Body Composition in Early Childhood

Parental, Fetal, Postnatal and Genetic determinants of fat, lean and bone mass

The Generation R study

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Lichaamsstelling in de vroege jeugd
Ouderlijke, foetale, postnatale en genetische determinanten van vet-, spier- en bot massa

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Chapter 1

Introduction

INTRODUCTION

Maternal and fetal determinants of adult disease

The prevalence of childhood overweight and obesity has increased dramatically in developed countries over the past two decades. (1, 2) Childhood obesity is associated with short-term morbidity such as asthma and psychological problems and with an increased risk for chronic morbidity and mortality in adulthood. (3, 4) Previous studies have shown that both parental anthropometrics and anthropometrics at birth are associated with obesity in childhood. (5, 6)

Birth weight is strongly associated with perinatal morbidity and mortality. (7) Low birth weight is related to impaired growth and development, and increased mortality in infancy. (8) High birth weight is related to complications during delivery (such as shoulder dystocia and caesarean sections) and to obesity during child- and adulthood. (9, 10) Low birth weight seems also to be associated with diseases in adulthood such as obesity in later life. (11-13)

Determinants of adult disease have been suggested to be: parental anthropometrics, fetal growth and genetics. Until now birth weight was used as a proxy for fetal growth. Although birth weight is the result of fetal growth during pregnancy, different fetal growth patterns may lead to different health consequences.

The *developmental origins of health and disease* hypothesis poses that an adverse fetal environment leads to adaptations that program the fetus' metabolism. These adaptations predispose the individual to increased fat mass and insulin resistance postnatally. (11, 12) (Figure 1) However, studies relating these early life factors with more detailed measures of fat mass are scarce. (5, 14)

Maternal anthropometrics seem to be important determinants of birth weight (5, 15). Maternal height and weight have been related to offspring weight and length at birth. (6) Previous studies have suggested that both pre-pregnancy body mass index and gestational weight gain are positively associated with birth weight in the offspring and are related to risks of both low and high offspring birth weight. (16-22) However, these studies were conducted in small or selected populations and results obtained in larger cohorts were merely based on questionnaire data (10, 19, 23, 24).

Pre-pregnancy body mass index reflects nutritional status, whereas gestational weight gain reflects both nutritional status and tissue expansion. (25) It has been suggested that about 30% of gestational weight gain comprises the fetus, amniotic fluid and the placenta, whereas the remaining 70% comprises uterine and mammary tissue expansion, increased blood volume and fat stores and extracellular fluid. However, most studies have focused on one maternal determinant, so the combined effects of pre-pregnancy anthropometrics and gestational weight gain are not well known.

To our knowledge, no information is available about the effects of maternal anthropometrics on fetal growth in different periods of pregnancy. Adverse exposures might affect early placentation and placental and subsequently fetal growth and development. This may be important as development of the placenta and fetus might have critical periods, in which an adverse fetal

environment might lead to developmental adaptations, which in turn affect both fetal growth and the risk of disease in later life (11, 26).

On the other hand, it was suggested that the associations between low birth weight and adult disease could be explained by genetic variants playing a larger role in susceptibility to insulin during fetal life leading to low birth weight and also leading to a metabolically unhealthy profile. (Figure 1)

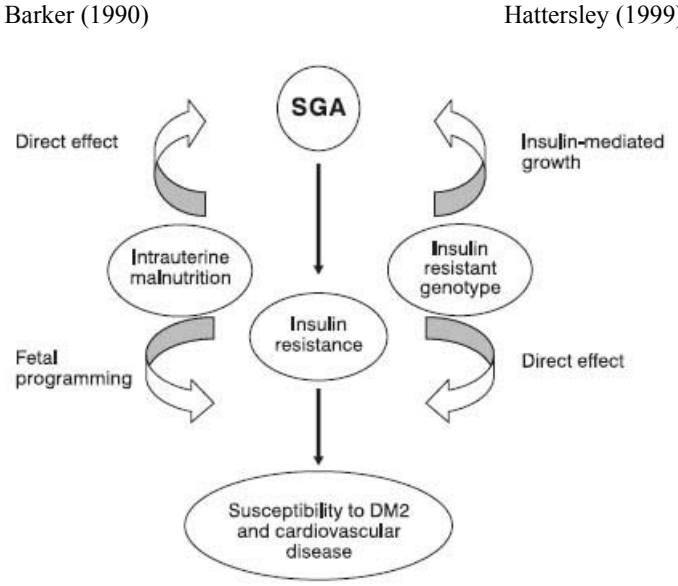

Figure 1. Hypotheses to explain the relation between SGA and metabolic syndrome

Influence of postnatal growth acceleration on determinants of adult disease

More recently, it has been postulated that not birth weight, but postnatal catch-up in weight (growth acceleration) is related to overweight. (Figure 2) Children with catch-up in weight within the first 2 years of life had more fat mass measured by skin folds at the age of 5 years. Also, adults with rapid catch-up in weight in childhood had the greatest risk for cardiovascular disease. (27-29) However, the exact timing of the rapid weight gain that contributes to these long-term risks is unknown. It is also unclear whether this unfavorable body composition is solely due to an excess of fat mass or due to a combination of higher fat mass and diminished lean mass. (30) Previously it was shown that weight gain within 9 months after birth was associated with body fat in girls at the age of ten years old. (31) Others have found that rapid weight gain in the first 3 months of life increases the risk of an unfavorable metabolic profile in adulthood. (32-35)

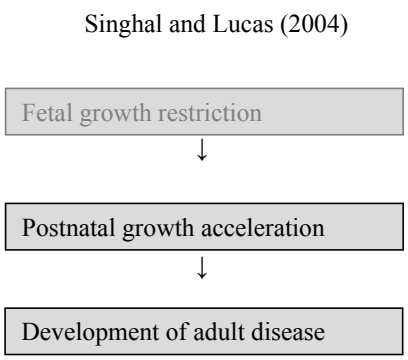

Figure 2. Postnatal growth acceleration hypothesis

Tracking of adiposity in childhood to obesity in adulthood

Childhood obesity tends to track into adulthood, meaning that subjects keep their ranking position in body mass index distribution over time. (36) Obesity in childhood is not only associated with short-term morbidity such as asthma and psychological problems but also with an increased risk for chronic morbidity and mortality in adulthood, as childhood obesity tends to track into adulthood. (3, 4, 36-38)

