking during pregnancy is a common and preventable specific adverse environmental exposure for the fetus.[1-3] Maternal smoking during pregnancy is associated with fetal growth retardation and increased risks of preterm birth and lower birth weight.[4-6] Maternal smoking during pregnancy also seems to increase the risk of obesity in the offspring.[7-8] The mechanisms underlying these associations may include developmental adaptations leading to changes in body composition and appetite behavior.[9-10] A recent systematic review suggested that prenatal smoke exposure leads to a higher body mass index (BMI) in childhood.[11] However, using BMI as outcome does not give information about body composition.[12-13] Studies relating maternal smoking during pregnancy with direct measures of body composition in the offspring might be important since adverse body composition and especially unfavorable fat distribution are important contributors to metabolic syndrome outcomes.[14] Thus far, studies on the association between maternal smoking during pregnancy and measures of body composition are scarce and showed inconsistent results.[15-17] Skinfold thickness is a valid and easy to perform measurement for subcutaneous fat mass assessment in young children in epidemiological studies.[12-13]

For the present study, we hypothesized that active maternal smoking during pregnancy leads to higher peripheral, central and total subcutaneous fat mass in young childhood. We examined in a population-based prospective cohort study in 907 Dutch mothers and children followed from early fetal life onwards, the associations of exposure to maternal smoking during pregnancy with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands.[18-19] Enrollment in the study was aimed at early pregnancy (gestational age <18 weeks) but was possible until birth of the child. All children were born between April 2002 and January 2006, and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Of all eligible children in the study area, 61% were participating in the study at birth.[19] Additional detailed assessments of fetal and postnatal

growth and development were conducted in third trimester of pregnancy in a subgroup of 1,232 Dutch mothers and their children in order to exclude bias due to ethnicity.[19] Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands.[19-20] Pregnant mothers participating in the Generation R study, who met this criterion, were approached for the additional measurements.[18-19] Of all approached women, 80% agreed to participate in the subgroup study. In total, 1,039 children participated in at least one of the postnatal assessments at the ages of 1.5, 6 and 24 months. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

Maternal smoking during pregnancy

Information on maternal smoking was obtained by postal questionnaires sent in first, second and third trimester of pregnancy. Response rates for these questionnaires were 91%, 80%, and 77%, respectively.[19] Active maternal smoking at enrollment was assessed in the first questionnaire by asking the mother whether she smoked during her pregnancy. We grouped mothers into 3 categories: 1) never smoked; 2) smoked only until their pregnancy was acknowledged (first trimester only); and 3) continued to smoke during pregnancy. This first questionnaire was sent to all mothers, independently of gestational age at enrollment. In the second and third questionnaires, the mothers were asked whether they had smoked during the past 2 months (yes, no). Mothers who reported in the first questionnaire not to smoke or to have smoked until their pregnancy was acknowledged, but reported to have smoked in the second or third questionnaire were recategorized as ''continued smoking''. Among smokers, the number of cigarettes daily smoked was categorized into the categories: no smoking, <5 cigarettes/day and >5 cigarettes/day.

Subcutaneous fat mass measurements and anthropometrics

Subcutaneous fat mass was measured as skinfold thickness (SFT) at the ages of 1.5, 6 and 24 months on the left side of the body at four different sites (biceps, triceps, suprailiacal and subscapular) according to standard procedures by using a skinfold caliper (Slim Guide, Creative Health Products).[21] Four well-trained medical assistants performed all measurements.[22] The consensus between and among observers for the medical assistants was analyzed using the intraclass correlation coefficient (ICC).[23-24] Intraobserver ICC was 0.88 and interobserver ICC was 0.76. Total subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT + suprailiacal SFT + subscapular SFT. Central subcutaneous

fat mass was calculated from the sum of suprailiacal SFT + subscapular SFT. Peripheral subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT.[25,26] Body length was measured in supine position to the nearest millimeter (mm) using a neonatometer and at the age of 24 months, height was measured by a Harpenden stadiometer (Holtain Limited, Dyfed, UK) in standing position. Weight was measured in naked infants to the nearest grams at the ages of 1.5 and 6 months by an electronic infant scale and at the age of 24 months by a mechanical personal scale (SECA, Almere, The Netherlands).

Covariates

Birth weight, date of birth and sex were obtained from midwife and hospital registries at birth. Information on the highest attained maternal educational level (low, moderate and higher), and parity (primipara, multipara) were obtained at enrollment in the study.[27] Educational level of the mother was defined according to the classification of Statistics Netherlands.[28] Maternal height and weight were assessed at enrollment. Height and weight were measured while the mother stood without shoes and without heavy clothing. BMI was calculated (kg/m^2). Information on breastfeeding (yes, no) was obtained by postnatal questionnaires at the ages of 2, 6, and 12 months. Mothers were asked whether they ever breastfed their child (yes, no) and at what age they quitted breastfeeding.

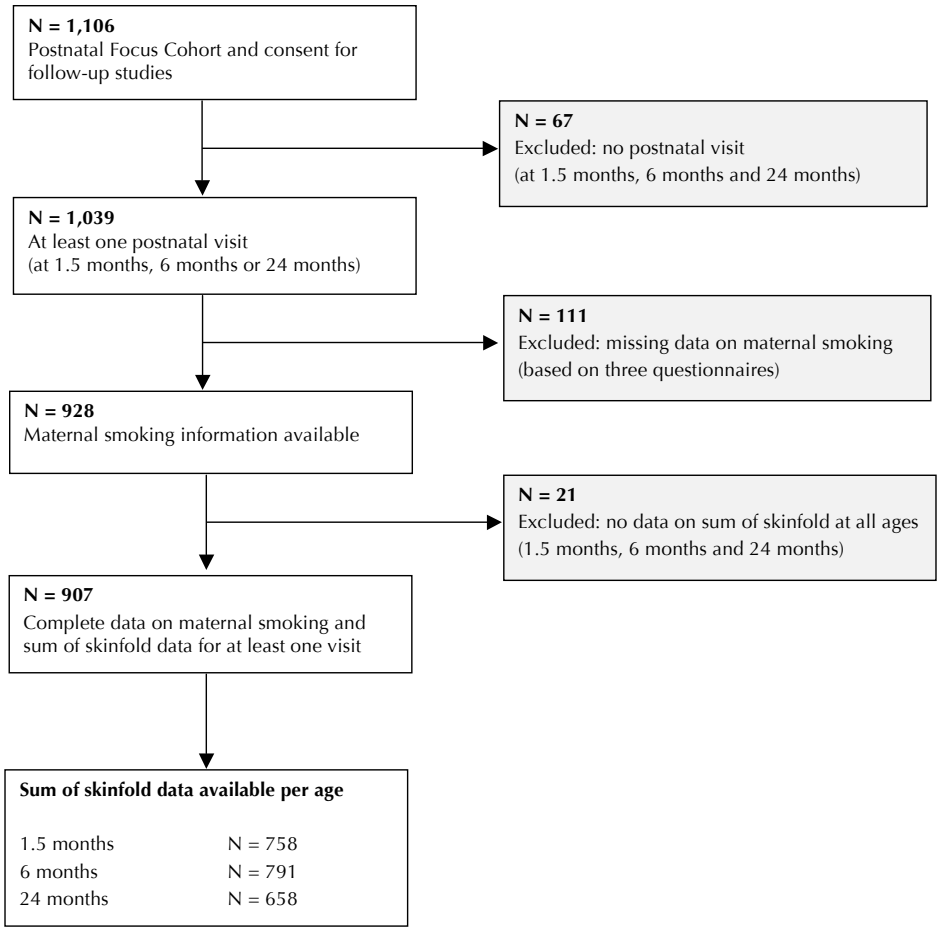
Population for analysis

From the total of 1,106 children and their mothers who gave consent for participating in the postnatal phase, 1,039 children participated in at least one of the postnatal assessments at the ages of 1.5, 6 and 24 months (**Figure 1**). Children without complete information on maternal smoking during pregnancy (N = 111) were excluded from the analyses. Of the remaining 928 live births with complete data on maternal smoking, information on the sum of skinfold thickness measurements in at least one of the three visits was available in 907 children. Twins (N = 24) were not excluded from the analyses, as they did not differ in the outcome measure from the singletons and no differences in results were observed after excluding them. Missing skinfold measurements were mainly due to crying behavior. Percentage of maternal smoking was not different between participating mothers (N = 907) and those lost to follow- up (N = 325).

Statistical analysis

Differences in baseline characteristics between the maternal smoking categories were compared with Student's t-tests for continuous variables and Chi-square tests for cat-

Figure 1 Flow chart of participants in study



egorical variables. Similarly, we tested the differences in peripheral, central and total subcutaneous fat mass between the maternal smoking categories at the ages of 1.5, 6 and 24 months. The associations between period of maternal smoking during pregnancy (no, first trimester only, continued) and peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months, were assessed using multiple linear regression models. The models were adjusted for potential confounders including child's age at visit, sex, maternal education, maternal height and weight, breastfeeding (yes, no), current height and observer of the skinfold measurement. Postnatal smoking, birth weight, parity, maternal alcohol use and maternal age were not included in the final models since they did not materially change the effect estimates. Similar regression models were used for assessing the associations of the reported number of cigarettes smoked in both first and

third trimester of pregnancy and peripheral, central, and total subcutaneous fat mass. The associations between period of maternal smoking during pregnancy and anthropometrics like height, weight and BMI at the ages of 1.5, 6 and 24 months, were also assessed using multiple linear regression models. These models were adjusted for potential confounders like age at visit, sex, maternal education, maternal height and weight, and breastfeeding (yes, no). Tests for trends were performed by treating each categorized variable as a continuous term and by entering the variable into the fully adjusted linear regression model. To handle missing values in covariates (< 20% missing), we performed multiple imputations with a software package by generating 5 independent datasets for all analyses. Imputations were based on the relationships between covariates included in this study using the Markov Chain Monte Carlo (MCMC) method. All measures of association are presented with their 95% confidence interval (CI). Statistical analyses including the multiple imputations were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Of all mothers included in the analyses, 9.2% (N = 83) reported smoking in first trimester only and 13.8% (N = 125) reported continued smoking during pregnancy (**Table 1**). Mothers who continued smoking were younger and lower educated than mothers who never smoked during pregnancy. Mean birth weights of children from mothers who never smoked during pregnancy and who continued smoking were 3,533 grams (standard deviation (SD) 545) and 3,267 grams (SD 573), respectively. In children of mothers who continued smoking, the highest rate for small size for gestational age (14.4%) and lowest rate for ever breastfeeding (82.5%) were observed. As compared to mothers who did participate, those who did not participate were younger ($P < 0.01$). Furthermore, they had a lower educational level and were more likely to smoke ($P < 0.01$). Their children were also born more frequently preterm and with a lower birth weight ($P < 0.05$) (data not shown).

Table 2 shows that we observed no differences in the unadjusted peripheral, central and total subcutaneous fat mass measurements during childhood between the offspring of nonsmoking mothers, mothers who smoked in first trimester and mothers who continued smoking. **Table 3** gives the associations between period of maternal smoking, and the number of cigarettes smoked per day in mothers who smoked in first trimester only and those who continued smoking with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months. Smoking in first trimester only and continued

Table 1 Characteristics of mothers and their children according to category of maternal smoking during pregnancy (N = 907)[1,2]

	No smoking (N = 699, 77.1%)	Smoked in first trimester only (N = 83, 9.2%)	Continued smoking (N = 125, 13.8%)
Maternal characteristics			
Age (years)	31.9 (25.3 – 37.8)	31.2 (22.1 – 38.4)	30.8 (21.5 – 38.0)**
Height (cm)	171.3 (6.4)	171.2 (6.6)	169.5 (6.2)**
Weight (kg)	71.7 (13.3)	69.7 (11.3)	71.1 (13.5)
Body mass index (kg/m^2)	24.4 (4.3)	23.7 (3.4)	24.7 (4.5)
Highest completed education (%)			
Primary school	1.0	4.9	6.4**
Secondary school	30.7	34.9	57.6**
Higher education	68.3	60.2	36.0**
Alcohol consumption in pregnancy (%)			
Ever	69.0	85.5**	68.0
Never	31.0	14.5**	31.0
Parity (%)			
0	60.9	71.1	57.6
>=1	39.1	28.9	42.4
Birth			
Males (%)	51	46	58*
Gestational age (weeks)	40.0 (37.0 – 42.1)	39.7 (33.9 – 42.0)	39.8 (35.7 – 42.1)
Weight (grams)	3,533 (545)	3,458 (659)	3,267 (573)**
Small size for gestational age[3] (<5%)	3.1	3.6	14.4**
Low birth weight (<2500 g) %	3.9	8.4	7.2
Preterm birth (%)	5.2	9.6	6.4
Breastfeeding			
Ever (%)	90.6	94.0	82.5**
Duration (months)	5.4 (0.5 – 12.0)	4.6 (0.5 – 12.0)	2.5 (0.5 – 8.5)**

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.
[2]Differences in maternal and child characteristics (compared with the nonsmoking category) are evaluated using Students t-tests for continuous variables and Chi-squared tests for categorical variables. Gestational age is log-transformed for the t-tests. Birth weight in SDS is used for the t-tests.
[3]Small size for gestational age is defined as the lowest 5% of gestational age- and sex- adjusted birth weight.
*P < 0.05 and **P < 0.01

smoking were not significantly associated with subcutaneous fat mass at any age. Offspring of mothers who continued to smoke more than 5 cigarettes per day tended to have higher peripheral, central and total subcutaneous fat mass at the age of 24 months (P for trend > 0.05). However, it is likely a chance finding given it didn't seem to be an a

Table 2 Subcutaneous fat mass measurements according to maternal smoking during pregnancy[1,2]

N = 907	No smoking (N = 699, 77.1%)	Smoked in first trimester only (N = 83, 9.2%)	Continued smoking (N =125, 13.8%)
1.5 months (N = 758)			
Age (months)	1.60 (1.12 – 2.47)	1.59 (1.08 – 2.49)	1.63 (1.03 – 2.66)
Triceps (mm)	6.6 (4.0 – 12.0)	6.5 (4.0 – 12.0)	6.6 (3.5 – 12.0)
Biceps (mm)	5.5 (3.0 – 11.0)	5.5 (3.0 – 10.0)	5.6 (3.0 – 10.0)
Suprailiacal (mm)	5.8 (3.0 – 10.0)	5.8 (3.0 – 10.4)	5.7 (3.0 – 9.9)
Subscapular (mm)	6.2 (4.0 – 9.6)	6.3 (4.0 – 10.0)	6.1 (3.0 – 9.0)
Peripheral fat mass (mm)	12.2 (7.0 – 22.2)	12.0 (7.0 – 22.3)	12.2 (7.0 – 21.0)
Central fat mass (mm)	12.0 (7.0 – 19.0)	12.1 (7.8 – 20.0)	11.8 (7.0 – 19.0)
Total fat mass (mm)	24.2 (15.0 – 39.9)	24.1 (15.5 – 41.4)	24.0 (14.0 – 40.5)
6 months (N = 791)			
Age (months)	6.49 (5.61 – 7.88)	6.52 (5.70 – 7.84)	6.48 (5.46 – 7.91)
Triceps (mm)	7.9 (5.0 – 13.0)	8.1 (4.7 – 14.0)	7.8 (5.0 – 11.4)
Biceps (mm)	6.5 (4.0 – 11.0)	6.9 (4.0 – 11.3)	6.6 (4.0 – 10.0)
Suprailiacal (mm)	6.3 (3.5 – 10.0)	6.7 (3.5 – 9.3)	6.1 (3.1 – 9.8)
Subscapular (mm)	6.3 (4.0 – 9.0)	6.6 (4.0 – 9.6)	6.3 (4.0 – 9.0)
Peripheral fat mass (mm)	14.5 (9.0 – 22.0)	14.9 (9.0 – 25.3)	14.4 (9.1 – 21.0)
Central fat mass (mm)	12.6 (8.0 – 19.0)	13.3 (8.0 – 19.0)	12.4 (7.6 – 18.0)
Total fat mass (mm)	27.1 (19.0 – 39.0)	28.2 (17.0 – 43.3)	26.8 (17.1 – 37.4)
24 months (N = 658)			
Age (months)	25.29 (23.79 – 27.61)	25.45 (23.92 – 29.16)	25.51 (23.95 – 28.04)
Triceps (mm)	8.8 (5.0 – 14.0)	8.7 (4.0 – 13.3)	9.0 (5.0 – 13.9)
Biceps (mm)	6.7 (4.0 – 11.0)	6.6 (3.4 – 11.7)	6.9 (4.0 – 11.0)
Suprailiacal (mm)	5.6 (3.0 – 9.0)	5.8 (3.0 – 11.3)	5.7 (3.0 – 9.0)
Subscapular (mm)	6.0 (3.5 – 9.0)	6.2 (4.0 – 10.0)	6.3 (4.0 – 9.8)
Peripheral fat mass (mm)	15.5 (9.0 – 24.0)	15.3 (9.0 – 24.8)	15.8 (9.6 – 24.0)
Central fat mass (mm)	11.6 (7.0 – 18.0)	12.0 (7.2 – 18.7)	12.0 (7.0 – 18.0)
Total fat mass (mm)	27.1 (17.5 – 40.0)	27.3 (18.0 – 44.2)	28.0 (18.0 – 41.1)

[1]Values are medians (90% range). Differences in child characteristics (compared with the nonsmoking category) are evaluated using Students t-tests.

[2]Peripheral subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT. Central subcutaneous fat mass was calculated from the sum of suprailiacal SFT + subscapular SFT. Total subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT + suprailiacal SFT + subscapular SFT.

priori association from the methods and multiple comparisons. **Table 4** shows the associations between period of maternal smoking, and the number of cigarettes smoked per day in mothers who smoked in first trimester only and those who continued smoking

Table 3 Associations of maternal smoking during pregnancy with subcutaneous fat measures in children aged 1.5, 6 and 24 months[1-3]

	1.5 months			6 months			24 months		
	Peripheral fat (mm)	**Central fat (mm)**	**Total fat (mm)**	**Peripheral fat (mm)**	**Central fat (mm)**	**Total fat (mm)**	**Peripheral fat (mm)**	**Central fat (mm)**	**Total fat (mm)**
Maternal smoking	**N = 781**	**N = 759**	**N = 759**	**N = 794**	**N = 792**	**N = 803**	**N = 672**	**N = 660**	**N = 659**
No (N = 699)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
First trimester only (N = 83)	-0.30 (-1.30, 0.70)	-0.02 (-0.90, 0.86)	-0.32 (-2.09, 1.45)	0.61 (-0.35, 1.56)	0.62 (-0.19, 1.43)	1.22 (-0.35, 2.79)	0.07 (-1.08, 1.23)	0.40 (-0.52,1.32)	0.47 (-1.36, 2.30)
<5 cigarettes/ day (N = 53)	-1.06 (-2.27, 0.15)	-0.27 (-1.32, 0.79)	-1.29 (-3.41, 0.83)	0.29 (-0.89, 1.46)	0.50 (-0.50, 1.50)	0.78 (-1.16, 2.71)	0.63 (-0.76, 2.01)	0.85 (-0.26,1.96)	1.48 (-0.71, 3.68)
>5 cigarettes/ day (N = 30)	1.10 (-0.50, 2.70)	0.45 (-0.97, 1.87)	1.52 (-1.34, 4.38)	1.17 (-0.38, 2.72)	0.81 (-0.52, 2.14)	1.96 (-0.62, 4.53)	-0.95 (-2.90,0.99)	-0.41(-1.97,1.15)	-1.39 (-4.47, 1.69)
P for trend	P = 0.82	P = 0.80	P = 0.82	P = 0.15	P = 0.14	P = 0.10	P = 0.77	P = 0.69	P = 0.99
Continued (N = 125)	0.13 (-0.78, 1.03)	0.07 (-0.73, 0.87)	0.23 (-1.38, 1.84)	0.02 (-0.82, 0.87)	-0.28 (-0.99, 0.44)	-0.26 (-1.65, 1.12)	0.54 (-0.55, 1.63)	0.44 (-0.44,1.31)	1.03 (-0.72, 2.78)
<5 cigarettes/ day (N = 51)	-0.10 (-1.42, 1.22)	-0.41 (-1.57, 0.75)	-0.47 (-2.81, 1.87)	-0.01 (-1.23, 1.20)	-0.30 (-1.32, 0.73)	-0.32 (-2.31, 1.67)	-1.04 (-2.64,0.56)	-0.89 (-2.21, 0.43)	-1.85 (-4.45, 0.76)
>5 cigarettes/ day (N = 53)	0.15 (-1.12, 1.41)	0.04 (-1.06, 1.14)	0.22 (-2.01, 2.44)	-0.22 (-1.44, 0.99)	-0.54 (-1.57, 0.49)	-0.77 (-2.77, 1.23)	1.46 (-0.08, 3.01)	1.21(-0.04, 2.46)	2.67 (0.20, 5.14)*
P for trend	P = 0.88	P = 0.86	P = 0.97	P = 0.75	P = 0.26	P = 0.43	P = 0.24	P = 0.23	P = 0.17

[1]Values are regression coefficients (95% confidence interval) using multiple linear regression models.

[2]Models are adjusted for child's age at visit, sex, maternal education, maternal height and weight, ever breastfeeding, current height and observer.

[3]Peripheral subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT. Central subcutaneous fat mass was calculated from the sum of suprailiacal SFT + subscapular SFT. Total subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT + suprailiacal SFT + subscapular SFT.