Tracking of obesity, defined by body mass index (> 95th percentile), has previously been shown from the age of 2 years into adulthood. (39, 40) In a cohort of 474 boys and 448 girls in New Zealand, the correlations between body mass index at the age of 7 years and body mass index at the age of 21 years were 0.61 for boys and 0.52 for girls. (41) In China, 1455 children were measured twice. Of all children with overweight at enrolment 36.8% remained overweight 2 years later. (42) Also, in children from the age of 6-9 years old followed up for 6 years, the body mass index of thin and fat children were more likely to track: 51% and 46% remained in the bottom and upper quartiles, respectively. Overweight children were 2.8 times as likely as other children to become overweight as adolescents. Underweight children were 3.6 times as likely to remain underweight as adolescents. (43) These studies strongly suggest that the risk of development of obesity and its main health consequences are at least partly established in fetal and early postnatal life. However, to our knowledge, no data are present on tracking of adiposity from early infancy into childhood or into adulthood.

Role of Glucocorticoid Receptor (GCR) gene polymorphisms on body composition in childhood

Studies have postulated that genetic factors or epigenetic phenomena may explain the relation between low birth weight and cardiovascular diseases. (11, 12, 27, 44-49) This association might be explained by altered fetal programming of the hypothalamic-pituitary-adrenal (HPA)-axis. (50) Exogenous glucocorticoids (GC) lead to fetal growth retardation and lower birth weight.

Figure 3. Tracking of fat mass from infancy to childhood and beyond

(44, 50) Also, the major systems affected by early life programming are GC sensitive, namely blood pressure and blood glucose/insulin resistance. (44)

As glucocorticoids are important regulators of growth, development and metabolism and their effects are mediated by glucocorticoid receptors, polymorphisms in the glucocorticoid receptor (GCR) gene may contribute to a difference in sensitivity and thereby to associations between growth characteristics in early life and disease in adult life. (51, 52)

The GCR is a member of the nuclear receptor family and is expressed in most fetal tissues from the early embryonic stages. (44, 50) Five different variants in the glucocorticoid receptor gene have been described to be associated with cortisol sensitivity in adults. (53, 54) (Figure 4)

Haplotype	Polymorphism	Nucleotides	Allele frequency, %
0	Wild type	T GG A C A	42.4
1	*Bcl*I	T GG A **G** A	22.7
2	N363S	T GG **G** C A	4.1
3	ER22/23EK + GR9B+ *TthIII* I	**C** **AA** A C **G**	3.1
4	GR9B+ *TthIII* I	**C** GG A C **G**	13.4
5	*TthIII* I+ *Bcl*I	**C** GG A **G** A	14.3

Figure 4. Schematic overview of the Glucocorticoid Receptor Gene polymorphisms

The *Bcl*1 polymorphism is a C→G substitution located in intron 2. (54) The N363S polymorphism is located in codon 363 and causes an AAT→AGT nucleotide change. This change results in an amino acid change from aspargine to serine. The ER22/23EK polymorphism consists of two linked single nucleotide polymorphisms in codons 22 and 23 in exon 2. The alteration at the DNA level is GAG AGG to GAA AAG change, which leads to a glutamic acid-arginine (E-R) to glutamic acid- lysine (E-K) change. (55)

The GR-9β polymorphism is an ATTTA to GTTTA change in exon 9β, resulting in an increased expression and stability of GR-β in vivo. (56-58)

The *Bcl*1 and the N363S polymorphisms are associated with increased sensitivity to GCs, visceral obesity and type 2 diabetes. (54) GR-9β has been associated with decreased GC transrepressive activity, (59) decreased microbial colonization, (60) and with increased inflammatory mediators leading to an increased risk of cardiovascular disease. (56-58, 61) The ER22/23EK polymorphism is associated with a relative GC resistance, a healthier metabolic profile and increased insulin sensitivity; however, it is an uncommon polymorphism in the general population. (55, 62, 63) The *TthIII*I polymorphism was associated with elevated diurnal cortisol levels, but not with any anthropometric or glucose related phenotype in adults. (61, 64)

Previous studies suggest that variants of the glucocorticoid receptor gene may also affect body composition. (62, 65-68) The effects of some GCR gene polymorphisms on risk factors of CVD were different for men and women. (69) Thus far no studies were performed on the effects of the GCR gene polymorphisms on body composition in young children and whether these effects are different in boys and girls.

Role of Glucocorticoid Receptor (GCR) gene polymorphisms on subcutaneous fat mass and overweight in preschool children

It is likely that the Glucocorticoid Receptor Gene polymorphisms are to some extent responsible for the variability in the sensitivity to glucocorticoids. As glucocorticoids are important regulators of many processes involved in fat and glucose metabolism, these polymorphisms in the glucocorticoid receptor gene could lead to intrauterine growth retardation and metabolic and cardiovascular diseases in adulthood. Previous studies examined the potential role of glucocorticoids in the development of adult disease. (70, 71) Genetically established differences between individuals in glucocortcoid sensitivity may also be associated with these diseases. Previous studies have examined the associations of different polymorphisms in the glucocorticoid receptor gene and sensitivity to glucocorticoids. The results of these studies are conflicting. (53, 62, 64-68, 72, 73) Some studies suggest that genetically established differences in glucocortcoid sensitivity are important for various health related outcomes. In addition, it is known that environmental, dietary, and socioeconomic factors also play an important role in determinants of body composition and metabolic. (47) The effect of these GCR gene polymorphisms might be stronger on body composition and overweight in early life because of the limited life style influences.

Pre- and postnatal determinants of bone development

It was suggested that poor growth during fetal life and infancy is also associated with decreased bone mineral density in adulthood. (82-84) Reduced growth during intrauterine and early postnatal life was directly linked with an increased risk of hip fracture 6 to 7 decades later. (84-87) These associations may be explained by an adverse uterine environment, which may affect both early skeletal development and the acquisition of bone mineral density in childhood. (87) Con-

sistent with the programming hypothesis, maternal diet in pregnancy was found to be associated with 'areal' BMD in 9-year-old children in the ALSPAC study. (87) However, this was based on relatively small numbers of subjects as studies in young children are limited.