* P < 0.05

Table 4 Associations of maternal smoking during pregnancy with anthropometrics in children aged 1.5, 6 and 24 months[1,2]

	1.5 months			6 months			24 months		
	Height (cm)	**Weight (g)**	**Body mass index (kg/m²)**	**Height (cm)**	**Weight (g)**	**Body mass index (kg/m²)**	**Height (cm)**	**Weight (g)**	**Body mass index (kg/m²)**
Maternal smoking	**N = 765**	**N = 767**	**N = 765**	**N = 774**	**N = 776**	**N = 774**	**N = 671**	**N = 692**	**N = 671**
No (N = 699)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
First trimester only (N = 83)	-0.51 (-1.06, 0.03)	-5 (-152, 142)	0.22 (-0.13, 0.56)	-0.24 (-0.80, 0.33)	107 (-87, 300)	0.35 (0.03, 0.67)*	0.14 (-0.60, 0.88)	12 (-310, 334)	-0.11 (-0.44, 0.21)
<5 cigarettes/day (N = 53)	-0.62 (-1.11, -0.13)*	-101 (-235, 32)	0.01 (-0.30, 0.32)	-0.20 (-0.70, 0.30)	18 (-158, 194)	0.14 (-0.16, 0.43)	-0.19 (-0.88, 0.50)	-42 (-343, 258)	-0.05 (-0.35, 0.25)
>5 cigarettes/day (N = 30)	-0.90 (-1.46, -0.34)**	-200 (-353, -46)*	-0.19 (-0.55, 0.17)	-0.63 (-1.18, -0.07)*	-52 (-246, 142)	-0.19 (-0.13, 0.52)	-0.42 (-1.19, 0.34)	-208 (-544, 127)	-0.15 (-0.49, 0.19)
P for trend	P < 0.01	P < 0.01	P = 0.38	P = 0.03	P = 0.71	P = 0.17	P = 0.25	P = 0.24	P = 0.38
Continued (N = 125)	-0.96 (-1.46, -0.47)**	-268 (-402,-134)**	-0.33 (-0.64, -0.01)*	-0.45 (-0.94, 0.05)	-103 (-274, 68)	-0.01 (-0.29, 0.28)	-0.69 (-1.38, 0)	-263 (-567,42)	-0.11 (-0.42, 0.19)
<5 cigarettes/day (N = 51)	-1.02 (-1.75, -0.30)**	-264 (-461, -67)**	-0.27 (-0.73, 0.19)	-0.41 (-1.12, 0.31)	-144 (-390, 102)	-0.10 (-0.51, 0.30)	-0.88 (-1.91, 0.16)	-524 (-974, -73)*	-0.44 (-0.89, 0.01)
>5 cigarettes/day (N = 53)	-1.14 (-1.83, -0.45)**	-363 (-550,-175)**	-0.53 (-0.97,-0.09)*	-0.54 (-1.24, 0.17)	-189 (-431, 53)	-0.14 (-0.54, 0.27)	-0.80 (-1.75, 0.16)	-229 (-653, 194)	0.02 (-0.40, 0.45)
P for trend	P < 0.01	P < 0.01	P = 0.01	P = 0.08	P = 0.07	P = 0.43	P = 0.04	P = 0.07	P = 0.54

[1]Values are regression coefficients (95% confidence interval) using multiple linear regression models.

[2]Models are adjusted for child's age at visit, sex, maternal education, maternal height and weight, and ever breastfeeding.

* P < 0.05

with height, weight and BMI at the ages of 1.5, 6 and 24 months. Children of mothers who continued smoking tended to have a lower height and weight at all ages compared to nonsmoking mothers. Offspring of mothers who smoked more than 5 cigarettes per day in those who smoked in first trimester only and those who continued smoking, tended to have a lower height and weight (P for trend <0.10). The association with BMI at these ages seems not to be consistent, but tends to a negative relation with anthropometrics.

DISCUSSION

This population-based prospective cohort study showed that maternal smoking in first trimester only and continued smoking during pregnancy were not associated with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months.

Methodological considerations

Major strengths of this study are the population-based prospective design with subjects being studied from early pregnancy onwards, and information about a large number of potential confounders available. However, some methodological issues need to be considered. Of the 1,039 children of this study who participated postnatally, information on smoking during pregnancy at enrollment was missing in 11% of all mothers. This nonresponse would lead to biased effect estimates if the associations of maternal smoking during pregnancy with skinfold measurement in early childhood would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline.[29,30] The percentage of mothers who smoked during pregnancy may have been higher in those not included in the current analyses than in those who were included. This might have led to loss of statistical power and some underestimation of the estimated effects. Of the postnatal participants, skinfold thickness measurements were performed in at least 80%. Missing skinfold measurements were mainly due to crying behavior. Information on maternal smoking during pregnancy was collected by questionnaires, without reference to any skinfold measurement. Although assessing smoking during pregnancy by questionnaire seems to be a valid method, misclassification may occur.[31] Underreporting of maternal smoking across the various smoking categories may be present and have led to misclassification. In general, underreporting would lead to underestimation of differences between children from smoking and nonsmoking mothers. Also, our study group was ethnic homogeneous and the mothers were highly educated.

This may limit the generalizability of our results.[30] Finally, we used skinfold thickness as a measure of subcutaneous fat mass. Skinfold thickness provides a simple, easy, and quick yet highly informative assessment of regional fatness in most age groups and can be used in large-scale epidemiological studies, but has a limitation in differentiating between lean and fat mass of the whole body.[12]

Comparison of main findings with other studies

Obesity in both childhood and adulthood lead to different risk factors for cardiovascular diseases, diabetes type II and overall mortality.[32-43] Various studies suggest that exposure to maternal smoking during fetal life leads to overweight and obesity in childhood. A systematic review by Oken *et al.*[11] suggested that prenatal maternal smoking exposure leads to a 50% increased risk of overweight in childhood at the ages of 3 to 33 years. Also, a recent meta-analysis using 17 studies showed that maternal smoking during pregnancy was consistently associated with obesity in children with a mean age of 9 years.[8] It has been suggested that there is a dose-response association between the number of smoked cigarettes and the risk of childhood obesity.[44] These studies used BMI as measure for childhood obesity. Using BMI does not provide information about fat distribution.[12] A limited number of studies focused on the associations of maternal smoking during pregnancy with direct measures of body composition instead of BMI. A study in Southampton showed in 448 neonates that maternal smoking in late pregnancy was weakly associated with a lower fat mass percentage and greater lean mass percentage.[45] Another study in Sweden and Norway observed in 315 children aged 5 years that maternal smoking was associated with an increased risk of skinfold thickness higher than the 85th percentile, however the associations attenuated after adjustment for confounders like total energy intake of the parents.[17] In contrast, a study in Boston, in 746 children at the age of 3 years, showed no association of maternal smoking during pregnancy with central adiposity, measured by the subscapular and triceps ratio, which has been found to be a valid proxy for intra-abdominal adipose tissue in children.[15]

A study in Bristol, the United Kingdom found in 5,689 children with mean age of 10 years that maternal smoking at any time during pregnancy is associated with higher offspring total fat mass measured with Dual X-ray Absorptiometry (DXA). Maternal smoking was also associated with higher lean mass, but to a lesser extent.[16] In our study, we used skinfold thickness as measure of subcutaneous fat mass at the ages of 1.5, 6 and 24 months. We did not find significant associations between maternal smoking during pregnancy and peripheral, central and total subcutaneous fat mass in early childhood. We also did not observe associations between the number of cigarettes smoked during pregnancy and peripheral, central and total subcutaneous fat mass in the offspring. Most previous studies

assessed the associations between maternal smoking and adiposity at older age. The lack of association in this study might be due to the younger age groups, or use less advanced measuring techniques. This might also be the explanation for the inconsistent associations between maternal smoking during pregnancy and BMI. In the future, genome wide association studies might identify new genetic loci related to skinfold thickness.[46]

Studies examining the influence of prenatal smoke exposure in different periods of pregnancy might identify critical time periods, which are important from both a developmental and preventive perspective. We showed that both maternal first trimester smoking and continued smoking were not associated with peripheral, central and total subcutaneous fat mass until the age of 2 years.

Conclusion

Our findings suggest that intrauterine exposure to maternal cigarette smoke in different periods during pregnancy does not influence peripheral, central and total subcutaneous fat mass development in early childhood. Further studies are needed in children at older ages and to identify associations of maternal smoking during pregnancy with other measures of body composition like DXA and Magnetic Resonance Imaging (MRI).

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CHAPTER 4.5

PARENTAL SMOKING DURING PREGNANCY, BODY FAT DISTRIBUTION AND CARDIOMETABOLIC RISK FACTORS IN CHILDHOOD

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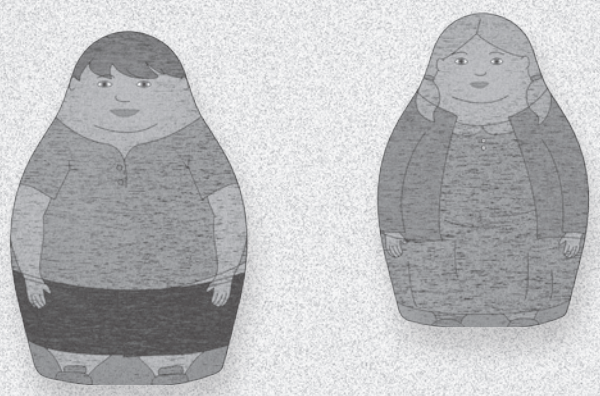

CHAPTER 5

Infant nutrition

CHAPTER 5.1

Breastfeeding and growth in preschool children

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ABSTRACT

Objective: Breastfeeding has been suggested to be associated with lower risks of obesity in older children and adults. We assessed whether the duration and exclusiveness of breastfeeding are associated with early postnatal growth rates and the risks of overweight and obesity in preschool children.

Methods: This present study was embedded in a population-based prospective cohort study from early fetal life onwards, in 5,047 children and their mothers in the Netherlands.

Results: Compared with children who were breastfed, those who were never breastfed had a lower weight at birth (difference 134 (95% confidence interval (CI) -190, -77) grams). No associations between breastfeeding duration and exclusivity with growth rates before the age of 3 months were observed. Shorter breastfeeding duration was associated with an increased gain in age- and sex-adjusted standard deviation scores (SDS) for length, weight and body mass index (BMI) (P for trend < 0.05) between 3 to 6 months of age. Similar tendencies were observed for the associations of breastfeeding exclusivity with change in length, weight and body mass index. Breastfeeding duration and exclusivity were not consistently associated with the risks of overweight and obesity at the ages of 1, 2 and 3 years.

Conclusions: Shorter breastfeeding duration and exclusivity during the first 6 months tend to be associated with increased growth rates for length, weight and BMI between the ages of 3 to 6 months but not with the risks of overweight and obesity until the age of 3 years.

INTRODUCTION

Current recommendations advise initiation and continuation of breastfeeding for more than 6 months to promote child health.[1-4] Previous studies suggested that breastfeeding has protective effects on the risks of cardiovascular diseases in adulthood.[5,6] Also, several studies suggested that breastfeeding leads to a lower risk of obesity in later life.[1-5] These associations have been shown in several studies and meta-analyses, also after adjustment for several potential confounders.[7] Furthermore, a dose-dependent association has been shown, suggesting that a longer duration of breastfeeding is associated with a lower body mass index (BMI) in older children and adulthood.[8]

Studies on the associations of breastfeeding with the risks of overweight and obesity in early childhood are scarce and have shown inconsistent results.[9-11] This inconsistency may be due to differences in study designs, indicators of overweight or obesity and assessment of breastfeeding.[12] Also, not all studies have data available about the exclusivity of breastfeeding. Assessing the associations of breastfeeding and childhood obesity at young ages is important since the risk of developing obesity may be partly explained by early postnatal growth patterns.[12] These growth patterns in early childhood might be intermediates in the associations of breastfeeding with obesity in later life.[13] Especially, high growth rates during first months of life are associated with metabolic syndrome outcomes.[14,15]

We hypothesized that prolonged duration and exclusivity of breastfeeding lead to lower growth rates during the first year of life and subsequently to lower risks of overweight and obesity in preschool children. We examined, in a population-based prospective cohort study in 5,047 children, the associations of breastfeeding duration and exclusivity with growth rates in infancy and the risks of overweight and obesity until the age of 3 years.

METHODS

Study design

The present study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands.[16,17] Enrollment in the study was aimed at early pregnancy (gestational age <18 weeks) but was possible until birth of the child. Assessments during pregnancy included physical examinations, fetal ultrasound examinations and adminis-

tration of questionnaires.[17] All children were born between April 2002 and January 2006 and form a prenatally enrolled birth cohort with a planned follow-up until young adulthood.

Postnatal growth data for the present study were available until the age of 3 years. Of all eligible children in the study area, 61% participated in the study at birth.[17] The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. Written informed consent was obtained from all parents.

Data collection and measurements

Duration and exclusiveness of breastfeeding

Information on breastfeeding initiation and continuation was obtained from delivery reports and postal questionnaires at the ages of 2, 6 and 12 months after birth.[17] Mothers were asked whether they ever breastfed their child (yes, no) and at what age they quitted breastfeeding. Subsequently, breastfeeding duration was categorized into 4 groups: 1) never; 2) <3 months; 3) 3-6 months; and 4) >6 months. Duration of exclusive breastfeeding was defined by using information about at what age other types of milk and/or solids were introduced in the first 6 months of life, according to a short food frequency questionnaire. The information on duration and exclusiveness of breastfeeding was combined and categorized into the following 3 categories: 1) never; 2) partial breastfeeding until 4 months; and 3) exclusive breastfeeding until 4 months. Never indicates infants who were never breastfed. Partial indicates infants receiving breastfeeding, formula feeding and/or solids in the first 4 months. Exclusive indicates infants who have been breastfed, without any other milk, solids or fluids during the first 4 months.

Postnatal growth characteristics

Postnatal growth was repeatedly measured at the Community Health Centers according to a standard schedule and procedures by a well-trained staff at the median ages of 3.1 months (95% range 1.1 – 4.5), 6.6 months (95% range 5.2 – 10.7), 13.0 months (95% range 11.1 – 15.9), 24.3 months (95% range 18.2 – 28.3) and 36.4 months (95% range 30.4 – 39.9)) months. Length was measured in the supine position to the nearest millimeter (mm) until the age of 14 months using a neonatometer, after which height was measured in standing position by a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight was measured using a mechanical personal scale (SECA, Almere, The Netherlands). BMI (kg/

m^2) was calculated. Standard deviation scores (SDS) for postnatal growth characteristics were obtained using Dutch reference growth charts (Growth Analyser 3.0, Dutch Growth Research Foundation, Rotterdam, The Netherlands). Relative overweight was defined as a BMI >1.1- 2.3 SDS (approximate adult BMI of 25-30 kg/m^2), and obesity was defined as a BMI >2.3 SDS (approximately adult BMI of >= 30).[18] Growth rates were defined as the change in SDS in age intervals between 0 to 3 months, 3 to 6 months and 6 to 12 months.

Covariates

Gestational age, sex and birth weight were obtained from midwife and hospital registries at birth. Information on the highest attained maternal educational level (low, moderate and higher), maternal ethnicity (European, non-European) and parity (primiparity, multiparity) were obtained at enrollment in the study. Ethnicity and educational level of the parents were defined according to the classification of Statistics Netherlands.[19,20] Information on maternal smoking (yes, no) and alcohol consumption during pregnancy (yes, no) was retrieved from prenatal questionnaires. Maternal height and weight were measured at enrollment while the mother stood without shoes and heavy clothing, and BMI was calculated (kg/m^2). Maternal age was registered at enrollment.

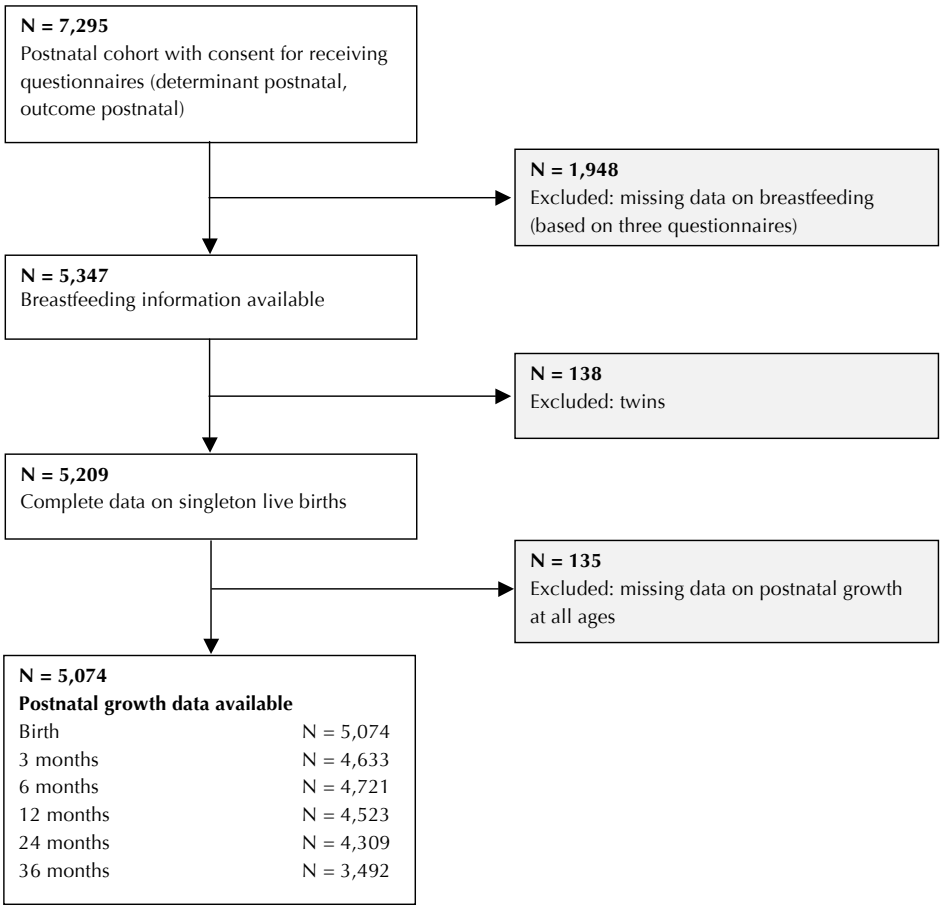
Population for analysis

In total, 7,295 children and their parents participated in the postnatal phase of the study and gave consent for participating in the questionnaire studies (**Figure 1**). Children without complete information on breastfeeding and twins were excluded from the analyses. Of the remaining singleton live births with complete data on breastfeeding, information on postnatal growth characteristic measures at at least one age was available in 5,074 children.

Statistical analysis

Differences in baseline characteristics between the breastfeeding duration categories were compared with ANOVA for continuous variables and Chi-square tests for categorical variables. The associations of breastfeeding (never, ever), breastfeeding duration (never, 0-3 months, 3-6 months and >6 months) and breastfeeding exclusivity (never, partial until 4 months and exclusive until 4 months) with the change in postnatal growth characteristics (length, weight and BMI) in SDS for different age periods (0 to 3, 3 to 6, and 6 to 12 months) were assessed using multiple linear regression models. Both dependent and independent variables were quantitative, and categorical variables were recoded to

Figure 1 Flow chart of participants in study



binary variables. The models were adjusted for potential confounders including child's age at visit, sex, birth weight, gestational age, maternal ethnicity, maternal education, maternal BMI, parity and smoking. Gestational age at enrollment was not included in the models since it did not materially change the results. Confounders were included in the models based on the literature or a change in effect estimates of more than 10%. We used the 'enter' method for including and excluding the independent variables.

For the analyses focused on the associations of breastfeeding duration with growth characteristics until the age of 3 months, we combined the breastfeeding groups into never and ever (0-3 months, 3-6 months and >6 months). For the analyses focused on growth characteristics at the ages of 3 to 6 months, we combined the breastfeeding duration groups into never, 0-3 months and 3-6 months (3-6 months and >6 months). Furthermore, we examined the associations of breastfeeding duration and exclusivity with

differences in BMI at the ages of 1, 2 and 3 years, and the risks of overweight and obesity at the same ages, using linear regression and logistic regression models, respectively. Finally, we assessed the associations of breastfeeding duration and exclusivity with longitudinally measured SDS of BMI in terms of overweight or obesity (combined outcome BMI >1.1 SDS) using the generalized estimating equations (GEE), taking into account correlations within subjects and assessing both time-dependent and -independent associations. Tests for trends were performed by treating each categorized variable as a continuous term and by entering the variable into the fully adjusted linear regression model. To handle missing values in covariates, we performed multiple imputations by generating 5 independent datasets for all analyses. For the GEE analyses, we performed multiple imputations of the data using Proc MI and Proc MIAnalyze. Imputations were based on the relationships between covariates included in the present study. All measures of association are presented with their 95% confidence intervals (CI). Cross-sectional analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The GEE analysis, including the Prox Genmod module, was performed with the Statistical Analysis System (version 9.1; SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the total group of 5,074 children, 89.8% had ever been breastfed (**Table 1**). Compared with mothers who breastfed their children for >6 months, those who never breastfed their children tended to have a younger age, higher BMI, higher rate of obesity, lower educational level, Dutch or European background and were more likely to smoke during pregnancy. Also, children who were never breastfed had a lower weight at birth and a higher prevalence of small size for gestational age and preterm birth (all $P < 0.05$). The median duration of breastfeeding was 4.4 (95% range 0.5 – 12.0) months. In total, 65.7% of all children were breastfed partially until the age of 4 months, and 24.1% of all children were breastfed exclusively until the age of 4 months.

Table 2 shows the associations of breastfeeding, breastfeeding duration and breastfeeding exclusivity with postnatal growth rates (length, weight and BMI) in different time periods presented as changes in SDS. Breastfeeding duration and exclusivity were not associated with growth rates before the age of 3 months. Compared with children who were ever breastfed, those who were never breastfed had a higher gain in length and weight between the ages of 3 to 6 months (difference 0.07 (95% CI 0.01, 0.14) SDS) and (0.06 (95% CI 0.01, 0.12) SDS), respectively. Compared with children who were breastfed for >3 months, children who were breastfed <3 months had also a higher gain in length and weight between

Table 1 Subject characteristics according to the duration of breastfeeding[1,2]

N = 5,074	Total	Never breast fed	0-3 months	3-6 months	>6 months
Maternal characteristics					
Age (years)	30.9 (21.9 – 38.5)	30.6 (21.3 – 38.5)	29.9 (20.9 – 37.8) **	31.5 (23.0 – 38.3) **	32.1 (24.2 – 39.2) **
Gestational weight change per week (kg)	0.45 (0.2)	0.45 (0.2)	0.44 (0.2)	0.45 (0.2)	0.45 (0.2)
Height (cm)	168.2 (7.3)	168.3 (6.9)	167.6 (7.4)	168.9 (7.1)	168.7 (7.3)
Weight (kg)	69.5 (12.8)	72.1 (15.4)	70.2 (13.7)*	68.9 (11.4) **	68.1 (11.2) **
Body mass index (kg/m^2)	24.6 (4.3)	25.4 (5.0)	25.0 (4.8)	24.2 (3.8) **	23.9 (3.7) **
Overweight (%)[3]	23.0	26.7	27.2	24.1	23.8
Obesity (%)[3]	11.0	17.1	13.9	8.7**	6.8**
Highest educational level (%)					
Low	7.5	7.4	9.4**	3.8**	6.0**
Moderate	41.1	62.4	50.0**	33.3**	26.5**
Higher	51.4	30.2	40.6**	62.9**	67.5**
Ethnicity (%)					
Dutch and other Europeans	67.6	79.7	62.2**	71.6**	71.1**
Non-European	32.4	20.3	37.8**	28.4**	28.9**
Smoking during pregnancy (%)					
Ever	25.4	40.1	31.1**	20.3**	17.1**
Never	74.6	59.9	68.9**	79.7**	82.9**
Alcohol consumption during pregnancy (%)					
Ever	59.9	49.2	55.7	69.2**	63.8**
Never	40.1	50.8	44.3	30.8**	36.2**
Parity (%)					
0	57.5	47.3	62.0**	60.1**	53.8*
>=1	42.5	52.7	38.0**	39.9**	46.2*
Birth characteristics					
Males (%)	50	52	50	50	47
Gestational age (weeks)	39.9 (37.1 – 42.1)	39.7 (37.0 – 42.0)	39.9 (36.9 – 42.0)	39.9 (37.1 – 42.1) *	40.1 (37.7 – 42.1) **
Weight (grams)	3,449 (546)	3,391 (582)	3,409 (548)	3,456 (554)*	3,525 (506) **
Small size for gestational age[3] (<5%)	5.0	6.5	5.8	5.0	3.9*
Low birth weight (<2 500 g) %	4.1	5.8	5.2	4.1	2.0**
Preterm birth (%)	4.8	5.6	5.5	4.6	3.4*

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.