BMD is the result of the equilibrium between bone formation and bone resorption. Bone mineral acquisition is thought to be associated with genetic and environmental factors. (88, 89) Low bone mineral density (BMD) is associated with a higher risk of fractures. (90) Bone mineral density is different for total body and for lumbar spine. Lumbar spine (LS) mainly consists of trabecular bone, and BMD_{LS} is mostly affected by weight-bearing. The bone of the total body (TB) consists of 80% cortical bone and BMD_{TB} is mostly affected by nutrition and physical activity. (91-95)

Most studies were performed in an elderly population; however, accrual of bone mineral density during childhood is a major determinant of bone mineral density in later life. (84, 96) Bone mineral density increases rapidly during childhood and adolescence. (94, 97, 98) Among adolescents, childhood weight and height was found to be associated with BMD. (99, 100) However, to our knowledge very limited data is available on anthropometrics or pre- and postnatal growth characteristics and BMD development.

Aims of this study

This thesis describes results of 6 studies performed in The Generation R study. A population-based, prospective cohort study from early fetal life onwards. The studies were designed to investigate the associations between maternal anthropometrics before and during pregnancy with fetal growth, body composition and bone mineral density (BMD) in early infancy and to determine whether catch-up in weight during early infancy will influence fat mass and BMD at the age of 6 moths. Also, we wanted to investigate whether the GCR haplotypes are associated with fat mass (FM) in early infancy and whether catch-up in weight modifies the effect of the polymorphism on body composition. Finally, we investigated the development and tracking of fat mass from infancy to early childhood. (Figure 5)

The specific aims of this thesis were to study:

1

The associations of maternal anthropometrics before and during pregnancy with fetal growth measured in different periods of pregnancy and the risks of small and large size for gestational age at birth.

2

The relation of maternal pre-pregnancy BMI, height, blood pressure and smoking during pregnancy with fetal growth restraint, and whether this will lead to a diminished fat mass percentage in early infancy and finally, whether catch-up in weight during early infancy will result in a higher fat mass percentage at the age of 6 months.

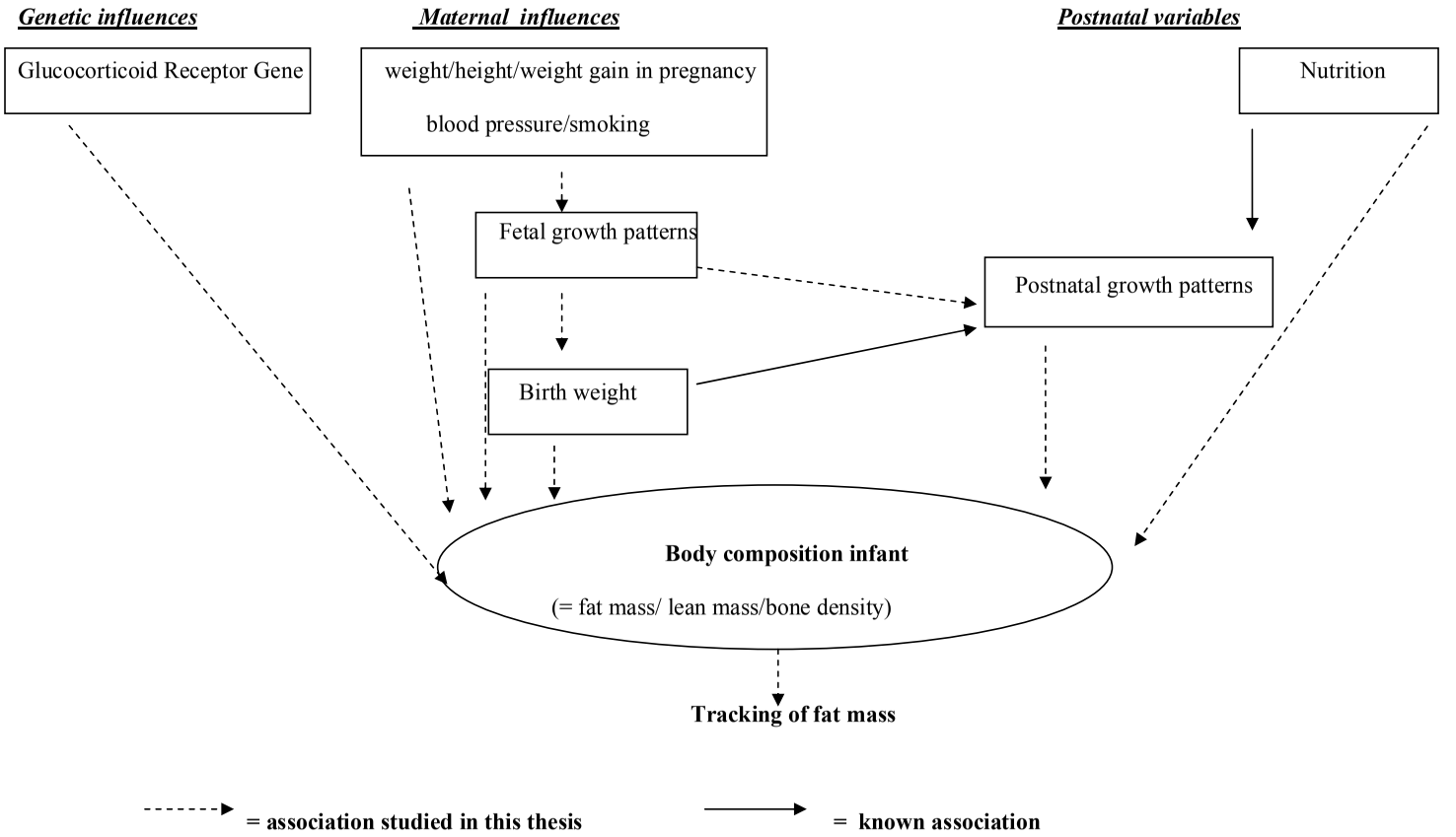

Figure 5. Associations studied in this thesis

3

The development of subcutaneous fat mass, measured by skinfold thickness, in the first 2 years of life. Additionally, the tracking of subcutaneous fat mass from infancy to early childhood and whether parental, fetal and postnatal growth characteristics are associated with subcutaneous fat mass at the age of 2 years.