[2]Differences in maternal and child characteristics for the breastfeeding duration groups were evaluated using ANOVA for continuous variables and Chi-squared tests for categorical variables.

[3]Overweight is defined as body mass index > 25–29.9 kg/m^2. Obesity is defined as body mass index >30 kg/m^2. Small size for gestational age is defined as the lowest 5% of gestational age- and sex-adjusted birth weight.

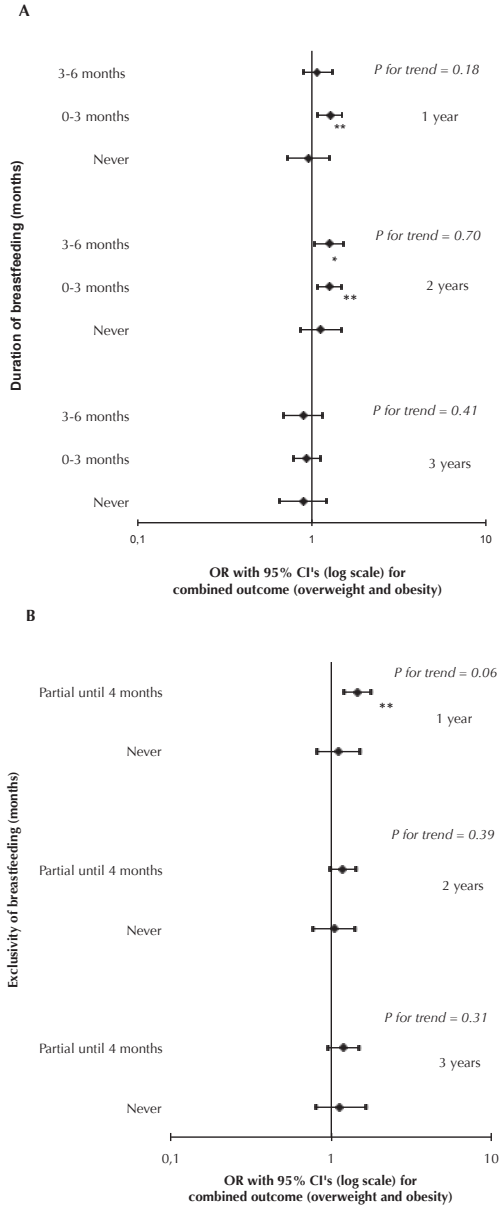
* P < 0.05 and ** P < 0.01

Table 2 Breastfeeding duration and exclusivity and growth rates in different intervals during the first year of infancy[1-3]

N = 5,074	Length (change in SDS)			Weight (change in SDS)			Body mass index (change in SDS)	
	0-3 months	3-6 months	6-12 months	0-3 months	3-6 months	6-12 months	3-6 months	6-12 months
Ever breastfed	N = 2,983	N = 4,212	N = 4,191	N = 4,371	N = 4,459	N = 4,283	N = 4,203	N = 4,167
Never (N = 516)	-0.01 (-0.14, 0.12) N = 309	0.07 (0.01, 0.14)* N = 417	-0.03 (-0.09, 0.03) N = 420	0.01 (-0.08, 0.09) N = 419	0.06 (0.01, 0.12)* N = 434	-0.03 (-0.09, 0.03) N = 426	0.02 (-0.06, 0.11) N = 414	-0.03 (-0.11, 0.06) N = 414
Ever (N = 4,555)	Reference N = 2,674	Reference N = 3,795	Reference N = 3,771	Reference N = 3,952	Reference N = 4,025	Reference N = 3,857	Reference N = 3,789	Reference N = 3,753
Duration of being breastfed	N = 2,709	N = 3,804	N = 3,776	N = 3,942	N = 4,027	N = 3,859	N = 3,795	N = 3,755
Never (N = 516)	-0.02 (-0.15, 0.12) N = 310	0.17 (0.10, 0.24)** N = 418	-0.04 (-0.11, 0.03) N = 421	0.01 (-0.08, 0.09) N = 420	0.14 (0.08, 0.20)** N = 435	-0.03 (-0.10, 0.03) N = 427	0.03 (-0.06, 0.12) N = 415	-0.01 (-0.10, 0.08) N = 415
0-3 months (N = 1,832)	Reference N = 2,399	0.19 (0.15, 0.24)** N = 1,522	-0.03 (-0.08, 0.02) N = 1,497	Reference N = 3,522	0.14 (0.10, 0.18)** N = 1,615	-0.01 (-0.05, 0.04) N = 1,528	0.02 (-0.03, 0.08) N = 1,518	0.02 (-0.04, 0.08) N = 1,488
3-6 months (N = 1,039)	N.A.	Reference N = 1,864	0.01 (-0.05, 0.06) N = 892	N.A.	Reference N = 1,977	0.02 (-0.03, 0.07) N = 912	Reference N = 1,862	0.03 (-0.04, 0.10) N = 889
>6 months (N = 1,149)	N.A.	N.A.	Reference N = 966	N.A.	N.A.	Reference N = 992	N.A.	Reference N = 963
P for trend	P = 0.48	P < 0.01	P = 0.15	P = 0.98	P < 0.01	P = 0.28	P = 0.03	P = 0.96
Exclusively breastfed	N = 2,986	N = 4,216	N = 4,195	N = 4,375	N = 4,463	N = 4,287	N = 4,207	N = 4,171
Never (N = 516)	-0.05 (-0.20, 0.10) N = 310	0.24 (0.17, 0.32)** N = 418	-0.05 (-0.12, 0.02) N = 421	0.01 (-0.09, 0.11) N = 420	0.22 (0.16, 0.29)** N = 435	-0.02 (-0.08, 0.05) N = 427	0.09 (-0.01, 0.18) N = 415	0.01 (-0.08, 0.10) N = 415
Partial until 4 months (N = 3,333)	-0.05 (-0.15, 0.04) N = 1,898	0.23 (0.18, 0.27)** N = 2,745	-0.02 (-0.07, 0.02) N = 2,741	0.01 (-0.05, 0.07) N = 2,875	0.21 (0.17, 0.25)** N = 2,471	0.02 (-0.02, 0.06) N = 2,806	0.08 (0.02, 0.14)** N = 2,738	0.05 (-0.01, 0.11) N = 2,726
Exclusive until 4 months (N = 1,225)	Reference N = 778	Reference N = 1,053	Reference N = 1,033	Reference N = 1,080	Reference N = 933	Reference N = 1,045	Reference N = 1,045	Reference N = 1,030
P for trend	P = 0.33	P < 0.01	P = 0.17	P = 0.82	P < 0.01	P = 0.96	P = 0.01	P = 0.37

[1]Values are standardized regression coefficients (95% confidence interval). * P < 0.05 and ** P < 0.01 using multiple linear regression models.
[2]Models are adjusted for child's age at visit, sex, birth weight, gestational age, maternal ethnicity, maternal education, maternal body mass index, smoking and parity.
[3]For the analyses focused on growth characteristics at the ages of 0 to 3 months, we combined the breastfeeding duration groups into never and ever (0-3, 3-6 and >6 months). For the analyses focused on growth characteristics at the ages of 3 to 6 months, we combined the breastfeeding duration groups into never, 0-3 and 3-6 months (3-6 and >6 months).
SDS: standard deviation score; NA: not applicable

Figure 2 Breastfeeding and the risks of overweight and obesity in the first 3 years (N = 5,074)[1,2]



[1]Values are odds ratios (95% confidence interval represented by horizontal bars). Breastfeeding duration > 6 months and breastfeeding exclusivity until 4 months, are considered as the reference groups in A and B, respectively.

[2]* P < 0.05 and ** P < 0.01 using generalized estimating equations (GEE). Models are adjusted for child's age at visit, sex, birth weight, gestational age, maternal ethnicity, maternal education, maternal body mass index, smoking and parity.

[3]Outcome is defined as age- and sex-adjusted body mass index > 1.1 SDS.

the ages of 3 to 6 months (all P-value for trend < 0.01). The highest gain in length was observed in children who were breastfed for only 0-3 months. We observed similar tendencies for the associations between breastfeeding exclusivity and gain in length, weight and BMI between the ages of 3 of 6 months. Children who were never breastfed or breastfed partially until 4 months showed a higher increase in length, weight and BMI. The highest effects were observed for children who were never breastfed. Breastfeeding duration and exclusivity were not associated with growth between the ages of 6 of 12 months.

Figure 2 shows the results from the longitudinal GEE models, which indicate no consistent associations between breastfeeding duration and exclusivity with the risks of overweight and obesity (BMI >1.1 SDS) at the ages of 1, 2 and 3 years. In addition, there was no consistent association between breastfeeding duration and exclusivity with BMI, overweight or obesity (**Supplemental Table 1**).

DISCUSSION

Breastfeeding duration and exclusivity were inversely associated with growth rates in length, weight and BMI between the ages of 3 of 6 months. We did not observe associations between breastfeeding duration and exclusivity and the risks of overweight and obesity in the first 3 years of life.

Methodological considerations

An important strength of the present study was the population-based cohort, with a large number of subjects being studied from early pregnancy onwards and information about a large number of potential confounders available. Information was available about duration and exclusivity of breastfeeding. Some methodological issues need to be considered. Of all children in the present study, questionnaires with breastfeeding information were available in 68%. This nonresponse would lead to biased effect estimates if the associations of breastfeeding duration and exclusivity with postnatal growth characteristics would be different between those included and not included in the analyses. However, this seems unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline.[21] In the present analysis, loss to follow-up was < 10%. However, the number of follow-up measurements was smaller with increasing age. Information on breastfeeding was prospectively collected by questionnaires without direct reference to any growth characteristic. Although assessing breastfeeding by questionnaires seems to be a valid method, misclassification

may occur.[22,23] We estimated breastfeeding exclusivity according to whether the child received breastfeeding without any other infant formula, milk or solids according to the short food frequency questionnaire. This definition does not cover the strict criteria used by the World Health Organization (WHO), which suggest that even the use of water in combination with breastfeeding does not fulfill the definition of exclusivity. However, we did ask for the most commonly introduced solids and fluids. Furthermore, in the Netherlands, it is not common that children receive breastfeeding in combination with the use of water to prevent dehydration. Therefore, we think that our measurement of exclusive breastfeeding is a good proxy for exclusive breastfeeding according to the WHO criteria.

Finally, we used BMI for defining overweight and obesity in early childhood. We should be careful with these definitions, as at this young age there is no clear cut-off point to define obesity, and BMI cannot differentiate between fat and lean mass.

Comparison of main findings with other studies

In line with previous studies, we observed differences in maternal characteristics between breastfeeding duration groups.[24-26] We previously showed socioeconomic and ethnic differences in breastfeeding duration.[27,28] In the present study group, mothers who never breastfed their children were also more likely to have a younger age, higher BMI, Dutch or European background and were more likely to smoke during pregnancy. We additionally observed that children who were never breastfed had a lower weight at birth and a higher risk of small size for gestational age and preterm birth. The associations of maternal and birth characteristics with breastfeeding initiation and duration show that these characteristics should be considered as potential confounders when studying the associations between breastfeeding and childhood growth.

It has been shown that after the first week of life, growth patterns appear to be similar between breastfed and formula-fed children for the first 2 to 3 months.[29] However, thereafter, the growth rates between breastfed and formula-fed children diverge with less distinct differences in length gain than weight gain. Previous studies have suggested that breastfed children have a slower growth between 3 to 12 months of life.[30–33] The present results are in line with the findings, but we showed that children who were never breastfed have higher growth rates in length and weight only between the ages of 3 to 6 months. After the age of 6 months, it is very likely that complementary foods such as fruit and vegetable snacks are introduced. This may explain why we did not observe any effects in growth after the age of 6 months. We also showed that exclusive breastfeeding for 4 months was associated with a lower gain in length, weight and BMI during the first 3 to 6 months. This is in line with a previous study in randomly selected healthy newborns from Denmark and Iceland, which showed that exclusive breastfeeding, influenced growth

rates during infancy.[33] The authors suggested that exclusive breastfeeding until 2 months is related to lower weight gain from 2 to 6 months as well as from 6 to 12 months.

The biological mechanisms by which breastfeeding might protect against high growth rates are not well understood. One suggested mechanism is that high protein intake in formula feeding stimulates the secretion of insulin-like growth factor I (IGF-I), which accelerates growth and increases muscle mass and adipose tissue.[34] Prolonged breastfeeding duration might also reduce plasma levels of appetite-related peptide and ghrelin.[34,35] Furthermore, formula-fed infants have higher plasma insulin concentrations, which might result in increased insulin resistance.[35]

Studies that focused on the associations between breastfeeding and the risks of overweight and obesity in early childhood have shown inconsistent results.[9-11] In the present study, breastfeeding duration and exclusivity were not consistently associated with the risks of overweight and obesity in the first 3 years of life. We observed that partial feeding until 4 months may increase the risks of overweight and obesity. However, this association was not consistent with the other results. We cannot explain this specific association, which might also be a chance finding. Similar results in this age range have been observed in previous studies.[36-38] Furthermore, high postnatal growth rates in the first 6 months of life are independently associated with the risks of overweight and obesity in later life.[39] Therefore, the associations between shorter duration of breastfeeding and the risks of overweight and obesity might appear at older ages.

Conclusion

The present results suggest that shorter breastfeeding duration and exclusivity are associated with increased postnatal growth rates for height, weight and BMI in the first 3 to 6 months of life. Breastfeeding duration and exclusivity are not associated with the risks of overweight and obesity in the first 3 years. Further research is needed to assess whether and from which age breastfeeding duration and exclusiveness are associated with childhood obesity.

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Supplemental Table 1 Breastfeeding duration and exclusivity and risks of overweight and obesity in the first 3 years of infancy[1,2]

	Age 1 year			Age 2 years			Age 3 years		
N = 5,074	Body mass index difference kg/m² (95% CI)	Overweight odds ratio (95% CI)[3]	Obesity odds ratio (95% CI)[3]	Body mass index difference kg/m² (95% CI)	Overweight odds ratio (95% CI)[3]	Obesity odds ratio (95% CI)[3]	Body mass index difference kg/m² (95% CI)	Overweight odds ratio (95% CI)[3]	Obesity odds ratio (95% CI)[3]
Ever breastfed	N = 4,463	N = 4,463 (755)	N = 4,463 (71)	N = 4,257	N = 4,257 (729)	N = 4,257 (107)	N = 3,406	N = 3,404 (456)	N = 3,404 (72)
Never (N = 516)	-0.03 (-0.16, 0.10) N = 451	0.81 (0.61, 1.08) N = 451 (65)	1.26 (0.60, 2.62) N = 451 (9)	0.02 (-0.12, 0.16) N = 431	0.77 (0.58, 1.04) N = 431 (61)	2.03 (0.66, 1.84)* N = 431 (18)	-0.01 (-0.16, 0.14) N = 360	0.90 (0.63, 1.27) N = 359 (45)	1.44 (0.73, 2.83) N = 359 (11)
Ever (N = 4,555)	Reference N = 4,012	Reference N = 4,012 (690)	Reference N = 4,012 (62)	Reference N = 3,826	Reference N = 3,826 (668)	Reference N = 3,826 (89)	Reference N = 3,046	Reference N = 3,045 (411)	Reference N = 3,045 (61)
Duration of being breastfed	N = 4,009	N = 4,009 (676)	N = 4,009 (59)	N = 3,822	N = 3,822 (666)	N = 3,822 (95)	N = 3,040	N = 3,038 (402)	N = 3,038 (66)
Never (N = 516)	0.10 (-0.05, 0.25) N = 452	0.92 (0.66, 1.27) N = 452 (65)	2.25 (0.83, 6.02) N = 452 (9)	0.03 (-0.12, 0.20) N = 432	0.86 (0.61, 1.19) N = 432 (61)	1.76 (0.92, 3.41) N = 432 (18)	0.02 (-0.15, 0.20) N = 361	1.03 (0.69, 1.53) N = 360 (45)	1.08 (0.48, 2.44) N = 360 (11)
0-3 months (N = 1,832)	0.19 (0.08, 0.29)** N = 1,590	1.27 (1.02, 1.58)* N = 1,590 (298)	1.96 (0.88, 4.37) N = 1,590 (29)	0.02 (-0.09, 0.14) N = 1,503	1.19 (0.95, 1.48) N = 1,503 (276)	0.94 (0.55, 1.61) N = 1,503 (38)	0.04 (-0.09, 0.16) N = 1,214	1.17 (0.88, 1.55) N = 1,213 (169)	0.73 (0.38, 1.39) N = 1,213 (27)
3-6 months (N = 1,039)	0.10 (-0.01, 0.22) N = 941	1.07 (0.84, 1.36) N = 941 (152)	1.75 (0.72, 4.25) N = 941 (13)	0 (-0.13, 0.12) N = 894	1.19 (0.94, 1.52) N = 894 (165)	0.68 (0.35, 1.31) N = 894 (15)	0.06 (-0.08, 0.20) N = 681	1.20 (0.88, 1.64) N = 681 (92)	0.71 (0.33, 1.55) N = 681 (11)
>6 months (N = 1,149)	Reference N = 1,026	Reference N = 1,026 (161)	Reference N = 1,026 (8)	Reference N = 993	Reference N = 993 (164)	Reference N = 993 (24)	Reference N = 784	Reference N = 784 (96)	Reference N = 784 (17)
P for trend	P < 0.01	P = 0.96	P = 0.09	P = 0.61	P = 0.92	P = 0.17	P = 0.72	P = 0.61	P = 0.87
Exclusively breastfed	N = 4,467	N = 4,467 (756)	N = 4,467 (71)	N = 4,261	N = 4,261 (729)	N = 4,261 (107)	N = 3,409	N = 3,407 (456)	N = 3,407 (72)
Never (N = 516)	0.11 (-0.04, 0.26) N = 452	1.09 (0.78, 1.50) N = 452 (65)	1.60 (0.64, 3.95) N = 452 (9)	0.06 (-0.09, 0.22) N = 432	0.89 (0.64, 1.24) N = 432 (61)	2.32 (1.18, 4.55)* N = 432 (18)	-0.02 (-0.19, 0.15) N = 361	1.02 (0.68, 1.52) N = 360 (45)	1.12 (0.49, 2.54) N = 360 (11)
Partial until 4 months (N = 3,333)	0.18 (0.09, 0.27)** N = 2,922	1.46 (1.20, 1.79)** N = 2,922 (539)	1.36 (0.71, 2.60) N = 2,922 (50)	0.07 (-0.03, 0.16) N = 2,799	1.20 (0.98, 1.47) N = 2,799 (504)	1.19 (0.71, 1.99) N = 2,799 (69)	-0.01 (-0.12, 0.10) N = 2,234	1.19 (0.92, 1.52) N = 2,233 (314)	0.73 (0.40, 1.32) N = 2,233 (45)

Exclusive until 4 months (N = 1,225)	Reference N = 1,093	Reference N = 1,093 (152)	Reference N = 1,093 (12)	Reference N = 1,030	Reference N = 1,030 (164)	Reference N = 1,030 (20)	Reference N = 814	Reference N = 814 (97)	Reference N = 814 (16)
P for trend	P = 0.01	P = 0.07	P = 0.79	P = 0.26	P = 0.83	P = 0.03	P = 0.77	P = 0.59	P = 0.99

[1]Values are unstandardized regression coefficients and odds ratios (95% confidence interval). * P < 0.05 and ** P < 0.01 using multiple linear and logistic regression models.

[2]Models are adjusted for child's age at visit, sex, birth weight, gestational age, maternal ethnicity, maternal education, maternal body mass index, smoking and parity.

[3]Overweight is defined as age- and sex-adjusted body mass index > 1.1–2.3 SDS; obesity is defined as age- and sex-adjusted body mass index > 2.3 SDS. Parentheses represent the cases of overweight and obesity.

CHAPTER 5.2

Infant diet and subcutaneous fat mass in early childhood

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ABSTRACT

Objective: Breastfeeding has a protective effect on childhood obesity, but the influences on body composition in early childhood are not known. We assessed whether the duration and exclusiveness of breastfeeding, and the timing of introduction of solid foods are associated with the subcutaneous fat mass in early childhood.

Methods: This study was embedded in a population-based prospective cohort study in 779 children. Peripheral (biceps, triceps) and central (suprailiacal and subscapular) subcutaneous fat mass was measured as skinfold thickness at the ages of 1.5, 6 and 24 months.

Results: Breastfeeding duration was not associated with subcutaneous fat mass at the age of 1.5 months. Shorter breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months (P-value for trend <0.05), but not at the age of 24 months. As compared to children who were exclusively breastfed for 4 months, those who were non-exclusively breastfed had a higher central fat mass at the age of 24 months (P-value for trend <0.01). Timing of introduction of solid foods was not associated with subcutaneous fat mass.

Conclusions: Our results suggest that a shorter duration and non-exclusive breastfeeding affect early body composition during the first 2 years of life. Follow-up studies at older ages are needed to explore the long-term consequences.

INTRODUCTION

Several studies showed a protective effect of breastfeeding on the risk of overweight in children and adults.[1-4] Although the effect estimates for the associations of breastfeeding with mean body mass index (BMI) are generally small, they seem to be consistent.[1-5] An inverse dose-dependent association has been shown, suggesting that longer duration of breastfeeding is associated with a lower BMI.[1,2] Some studies have also suggested that early weaning may increase BMI in childhood, but results are inconsistent.[6-9] BMI provides only information about body weight, whereas it does not distinguish between fat and lean mass.[10,11] Because an unfavorable fat distribution may be stronger related to cardiovascular and metabolic diseases, it is important to explore the associations of breastfeeding with measures of fat distribution. Only a few studies have examined the relationships between breastfeeding in infancy and direct measures of adiposity in childhood, but no consistent associations were observed.[12,13] Skinfold thickness is a valid measurement for subcutaneous fat mass assessment in epidemiological studies.[10,11] These measurements are quick and simple to obtain in most age groups, including young infants.[11] We have previously shown that birth weight is associated with subcutaneous fat mass in early childhood.[14] Subcutaneous fat mass also tends to track throughout early childhood.[15]

We examined in a population-based prospective cohort study in 779 children, the associations of breastfeeding duration and exclusivity, and the timing of introduction of solid foods with peripheral, central and total subcutaneous fat mass in early childhood.