4

Whether some GCR polymorphisms (*Bcl*1, N363S and the GR-9β polymorphisms) are associated with increased fat mass (FM) in early infancy, the ER22/23EK polymorphism with lower FM and whether the *TthIII*I polymorphism has indeed no effect on fat mass. Additionally, whether there is a critical period in the first year of life in which catch-up in weight changes the effect of the polymorphism on body composition. Also, whether some GCR polymorphisms are related to overweight and obesity in pre-school children.

5

The associations of parental anthropometrics and fetal growth patterns with BMD_{TB} ,BMD_{LS} and BMC, and whether the associations will be different for the bone types. Additionally, whether higher gain in weight and height during early infancy will lead to a higher BMD_{TB} and BMD_{LS} at the age of 6 months.

General design of the Generation R study

The studies in this thesis were embedded in the Generation R study. This a prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and biological determinants of growth, development and health from fetal life until young adulthood. (101, 102)

The cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. (Figure 6) Mothers were informed about the study by health-care workers in pregnancy (midwives, obstetricians). Enrollment in the study was aimed at early pregnancy (gestational age <18 weeks) at a routine fetal ultrasound examination, but was possible until the first month after delivery during routine visits at child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations, and administration of questionnaires, were planned for early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18–25 weeks), and late pregnancy (gestational age >25 weeks). (101) All children were born between April 2002 and January 2006. Detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch mothers and their children from 30 weeks of gestation. This subgroup is ethnically homogeneous to exclude possible confounding or effect modification by ethnicity. Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands. (101) No other exclusion criteria were used.

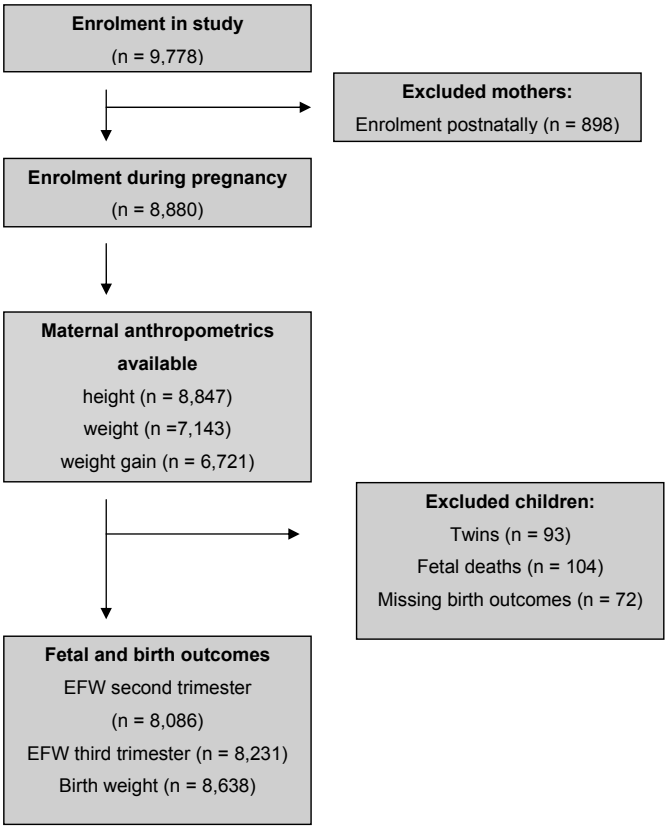

Figure 6. Enrolment and fetal and birth outcomes of the Generation R study

Outline of the thesis

Chapter 1 gives an introduction to the topics described in this thesis. In **Chapter 2** the influences of fetal and parental anthropometrics on birth weight are presented. In **Chapter 3** we report the effects of parental anthropometrics and fetal growth patterns on subcutaneous fat mass and additionally the tracking of subcutaneous fat mass in childhood. **Chapter 4** describes the effect of fetal and fetal growth patterns and the effect of catch up in weight on body composition in early infancy. In **Chapter 5** we report the effects of GCR polymorphisms on body composition. The effects of GCR polymorphisms on fat mass and overweight are reported in **Chapter 6.** In **Chapter 7** we describe the effects of parental anthropometrics and growth patterns on bone mineral density. **Chapter 8** provides a general discussion of the findings and the clinical implications**.** This discussion concludes with implications for future research and clinical practice. **Chapter 9** summarizes the findings in English. Finally, **Chapter 10** provides a summary of the findings in Dutch. **Chapter 11** contains the list of abbreviations and after word.

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Chapter 2

Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth

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ABSTRACT

Objective

We aimed to examine the associations of maternal anthropometrics with fetal weight measured in different periods of pregnancy and with birth outcomes.

Design

Population-based birth cohort study.

Setting

Data of pregnant women and their children in Rotterdam, the Netherlands.

Population

In 8,541 mothers, height, pre-pregnancy body mass index (BMI) and gestational weight gain were available.

Methods

Fetal growth was measured by ultrasound in mid- and late pregnancy. Regression analyses were used to assess the impact of maternal anthropometrics on fetal weight and birth outcomes.

Main outcome measures

Fetal weight and birth outcomes: weight (grams) and the risks of small (<5th percentile) and large (>95th percentile) size for gestational age at birth.

Results

Maternal BMI in pregnancy was positively associated with estimated fetal weight during pregnancy. The effect estimates increased with advancing gestational age. All maternal anthropometrics were positively associated with fetal size (*P*-values for trend <0.01). Mothers with both their pre-pregnancy BMI and gestational weight gain quartile in the lowest and highest quartiles showed the highest risks of having a small and large size for gestational age child at birth, respectively. The effect of pre-pregnancy body mass index was strongly modified by gestational weight gain.

Conclusions

Fetal growth is positively affected by maternal BMI during pregnancy. Maternal height, pre-pregnancy BMI and gestational weight gain are all associated with increased risks of small and large size for gestational age at birth in the offspring, with an increased effect when combined.