METHODS

Study design

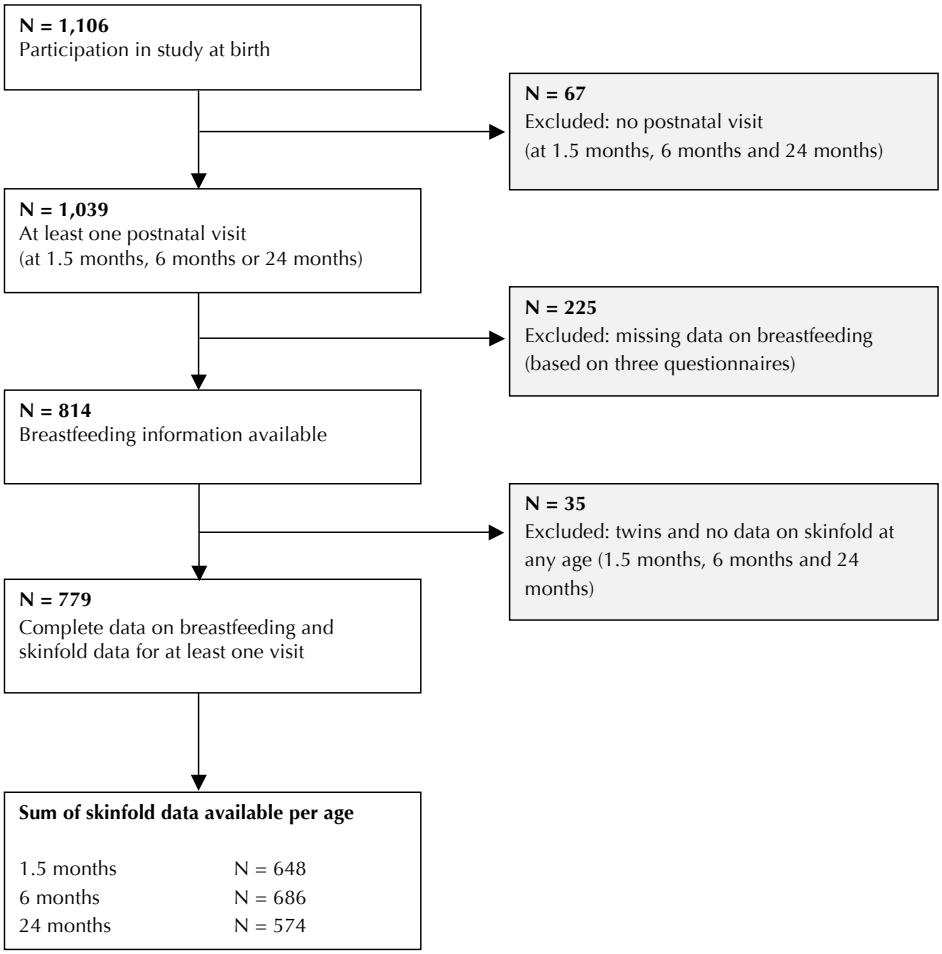
This study was embedded in the Generation R Study, a population-based prospective cohort study of pregnant women and their children from fetal life onwards in Rotterdam, the Netherlands.[16] Enrollment in this study was aimed at early pregnancy (gestational age <18 weeks) but was possible until birth of the child. All children were born between April 2002 and January 2006, and form a prenatally enrolled birth-cohort that is currently followed until young adulthood. Additional detailed assessments of postnatal growth and development were conducted in a subgroup of 1,106 Dutch mothers and their children from late pregnancy.[16] Between February 2003 and April 2005, all pregnant mothers participating in the Generation R study, who met this criterion, were approached for

additional measurements.[16] Of all approached women, 80% agreed to participate in the subgroup study. The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Population for analysis

From the total of 1,106 children, 1,039 children participated in at least one of the postnatal assessments at the ages of 1.5, 6 and 24 months (**Figure 1**). Children without complete information on breastfeeding (N = 225) were excluded from the analyses. Of the remaining 814 live births with complete data on breastfeeding, skinfold measurements were measured

Figure 1 Flow chart of participants in study



in at least one of the 3 visits in 799 children. Next, twins (N = 20) were excluded from the analyses because twins are correlated and may differ from other children in the relation between breastfeeding and subcutaneous fat mass. Missing skinfold measurements were mainly due to crying behavior. No differences in child and maternal characteristics were found between children with and without skinfold measurements (N = 779 versus 1,106).

Data collection and measurements

Duration and exclusiveness of breastfeeding and solids foods introduction

Information on breastfeeding initiation and continuation was obtained from delivery reports and postal questionnaires at the ages of 2, 6 and 12 months after birth, as described previously.[17] Mothers were asked whether they ever breastfed their child (yes, no) and at what age they quitted breastfeeding. Subsequently, breastfeeding duration was categorized into 3 groups: 1) never; 2) <4 months; 3) >=4 months. Duration of exclusive breastfeeding was defined by using information about at what age other types of milk and/or solids were introduced in the first 6 months of life, according to a short food frequency questionnaire. The information on duration and exclusiveness of breastfeeding was combined and categorized into the following 3 categories: 1) never; 2) non-exclusively breastfed until 4 months; and 3) exclusively breastfed until 4 months. Never indicates infants who were never breastfed. Non-exclusive indicates infants receiving both breastfeeding, and formula feeding or solids during the first 4 months. Exclusive indicates infants who have been breastfed, without any other milk, solids or fluids during the first 4 months. Information on the timing of introduction of solid foods included fruit and vegetable snacks and was obtained from the same short food frequency questionnaire. The timing of solid foods was defined as the age at which a fruit or vegetable snack was given for the first time (<4 months; 4-5 months; and >5 months).

Subcutaneous fat mass measurements and anthropometrics

Subcutaneous fat mass was measured as skinfold thicknesses (SFT) in millimeters (mm) at the ages of 1.5, 6 and 24 months on the left side of the body at four different sites (biceps, triceps, suprailiacal and subscapular) according to the standard procedures by using a skinfold caliper (Slim Guide, Creative Health Products, USA).[18] Four well-trained medical assistants performed all measurements.[19] The consensus between and among observers for the medical assistants was analyzed using the intraclass correlation coefficient (ICC).[20,21] Intraobserver ICC was 0.88 and interobserver ICC was 0.76. The total subcutaneous fat mass was calculated from the sum of biceps SFT, triceps SFT, suprailia-

cal SFT, subscapular SFT. Central subcutaneous fat mass was calculated from the sum of suprailiacal SFT + subscapular SFT. Peripheral subcutaneous fat mass was calculated from the sum of triceps SFT, biceps SFT.[22,23] Body length at the age of 1.5 and 6 months was measured in supine position to the nearest mm using a neonatometer and in 24-month olds height was measured in standing position by a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight was measured to the nearest grams in naked infants at the age of 1.5 and 6 months by using an electronic infant scale (SECA, Almere, The Netherlands) and in 24-month olds by a mechanical personal scale (SECA).

Covariates

Gestational age at birth, birth weight and sex were obtained from midwife and hospital registries at birth. Information on the highest attained maternal educational level and parity were obtained at enrollment in the study. Educational level of the parents was defined according to the classification of Statistics Netherlands.[24] Information on maternal smoking during pregnancy (yes, no) was retrieved from the prenatal questionnaire. Maternal height and weight were measured at enrollment while the mother stood without shoes and heavy clothing, and BMI (kg/m^2) was calculated.

Statistical analysis

Differences in baseline characteristics between the breastfeeding variables ever (never; ever), duration (<4 months; >4 months) and exclusivity (non-exclusively breastfed until 4 months; exclusively breastfed until 4 months) were compared with Student's t-tests for continuous variables and Chi-square tests for categorical variables. The associations of breastfeeding (never; ever), breastfeeding duration (never; <4 months; and >4 months), breastfeeding exclusivity (never; non-exclusive until 4 months; and exclusive until 4 months) and the timing of introduction of fruit and vegetable snacks (<4 months; 4- 5 months; and >5 months) with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months, were assessed using multiple linear regression models. The reference groups of these regression models were ever breastfed, breastfeeding duration of >4 months, exclusively breastfed until 4 months, respectively. In these models, breastfeeding categories were used as predictor variables and peripheral, central and total fat as outcome variables. The models were adjusted for potential confounders including child's age at visit, sex, maternal education, maternal BMI, smoking and parity, gestational age, birth weight, current height and observer. The models focused on the role of the timing of introduction of solid foods, were additionally adjusted for breastfeeding duration. Tests for trends were performed by treating each categorized variable as a continuous

term and by entering the variable into the fully adjusted linear regression model. To handle missing values in covariates, we performed multiple imputations by generating 5 independent datasets for all analyses using the Markov Chain Monte Carlo (MCMC) method. Imputations were based on the relationships between covariates included in this study. All measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Subject characteristics are given in **Table 1**. Of the total group of 779 children, 87.9% had ever been breastfed with a mean duration of 4.5 months (range 0.5 – 12.0). Differences between the breastfeeding categories are given in Supplemental Tables 1-3. As compared to mothers who never breastfed their children, those who breastfed their children had more frequently a higher educational level, were more likely to consume alcohol during pregnancy but less likely to smoke (**Supplemental Table 1**). Mothers who breastfed their children for >=4 months, compared with mothers who breastfed their children <4 months, were older and had a lower BMI (**Supplemental Table 2**). Mothers who exclusively breastfed their children had a lower weight, were more likely to have >1 child and had children with a higher birth weight (**Supplemental Table 3**).

The associations of ever breastfeeding, breastfeeding duration and exclusivity with peripheral, central and total fat mass at the ages of 1.5, 6 and 24 months are shown in **Table 2**. Breastfeeding duration was not associated with subcutaneous fat mass at the age of 1.5 months. Shorter breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months (P-value for trend <0.05), but not at the age of 24 months. As compared with children who were breastfed exclusively for 4 months, those who were never breastfed had a higher peripheral and total subcutaneous fat mass at the age of 6 months and higher central fat mass at the age of 24 months (P-value for trend <0.01).

Table 3 presents the associations between the timing of introduction of solid foods and peripheral, central and total fat mass at the ages of 1.5, 6 and 24 months. As compared with children who received solid foods after the age of 5 months, those who received solid foods before the age of 5 months, tended to have increased subcutaneous fat mass measures, but these associations were not significant.

Table 1 Subject characteristics[1]

	Total (N = 779)
Maternal characteristics	
Age (years)	32.3 (24.9 – 37.9)
Height (cm)	170.8 (6.3)
Weight (kg)	72.0 (13.4)
Body mass index (kg/m^2)	24.7 (4.4)
Highest educational level (%)	
Low	1.8
Moderate	34.5
High	63.7
Smoking in pregnancy (%)	
Ever	22.2
Never	77.8
Alcohol consumption in pregnancy (%)	
Ever	70.1
Never	29.9
Parity (%)	
0	62.5
≥1	37.5
Birth characteristics	
Males (%)	51
Gestational age (weeks)	40.3 (37.1 – 42.1)
Weight (grams)	3,515 (549)
Small size for gestational age (<5%)	4.9
Low birth weight (<2500 g) (%)	3.6
Preterm birth (%)	4.1
Breastfeeding	
Ever (%)	87.5
Duration (months)	4.5 (0.0 – 12.0)
Timing of introduction of solid foods (%)	
< 4 months introduced	7.7
> 4-5 months introduced	60.4
> 5 months introduced	31.9

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution. Of the total group, data were missing on maternal anthropometrics (N = 5), maternal education (N = 9), maternal smoking (N = 98), maternal alcohol use (N = 93), parity (N = 1), breastfeeding duration (N = 141), and introduction of solid foods (N = 153).

Table 2 Associations of ever breastfeeding, breastfeeding duration and exclusivity of breastfeeding with subcutaneous skinfolds (mm) at different ages in early childhood[1,2]

	Age 1.5 months			Age 6 months			Age 24 months		
N = 779	**Peripheral fat (mm) (95% CI)**	**Central fat (mm) (95% CI)**	**Total fat (mm) (95% CI)**	**Peripheral fat (mm) (95% CI)**	**Central fat (mm) (95% CI)**	**Total fat (mm) (95% CI)**	**Peripheral fat (mm) (95% CI)**	**Central fat (mm) (95% CI)**	**Total fat (mm) (95% CI)**
Ever breastfed	N = 653	N = 648	N = 648	N = 688	N = 686	N = 686	N = 584	N = 575	N = 575
Never (N = 97)	-0.24 (-1.02,0.54)	-0.22 (-0.92, 0.49)	-0.45 (-1.82, 0.93)	1.00 (0.10,1.89)*	0.25 (-0.51, 1.02)	1.24 (-0.22, 2.70)	0.23 (-0.98, 1.44)	0.62 (-0.34, 1.57)	0.86 (-1.07, 2.80)
Ever (N = 682)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
Duration of being breastfed	N = 615	N = 611	N = 611	N = 647	N = 645	N = 645	N = 553	N = 544	N = 544
Never (N = 97)	-0.16 (-0.92,0.62)	-0.12 (-0.80, 0.57)	-0.26 (-1.60, 1.08)	1.49 (0.51, 2.47)**	0.50 (-0.35, 1.35)	1.99 (0.37, 3.61)*	0.21 (-1.13, 1.55)	0.90 (-0.14, 1.94)	1.14 (-0.98, 3.26)
< 4 months (N = 354)	Reference	Reference	Reference	0.69 (0.02, 1.37)*	0.34 (-0.24, 0.92)	1.04 (-0.06, 2.15)	0.04 (-0.82, 0.91)	0.50 (-0.17, 1.17)	0.55 (-0.82, 1.92)
>= 4 months (N = 283)	N.A	N.A	N.A	Reference	Reference	Reference	Reference	Reference	Reference
P for trend	P = 0.38	P = 0.79	P = 0.50	P < 0.01	P = 0.18	P = 0.01	P = 0.79	P = 0.06	P = 0.26
Exclusively breastfed	N = 653	N = 648	N = 648	N = 688	N = 686	N = 686	N = 584	N = 575	N = 575
Never (N = 97)	0.46 (-0.43, 1.35)	0.14 (-0.67, 0.95)	0.58 (-0.99, 2.15)	1.40 (0.37, 2.42)**	0.57 (-0.30, 1.44)	1.96 (0.29,3.63)*	0.50 (-0.86, 1.86)	1.24 (0.17, 2.30)*	1.75 (-0.42, 3.91)
Non-exclusive until 4 months[3] (N = 472)	0.96 (0.36,1.56)**	0.49 (-0.05, 1.04)	1.42 (0.36, 2.48)**	0.55 (-0.14, 1.24)	0.44 (-0.15, 1.03)	1.00 (-0.13, 2.12)	0.37 (-0.49, 1.23)	0.86 (0.19, 1.53)*	1.23 (-0.14, 2.59)
Exclusive until 4 months[3] (N = 210)	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Reference
P for trend	P = 0.07	P = 0.41	P = 0.16	P < 0.01	P = 0.13	P = 0.02	P = 0.38	P < 0.01	P = 0.06

[1]Values are unstandardized regression coefficients (95% confidence interval). * P < 0.05 and ** P < 0.01 using multiple linear regression models.

[2]Models are adjusted for child's age at visit, sex, birth weight, maternal education, maternal body mass index, smoking and parity, gestational age, current height and observer.

[3]Non-exclusive breastfeeding until 4 months includes partial until 4 months, partial thereafter; and partial until 4 months, not thereafter. Exclusive breastfeeding until 4 months includes exclusive until 6 months; exclusive until 4 months, partial thereafter; exclusive until 4 months, not thereafter.

Table 3 Timing of introduction of solid foods and subcutaneous skinfolds (mm) in early childhood[1,2]

Solid foods (fruit or vegetables)	Age 6 months			Age 24 months		
N = 779	Peripheral fat (mm) (95% CI)	Central fat (mm) (95% CI)	Total fat (mm) (95% CI)	Peripheral fat (mm) (95% CI)	Central fat (mm) (95% CI)	Total fat (mm) (95% CI)
	N = 486	N = 484	N = 484	N = 476	N = 469	N = 469
< 4 months (N = 48)	0.23 (-1.11, 1.56)	0.96 (-0.30, 2.21)	1.18 (-1.04, 3.40)	1.66 (-0.11, 3.42)	-0.09 (-1.39, 1.21)	1.29 (-1.46, 4.04)
> 4-5 months (N = 378)	-0.06 (-0.79, 0.67)	0.45 (-0.24, 1.14)	0.40 (-0.82, 1.61)	0.82 (-0.09, 1.73)	0.66 (-0.01, 1.33)	1.46 (0.05, 2.88)*
>5 months (N = 200)	Reference	Reference	Reference	Reference	Reference	Reference
P for trend	P = 0.89	P = 0.10	P = 0.31	P = 0.03	P = 0.29	P = 0.08

[1]Values are unstandardized regression coefficients (95% confidence interval) using multiple linear regression models.

[2]Models are adjusted for child's age at visit, sex, maternal education, maternal body mass index, smoking and parity, gestational age, birth weight, current height, observer and breastfeeding duration.

* P < 0.05

DISCUSSION

Results from this study suggest that a shorter duration and non-exclusive breastfed affect early body composition during the first 2 years of life. A shorter duration of breastfeeding was associated with higher peripheral and total subcutaneous fat mass at the age of 6 months, whereas non-exclusive breastfed was associated with higher central subcutaneous fat mass at the age 24 months. Early introduction of solid foods was not associated with subcutaneous fat mass measures.

Methodological considerations

The most important strengths of this study are the population-based prospective design with a relatively large number of subjects being studied from early pregnancy onwards, and information about a large number of potential confounders available. Our analyses were based on 779 children with SFT measurements. Furthermore, information was available about duration and exclusivity of breastfeeding. Some methodological issues need to be considered. Of the total group of 1,039 children, breastfeeding information was available in 78%. This nonresponse would lead to biased effect estimates if the associations of breastfeeding duration and exclusivity with SFT measurements would be different between those included and not included in the analyses. This seems unlikely because biased estimates in cohort studies mainly arise from loss to follow-up rather than from

nonresponse at baseline.[25] Information on breastfeeding was prospectively collected by questionnaires without direct reference to any skinfold measurement. Although assessing breastfeeding by questionnaires seems to be a valid method, misclassification may occur.[26] We estimated breastfeeding exclusivity according to whether the child received breastfeeding without any other infant formula, milk or solids according to the short food frequency questionnaire. This definition does not cover the strict criteria used by the World Health Organization (WHO), which suggest that even the use of water in combination with breastfeeding does not fulfill the definition of exclusivity. However, we did ask for the most commonly introduced solids and fluids. Furthermore, in the Netherlands it is not common that children receive breastfeeding in combination with the use of water to prevent dehydration. Therefore, we consider our measurement of exclusive breastfed as a good proxy for exclusive breastfed according to the WHO criteria. Our definition of solid foods included only fruit or vegetable snacks. However, in the first months of life it is not likely that other products were introduced. We created our specific duration, exclusivity and solid foods categories based on the collected data and growth measurements. These categories do not enable direct comparison with the widely used categories (6 months) of the WHO. Furthermore, our study group was ethnically homogenous and the mothers were highly educated. We were not able to assess the effect of breastfeeding on subcutaneous fat mass development in children with different ethnic and social backgrounds. Finally, we used SFT as a measure of subcutaneous fat mass because of the limited use of BMI as a direct measure of adiposity in early childhood. SFT provides a simple, easy, and quick yet highly informative assessment of regional fatness in most age groups and can be used in large-scale epidemiological studies.[11] In general, intraobserver and interobserver error are low compared with between-subject variability, but in obese children accuracy and precision are poorer.[10,11] Furthermore, SFT has a limitation in assessing lean and fat mass of the whole body.[11]

Comparison of main findings with other studies

Several studies have shown that breastfeeding is associated with a lower risk of later overweight and obesity.[1-4] BMI is a poor outcome due to its low predictive value and lack of information about fat distribution.[27] Studies focusing on the association between breastfeeding and body composition instead of BMI did show inconsistent associations.[12,13] This may be due to differences in body measurements, ages and samples sizes. A study in adult males from Brazil did not show an association between breastfeeding and adult body fat, measured by skinfolds and fat mass using a bio-impedance scale[28], while a large study in the United Kingdom reported a negligible protective effect of breastfeeding duration for >6 months on mean body fat measured with dual-energy X-ray absorptiometry

(DXA) in children aged 9-10 years.[29] Any association was attenuated after adjustment for confounders. One study in Southampton reported a graded association between shorter breastfeeding duration and higher DXA-derived fat mass in children aged 4 years.[30] In contrast, two studies, assessing the associations of breastfeeding and direct measures of body composition using DXA at ages of 2 and 5 years, did not show any association.[12,31] We used SFT as measure of subcutaneous fat mass at younger ages, but the results are in line with these previous studies. Only at the age of 6 months we found that shorter breastfeeding duration leads to higher peripheral and total fat mass. Our study was the first study that used both breastfeeding duration and exclusivity, to examine the effect on subcutaneous fat mass. We showed that non-exclusive breastfed was associated with higher peripheral and total subcutaneous fat mass at the age of 1.5 months and higher central fat mass at the age of 24 months. We cannot explain these differences. However, central fat mass might be stronger related to adverse cardiovascular and metabolic health outcomes.[32,33] Although the effect estimates were small, exclusive breastfed might affect subcutaneous fat mass development in early childhood.

The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Nutrition Committee recommended in 2008 that complementary foods (solid foods and liquids other than breastfeeding or formula) should not be introduced before 4 months and not later than 6 months.[34] Many studies in industrialized countries showed non-adherence to these recommendations.[35,36] In our Dutch study population, we found that most mothers introduced solid foods mainly after the age of 4 months. There is conflicting evidence about the relation between the timing of introduction of solid foods and adiposity in childhood. A few studies did not show differences in adiposity between early and delayed introduction of solid foods.[37,38] One recent study suggested that a diet based on fruit, vegetables, and home-prepared foods in the first year of life is associated with a higher lean and lower fat mass measured with DXA at the age of 4 years.[30] The same study group reported a greater gain in both weight and SFT between 6 and 12 months in infants who received the same diet with fruit, vegetables and home-prepared foods at the age of 6 months.[39] We did not observe significant associations between early introduction of solid foods and subcutaneous fat mass measures.

Conclusion

Our results suggest that a shorter duration and non- exclusive breastfed affect early body composition during the first 2 years of life. Early introduction of solid foods is not associated with subcutaneous fat mass measures. Follow-up studies are needed to assess whether breastfeeding duration and exclusivity affect subcutaneous fat mass and other measures of body composition at older ages.