INTRODUCTION

Birth weight is strongly associated with perinatal morbidity and mortality. (1) Low birth weight is associated with impaired growth and development, and increased mortality in infancy. (2) Low birth weight seems also to be related to diseases in adulthood. (3) High birth weight is related to complications during delivery (such as shoulder dystocia and caesarean sections) and to obesity during child- and adulthood. (4, 5)

Maternal anthropometrics seem to be important determinants of birth weight. (6, 7) Maternal height and weight have been related to offspring weight and length at birth. (8) Previous studies have suggested that both pre-pregnancy body mass index and gestational weight gain are positively associated with birth weight in the offspring and are related to risks of both low and high offspring birth weight. (9-18) However, these studies were conducted in small or selected populations and results obtained in larger cohorts were merely based on questionnaire data. (5, 11, 19, 20) Pre-pregnancy body mass index reflects nutritional status, whereas gestational weight gain reflects both nutritional status and tissue expansion. (21) It has been suggested that about 30% of gestational weight gain comprises the fetus, amniotic fluid and the placenta, whereas the remaining 70% comprises uterine and mammary tissue expansion, increased blood volume and fat stores and extracellular fluid. Recent studies have shown that several dietary factors and micronutrients are related to birth weight. (22, 23) However, results from recent studies showed conflicting results. The combined effects of pre-pregnancy anthropometrics and gestational weight gain are not well known.

To our knowledge, no information is available about the effects of maternal anthropometrics on fetal growth in different periods of pregnancy. This may be important as development of the placenta and fetus might have critical periods, in which an adverse fetal environment might lead to developmental adaptations, which in turn affect both fetal growth and the risk of disease in later life. (3, 24) Adverse nutritional exposures might also affect early placentation and placental and subsequently fetal growth and development. In a population-based prospective cohort study from early fetal life onwards, we examined the associations of maternal anthropometrics before and during pregnancy with fetal growth measured in different periods of pregnancy and the risks of small and large size for gestational age at birth.

METHODS

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards. This study was designed to identify early environmental and genetic determinants of growth, development, and health in fetal life, child- and adulthood and has been described previously in detail. (25, 26)

Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Mothers were informed about the study by health-care workers in pregnancy (midwives, obstetricians). Enrollment in the study was aimed at early pregnancy (gestational age <18 weeks) at a routine fetal ultrasound examination, but was possible until the first month after delivery during routine visits at child health centers. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations, and administration of questionnaires, were planned for early pregnancy (gestational age <18 weeks), mid-pregnancy (gestational age 18–25 weeks), and late pregnancy (gestational age >25 weeks). Mothers enrolled in early pregnancy (69%) had three assessments (early, mid-, and late pregnancy), those enrolled in mid-pregnancy (19%) had two assessments and those enrolled in late pregnancy (3%) had one assessment. (25)

All children were born between April 2002 and January 2006. Of all eligible children, 61% were participating in the study at birth. (25) The study protocol was approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Maternal anthropometrics

Maternal anthropometrics were measured at one of the research centers at the visits in early, mid-, and late pregnancy. The medians (95% range) of gestational age for these assessments were 12.6 (9.6–16.9) weeks, 20.4 (18.6–22.5) weeks, and 30.2 (28.5–32.5) weeks, respectively. We measured weight (kg) and height (cm) lightly clothed without shoes and we calculated maternal body mass index (kg/m^2) for each pregnancy period. Information about maternal weight just before pregnancy was obtained by questionnaires. Since enrolment in our study was in pregnancy, we were not able to measure maternal weight before pregnancy. However, in our population for analysis, 48% and 76% of all women were enrolled before a gestational age of 14 and 18 weeks, respectively. Correlation of pre-pregnancy weight obtained by questionnaire and weight measured at enrolment was 0.97 (p <0 .001). No differences in results were found when we used weight measured at enrolment instead of pre-pregnancy weight obtained by questionnaire. Maximum weight during pregnancy was available in only 42% (n =3,106; mean (standard deviation (SD) score) 81.4 kg (13)). Because of the number of missings of maximum weight, we defined weight gain as the difference between weight before pregnancy and weight in late pregnancy. This is actually a measure of weight gain during the first two trimesters, and was strongly correlated with weight gain during the entire pregnancy (r = 0.80, P <0.01). For presentation purposes, we constructed quintiles of maternal height (cm) (<160, 160-165, 165.1-169, 169.1-174, >174) pre-pregnancy body mass index (kg/m2) (<20.3, 20.3-21.8, 21.9-23.4, 23.5-26.4, >26.4) and gestational weight gain (kg) (<7, 7-9, 9.1-11, 11.1-13, >13).

Fetal ultrasonography

Fetal ultrasound examinations were carried out at the research centers in early, mid-, and late pregnancy. Gestational age was established by ultrasound examination since using the last menstrual period has limitations, including the large number of women who do not know the exact date of their last menstrual period or have irregular menstrual cycles. (27-29) Fetal measurements in early pregnancy were not included as growth characteristics since these examinations were performed to establish gestational age. Fetal growth was measured by head circumference (HC), abdominal circumference (AC), and femur length (FL) in mid- and late pregnancy. All growth characteristics were measured to the nearest millimeter using standardized procedures. (29) Estimated fetal weight (EFW) was calculated using the formula by Hadlock:

$(\log_{10} EFW = 1.5662 - 0.0108\ (HC) + 0.0468\ (AC) + 0.171\ (FL) + 0.00034\ (HC)^2 - 0.003685\ (AC * FL))$. (30)

With all estimated fetal weight data, we constructed longitudinal growth curves and gestational-age-adjusted standard deviation (SD) scores curves. (29) These SD-scores were used in the analyses. Although this variable is more difficult to interpret than estimated fetal weight measures in grams, use of SD-scores enables comparison of the relative and gestational age adjusted effects of fetal weight during pregnancy.

Birth outcomes

Date of birth, birth weight, and gender were obtained from midwife and hospital registries. Small and large size for gestational age at birth was defined as the lowest and highest 5% of birth weight in our study population, respectively. Main outcomes were birth weight (grams), small size for gestational age and large size for gestational age at birth. (31, 32) Gestational age-adjusted-SD-scores for birth weight were based on previously published reference charts from a large North European birth cohort (33).