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Supplemental Table 1 Subject characteristics[1-3]

	Never breastfed (12.1%, N = 97)	Ever breastfed (87.9%, N = 682)
Maternal characteristics		
Age (years)	32.4 (23.2 – 36.6)	32.3 (24.9 – 38.0)
Height (cm)	169.7 (6.2)	170.9 (6.3)
Weight (kg)	73.0 (16.7)	71.9 (12.9)
Body mass index (kg/m^2)	25.3 (5.3)	24.6 (4.2)
Highest educational level (%)		
Low	4.1	1.5**
Moderate	58.8	31.1**
High	37.1	67.4**
Smoking in pregnancy (%)		
Ever	27.8	21.4*
Never	72.2	78.6*
Alcohol consumption in pregnancy (%)		
Ever	48.5	73.2**
Never	51.5	26.8**
Parity (%)		
0	55.7	63.5
≥1	44.3	36.5
Birth characteristics		
Males (%)	55	51
Gestational age (weeks)	40.1 (34.8 – 42.0)	40.3 (37.1 – 42.1)
Weight (grams)	3,475 (585)	3,520 (544)
Small size for gestational age (<5%)	7.2	4.5
Low birth weight (<2500 g) (%)	5.2	3.4
Preterm birth (%)	6.2	3.8
Breastfeeding		
Duration (months)	-	4.5 (0.07 – 12.0)
Timing of introduction of solid foods (%)		
< 4 months introduced	10.3	7.4
> 4-5 months introduced	67.2	59.7
> 5 months introduced	22.4	32.9

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.

[2]Differences in maternal and child characteristics for the breastfeeding groups (never; ever) were evaluated using t-tests for continuous variables and Chi-squared tests for categorical variables. Gestational age was log-transformed for the t-tests.

[3]Of the total group, data were missing on maternal anthropometrics (N = 5), maternal education (N = 9), maternal smoking (N = 98), maternal alcohol use (N = 93), parity (N = 1), breastfeeding duration (N = 141), and introduction of solid foods (N = 153).

* P < 0.05 and ** P < 0.01

Supplemental Table 2 Subject characteristics[1-3]

	Breastfed for < 4 months (45.4%, N = 354)	Breastfed for >= 4 months (36.3%, N = 283)
Maternal characteristics		
Age (years)	31.8 (24.1 – 38.0)	32.6 (26.7 – 38.3)**
Height (cm)	170.5 (6.2)	171.6 (6.3)*
Weight (kg)	73.0 (14.4)	70.7 (10.7)
Body mass index (kg/m^2)	25.1 (4.8)	24.0 (3.5)**
Highest educational level (%)		
Low	2.8	-
Moderate	39.0	23.0**
High	58.2	77.2**
Smoking in pregnancy (%)		
Ever	27.4	14.5**
Never	72.6	85.5**
Alcohol consumption in pregnancy (%)		
Ever	69.5	76.3**
Never	30.5	23.7**
Parity (%)		
0	65.5	60.1
≥1	34.5	39.9
Birth characteristics		
Males (%)	50	50
Gestational age (weeks)	40.3 (37.3 – 42.1)	40.3 (37.1 – 42.3)
Weight (grams)	3,501 (512)	3,553 (561)
Small size for gestational age (<5%)	5.9	3.5
Low birth weight (<2500 g) (%)	3.7	2.5
Preterm birth (%)	4.0	3.2
Breastfeeding		
Duration (months)	1.5 (0.07 – 3.5)	7.0 (4.5 – 12.0)**
Timing of introduction of solid foods (%)		
< 4 months introduced	12.8	2.2**
> 4-5 months introduced	68.8	49.8**
> 5 months introduced	18.4	48.0**

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.

[2]Differences in maternal and child characteristics for the breastfeeding duration groups (breastfed for <4 months; breastfed for >=4 months) were evaluated using t-tests for continuous variables and Chi-squared tests for categorical variables. Gestational age was log-transformed for the t-tests.

[3]Of the total group, data were missing on maternal anthropometrics (N = 5), maternal education (N = 9), maternal smoking (N = 98), maternal alcohol use (N = 93), parity (N = 1), breastfeeding duration (N = 141), and introduction of solid foods (N = 153).

* P < 0.05 and ** P < 0.01

Supplemental Table 3 Subject characteristics[1-3]

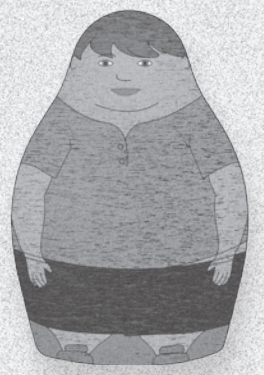
	Non-exclusively breastfed (60.6%, N = 472)	Exclusively breastfed (27.0%, N = 210)
Maternal characteristics		
Age (years)	31.9 (24.5 – 37.9)	32.6 (26.1 – 38.3)*
Height (cm)	170.9 (6.4)	171.1 (6.2)
Weight (kg)	72.6 (13.7)	70.2 (10.9)*
Body mass index (kg/m^2)	24.8 (4.5)	24.0 (3.5)*
Highest educational level (%)		
Low	2.1	-
Moderate	33.9	25.2**
High	64.0	74.8**
Smoking in pregnancy (%)		
Ever	24.2	14.8**
Never	75.8	85.2**
Alcohol consumption in pregnancy (%)		
Ever	72.0	75.7**
Never	28.0	24.3**
Parity (%)		
0	66.5	56.7*
≥1	33.5	43.3*
Birth characteristics		
Males (%)	50	51
Gestational age (weeks)	40.4 (37.1 – 42.1)	40.1 (37.1 – 42.3)
Weight (grams)	3,493 (554)	3,581 (518)*
Small size for gestational age (<5%)	5.1	3.3
Low birth weight (<2500 g) (%)	4.2	1.4
Preterm birth (%)	4.0	3.3
Breastfeeding		
Duration (months)	2.5 (0.07 – 8.5)	8.5 (2.5 – 12.5)**
Timing of introduction of solid foods (%)		
< 4 months introduced	11.7	-
> 4-5 months introduced	66.8	47.6**
> 5 months introduced	21.5	52.4**

[1]Values are means (standard deviation), percentages, or medians (90% range) for variables with skewed distribution.

[2]Differences in maternal and child characteristics for the breastfeeding exclusivity groups (non-exclusively breastfed; exclusively breastfed) were evaluated using t-tests for continuous variables and Chi-squared tests for categorical variables. Gestational age was log-transformed for the t-tests.

[3]Of the total group, data were missing on maternal anthropometrics (N = 5), maternal education (N = 9), maternal smoking (N = 98), maternal alcohol use (N = 93), parity (N = 1), breastfeeding duration (N = 141), and introduction of solid foods (N = 153).

* P < 0.05 and ** P < 0.01



CHAPTER 5.3

INFANT DIET, BODY FAT DISTRIBUTION AND CARDIOMETABOLIC RISK FACTORS IN CHILDHOOD

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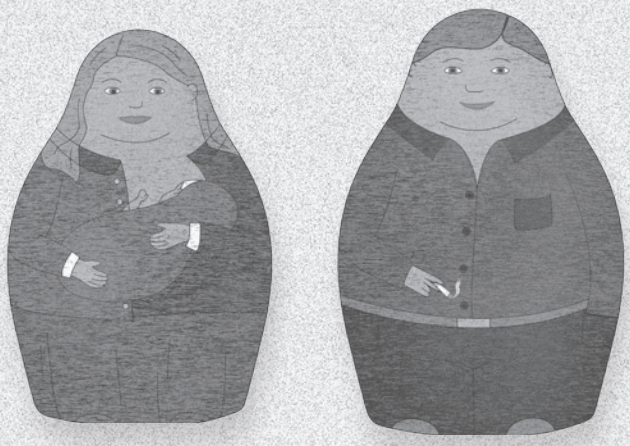
Submitted



CHAPTER 6

General discussion

INTRODUCTION

The World Health Organization defines childhood overweight and obesity as abnormal or excessive accumulation of adipose tissue, which is an established risk factor for harmful health.[1] Currently, overweight and obesity are the fifth leading cause of global deaths.[1] The dramatic increase in the worldwide prevalence of overweight and obesity might be designated as a 'global epidemic'. In the Netherlands, the prevalence of childhood overweight and obesity is fluctuating, but has an overall increasing trend.[2,3] Also, the prevalence of cardiometabolic risk factors associated with overweight and obesity is increasing in children.[4,5]

Childhood overweight and obesity are important risk factors for overweight and obesity in adulthood. The concept of persistence of overweight over time is often referred to as 'tracking'.[6,7] Tracking is the phenomenon that children keep their body mass index (BMI) position in the population distribution from childhood to adulthood.[8,9] Several studies have demonstrated that overweight in childhood tracks into adulthood.[7] It has also been suggested that clustered cardiometabolic risk factors associated with overweight and obesity track from childhood into adolescence.[10]

A growing body of research suggests that overweight and cardiometabolic diseases at least partly originate in intrauterine life. The *developmental origins of health and disease (DOHaD)* hypothesis proposes that the developing fetus responds to suboptimal conditions during critical periods of cellular proliferation and differentiation by enabling structural and functional adaptations in cells, tissues and organ systems.[11,12] These changes may have long-term consequences for body composition, and cardiovascular and metabolic dysfunction and disease in later life.[11]

Several environmental and behavioral factors during the intrauterine period have been identified that might lead to overweight, obesity and cardiometabolic dysfunction.[11,12] Fetal and early infant growth patterns seem to be strongly associated with later risk of obesity and cardiometabolic diseases.[13] However, the specific fetal and infant growth patterns that might contribute to an adverse body fat distribution in children are not well-known.

The majority of studies assessing childhood adiposity used BMI as outcome measure. However, BMI lacks good accuracy and reproducibility.[14,15] A high BMI is difficult to interpret when the relative proportions of fat, muscle, bone and organ mass are changing, especially during childhood and adolescence.[16]

Previous studies suggested that body fat distribution rather than BMI is related to the risks of cardiovascular and metabolic diseases.[16,17] Overall, the cardiometabolic risks seem to be mainly related to the visceral fat tissue compartments, while subcutaneous fat tissue plays a controversial role.[14,19] Most studies focused on total body and abdominal

fat distribution have been performed in adults. Not much is known about adipose tissue development and fat distribution in children.

From these perspectives, we can conclude that it is important to gain more knowledge on the associations of early life factors with detailed measures of body fat distribution and cardiometabolic risk factors in childhood. The key objectives for this thesis were to examine the associations of repeatedly measured fetal and infant growth patterns, parental factors, and infant diet with childhood body fat distribution and cardiometabolic outcomes. All studies described in this thesis were embedded in the Generation R Study, an ongoing prospective population-based cohort study from early fetal life onwards, in the city of Rotterdam, the Netherlands. All pregnant women living in the study area and with a delivery date from April 2002 until January 2006 were eligible for enrollment in the study. In total, 9,778 mothers and 71% of their partners were enrolled.[20]

MAIN FINDINGS, COMPARISON WITH RECENT STUDIES, POSSIBLE MECHANISMS

Early growth

Early growth and overweight in childhood

Fetal and infant growth are recognized as critical for the development of overweight and obesity in later life.[21] Individuals who show poor growth *in utero* are at increased risk for obesity and cardiometabolic diseases in later life.[13] Recent studies suggest that especially the pattern of weight gain after birth is a more key determinant of one's cardiometabolic health. This effect may be stronger in children born with a small size for gestational age (SGA).[13] Adaptations in infant growth patterns are influenced by the drive to compensate for fetal growth restriction or growth acceleration caused by changes in the maternal-uterine environment.[22]

We examined whether fetal growth characteristics are associated with infant growth rates, and whether these infant growth rates influence the risks of overweight and obesity in early childhood. Previous studies suggested that changes in infant growth patterns affect the risk of obesity in later life.[23] From the repeated growth measurements in childhood, we derived longitudinal growth curves and estimated the infant peak weight velocity (PWV), peak height velocity (PHV), and body mass index at peak (BMIAP). We observed that slower fetal weight and height gain between the third trimester and birth were associated with higher PWV and PHV, respectively. In contrast, growth in weight

and height measured at the second trimester of pregnancy was not inversely but positively associated with PWV and PHV, respectively. Body stature and size are known to be a highly heritable trait, with a large genetic component.[24,25] A possible mechanism for the different effects of early and late fetal growth on childhood growth could be that the fetus tends to grow along its growth curve until the second trimester of pregnancy, but that this curve is more susceptible to maternal-uterine environmental factors during the third trimester of pregnancy. In the period after birth, the child may continue along its original genetically determined growth curve, or may deviate by showing compensatory accelerated or decelerated growth as response to decreased or increased, respectively, third trimester fetal growth.

Birth weight itself was strongly associated with a higher BMI at the age of 4 years. Similar associations between birth weight and BMI were demonstrated in children aged 11 years, and in adults aged 19 and 33 years.[26-28] We observed that higher PWV and BMIAP during infancy were associated with increased risks of overweight and obesity at the age of 4 years. Similar results were found by the Northern Finnish Birth Cohort Study 1966 in adults aged 31 years.[29] Also, a study in the UK showed that increased weight velocity in childhood between 1 year and 9 months and 5 years was the most important predictor of BMI in young adulthood.[30] A recent study in 8-year old children suggested that larger size during birth and 24 months, rather than velocity or timing of peak velocity, was related to increased odds of overweight.[31] From these studies, we can conclude that postnatal weight gain patterns are important for predicting overweight and obesity in both childhood and adulthood. Whether these associations are explained by environmental exposures or just reflect common genetic variants remains to be studied.

Early growth and body fat distribution in childhood

Although the positive associations of birth weight with BMI in later life seem to be consistent across studies, not much is known about the associations of birth weight with subsequent body fat distribution outcomes in different stages of later life. Results from previous studies remain inconsistent in both children and adults.[32,33] The differences in results from previous studies may be explained by differences in adjustment for current size or numerous potential confounders, and the variety of methods used to assess body fat distribution.[34-36] Most studies relied on estimates of abdominal fat, such as waist circumference, waist-to-hip ratio and skinfold thickness. The limitation of these estimates is that they depend on both lean and fat mass.[37] Noninvasive methods to perform more direct measurements of total and abdominal body fat distribution have become available by the use of Dual-energy X-ray absorptiometry (DXA) and abdominal ultrasound. DXA quantifies fat content with high precision and has the capacity for regional analysis but

cannot differentiate the amount of the two abdominal fat compartments.[15,38,39] Ultrasound is a reliable method to differentiate between the abdominal visceral and subcutaneous fat compartments.[15] Both DXA and abdominal ultrasound have been validated against CT, which is assumed to be the reference standard for measuring body fat distribution.[14]

A large population-based cohort study in the UK in 6,086 children aged 9 years, suggested that higher birth weight predict higher DXA-derived total fat mass, but not truncal fat mass.[40] In 242 overweight children aged 11 years, birth weight predicted DXA-derived truncal and total fat, but not MRI-derived visceral or subcutaneous fat.[26] In our study in 5,900 children, we observed that birth weight was positively associated with childhood BMI but not with DXA-derived total fat. Results from our study also suggested that fetal growth characteristics from second trimester onwards influence BMI and body fat distribution in childhood. Third trimester abdominal circumference influenced android/gynoid fat ratio in childhood, suggesting that central fat distribution partly origins in fetal life. Infant weight measurements were positively associated with BMI and body fat distribution in childhood, with stronger effect estimates at older ages. We observed that rapid infant growth in weight leads to higher total fat and an adverse body fat distribution reflected as higher android/gynoid fat ratio.

We measured abdominal preperitoneal and subcutaneous fat at the ages of 2 and 6 years. Rapid infant weight gain from birth to 2 years was associated with higher abdominal preperitoneal fat at the age of 2 years. Less consistent associations of rapid fetal and early infant growth rates with abdominal preperitoneal and subcutaneous fat at the age of 6 years were found. Rapid growth in early infant length and weight had stronger effects on total fat than on abdominal fat in childhood. The lack of strong associations may mainly be explained by the relative narrow range of preperitoneal fat in children and a larger measurement error for this measurement.[41,42]

Most children with fetal growth deceleration or children born small for gestational age (SGA) show a rapid catch-up growth during the first year of life.[43] This growth acceleration encompasses growth in height and weight as well as fat mass.[44] Children born SGA may have a similar BMI, but a greater percentage of body fat than those born appropriate for gestational age (AGA), either as young adults or at older ages.[44] Also body fat distribution seems to be affected since poor fetal growth has been related to increased abdominal fat in later life.[44] Studies assessing the associations of fetal growth patterns instead of size at birth, with abdominal fat in later life are scarce. A study in matched pairs showed that SGA children tended to be viscerally adipose at the age of 6 years, even if they are not overweight.[45] It has also been suggested that SGA children had higher total and abdominal fat, measured by DXA and Magnetic Resonance Imaging (MRI), between 2 to 6 years.[46]

The combination of fetal growth deceleration or SGA and infant growth acceleration may lead to higher total and abdominal body fat in childhood and adulthood.[34,43] We

explored the associations of third trimester fetal growth and infant growth with body fat distribution outcomes in childhood. We observed that children with third trimester growth deceleration followed by infant growth acceleration had higher body fat distribution outcomes. This finding is in line with the developmental origins of health and disease (DOHaD) hypothesis and suggests that decelerated fetal growth and rapid infant growth are important for the programming of later body composition. The highest body fat outcomes were observed in children with both third trimester and infant growth acceleration. We also explored the associations of size at birth and infant growth with body fat distribution outcomes. Children born AGA and large for gestational age (LGA) with infant growth acceleration had the highest body fat outcomes in childhood. In children born SGA, infant growth acceleration tended to be associated with an adverse body fat distribution, but these associations were not strong. This might be in line with a study proposing that infant growth acceleration seems to be an endogenous and physiological process in order to restore body size, fat stores and body composition that were altered during fetal life, without negative consequences on insulin metabolism.[47]

Parental factors

Parental anthropometrics, early growth and overweight in early childhood

Parental BMI is strongly associated with BMI in childhood.[48,49] We examined whether and to what extent parental anthropometrics also correlate with corresponding fetal and postnatal anthropometrics until the age of 4 years. We observed that parental anthropometrics were strongly related to fetal and postnatal anthropometrics, with higher effect estimates at older ages. The highest effect estimates were observed for length or height measurements. These findings are in line with studies showing increasing hereditability for height and weight from the second trimester of pregnancy to infancy.[50]

One of the strongest risk factors of childhood obesity is parental BMI.[51] However, it is not well-known whether the associations of parental BMI with childhood BMI are explained by direct intrauterine programming effects, family-shared environmental exposures or genetic influences. Observing stronger effect estimates for the associations of maternal BMI, than paternal BMI with offspring BMI, might suggest direct intrauterine effects according to the developmental overnutrition hypothesis, or a consequence of mother's dominant role in feeding decisions.[52] If the associations are of similar magnitude for both maternal and paternal BMI, environmental and behavioral lifestyle-related factors and genetic factors might be involved in the underlying pathways.[52-54] Recent data from the Health Surveys for England suggested stronger associations of maternal weight than paternal weight with the risk of obesity in 7,078 children between the ages

of 2 and 15 years.[54] A large population-based study in the UK in 4,091 children aged 9 to 11 years compared the associations of maternal and paternal pre-pregnancy BMI with DXA-derived fat mass.[55] Although the maternal-paternal difference was small, effect sizes for maternal associations were larger than for paternal associations. The fat mass and obesity associated (FTO) gene, previously related to type 2 diabetes via an effect on BMI, was shown to have an association with fat mass in their cohort.[55] In a Mendelian Randomization study a genetic variant is used that serves as a proxy for an environmentally modifiable exposure in order to make causal inferences about the outcomes of this modifiable exposure. No effect was observed when maternal FTO, controlling for offspring FTO, was used as instrument to assess any causal association between maternal and offspring adiposity, suggesting that an intrauterine mechanism is unlikely.[55] A cohort study in the UK in 4,654 children aged 7.5 years found similar associations for maternal and paternal BMI in relation to offspring BMI, indicating similar contributions of genetic or environmental factors to BMI of the offspring.[56] Similar parental BMI associations with BMI in offspring aged 3 years were also reported by a study conducted in Norway in 29,216 children.[53] Contrary, one study suggested that paternal and not maternal BMI is a independent contributing factor to early childhood BMI and that the association of paternal BMI with childhood BMI gets stronger with increasing age of the children, whereas the association of maternal BMI with childhood BMI stays more stable.[53,57] Thus, potential differences in parental-offspring associations for BMI might appear at older ages. Other explanations for differences in results between studies may be due to varying reference groups and outcomes (BMI or overweight/obesity), different confounding variables and sample sizes, varying ages of the studied population and the used statistical methods to test differences in maternal and paternal associations.[53,54] Also, the use of self-reported BMI data and undeclared non-paternity might have biased the results.[54]

We tested the associations of maternal and paternal BMI with longitudinally measured childhood BMI until the age of 4 years. We observed that maternal BMI had overall a stronger effect on BMI during early childhood than paternal BMI. This effect was already present at early infant ages and might indicate a direct intrauterine effect at least in early childhood, or mother's prominent role in infant feeding practices. The associations of maternal height and weight with corresponding measures in the offspring in fetal life were also higher than for paternal height and weight. We observed that both maternal and paternal overweight and obesity were associated with an increased risk of childhood overweight. The highest risk was observed in children with two obese parents. Parental obesity might also create and sustain on obesogenic environment for their offspring besides specific genes or intrauterine factors that are involved.[58-60]

Gestational weight gain, early growth and overweight in early childhood

Gestational weight gain (GWG) includes growth of the fetus, placenta and uterus, amniotic fluid, maternal plasma volume and maternal adipose tissue.[61] An emerging body of evidence suggests that excessive GWG is associated with higher birth weight and body fat, and an increased risk of obesity in children and young adults.[62] In line, we observed that excessive GWG is associated with an increased risk of LGA, and an increased risk of overweight at the age of 4 years. The mechanisms underlying these associations are not well-known. GWG might predispose the mother and fetus to increased concentrations of leptin and insulin, which can affect neurodevelopment in the hypothalamus leading to a permanent influence on energy balance.[63] It appears that these processes occur in early pregnancy and that such processes are sensitive to maternal nutrition.[64] GWG in early pregnancy is mainly due to expansion and deposition of maternal tissues.[65] Accordingly, we and other recent studies observed that GWG in only early and mid-pregnancy was associated with childhood overweight.[64,65]

Recent studies are increasingly revealing that the effects of GWG on offspring BMI and risks of overweight and obesity are strongest in underweight or normal weight mothers.[66,67] Our findings were in line with these studies. The results of a large sibling study by Lawlor *et al.*[67] were partly in line by suggesting that shared familial (genetic and environmental) factors explain the associations of GWG in normal weight mothers, whereas intrauterine mechanisms might contribute to the associations of GWG in overweight and obese mothers.