Covariates

Information about educational level, ethnicity, parity, and smoking and alcohol consumption during pregnancy was obtained from a questionnaire at enrollment. (25, 26) Of all mothers who were enrolled in pregnancy, 91% completed the questionnaire. (25)

Sample for analysis

In total, 61% of all children born in the study area were enrolled in the study. Of the total of 9,778 mothers, 91% (n = 8,880) were enrolled during pregnancy (Figure 1). Of these mothers, height was measured in 99% (n =8,847) and information about pre-pregnancy weight and gestational weight gain was available in 80% (n = 7,143) and 76% (n = 6,721), respectively. Analyses were restricted to live births with known weight and gestational age at birth (n = 8,638). Twin

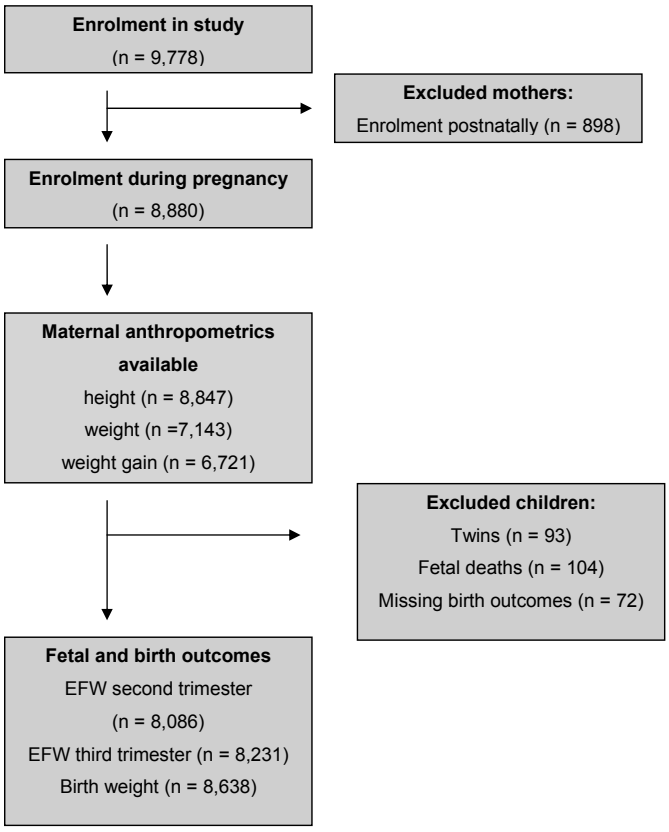

Figure 1. Enrolment and Fetal and Birth Outcomes

pregnancies (n = 93), fetal deaths (n = 104), or children with missing birth outcomes (n = 72) were excluded. From all mothers with at least one anthropometric measure, infant birth weight was available in 97% (n = 8,541) and estimated fetal weight was available in 91% (n = 8,086) and 93% (n = 8,231) in mid- and late pregnancy, respectively. Due to missing data on covariates, analyses with the adjusted models were based on 7,514 to 7,939 subjects. Of all mothers, 5.7% had a second (n = 492) or third (n = 4) pregnancy in our study. Since there were no differences in results after exclusion, these pregnancies were included in the analyses.

Data analysis

First, we assessed in which period during pregnancy differences in estimated fetal weight between body mass index groups appeared. For this, we constructed quintiles of maternal body mass index for mid-pregnancy, late pregnancy and delivery, and assessed the associations with SD-score of estimated fetal weight at each period using univariate regression models. Since maternal weight at delivery was not available, we used maximum weight in pregnancy to calculate body mass index at delivery. The effects of pre-pregnancy maternal body mass index on fetal and

birth weight were also assessed with unbalanced repeated-measurements regression analysis. The best fitting models were constructed using fractional polynomials of gestational age. (34) We included body mass index as interaction with gestational age period. The models have an optimal use of data and take the correlations within subjects into account.

Second, we used scatterplots and Pearson's correlation coefficients (*r*) to assess the relationships of maternal height, pre-pregnancy body mass index and gestational weight gain with SD-scores of birth weight. The associations of height, pre-pregnancy body mass index and gestational weight gain in quintiles with continuous birth weight were further explored using multiple linear regression models. These models were adjusted for gestational age, maternal age, smoking, alcohol consumption, educational level, ethnicity, parity, pregnancy complications (gestational diabetes, pre-eclampsia, hypertension) and infant gender. Selection of covariates was based on results from previous studies, change of effect estimate of interest of > 10% and strong correlations with birth weight. The associations of maternal height, pre-pregnancy body mass index and gestational weight gain with the risks of small and large size for gestational age at birth were assessed using multivariate logistic regression models. For all analyses with quintiles of anthropometric measures as determinant, the middle (3rd quintile) group was considered as reference. Tests for trend were performed by treating each categorized variable as continuous and entering it in the adjusted linear or logistic regression models.

Third, we explored the additional effects of pre-pregnancy body mass index and gestational weight gain on the risks of small and large size for gestational age at birth by combining pre-pregnancy body mass index and gestational weight gain categories and using multivariate logistic regression models. To increase the number of subjects per category, we used quartiles instead of quintiles of body mass index and gestational weight gain (Pre-pregnancy body mass index quartile categories < 20.5, 20.5– 22.5, 22.6- 25.5, > 25.5 kg/m2 and gestational weight gain quartile categories < 7, 7-10, 10,1-13, > 13 kg). These models were adjusted for the same potential confounders. The interactions of pre-pregnancy body mass index and weight gain were significant ($p < 0.05$) in the linear regression analyses and were presented as stratified analysis. For the analysis focused on the risk of small size for gestational age, the reference group comprised women in the highest body mass index and weight gain quartile. For the analysis focused on the risk of large size for gestational age at birth, the reference group consisted of women in the lowest body mass index and weight gain quartile. Analyses were performed using the Statistical Analysis System version 9.1 (SAS, Stata corporation, College station, TX, USA), including the Proc Mixed module for unbalanced repeated measurements and the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject characteristics

Characteristics of the participants are presented in Table 1. Of all participants, 51% were of Dutch origin and 42% had higher educational level. Of all mothers, 76% were enrolled in early pregnancy.