Parental smoking during pregnancy, early growth and overweight in early childhood

Cigarette smoke contains more than 47,000 toxic substances. It is even reported that each puff of cigarette smoke contains about the 10^{17} oxidant molecules.[68,69] Nicotine fully crosses the placenta during pregnancy resulting in fetal concentrations that are generally 15% higher than maternal concentrations.[70] Fetal smoke exposure is an important risk factors for adverse pregnancy outcomes such as intrauterine growth restriction, preterm birth and low birth weight.[71,72] Children of mothers who smoked during pregnancy have also increased risks of overweight and obesity in childhood and adulthood.[73] A systematic review by Oken *et al.*[73] suggested that prenatal smoke exposure led to a 50% increased risk of overweight in offspring aged 3 to 33. Also in our study, we showed that children of mothers who smoked during pregnancy had an increased risk of obesity at the ages of 4 and 6 years. The strongest association was observed at the age of 4 years. Very few studies have assessed the consequences of fetal smoke exposure in different

periods of pregnancy. In line with previous studies, we observed that continued smoking during pregnancy, and not first trimester only smoking was associated with an increased risk of obesity.[74,75] In addition, continued smoking during pregnancy led to persistently smaller height from fetal life until 4 years and lower weight until 3 months - that tended to be higher thereafter - which resulted in a higher BMI from 3 months onwards. First trimester only smoking did not affect fetal and postnatal growth in the offspring. It could be that first trimester only smoking does not expose the fetus to enough smoke exposure to form a risk of overweight: there might be a gradual relationship.[75] It is also possible that mothers who stopped smoking when there pregnancy was acknowledged, are more health-conscious than mothers who continued smoking during pregnancy.[74]

Previous studies suggested that the observed associations between maternal smoking during pregnancy and childhood obesity were not affected by adjusting for several lifestyle-related confounders. Even after additional adjustment for child's diet, sedentary habits and physical activity, the associations remained.[74] However, given the design of observational studies residual confounding cannot be ruled out. To overcome this limitation, we assessed the associations of paternal smoking during pregnancy with early childhood growth and the risk of obesity, and compared this with the associations of maternal smoking. In line with other studies, we did not observe consistent associations between paternal smoking during pregnancy and these outcomes, which might be suggestive for an intrauterine effect of maternal smoking during pregnancy on early growth and the risk of obesity.[76,77] Exact mechanisms are difficult to provide as cigarettes contain several toxins other than nicotine.[74] The observed associations are possibly explained by a combination of factors: placental dysfunction by nicotine induced vasoconstriction, increased production of catecholamines in infancy, altered appetite control in the central nervous system or altered fetal regulation of leptin and ghrelin responsive pathways, and nicotine induced infant catch-up growth.[74,78,79] Further research is needed to reveal the exact mechanisms.

Parental smoking during pregnancy and body fat distribution in childhood

Epidemiological studies are increasingly demonstrating that maternal smoking during pregnancy is associated with higher body fat distribution outcomes in the offspring.[79-81] A study in newborns from smoking mothers during pregnancy has shown that lean body mass appears to be more affected than subcutaneous body fat measured as skinfold thickness.[82] We also did not observe associations of maternal smoking during pregnancy with peripheral, central and total subcutaneous fat mass in infants aged 1.5, 6 and 24 months. As another study found an association of maternal smoking during pregnancy

with subcutaneous fat mass in children aged 5 years, it seems that a potential effect of maternal smoke exposure on offspring's subcutaneous body fat appears later in childhood.[83]

We observed that children of mothers who continued smoking during pregnancy had a higher BMI and abdominal subcutaneous fat at the age of 6 years, compared to mothers who never smoked. This is partly in line with a study in pubertal children, in which maternal smoking during pregnancy was associated with higher abdominal subcutaneous and visceral fat, measured by MRI.[84] A possible explanation why we did not find an association between continued maternal smoking during pregnancy and preperitoneal fat, might be that there is little intra-abdominal fat proportional to total body fat in children when compared to adolescents or adults. More of the fat depot in children is subcutaneous fat.[41,85]

We also observed that continued maternal smoking during pregnancy was associated with higher DXA-derived android/gynoid fat ratio, but not with total fat. All associations with body fat distribution outcomes reported in our study were only observed in girls. Stronger associations in girls than in boys have previously been demonstrated in a few other studies, but tests for interactions were not always presented.[79] In the normal physiologically state, girls tend to accrue subcutaneous fat, especially over the thighs and boys accumulate both subcutaneous and visceral fat, mainly in the upper body.[39] These processes do mainly occur in puberty and as we performed our study at prepubertal age, the associations in boys may be appear at older ages. It might also be that girls are more susceptible to the adverse effects of fetal smoke exposure. Further studies are needed to explore the mechanisms underlying these sex differences.

We observed that both maternal and paternal smoking during pregnancy were associated with higher BMI and body fat distribution outcomes, and increased risk of overweight at the age of 6 years. This finding is partly in line with the study by Leary *et al.*[79] in 5,689 children at the age of 10 years, showing that the effect estimates for paternal smoking were only somewhat smaller than those for maternal smoking in relation to DXA-derived total fat. Overall, the effect of paternal smoking should be much lower than for maternal smoking assuming that the effect of paternal smoking is due to passive smoke inhaled by the mother during pregnancy, that the fathers were the biological fathers and partners during pregnancy and that the fathers smoked during pregnancy.[86] The finding that both maternal and paternal smoking during pregnancy increase the risk of higher BMI and adverse body fat distribution in childhood, might therefore suggest that residual confounding by unmeasured family based lifestyle-related factors is more likely than direct intrauterine effects. Given the general habit that children may spend more time with their mothers than their fathers, maternal smoking will be more strongly related to unmeasured confounders than paternal smoking.[79] Mothers who continued smoking during pregnancy might have a unhealthier lifestyle and might be generally less vigilant

about their children's diet and lifestyle.[87] Alternatively, the associations for paternal smoking may reflect the detrimental effects of passive smoke exposure in early childhood.[88] However, thus far there is no consistent evidence that passive smoke exposure causes childhood overweight and an adverse body fat distribution.[86]

The discrepancy in maternal and paternal associations for childhood BMI during the preschool period and the school-age period in our study might be explained by different mechanisms.

Parental smoking during pregnancy and cardiometabolic risk factors in childhood

Maternal smoking during pregnancy has been found to be associated with elevated blood pressure at the ages of 2.5 months, 3 years and 5 years, and with increased insulin resistance at the age of 10 years.[70,,83,89,90] Also, associations between maternal smoking during pregnancy and lower HDL-cholesterol and higher total-to-HDL cholesterol concentrations in 8-year old children were found.[91,92] LDL-cholesterol was significantly higher in 9-10 year old children whose mothers smoked until late pregnancy.[93] In contrast to these studies, we did not observe - after full adjustments - any association of maternal or paternal smoking during pregnancy with blood pressure, and insulin and cholesterol concentrations in children at the age of 6 years. It may be that differences in associations attributed to smoking during pregnancy and cardiometabolic risk factors in the offspring may at least partly due to residual confounding.

Infant diet

Breastfeeding, growth and overweight in early childhood

As infant diet is a key factor in infant growth, infant diet habits have frequently been studied in predicting the risk of later overweight.[94] A large number of studies indicated that breastfeeding protects against the development of later overweight and obesity, but results are inconsistent.[95,96] Differences in results from studies may be explained by differences in age, confounding variables, study size, classification and definition of breastfeeding, and the studied population. Results from meta-analyses and systematic reviews suggest that breastfeeding is weakly associated with a lower risk of overweight and obesity.[94,95] A cluster-randomized trial of a breastfeeding promotion intervention found no effect on BMI at the age of 6.5 years.[96] Therefore, reported associations of breastfeeding with childhood BMI may be biased by confounding, reverse causality or publication bias.[97] Recently, two studies reported that breastfeeding reduces the upper tail of the BMI distribution and tends to increase the lower tail, while having a null effect

on subjects near the mean.[98,99] Consequently, it might be that breastfeeding may shift individual BMI to the mean and prevent overweight, but also underweight. Similar findings were suggested in 9,698 children aged 4.5 to 7.3 years in Germany by using quantile regression.[100]

In our study, we observed that breastfeeding duration and exclusivity were not consistently associated with the risks of overweight and obesity in infants in the first 3 years. It is possible that a potential effect of breastfeeding appears at older ages. However, in another study at longer follow-up, we observed that breastfeeding duration and exclusivity were also not associated with BMI in school-age children. The differences in results from our and previous studies, which suggested inverse associations between breastfeeding duration and later BMI, might be explained by extensive adjustments for confounders in our study. It is also possible that the variation in the timing of adiposity rebound at this age might be quite high, and that therefore no associations were observed.

Breastfed infants differ in their growth patterns from formula-fed infants. By 12 months of age, formula-fed infants weight on average 400-600 gram more than the breastfed infants.[101] Differences in susceptibility of the risk of obesity between breastfed and formula-fed infants are possibly mediated by differences in these early growth patterns. Both observational studies and randomized trials support the hypothesis that a rapid infant growth, which can be the result of higher levels of infant nutrition (e.g. nutrient-enriched formula), is related to the risk of obesity.[102] We observed that never breastfed and non-exclusively breastfed infants had an increased gain in age- and sex-adjusted standard deviation scores (SDS) for length, weight and BMI between 3 to 6 months of age. These findings are to some extent in line with the previous statement. We did not observe associations of breastfeeding duration and exclusivity with growth after the age of 6 months.

Breastfeeding and body fat distribution in childhood

In both children and adults, the association of breastfeeding duration with body fat distribution outcomes has been inconsistent.[103] Due to limited accurate measuring techniques, small sample sizes and the nonlinearly change of body composition during the first year of life, studies have reported conflicting results.[101,103] Breastfeeding might program childhood adiposity.[94] Higher plasma concentrations of insulin have been shown in formula-fed infants compared to breastfed infants, which may stimulate fat deposition. Also, breast milk contains several bioactive factors that may modulate inhibitors of adipocyte differentiation, such as adipokines.[94,104] The protein intake of breastfed infants seem to be lower than the intake of formula-fed infants.[104] High intakes of protein, rather than high intakes of energy, may predict early adipose rebound and BMI in childhood.[105]

Behavioral explanations postulate that breastfeeding mothers may response to children's cues indicating satiety or that breastfed children may regulate the milk production of their mother.[105,106]

Previously, a systematic review and meta-analysis showed that formula-fed infants compared to breastfed infants had higher fat-free mass in the first year of life, after which the associations reversed with higher fat mass in formula-fed infants.[101] This switch might indicate that there is a programming effect of infant diet on intermediary metabolism or appetite regulation.[101] Two smaller studies found no association between breastfeeding duration and DXA-derived body fat in children aged 5 years and from 12 months onwards.[107,108] Similarly, a large population-based cohort study in the UK in 4,325 children showed a protective effect of breastfeeding on DXA-derived total body fat at the ages of 9-10 years.[109] However, in line with results from our study, the associations of breastfeeding duration with all body fat outcomes in that study, attenuated after adjustment for confounders. These results are in contrast with a study providing evidence that breastfeeding reduces abdominal visceral and subcutaneous fat mass, measured with MRI, and protects against central fat patterns in children who are at upper percentiles of adiposity measures.[97] The difference in results for abdominal fat between our and the latter study might be explained by differences in abdominal fat measurements. Measurement error might be larger by using ultrasound instead of MRI. We also adjusted our analyses for a wide range of potential confounders. Especially adjustment for maternal education did attenuate the effect estimates to the largest extent. Mothers who do not breastfed their infants might have less healthier family related lifestyle habits.[110] After adjustment for multiple testing, no associations remained significant. The association of breastfeeding with body fat distribution may also be weaker in early childhood and become stronger around the timing of adiposity rebound or thereafter.[105]

In a study in 14,726 2 to 9 year old children from eight European countries, exclusive breastfeeding for 4-6 months was related to lower fat mass, measured as high BMI, waist-to-hip ratio and skinfold thicknesses.[111] We observed that as compared to children who were exclusively breastfed for 4 months, those who were non-exclusively breastfed had a higher central fat mass, measured by skinfold thicknesses, at the age of 24 months. Thus, the association of breastfeeding with body composition in early childhood seems to depend on the age of the child and the method that is being used.

Introduction of solid foods and body fat distribution in childhood

Complementary food is defined by the World Health Organization as any food or liquid given along with breast milk.[112] Possible short-term and long-term effects of early introduction of solid foods concern growth patterns, and the risk of diabetes and adiposity

in later life.[113] Studies have been reporting inconsistent associations between the timing of introduction of solid foods and body fat distribution outcomes. An overall evidence for an independent effect of the timing of introduction of solid foods on body fat in later life is insufficient.[113] The study conducted in eight European countries in 14,726 children mentioned in the previous paragraph, found no association between the timing of introduction of solid foods and adiposity measures such as skinfold thickness and WtHR.[111] In our study, the definition of timing of introduction of solid foods was defined as the age at which a fruit or vegetable snack was given for the first time, independent of whether the infant was breastfed or formula-fed. In line with the previous study, we did not find any association of the timing of introduction of solid foods with skinfold thicknesses at 24 months and body fat distribution outcomes at the age of 6 years. Thus, to date the evidence that the timing of introduction of solid foods is associated with childhood BMI and body fat distribution is not consistent.

Infant diet and cardiometabolic risk factors in childhood

Breastfeeding has been suggested to have protective cardiometabolic effects in addition to the already known health advantages.[4] Yet, the evidence concerning the role of breastfeeding in infancy with the development of cardiometabolic risk factors in children is not consistent.[114] No apparent beneficial effect of breastfeeding on blood pressure was observed after a 6-year follow-up in the PROBIT cohort[96], while two meta-analyses showed a difference of -1.1 and -1.4 mmHg in childhood and adulthood, respectively, for systolic blood pressure in breastfed subjects.[115,116] Similarly, Lawlor *et al.*[114] showed that children who had ever been exclusively breastfed had a reduction in systolic blood pressure in childhood compared to children who had not exclusively been breastfed. The potential mechanisms that underlie the associations of breastfeeding with lower childhood blood pressure may include lower levels of sodium compared to formula or a protective effect of long chain polyunsaturated fatty acids (LC-PUFA) in breast milk.[114] The effect of breastfeeding on cardiovascular function may also be attributed to a better appetite regulation or lower weight gain in infancy.[117] In line with results from the PROBIT cohort, our study showed after full adjustment, that the associations of breastfeeding duration and exclusivity with blood pressure in childhood were attenuated. The only association that remained significant after adjustment for potential confounders was the association of the timing of introduction of solid foods between the ages of 4 and 5 months with systolic and diastolic blood pressure at the age of 6 years. However, after additional adjustment for multiple testing, these associations were not significant anymore.

A meta-analysis showed that as compared to formula-fed infants, breastfed infants have marginally lower serum insulin levels in infancy, although associations were not found in

childhood.[118] A systematic review and meta-analysis revealed that breastfed infants had higher total cholesterol in infancy than those formula-fed.[119] Infant feeding was not associated with total cholesterol in childhood.[119] Breast milk is a source of cholesterol, which might explain why breastfed infants have higher cholesterol levels during the period of breastfeeding only.[120] Our results concerning insulin and cholesterol concentrations are in line with the previous mentioned meta-analyses. The lack of association could be explained by the fact that any beneficial effect of breastfeeding on cardiometabolic health might only become evident at older ages, or that the effect might be confined to specific populations or explained by potential confounders.[102] Overall, our results seem to be suggest that the previously reported associations of infant feeding with cardiometabolic outcomes in childhood may be explained by (residual) confounding. Further studies are needed.

METHODOLOGICAL CONSIDERATIONS

Studies in this thesis were embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards. A prospective design enables to measure a broad set of baseline characteristics, to plan frequent new measurements over time and to examine temporal associations between exposure and outcome. However, there are also some limitations to our studies. In the following paragraphs general methodological considerations will be discussed. These considerations will be related to the study design and assessment of the exposures and outcomes. Types of bias that could have affected the validity of our results are selection bias, information bias and confounding.

Selection bias

Of all children eligible at birth within the study area, 61% participated in the Generation R Study. When the relation between exposure and outcome is different for those who participate and those who were eligible but do not participate, selection bias may occur.[121] This may occur if the decision to participate is associated with sociodemographic factors or health conditions that are related to certain risk factors.[122] In the Generation R Study, nonresponse due to non-participation is not likely to be at random. For example, the percentage of mothers from ethnic minorities and lower educational level or children with medical complications are lower in the group that participated in the Generation R Study than would be expected from the population statistics in Rotterdam.[123] Furthermore, the percentages of children born preterm or with a low birth weight were relatively low. We do not expect that this selection towards a healthier and higher educated study

population affected our study results, since these selection mechanisms were not related to both the exposures and outcomes. However, the selection mechanisms at baseline will probably affect the statistical power and generalizability of our results due to differences in frequency rates in exposures and outcomes. Several studies have shown that effect estimates in association studies are not markedly affected by selective non-participation at baseline in large cohort studies.[124-125] Selection bias in our studies might mainly arise from selective loss to follow-up and selective nonresponse at visits to the research center and questionnaires. In studies regarding postnatal growth, children who were included in the follow-up measurements had a higher birth weight and gestational age than those who were lost to follow-up. Children born with a low birth weight or born preterm have different postnatal growth patterns than children born with a normal birth weight and born term. This selective loss to follow-up towards a healthier population might have biased our results. However, in general, due to the prospective nature of this cohort study, selection on the outcome at baseline is not an issue. Of the children participating in the Generation R Study at the age of 6 years, 90% and 81% participated in the DXA and abdominal ultrasound measurements, respectively. This loss to follow-up would lead to selection bias if the associations of early factors with body fat distribution outcomes differ between those who participate and those who were loss to follow-up. This seems unlikely but cannot be studied. Nonresponse to questionnaires was the main source of missing data in our studies. There are three types of patterns of missing data: missing completely at random (MCAR), missing at random (MAR) and missing not at random (MNAR). MCAR indicates missing data that is not related to any subject characteristic. MAR indicates missing data that is related to subject characteristics measured in the study. MNAR indicates missing data that is related to subject characteristics not measured in the study. In order to reduce the potential risks caused by selective missingness, we applied multiple imputations on the covariates in the majority of the studies within this thesis. Multiple imputations reduce the potential bias and loss of information that may occur in analyses restricted to subjects with complete data ('complete-case analyses'). Complete-case analysis is only valid when data is MCAR, or when data is MAR but not related to the outcome that is studied. Results of a multiple imputation procedure are only valid under the assumption that data is MAR.[126-127] However, the distinction between MAR and MNAR cannot statistically be tested. For the studies included in this thesis, we considered missing data to be random.

Information bias

Information on the exposures and outcomes in the studies described in this thesis was mainly prospectively collected by medical records, physical examinations, ultrasound

examinations, blood samples and parental self-reported questionnaires. Self-reported data are prone to misclassification: when misclassification of the exposure is related to the outcome, bias may occur. Measurement error can be divided into two types: differential and non-differential. Differential measurement error is related to the outcome of interest and can occur when individuals recall information about the exposure differently than individuals without the outcome of interest.[121] This type of error is known as recall bias and occurs mainly when the outcome is measured before or at the same time when information about the exposure is collected.[128] Information about the exposures in our studies, such as anthropometrics, infant diet and prenatal smoke exposure was mainly collected before the outcome measurements. The mothers and children were not aware of the specific research questions addressed in this thesis. Therefore, differential misclassification of the exposures seems unlikely.

Non-differential measurement error is not particularly related to the outcome, but this random misclassification may lead to bias towards null and loss of statistical power.[128] Especially assessment of adverse lifestyle habits may lead to under- or overreporting. For example, mothers might overreport the number of months that they breastfed their infant or introduced solid foods for the first time, while they underreport the number of cigarettes they smoked during pregnancy. To overcome this form of information bias, studies may focus on appropriate biomarkers and metabolites that can serve as objective markers of the exposures of interest. Misclassification of the outcome measurements is also possible. Main outcomes in our studies were fetal and postnatal growth, body fat distribution and cardiometabolic outcomes. Although, growth measurements in the conducted studies were standardized, random measurement error might still have occurred. Other outcomes were either derived from medical records, or measured by well-trained research nurses or standardized devices.

Confounding

Information about many variables related to growth and development was collected in the Generation R Study. Therefore many potential confounders were available for analyses. Confounding effects may be considered as biased effects due to an extraneous factor, which leads to an effect that is mistaken for or mixed with the real effects. A confounder must be related to both exposure and outcome and may not be in the causal pathway from exposure to outcome.[129] The choice whether to consider a factor as a confounder was mainly based on pre-existing knowledge, or a significant change in effect estimates. Although we had information about a wide range of potential confounders, it is still a possibility that we might have missed potential confounders or did not measure potential confounders appropriately. In some cases we did not use particular confounders due to

the insufficient or inappropriate responses based on questionnaires, or when confounders were not yet available. Residual confounding due to unmeasured effects by complex social or behavioral factors might still be possible and might have led to an overestimation of the results. Some of these unmeasured factors might include parental or child diet habits, physical activity levels and specific sedentary habits. In observational studies, residual confounding will always remain a limitation, which might only be addressed by performing randomized controlled trials (RCT) or a Mendelian Randomization (MR) approach. RCTs are not always feasible or ethical for many exposures such as toxins or complex nutritional regimes. MR provides an alternative approach of dealing with potential residual confounding in observational studies. MR is defined as any study that uses genetic variants that serves as a robust proxy for an environmentally modifiable exposure in order to make causal inferences about the outcomes of these modifiable exposures.[130,131]

CONCLUSIONS

Chapter	Conclusions
3	• Fetal growth characteristics strongly influenced infant growth rates. A higher PWV, which generally occurs in the first month after birth, was associated with an increased risk of overweight and obesity at the age of 4 years. • Growth characteristics and patterns in fetal and infant life were associated with body fat distribution outcomes at the age of 2 years. • Fetal and infant anthropometrics were positively associated with body fat distribution outcomes at the age of 6 years. Children with both fetal and infant growth acceleration had the highest body fat outcomes.
4	• Maternal BMI had a significantly stronger effect on childhood BMI than paternal BMI. Gestational weight gain in mothers without overweight and obesity was related to the risk of overweight in early childhood. • Maternal obesity was associated with increased risks of delivering large size for gestational age infants and childhood obesity. Gestational weight gain in early pregnancy was associated with an increased risk of childhood obesity. • Direct intrauterine exposure to smoke until late pregnancy led to different height and weight growth adaptations and increased risks of overweight and obesity in preschool children. • Fetal exposure to cigarette smoke during pregnancy did not influence subcutaneous fat mass in early childhood. • Continued maternal smoking during pregnancy was associated with an adverse body fat distribution in school-age girls, but not with other cardiometabolic risk factors. Similar associations with body fat distribution outcomes were observed for paternal smoking during pregnancy.
5	• Shorter breastfeeding duration and non-exclusive breastfeeding during the first 6 months tended to be associated with increased growth rates for length, weight and body mass index between the ages of 3 to 6 months but not with the risks of overweight and obesity until the age of 3 years. • Shorter breastfeeding duration and non-exclusive breastfeeding did affect subcutaneous fat mass during the first 2 years of life. • Breastfeeding duration and exclusiveness were not consistently associated with body fat distribution outcomes or cardiometabolic risk factors in school-age children. Earlier introduction of solid foods may be associated with higher childhood blood pressure.