Table 1. General Characteristics of the Participants*

	N = 8541
Age (yr)	29.6 (5.3)
Height (cm)	167.1 (7.4)
Weight (kg)	66.3 (12.8)
Body mass index (kg/m2)	
Pre-pregnancy	23.6 (4.4)
<18 weeks	24.5 (4.4)
18 - 25 weeks	25.7 (4.4)
>25 weeks	27.5 (4.4)
Parity ≥ 1 (%)	44.4
Smoking in pregnancy (%)	
No	80.9
Yes	19.1
Alcohol consumption in pregnancy (%)	
No	60.8
Yes	39.2
Education (%)	
Primary school	11.6
Secondary school	46.4
Higher education	42.0
Ethnicity† (%)	
Dutch	50.8
Other western	11.7
Non-western	37.5
First child of same mother in study (%)	94.1
Enrollment in study in early pregnancy (%)	76.0
Birth outcomes	
Birth weight (g)	3410 (562)
Gestational age (weeks)	40.1 (37.9,41.7)‡
Small for gestational age § (%)	4.8
Large for gestational age (%)	5

* Values presented are means (with standard deviations) or percentages unless otherwise noted. Some data were missing on height (n=31), body mass index before pregnancy (n=1656), body mass index<18 weeks (n=2090), body mass index 18-25 weeks (n=611), body mass index >25 weeks (n=934), parity (n=93), smoking in pregnancy (n=1052), alcohol in pregnancy (n=890), educational level (n=757), and ethnicity (n=627).

† Ethnicity: Non-western (Moroccan, Turkish, Antillean, Surinamese, Cape Verdian, African, American and Asian non-western), western (European, Asian and American Western, Australian)

‡ Median (90% range)

§ Small for gestational age = birth weight < 5th percentile
Large for gestational age = birth weight > 95th percentile

Body mass index and estimated fetal weight in different periods of pregnancy

Figure 2 presents the estimated differences in SD-score for estimated fetal weight and birth weight between mothers in quintiles of body mass index in mid- and late pregnancy and at delivery. With advancing gestational age, we found an increased difference in fetal weight per body mass index quintile. The relative effect of body mass index on fetal weight seemed to increase from mid-pregnancy onwards. The largest effects were found in late pregnancy and at birth. The repeated regression analyses showed that pre-pregnancy maternal body mass index was associated with an increase of fetal weight growth rate (difference in grams per week compared to first quintile of 1.99 (95%CI: 1.10, 2.88), 1.74 (95%CI: 0.81, 2.68), 2.83 (95%CI: 1.95, 3.72) and 4.39 (95%CI: 3.48, 5.29) for the second, third, fourth and fifth quintile respectively).

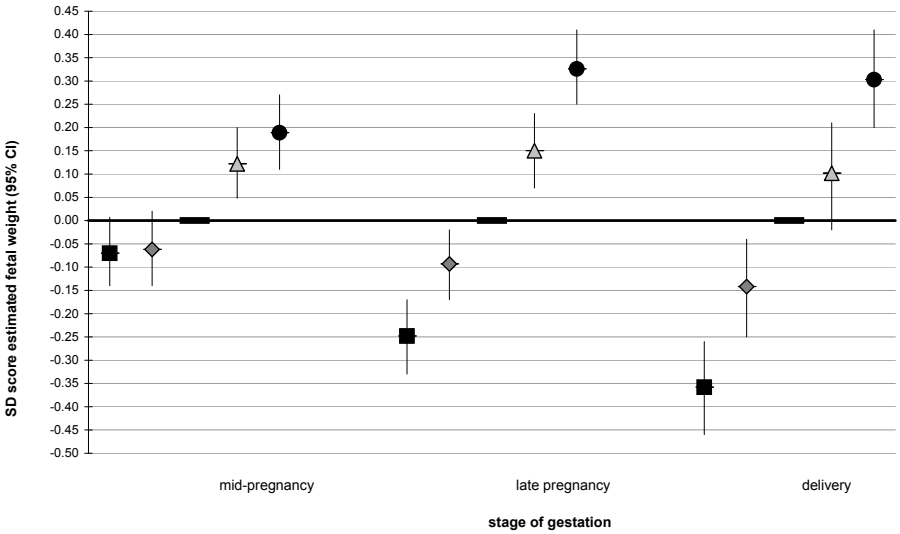

Figure 2. Quintiles of Body Mass Index in Pregnancy and the Difference in SD Score of estimated Fetal Weight mid-pregnancy=gestational age 18–25 weeks, late pregnancy= gestational age >25 weeks Regression coefficients for the associations of body mass index with estimated fetal and birth weight were in mid-pregnancy: increase of 0.02 (95%CI 0.01, 0.03) SD per unit body mass increase; in late-pregnancy: increase of 0.05 (95%CI 0.04-0.06) SD per unit body mass increase, and at delivery increase of 0.05 (95%CI 0.04, 0.06) SD per unit body mass increase.

Birth outcomes

Figure 3 (a,b,c) gives the correlations of maternal height, pre-pregnancy body mass index and gestational weight gain with SD-score of birth weight. Overall, very weak correlations were found for height ($r = 0.23$, $P < 0.01$), pre-pregnancy body mass index ($r = 0.14$, $P < 0.01$) and gestational weight gain ($r = 0.14$, $P < 0.01$).