FUTURE RESEARCH

Causality

Embedding the studies of this thesis in the large Generation R cohort study enabled us to take account for many potential confounders. However, as in any observational study, the question whether the observed associations are causal or not, is of great interest. Other study designs may be complementary to the current studies to assess the causality. Mendelian Randomization (MR) studies or randomized controlled trials (RCT) may help to establish whether the found associations are causal. MR studies use genetic variants that serve as a robust proxy for an environmentally modifiable exposure in order to make causal inferences about the outcomes of these modifiable exposures.[130,131] The main advantage of this approach is that the association of the genetic variant with the outcome of interest is not confounded. Especially, in studies assessing the associations of maternal smoke exposure during pregnancy with offspring outcomes, it is difficult to demonstrate whether intrauterine effects or residual confounding explain the observed associations. Even when comparing the maternal and paternal associations with the same outcome, this issue remains to some extent. A common genetic variant at chromosome 15q25, which is known to be involved in nicotine metabolism, modifies the associations of maternal smoking during pregnancy with fetal growth characteristics.[132] In mothers who smoked during pregnancy, the T-allele resulted in smaller femur length and lower estimated fetal weight from second trimester onwards. This genetic variant might be used as proxy for fetal smoke exposure in relation to its outcomes. For early life exposures, it is also possible to compare outcomes in siblings who are concordant or discordant for the exposure, which will control for many family-level confounding factors.[133]

Our studies assessing the associations of infant nutrition with body fat distribution and cardiometabolic outcomes are also limited in their causality interpretation due to the observational design of the studies. Results from well-conducted observational studies and trials of infant formula versus breastfeeding with adequate follow-up rates in adult life, will be able to establish whether associations of infant nutrition with body fat distribution and cardiometabolic risk factors are causal. However, it is not feasible to randomize healthy babies into breastfed and non-breastfed groups, whereas interventional studies that randomly assign a breastfeeding promotion would require enormously large sample sizes.[104]

Pathways

Epigenetics

The mechanisms underlying the associations of early life exposures with body fat distribution outcomes in childhood are not known. Future studies are needed to explore these mechanisms. It seems that neither genetics nor environmental factors alone explain the found associations. Epigenetic modifications, such as DNA methylation may affect the transcriptional read-out of the genome.[134] Epigenetic changes can promote the expression of a gene that has normally been silent or silence a gene that is normally active. Studies in animal models suggest that mechanisms underlying the associations of fetal exposures with outcomes in later life include altered epigenetic regulation of DNA methylation and covalent modifications of histones that bind to DNA.[135] These changes may contribute to the increasing prevalences of diabetes, obesity and cardiovascular diseases. The best-characterized epigenetic modification of DNA is the methylation of cytosine residues within CpG dinucleotides. Early nutrition may influence the establishment and maintenance of cytosine methylation.[136] Epigenetic studies assessing the effect of infant and maternal nutrition and gestational weight gain on offspring's cardiometabolic health should be extended. More research needs to be performed to determine whether and which critical periods are involved in these associations.

Detailed measures of body fat distribution

The associations of early growth factors with obesity, body fat distribution and metabolic derangements in adulthood seem to be established, but underlying mechanisms are not completely understood. Ectopic fat depots may contribute to hypotheses that are postulated to explain these associations.[137] Recent studies have been focused on specific fat depots, such as intrahepatic, intrathoracic and pericardial fat, and their associations with cardiovascular and metabolic diseases.[137] Many of these studies have been limited to animal models, but additional research in human subjects would be necessary. Intrathoracic and pericardial fat are for example demonstrated to be related with vascular calcification in adulthood.[138] However, the relative importance of various ectopic fat depots and their contributions to systemic cardiometabolic derangements is not clarified and should be investigated. It is not well-known when in early life intrathoracic and pericardial fat-related derangements occur in children.

Ectopic fat deposition in the liver may help to explain why some children do or do not have metabolic syndrome, as obesity does not sufficiently explain this difference.[139] In children, nonalcoholic fatty liver disease (steathosis) has been associated with obesity,

insulin resistance and hypertriglyceridemia.[140] Metabolic syndrome data in children with steathosis is however limited. A case-control study of overweight and obese children, with and without steathosis, showed that children with biopsy-proved steathosis had higher values of cardiovascular risk factors.[140] It is possible that fat deposition in the liver may play a more important role than obesity itself in determining cardiovascular risk factors. Longitudinal studies are needed to clarify to what extent steathosis in children is associated with longer-term cardiovascular outcomes. Biological mechanisms by which steathosis contributes to cardiovascular risk factors need to be studied. A recent study provided novel evidence by showing that smoke exposure may accelerate development of experimental steathosis.[141] Future studies should investigate the clinical relevance of these findings.

Associations of abdominal visceral and subcutaneous fat compartments with cardiometabolic health are widely addressed in both children and adults.[137] The mechanisms by which these fat compartments are causally related to cardiometabolic diseases are not fully understood. Studies have high-lightened the endocrine activity of adipose tissue, which is exerted through the synthesis and secretion of a wide variety of peptides and adipokines. Adipokines might play a role in the regulation of lipids and carbohydrates metabolism, appetite and energy expenditure.[142] Several experimental and human studies assessed the dynamics and effects of adipokines in obese children and adults. A dysregulation of adipokines might be implicated in obesity, type 2 diabetes and cardiovascular diseases.[143] Findings were not always consistent or sufficiently assessed. Further research is needed for a better understanding of the regulation of adipokines.

Implications

It seems that early life factors might influence the risk of overweight, obesity and body fat distribution in childhood. These associations may be important for the identification of high-risk children and to develop adapted preventive strategies or interventions in very early stages of life. Ethnic groups vary in their body composition and may therefore differ in cardiometabolic risks.[144] Probably, both genetic and lifestyle factors are involved in these differences. Identifying high-risk children may help to improve current screening practices. Specific interventions in order to promote infant weight gain by increasing caloric or protein intake should be revised based on our studies linking infant growth acceleration, independent of fetal growth, to increased adiposity in childhood.

Parental anthropometrics may be an essential target for childhood adiposity prevention as well, even before getting pregnant. The criteria for optimal gestational weight gain should be defined and used in prenatal care systems. Pregnancy is a period when women are more likely to be motivated to make lifestyle changes. Tobacco-induced morbidity is

extensive and research is needed for a better understanding of the toxic effects of fetal smoke exposure on childhood health. Smoking cessation programs should be improved before pregnancies in both mothers and fathers. Finally, families should be provided information about the benefits of breastfeeding in general.

MAIN CONCLUSION

Results in this thesis suggest that fetal and infant growth patterns and parental exposures are related to the risks of overweight, obesity and body fat distribution in childhood. The observed associations may be small, but may have an impact on the burden of chronic diseases later in life on a population level.

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CHAPTER 7

English summary

Nederlandse samenvatting

SUMMARY OF THE THESIS

A general introduction including background information and the study aims for this thesis are provided in **chapter 1**. The common health consequences of overweight and obesity are harmful and divers. Currently, overweight and obesity are the fifth leading cause of global deaths. On average, the percentage of overweight in children in the Netherlands has been increased with 40% in the last 30 years. Also, the prevalence of cardiometabolic risk factors associated with overweight and obesity is increasing in children. Childhood overweight and obesity are important risk factors for overweight and obesity in adulthood. Several studies have demonstrated that overweight and the related clustered cardiometabolic risk factors in childhood, 'track' into adolescence and adulthood. Tracking means that children keep their body mass index (BMI) position in the population distribution from childhood into adulthood. Childhood overweight is therefore a major health problem with adverse short- and long-term consequences.

Many studies have been performed on overweight or adiposity in childhood. The majority of these studies used BMI as outcome measure. BMI is defined as a person's weight in kilograms divided by the square of his height in meters. A high BMI is not only explained by the amount of body fat and might be difficult to interpret when the relative proportions of fat, muscle, bone and organ mass are changing, especially during childhood and adolescence.

Previous studies suggested that body fat distribution rather than BMI is related to the risks of cardiovascular and metabolic diseases. Most of these studies have been performed in adults and not much is known about adipose tissue development and fat distribution in children.

A growing body of research suggests that overweight and cardiometabolic diseases do not only originate in childhood but also in intrauterine life. The *'Developmental Origins of Health and Disease (DOHaD)'* hypothesis proposes that the developing fetus responds to adverse fetal exposures such as suboptimal nutrition and smoke exposure by enabling structural and functional adaptations in cells, tissues and organ systems in the earliest phase of life. These adaptations may have beneficial effects on the short-term but could also lead to diseases in later life. The DOHaD hypothesis has been supported by several studies that have shown an association between low birth weight and higher risk of cardiovascular disease in later life. A low birth weight is obviously not a causal factor in itself but might be the result of various fetal exposures and growth patterns. The specific fetal and infant growth patterns and exposures that might contribute to diseases in later life are not well-known.

The key objective for this thesis was to examine the associations of several exposures in utero en in early postnatal life, and repeatedly measured fetal and infant growth characteristics with body fat distribution and cardiometabolic outcomes in childhood.

Chapter 2 includes a description of the study design and the methods. All studies described in this thesis were embedded in the Generation R Study, an ongoing prospective population-based cohort study from early fetal life onwards, in the city of Rotterdam, the Netherlands. All pregnant women living in the study area and with a delivery date from April 2002 until January 2006 were eligible for enrollment in the study. In total, 9,778 mothers and 71% of their partners were enrolled. Assessments during pregnancy included physical examinations, questionnaires, fetal ultrasound examinations and blood and urine samples. Postnatal information about the participating children was obtained from hands-on measurements at the routine child health centers and by questionnaires. At the ages of 1.5, 6 and 24 months skinfold thicknesses were measured on the left side of the body at four different sites (biceps, triceps, suprailiacal and subscapular) according to standard procedures in a subgroup of approximately 1,000 children. At the age of 6 years, detailed total and abdominal body fat distribution measurements were performed using Dual-energy- X-ray absorptiometry (DXA) and abdominal ultrasound, respectively. DXA quantifies total and regional body fat content with high precision, low X-ray exposure and short scanning time. Abdominal ultrasound images were obtained using a Linear Array probe according to the method of Suzuki. Other cardiometabolic outcomes were assessed at the same time. Systolic and diastolic blood pressure was measured four times with one-minute intervals using a validated automatic sphygmanometer. Insulin and cholesterol concentrations in blood were determined in the laboratory.

In **chapter 3.1** fetal growth (femur length, estimated fetal weight) and birth characteristics (birth length and weight) were related to infant peak weight velocity (PWV), peak height velocity (PHV) and BMI at adiposity peak (BMIAP). We found that estimated fetal weight measured during the second trimester of pregnancy was positively associated with PWV and BMIAP. Subjects with a smaller weight gain between the third trimester and birth had a higher PWV. Femur length measured during the second trimester was positively associated with PHV and negative with BMIAP. Gradual length gain between the second and third trimester and between the third trimester and birth were associated with higher PHV. Compared to infants in the lowest quintile, the infants in the highest quintile of PWV and BMIAP had strongly increased risks of overweight and obesity at the age of 4 years. These analyses suggest that early growth characteristics during the first year of life are influenced by fetal growth characteristics and related to overweight in childhood.

Chapter 3.2 provides the associations of fetal and postnatal growth characteristics in the second and third trimester of pregnancy, at birth and at the age of 2 years with abdominal fat mass at the age of 2 years. Fetal and birth weight were not associated with abdominal subcutaneous fat mass. Estimated fetal weight in the second trimester of pregnancy was inversely associated with preperitoneal fat mass. Weight gain from birth to

the age of 2 years was positively associated with preperitoneal fat mass measures. These associations remained significant after adjustment for age, sex, breastfeeding and BMI. Positive associations were found between postnatal catch-up growth in weight - even in the normal range of birth weight - and abdominal fat mass measures at the age of 2 years.

In **chapter 3.3** anthropometrics in the second and third trimester of pregnancy, at birth, and until 2 years of age were related to BMI, total fat and android/gynoid fat ratio measured by DXA at the age of 6 years. Anthropometric measures were also related to abdominal preperitoneal and subcutaneous fat measured by ultrasound. Higher third trimester fetal abdominal circumference was associated with a higher BMI and android/gynoid fat ratio, but not with abdominal preperitoneal and subcutaneous fat. Birth weight was positively associated with BMI, but not with body fat distribution. Higher infant weight from 3 months onwards was associated with a higher BMI, total fat, android/gynoid fat ratio, and abdominal preperitoneal and subcutaneous fat at the age of 6 years. These effect estimates were stronger at older ages. Infant growth acceleration was in each stratum of fetal growth associated with higher body fat distribution outcomes, and children with both fetal and infant growth acceleration had the highest body fat outcomes. As compared to children with normal third trimester fetal growth and infant growth, third trimester growth deceleration followed by infant growth acceleration was also associated with higher body fat outcomes.

In summary, the results described in chapter 3 suggest that specific fetal and early postnatal growth patterns influence body fat distribution and the risk of overweight in childhood.

Associations between maternal anthropometrics before and during pregnancy and paternal anthropometrics with fetal and postnatal growth measures and the risk of overweight until the age of 4 years are presented in **chapter 4.1.** Pre-pregnancy maternal and paternal height, weight and BMI were associated with corresponding fetal and postnatal anthropometric measures. Maternal BMI had a significantly stronger effect on childhood BMI than paternal BMI. As compared to children from parents with normal BMI, children from two obese parents had an increased risk of overweight at the age of 4 years. We also observed that maternal gestational weight gain was only in mothers with a normal BMI associated with BMI and the risk of overweight in the children.

In **chapter 4.2** we evaluated the associations of maternal obesity and excessive gestational weight gain with the risks of maternal pregnancy complications, delivery and birth complications and overweight in the offspring. As compared to normal weight mothers, maternal obesity was associated with increased risks of gestational hypertension, pre-eclampsia, gestational diabetes, Caesarean delivery, premature rupture of membranes, preterm birth, delivering large size for gestational age infants, and childhood obesity at

the age of 4 years. Weaker associations of excessive gestational weight gain with maternal, fetal and childhood outcomes were observed, with the strongest effects for first-trimester weight gain. Thus, maternal overweight has major consequences for several complications in both mothers and their infants.

In **chapter 4.3** we assessed the associations of active parental smoking during pregnancy with early growth characteristics and the risks of childhood overweight and obesity at the age of 4 years. We showed that in comparison with children from nonsmoking mothers, children from mothers who continued smoking during pregnancy had persistently smaller head circumferences and heights until the age of 4 years, whereas their weights were only lower until the age of 3 months. The smaller length and normal weight led to an increased BMI and an increased risk of obesity at the age of 4 years. We observed that paternal smoking during pregnancy was not associated with postnatal growth characteristics or risk of obesity in offspring.

In **chapter 4.4** we assessed the associations of active maternal smoking during pregnancy with subcutaneous fat mass measured as peripheral, central and total skinfold thickness at the ages of 1.5, 6 and 24 months. We did not observe differences in peripheral, central and total subcutaneous fat mass between the offspring of nonsmoking mothers, mothers who smoked in first trimester only and mothers who continued smoking during pregnancy. Also, the reported number of cigarettes smoked by mothers in both first and third trimester of pregnancy was not associated with peripheral, central and total subcutaneous fat mass in the offspring.

Chapter 4.5 describes whether parental smoking during pregnancy was related to BMI, total fat and android/gynoid fat ratio at the age of 6 years. We also examined whether parental smoking was related to abdominal preperitoneal and subcutaneous fat, blood pressure, and insulin and cholesterol concentrations in blood. We observed that, in comparison with children of nonsmoking mothers, children of mothers who continued smoking during pregnancy had a higher BMI, android/gynoid fat ratio and abdominal subcutaneous fat. These associations were only significant in girls. Similar associations with body fat distribution outcomes were observed for paternal smoking during pregnancy. Both maternal and paternal smoking during pregnancy were associated with an increased risk of childhood overweight. Parental smoking during pregnancy was not associated with childhood blood pressure and insulin and cholesterol concentrations.

In summary, the results described in chapter 4 suggest that weight, height and BMI of parents are related to childhood growth and the risk of overweight. Parental smoking appears to increase the risk of overweight and an adverse body fat distribution in childhood.

Chapter 5.1 describes the results regarding the associations of breastfeeding duration and exclusiveness with early postnatal growth rates and the risks of overweight and obesity until the age of 3 years. We observed no associations between breastfeeding duration and exclusivity and growth rates before the age of 3 months. Shorter breastfeeding duration was associated with an increased gain in length, weight and BMI, adjusted for age and sex, between 3 to 6 months of age. Similar tendencies were observed for the associations of breastfeeding exclusivity with change in length, weight and BMI. Breastfeeding duration and exclusivity were not consistently associated with the risks of overweight and obesity at the ages of 1, 2 and 3 years.

In **chapter 5.2** we assessed whether the duration and exclusiveness of breastfeeding, and the timing of introduction of solid foods were associated with peripheral, central and total subcutaneous fat mass at the ages of 1.5, 6 and 24 months. Breastfeeding duration was not associated with subcutaneous fat mass at the age of 1.5 months. Shorter breastfeeding was associated with a higher peripheral and total subcutaneous fat mass at the age of 6 months, but not at the age of 24 months. As compared to children who were exclusively breastfed for 4 months, those who were non-exclusively breastfed had a higher central fat mass at the age of 24 months. The timing of introduction of solid foods was not associated with subcutaneous fat mass. Therefore, breastfeeding seems to have temporary effects on subcutaneous fat mass measured as skinfold thickness.

We performed further research on this study and in **chapter 5.3,** we assessed the associations of breastfeeding duration and exclusiveness, and the timing of introduction of solid foods with BMI, total fat and android/gynoid fat ratio at the age of 6 years. We also assessed the associations with abdominal preperitoneal and subcutaneous fat, blood pressure, and insulin and cholesterol concentrations. After additional adjustment for maternal and infant confounders, introduction of solid foods between the ages of 4 and 5 months tended to be associated with a higher systolic and diastolic blood pressure, as compared to introduction after the age of 5 months. All other associations did attenuate into not significant. Infant feeding habits were not consistently associated with BMI, and insulin and cholesterol concentrations at the age of 6 years. After correction for multiple testing, none of the adjusted associations remained significant.

In summary, the results described in chapter 5 suggest that breastfeeding and the timing of introduction of solid foods in the first year of life do not strongly influence growth and body fat distribution and the risk of overweight in childhood.

Finally, in **chapter 6** a general discussion has been included regarding the observed associations in this thesis. This chapter also provides recommendations for future research and policy.

SAMENVATTING VAN HET PROEFSCHRIFT

Een algemene inleiding inclusief achtergrondinformatie en de doelstellingen voor dit proefschrift zijn opgenomen in **hoofdstuk 1**. In het algemeen kan gesteld worden dat de gevolgen van overgewicht en obesitas uiteenlopend en zeer schadelijk zijn voor de gezondheid. Op dit moment zijn overgewicht en obesitas de vijfde grootste oorzaak van sterfgevallen wereldwijd. Het aantal kinderen met overgewicht is in Nederland in de afgelopen 30 jaar met gemiddeld 40% gestegen. Ook de prevalentie van cardiometabole risicofactoren, die geassocieerd zijn met overgewicht en obesitas, neemt toe bij kinderen. Kinderen met overgewicht en obesitas hebben een verhoogde kans op het ontwikkelen van overgewicht en obesitas op de volwassen leeftijd. Diverse studies hebben aangetoond dat overgewicht en de daarmee geclusterde cardiometabole risicofactoren op de kinderleeftijd, 'tracking' vertonen tot in de adolescentie en volwassenheid. Tracking is het verschijnsel dat kinderen die bijvoorbeeld een relatief hoge body mass index (BMI) hebben in vergelijking met leeftijdsgenoten, een grote kans hebben om ook op oudere leeftijd een hoge BMI te hebben. Overgewicht op de kinderleeftijd is dus een belangrijk gezondheidsprobleem met nadelige consequenties op de korte en lange termijn.

Er wordt veel onderzoek naar overgewicht of adipositas bij kinderen verricht. In de meeste studies wordt als uitkomstmaat voor adipositas de BMI gebruikt. BMI wordt gedefinieerd als het gewicht van een persoon in kilogrammen gedeeld door het kwadraat van zijn lengte in meters. Een hoge BMI wordt dus niet alleen verklaard door de hoeveelheid lichaamsvet en kan moeilijk te interpreteren zijn wanneer de relatieve verhouding van vet, spieren, botten en orgaanmassa verandert, zoals op de kinderleeftijd en in de adolescentie gebeurt.

Eerder verrichte onderzoeken suggereren dat de lichaamsvetverdeling en niet zozeer BMI gerelateerd is aan het risico op cardiovasculaire en metabole ziekten. De meeste studies zijn uitgevoerd bij volwassenen, waardoor niet veel bekend is over de ontwikkeling van vetweefsel en vetverdeling bij kinderen.

Steeds meer onderzoek suggereert dat niet alleen de kinderleeftijd maar ook de intrauteriene periode van belang is voor het latere risico op overgewicht en cardiometabole ziekten. De '*Developmental Origins of Health and Disease' (DOHaD)* hypothese suggereert dat ongunstige foetale blootstellingen, zoals suboptimale voeding of roken door moeder kan leiden tot adaptatie mechanismen die de structuur en functie van verschillende orgaansystemen in de vroegste fase van het leven beïnvloeden. Deze adaptatie mechanismen kunnen gunstig zijn op de korte termijn, maar op latere leeftijd tot ziekten leiden. De DOHaD hypothese wordt ondersteund door onderzoek dat laat zien dat kinderen met een laag geboortegewicht op latere leeftijd een hoger risico hebben op de ontwikkeling van hart- en vaatziekten. Een laag geboortegewicht is uiteraard niet een causale factor op

zich, maar is het resultaat van verschillende foetale blootstellingen en groeipatronen. Er is nog onvoldoende bekend over de specifieke groeipatronen en blootstellingen in het vroege leven die leiden tot ziekten op latere leeftijd.

Het belangrijkste doel van dit proefschrift was om de relaties te onderzoeken tussen verschillende blootstellingen in de baarmoeder en in de vroege postnatale periode, herhaaldelijk gemeten foetale en postnatale groeikenmerken en de lichaamsvetverdeling en cardiometabole uitkomsten bij kinderen.

Hoofdstuk 2 bevat een beschrijving van de onderzoeksopzet en de methoden. Alle studies beschreven in dit proefschrift zijn uitgevoerd binnen de Generation R Studie, een lopend prospectieve cohort studie vanaf de vroege foetale leeftijd in Rotterdam. Alle zwangere vrouwen in het studiegebied en met een bevallingsdatum tussen april 2002 en januari 2006 kwamen in aanmerking voor het onderzoek. In totaal deden 9778 moeders en 71% van hun partners mee. Metingen tijdens de zwangerschap bestonden voornamelijk uit lichamelijke onderzoeken, vragenlijsten, foetale echo-onderzoeken en afname van bloed- en urinemonsters. Postnatale informatie van de deelnemende kinderen werd verkregen uit metingen tijdens de routine bezoeken aan consultatiebureaus en door middel van vragenlijsten. Op de leeftijden van 1.5, 6 en 24 maanden zijn huidplooidikten gemeten aan de linkerkant van het lichaam op vier verschillende plaatsen (biceps, triceps, suprailiacaal en subscapulair) volgens standaardprocedures in een subgroep van ongeveer 1000 kinderen. Op 6-jarige leeftijd is de totale lichaamsvetverdeling en abdominale vetverdeling met behulp van Dual X-ray absorptiometry (DXA) en abdominale echografie gemeten. DXA kwantificeert de inhoud van totaal en regionaal lichaamsvet met hoge precisie, geeft een lage blootstelling aan röntgenstraling en heeft een korte scan tijd. Abdominale echobeelden zijn gemaakt met een lineaire echokop volgens de methode van Suzuki. Andere cardiometabole uitkomsten werden gelijktijdig gemeten. De systolische en diastolische bloeddruk werd vier keer gemeten met intervallen van een minuut, door middel van een gevalideerde automatische sphygmanometer. De concentraties van insuline en cholesterol in het bloed zijn in het laboratorium bepaald.

In **hoofdstuk 3.1** worden foetale groei (femurlengte, geschatte foetale gewicht) en geboortekarakteristieken (geboortelengte en -gewicht) gerelateerd aan piek gewichtssnelheid (PWV), piek lengtesnelheid (PHV) en BMI tijdens adipositas piek (BMIAP). Wij toonden aan dat het geschatte gewicht van de foetus, gemeten tijdens het tweede trimester van de zwangerschap, positief was geassocieerd met PWV en BMIAP. Individuen met een tragere gewichtstoename tussen het derde trimester en de geboorte hadden een hogere PWV. Femurlengte, gemeten tijdens het tweede trimester, was positief geassocieerd met PHV en negatief met BMIAP. Een tragere lengtetoename tussen het tweede en derde trimester en

tussen het derde trimester en de geboorte was geassocieerd met een hogere PHV. Kinderen in het hoogste kwintiel van PWV en BMIAP hadden sterk verhoogde risico's op overgewicht en obesitas op 4-jarige leeftijd vergeleken met kinderen in het laagste kwintiel. Uit deze analysen blijkt dat vroege groeikenmerken tijdens het eerste levensjaar worden beïnvloed door foetale groeikenmerken en gerelateerd zijn aan overgewicht op de kinderleeftijd.

Hoofdstuk 3.2 geeft de associaties weer tussen foetale en postnatale groeikarakteristieken in het tweede en derde trimester van de zwangerschap, bij de geboorte en op 2 jaar, met abdominaal vet dat gemeten is met de echo, op 2-jarige leeftijd. Foetaal gewicht en geboortegewicht waren niet geassocieerd met abdominaal subcutaan vet. Een laag foetaal gewicht in het tweede trimester van de zwangerschap was geassocieerd met verhoogd abdominaal preperitoneaal vet. Gewichtstoename vanaf de geboorte tot de leeftijd van 2 jaar leidde tot verhoogde preperitoneale vetmetingen. Ook als er rekening gehouden werd met leeftijd, geslacht, borstvoeding en BMI bleven deze relaties significant. Een snelle postnatale inhaalgroei in gewicht leidde tot verhoogd abdominaal vet op 2-jarige leeftijd, zelfs in het normale bereik van geboortegewicht. Een snelle vroege postnatale groei lijkt dus te leiden tot relatief meer abdominaal preperitoneaal vet.

In **hoofdstuk 3.3** wordt de antropometrie in het tweede en derde trimester van de zwangerschap, bij de geboorte, en tot de leeftijd van 2 jaar gerelateerd aan BMI, totaal vet en androïde/gynoïde vet ratio gemeten met de DXA op 6-jarige leeftijd. Tevens wordt de antropometrie gerelateerd aan abdominaal preperitoneaal en subcutaan vet gemeten met de echo. Een grotere foetale buikomtrek in het derde trimester was geassocieerd met een hogere BMI en androïde/gynoïde vet ratio, maar niet met abdominaal preperitoneaal en subcutaan vet. Een hoger geboortegewicht leidde tot een hogere BMI, maar niet tot meer lichaamsvet. Het gewicht vanaf 3 maanden was geassocieerd met een hogere BMI, totaal vet, androïde/gynoïde vet ratio en abdominaal preperitoneaal en subcutaan vet op 6-jarige leeftijd. Deze relaties werden sterker naarmate de kinderen ouder werden. Een snelle postnatale inhaalgroei leidde onafhankelijk van foetale groei tot een hogere BMI en vetuitkomsten. Kinderen met zowel een snelle foetale als postnatale inhaalgroei hadden de hoogste uitkomsten. Vergeleken met kinderen met een normale foetale en postnatale groei, hadden kinderen met een foetale groeivertraging in het derde trimester gevolgd door een snelle postnatale inhaalgroei, ook een hogere BMI en vetuitkomsten.

Samenvattend laten de resultaten van hoofdstuk 3 zien dat specifieke foetale en vroege postnatale groeipatronen de lichaamsvetverdeling en de kans op overgewicht op de kinderleeftijd beïnvloeden.

De associaties tussen maternale antropometrie voor en tijdens de zwangerschap en paternale antropometrie, en foetale en postnatale groeikenmerken en het risico op overgewicht tot de leeftijd van 4 jaar worden gepresenteerd in **hoofdstuk 4.1**. Zowel lengte,

gewicht als BMI van moeder en vader voor de zwangerschap waren geassocieerd met de corresponderende foetale en postnatale metingen bij het nageslacht. BMI van moeder had een significant sterker effect op BMI van het kind dan BMI van vader. Kinderen van twee ouders met obesitas hadden een verhoogd risico op overgewicht op 4-jarige leeftijd, vergeleken met kinderen van twee ouders met een normale BMI. We zagen ook dat gewichtstoename tijdens de zwangerschap alleen bij moeders met een normale BMI geassocieerd was met een hogere BMI en het risico op overgewicht bij hun kinderen.

In **hoofdstuk 4.2** onderzochten we de associaties tussen maternale obesitas en overmatige gewichtstoename tijdens de zwangerschap en de risico's op maternale zwangerschapscomplicaties, bevallings- en geboortecomplicaties en overgewicht bij het nageslacht. In vergelijking met moeders met een normaal gewicht, was maternale obesitas geassocieerd met een verhoogd risico op zwangerschapshypertensie, preeclampsie, zwangerschapsdiabetes, een keizersnede, het vroegtijdig breken van de vliezen, vroeggeboorte, hoog geboortegewicht voor de zwangerschapsduur en obesitas bij 4-jarige kinderen. Minder sterke associaties werden gevonden tussen overmatige gewichtstoename tijdens de zwangerschap en maternale, foetale en postnatale uitkomsten. De sterkste effecten werden gevonden voor gewichtstoename in het eerste trimester van de zwangerschap. Overgewicht van de moeder heeft dus grote consequenties voor verschillende complicaties bij zowel moeder als kind.

In **hoofdstuk 4.3** hebben we de associaties onderzocht tussen het actief roken door beide ouders tijdens de zwangerschap, vroege groeikarakteristieken en de risico's op overgewicht en obesitas op 4-jarige leeftijd. We toonden aan, dat in vergelijking met kinderen van niet rokende moeders, kinderen van moeders die rookten tijdens de gehele zwangerschap een persisterend kleinere hoofdomvang en lengte hadden tot de leeftijd van 4 jaar, terwijl hun gewicht alleen lager was tot de leeftijd van 3 maanden. De kleinere lengte en het normale gewicht leidde tot een hogere BMI en een verhoogd risico op obesitas op 4-jarige leeftijd. We zagen dat het roken van vader tijdens de zwangerschap niet geassocieerd was met postnatale groeikarakteristieken of met het risico op obesitas bij het nageslacht.

In **hoofdstuk 4.4** hebben we de associaties tussen het actief roken door moeder tijdens de zwangerschap en subcutane vetmassa onderzocht op de leeftijden van 1.5, 6 en 24 maanden. De subcutane vetmassa was gemeten als perifere, centrale en totale dikte van de huidplooien. We zagen geen verschillen in perifere, centrale en totale subcutane vetmassa tussen het nageslacht van niet rokende moeders, moeders die alleen rookten in het eerste trimester en moeders die rookten tijdens de gehele zwangerschap. Ook was het gerapporteerde aantal sigaretten gerookt door moeders, in zowel het eerste als derde trimester van de zwangerschap, niet geassocieerd met perifere, centrale en totale subcutane vetmassa bij het nageslacht.

In **hoofdstuk 4.5** wordt beschreven of het roken door beide ouders tijdens de zwangerschap gerelateerd was aan BMI, totaal vet en androïde/gynoïde vet ratio op 6-jarige leeftijd. We onderzochten ook of het roken door ouders gerelateerd was aan abdominaal preperitoneaal en subcutaan vet, bloeddruk en de concentraties van insuline en cholesterol in het bloed. We lieten zien dat in vergelijking met kinderen van niet rokende moeders, kinderen van moeders die rookten tijdens de gehele zwangerschap een hogere BMI, androïde/gynoïde vet ratio en abdominaal subcutaan vet hadden. Deze associaties bleken alleen significant te zijn bij meisjes. Er waren soortgelijke effecten te zien voor het roken van vader tijdens de zwangerschap. Het roken tijdens de gehele zwangerschap door zowel moeder als vader was gerelateerd aan een verhoogd risico op overgewicht op 6-jarige leeftijd. Het roken door ouders was niet geassocieerd met bloeddruk en de concentraties van insuline en cholesterol bij kinderen.

Samenvattend laten de resultaten van hoofdstuk 4 zien dat het gewicht, de lengte en BMI van ouders gerelateerd zijn aan de groei van het kind en het risico op overgewicht. Roken door ouders lijkt de kans op overgewicht en een ongunstige lichaamsvetverdeling bij kinderen te vergroten.

Hoofdstuk 5.1 beschrijft de resultaten met betrekking tot de associaties tussen duur en exclusiviteit van borstvoeding en vroege postnatale groei en de risico's op overgewicht en obesitas tot de leeftijd van 3 jaar. We zagen geen relaties tussen borstvoedingsduur en exclusiviteit en groeikenmerken voor de leeftijd van 3 maanden. Een kortere borstvoedingsduur was wel geassocieerd met toename in lengte, gewicht en BMI, gecorrigeerd voor leeftijd en geslacht, op de leeftijd van 3 tot 6 maanden. Vergelijkbare tendensen werden waargenomen voor de associaties tussen exclusiviteit van borstvoeding en verandering in lengte, gewicht en BMI. Borstvoedingsduur en exclusiviteit waren niet consistent geassocieerd met de risico's op overgewicht en obesitas op de leeftijden van 1, 2 en 3 jaar.

In **hoofdstuk 5.2** hebben we onderzocht of de duur en exclusiviteit van borstvoeding, en de leeftijd van introductie van vast voedsel geassocieerd waren met de perifere, centrale en totale subcutane vetmassa op de leeftijden van 1.5, 6 en 24 maanden. De duur van borstvoeding was niet geassocieerd met de subcutane vetmassa op de leeftijd van 1.5 maand. Een kortere borstvoedingsduur was wel gerelateerd aan een hogere perifere en totale subcutane vetmassa op de leeftijd van 6 maanden, maar niet op de leeftijd van 24 maanden. In vergelijking met kinderen die exclusief borstvoeding hebben gehad gedurende 4 maanden, hadden kinderen die niet exclusief borstvoeding hebben gehad een hogere centrale vetmassa op de leeftijd van 24 maanden. Leeftijd van introductie van vast voedsel was niet geassocieerd met subcutane vetmassa. Borstvoeding lijkt dus voorbijgaande effecten te hebben op de subcutane vetmassa, gemeten middels huidplooidikte.

Als vervolgonderzoek op deze studie, hebben we in **hoofdstuk 5.3** de relaties onderzocht tussen borstvoedingsduur en exclusiviteit, en de leeftijd van introductie van vast voedsel met BMI, totaal vet en androïde/gynoïde vet ratio op 6-jarige leeftijd. Tevens hebben we de relaties onderzocht met abdominaal preperitoneaal en subcutaan vet, bloeddruk en de concentraties van insuline en cholesterol in het bloed. Als we rekening hielden met maternale en kind leefstijlfactoren, leek introductie van vast voedsel voor de leeftijd van 5 maanden geassocieerd met een hogere systolische en diastolische bloeddruk, ten opzichte van introductie na de leeftijd van 5 maanden. Voedingsgewoonten waren niet consistent geassocieerd met BMI en de concentraties van insuline en cholesterol bij kinderen op 6-jarige leeftijd. Na correctie voor multiple testing verdwenen alle significante associaties.

Samenvattend laten de resultaten van hoofdstuk 5 zien dat borstvoeding en de leeftijd van introductie van vast voedsel in het eerste levensjaar geen groot effect hebben op de groei en lichaamsvetverdeling en het risico op overgewicht bij kinderen.

Tenslotte is in **hoofdstuk 6** een algemene discussie opgenomen over de bevindingen die zijn gedaan in dit proefschrift. Dit hoofdstuk bevat ook aanbevelingen voor toekomstig onderzoek en beleid.

CHAPTER 8

List of publications

Affiliations & authors

About the author

PhD portfolio

Dankwoord

LIST OF PUBLICATIONS

1. Mook-Kanamori DO, Holzhauer S, Hollestein LM, **Durmuş B**, Manniesing R, Koek M, Boehm G, van der Beek EM, Hofman A, Witteman JC, Lequin MH, Jaddoe VWV. Abdominal fat in children measured by ultrasound and computed tomography. *Ultrasound Med Biol* 2009;35(12):1938-46.

2. **Durmuş B**, Mook-Kanamori DO, Holzhauer S, van der Beek EM, Boehm G, Hofman A, Steegers EAP, Jaddoe VWV. Growth in fetal life and infancy is associated with abdominal adiposity at the age of 2 years: The Generation R Study. *Clin Endocrinol* 2010;72(5):633–640.

3. **Durmuş B**, Ay L, Hokken-Koelega AC, Raat H, Hofman A, Steegers EAP, Jaddoe VWV. Maternal smoking and subcutaneous fat mass in early childhood: The Generation R Study. *Eur J of Epidemiol* 2011;26(4):295-304.

4. **Durmuş B**, van Rossem L, Duijts L, Arends LR, Raat H, Hofman A, Steegers EAP, Jaddoe VWV. Breastfeeding and growth in children until the age of 3 years: The Generation R Study. *Br J of Nutrition* 2011;105(11):1704-11.

5. **Durmuş B**, Kruithof CJ, Gillman MW, Willemsen SP, Hofman A, Raat H, Eilers PHC, Steegers EAP, Jaddoe VWV. Parental smoking during pregnancy, early growth and the risk of obesity in preschool children: the Generation R Study. *Am J Clin Nutr* 2011;94(1):164-71.

6. Mook-Kanamori DO, **Durmuş B**, Sovio U, Hofman A, Raat H, Steegers EAP, Järvelin MR, Jaddoe VWV. Fetal and infant growth and the risk of obesity during early childhood. The Generation R Study. *Eur J Endocrinol* 2011;165(4):623-30.

7. **Durmuş B**, Ay L, Hokken- Koelega AC, Raat H, Hofman A, Steegers EAP, Jaddoe VWV. Infant diet and subcutaneous fat mass in early childhood: The Generation R Study. *Eur J Clin Nutr* 2012;66(2):253-60.

8. Heppe DHM, Kiefte-de Jong JC, **Durmuş B**, Moll HA, Raat H, Hofman A, Jaddoe VWV. Parental, fetal and infant risk factors for pre-school overweight. The Generation R Study. *Pediatr Res* 2013;73(1):120-7.

In press

9. **Durmuş B**, Arends LR, Ay L, Hokken- Koelega AC, Raat H, Hofman A, Steegers EAP, Jaddoe VWV. Parental anthropometrics, early growth and the risk of obesity in early childhood: The Generation R Study. *Pediatr Obes* 2012: DOI: 10.1111/j.2047-6310.2012.00114.x.

10. Gaillard R, **Durmuş B**, Hofman A, Steegers EAP, Jaddoe VWV. Risk factors and consequences of maternal obesity and excessive weight gain during pregnancy. A population-based prospective cohort study. *Obesity* 2013: DOI: 10.1002/oby.20088.

Submitted

11. **Durmuş B**, Heppe DHM, Taal HR, Manniesing R, Raat H, Steegers EAP, Hofman A, Gaillard R, Jaddoe VWV. Parental smoking during pregnancy, body fat distribution and cardiometabolic risk factors in school-age children. The Generation R Study.

12. **Durmuş B**, Heppe DHM, de Jonge LL, Manniesing R, Abrahamse M, Raat H, de Jongste JC, Hofman A, van der Beek EM, Duijts L, Jaddoe VWV. Infant diet, body fat distribution and cardiometabolic risk factors in school-age children. The Generation R Study.

13. **Durmuş B**, Kruithof CJ, de Jongste JC, Manniesing R, Raat H, Steegers EAP, Hofman A, Jaddoe VWV. Fetal and infant growth and body fat distribution in school-age children. The Generation R Study.

14. Fokkink WJ, Selman MH, Dortland JR, **Durmuş B**, Kuitwaard K, Huizinga R, van Rijs W, Tio-Gillen AP, van Doorn PA, Deelder AM, Wuhrer M, Jacobs BC. IgG Fc N-glycosylation in Guillain-Barre syndrome treated with IV immunoglobulin.

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ABOUT THE AUTHOR

Büşra Durmuş was born on 20th of May 1986 in Eskişehir, Turkey. In 2004 she graduated from secondary school (gymnasium) at the Melanchthon College in Rotterdam. In the same year, she entered medical school at the Erasmus Medical Center, and obtained her bachelor degree in 2008. During these four years she participated in many extra-curricular committees and workgroups. In 2009 she obtained her Master of Science degree in Clinical Research at the Netherlands Institute for Health Sciences in which she enrolled in the second year of medical school. During this Master of Science program she spent a month at the Johns Hopkins School of Medicine in Baltimore, USA, and attended a summer program at the Johns Hopkins Bloomberg School of Public Health. She performed her thesis in the Generation R Study in Rotterdam (2008-2009) and the topic was entitled 'Growth in fetal life and infancy is associated with abdominal adiposity at the age of 2 years'. After finishing her thesis, she started a PhD project in the Generation R Study, under the supervision of Dr. V.W.V. Jaddoe and Prof. dr. A.J. van der Heijden (Department of Pediatrics) and Prof. dr. A. Hofman (Department of Epidemiology) (2009-2010).

In the same year, she started with her internships, which she mainly conducted in the St. Elisabeth Hospital in Tilburg. In the summer of 2012, she graduated from medical school and returned to the Generation R Study to finish her PhD project. In June 2013 she will start as resident (ANIOS) at the Department of Neurology (head: Prof. dr. P.A.E. Sillevis Smitt) at the Erasmus MC.

PhD PORTFOLIO

Name PhD student:	Büşra Durmuş
Erasmus MC Department:	Epidemiology and Pediatrics
Research School:	Netherlands Institute for Health Sciences (NIHES)
PhD period:	March 2009 - March 2010 and Aug 2012 - February 2013
Promotors:	Prof. dr. A. Hofman and Prof. dr. A.J. van der Heijden
Supervisor:	Dr. V.W.V. Jaddoe

PhD training

	Year	Workload (ECTS)
Core and program specific courses		
Master's degree Clinical Research, NIHES, Erasmus University Rotterdam, the Netherlands	2006-2009	
Principles of Research in Medicine and Epidemiology	2006	0.7
Introduction to Data-analysis	2008	1.0
Regression Analysis	2008	1.9
Methods of Clinical Research	2006	0.7
Clinical Trials	2006	0.7
Topics in Meta-analysis	2007	0.7
Pharmaco-epidemiology	2006	0.7
Survival Analysis	2008	1.9
Topics in Evidence-based Medicine	2006	0.7
Case-control studies	2008	0.7
Study design	2006	4.3
Modern Statistical Methods	2008	4.3
Advanced courses		
Introduction to Clinical Research	2007	0.9
Advanced Topics in Decision-making in Medicine	2007	1.9
Intervention Research and Clinical Trial	2007	0.9
Diagnostic Research	2007	0.9
Repeated Measurements in Clinical Studies	2009	1.9
Advanced Topics in Clinical Trials	2009	1.9
Analysis of Time-varying Exposures	2009	0.9
Advanced Analysis of Prognosis Studies	2009	0.9
Prognosis Research	2007	0.9
Research Themes and Methodologies	2007	1.0
Research Seminars 1	2008	3.0
Research Seminars 2	2009	3.0
Johns Hopkins Bloomberg School of Public Health	2008	4.0

General academic skills		
Cursus presenteren, Erasmus University Rotterdam, the Netherlands	2009	1.4
Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC, the Netherlands	2009	0.7
Echocursus, Erasmus MC, the Netherlands	2009	1.0
Symposia and workshops		
Symposium Stichting Kind en Groei 2008: Small for gestational age, Erasmus University Rotterdam, The Netherlands	2008	0.3
Symposium Neuroimaging, Genetics, and Endophenotypes, Arminius Church Rotterdam, The Netherlands	2010	0.3
Lof der Geneeskunst 2012: de biologische klok, de Doelen Rotterdam, The Netherlands	2012	0.3
Workshop networking, Erasmus MC, the Netherlands	2012	0.3
(Inter)national congresses and presentations		
Generation R Research Meeting	2009	0.3
European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), Budapest, Hungary. *2x poster presentation*	2009	1.4
Developmental Origins of Health and Disease (DOHaD), Santiago de Chile, Chili. *1x poster presentation*	2009	1.4
Developmental Origins of Health and Disease (DOHaD), Rotterdam, The Netherlands. *1x oral presentation*	2012	0.7
Reviewed papers		
International Journal of Pediatrics	2011	0.4
International Journal of Obesity	2012	0.4
PLoS ONE	2012	0.4
European Journal of Epidemiology	2012	0.4

DANKWOORD

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