Associations of maternal height, pre-pregnancy body mass index and gestational weight gain with birth weight measured continuously and the risks of small and large size for gestational age at birth are presented in Table 2. Overall, all anthropometric measures were associated with outcomes at birth (all *P*-values for trend <0.01). The children of mothers in the lowest quintile of

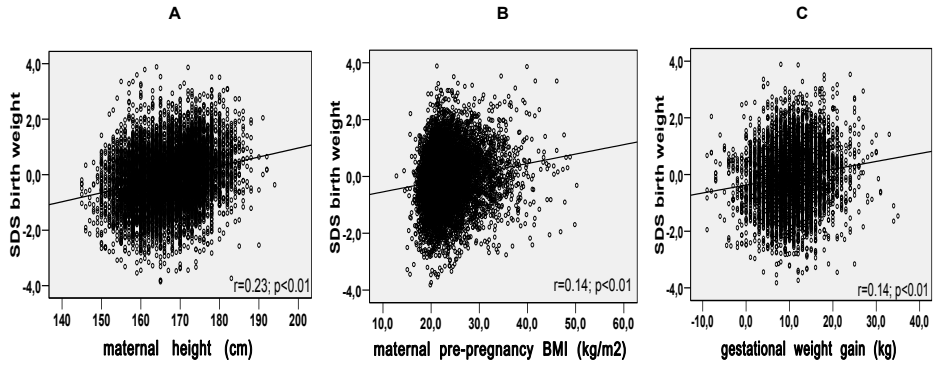

Figure 3. Correlations between Maternal Anthropometrics and SD Score of Birth Weight
(A) maternal height, (B) maternal pre-pregnancy BMI, (C) gestational weight gain until late pregnancy (= gestational age >25 weeks)
SDS= standard deviation score, BMI= body mass index

height were lighter than children in the reference group (difference -132 grams, 95% confidence interval (CI): -166, -99) while mothers in the highest quintile gave birth to the largest children (difference 180 grams, 95% CI: 147, 215), and had offspring with the lowest risk of small size for gestational age (odds ratio (OR) 0.54, 95% CI: 0.32, 0.90) but the highest risk of large size for gestational age at birth (OR 2.57, 95% CI: 1.83, 3.60). Pre-pregnancy body mass index was also associated with birth weight (differences in birth weight for the lowest and highest quintile compared to the reference group: -88 grams (95% CI: -120, -57) and 106 grams (95% CI: 74, 138), respectively). The highest quintile of pre-pregnancy body mass was associated with a lower risk of offspring with small size at birth (OR 0.61, 95% CI: 0.41, 0.91), but a higher risk of offspring with large size at birth (OR 1.74, 95% CI: 1.30, 2.40). Similar tendencies were found for gestational weight gain (differences in birth weight for the lowest and highest quintile compared to the reference group: -69 grams (95% CI: -101, -37) and 167 grams (95% CI: 135, 200), respectively). The highest quintile of gestational weight gain was associated with an increased risk of large size at birth in the offspring (OR 2.34, 95% CI: 1.70, 3.21).

Interaction between pre-pregnancy body mass index and gestational weight gain

The associations of the combined categories of pre-pregnancy body mass index and gestational weight gain with the risks of small and large size for gestational age at birth in the offspring are presented in Figures 4 and 5, respectively.

Figure 4 shows that, within each pre-pregnancy body mass index quartile, the risk of small size for gestational age at birth in the offspring decreased when weight gain increased. The highest risk was found for mothers with both body mass index and weight gain in the lowest quartile (OR 6.80, 95% CI: 2.70, 17.50). Among women in the second and third body mass index quartile, only those with the lowest weight gain were at increased risk for delivering small size infants (OR 5.60, 95% CI: 2.20, 14.60 and OR 4.30, 95% CI: 1.70, 11.1, respectively).

Table 2. Maternal Anthropometrics and Birth Weight and the Risks of Small Size and Large Size for Gestational Age at Birth*

	Beta for birth weight (grams) (95% CI)	OR† for small size for gestational age at birth‡ (95% CI)	OR for large size for gestational age at birth§ (95% CI)
Height quintiles			
1st (<160 cm)	-132 (-166, -99)	2.46 (1.70, 3.58)	0.46 (0.29, 0.74)
2nd (160-165 cm)	-61 (-93, -29)	1.81 (1.25, 2.62)	0.60 (0.40, 0.93)¶
3rd (165.1-169 cm)	Reference	Reference	Reference
4th (169.1-174 cm)	59 (27, 91)	1.06 (0.71, 1.59)	1.09 (0.75, 1.57)
5th (>174 cm)	180 (147, 215)	0.54 (0.32, 0.90)¶	2.57 (1.83, 3.60)
P-value for trend	<0.01	<0.01	<0.01
Pre-pregnancy body mass index			
1st (<20.3 kg/m2)	-88 (-120, -57)	1.51 (1.10, 2.07)¶	0.35 (0.21, 0.58)
2nd (20.3-21.8 kg/m2)	-11 (-42, 19)	1.11 (0.79, 1.55)	0.95 (0.67, 1.36)
3rd (21.9-23.4 kg/m2)	Reference	Reference	Reference
4th (23.5-26.4 kg/m2)	32 (1, 64)¶	0.89 (0.62, 1.28)	1.30 (0.94, 1.81)
5th (>26.4 kg/m2)	106 (74, 138)	0.61 (0.41, 0.91)¶	1.74 (1.30, 2.40)
P-value for trend	<0.01	<0.01	<0.01
Gestational weight gain			
1st (<7 kg)	-69 (-101, -37)	1.40 (1.01, 1.93)¶	0.63 (0.41, 0.97)¶
2nd (7-9 kg)	-29 (-59, 1)	0.93 (0.66, 1.32)	0.98 (0.69, 1.39)
3rd (9.1-11 kg)	Reference	Reference	Reference
4th (11.1-13 kg)	62 (32, 92)	0.83 (0.59, 1.17)	1.12 (0.80, 1.58)
5th (>13 kg)	167 (135, 200)	0.60 (0.41, 0.88)¶	2.34 (1.70, 3.21)
P-value for trend	<0.01	<0.01	<0.01

* Values presented are results of multiple linear regression or logistic regression. Adjusted data are shown. Adjusted model: controlled for gestational age (not for small and large for gestational age), gender, maternal age, educational level, ethnicity, parity, smoking, alcohol consumption, maternal complications (diabetes, pre-eclampsia, hypertension), mode of delivery

† OR = odds ratio

‡ Small for gestational age = birth weight < 5th percentile

§ Large for gestational age = birth weight > 95th percentile

‖ P-value<0.01

¶ P-value<0.05

Figure 5 shows that in each quartile of body mass index, the risk of large size for gestational age increased per weight gain group. Within each pre-pregnancy body mass index quartile, significantly increased risks of large size for gestational age were found for the highest quartiles of gestational weight gain. The highest risk of large size offspring was found for mothers in the highest body mass index and gestational weight gain group (OR 8.60, 95% CI: 3.30, 22.60).

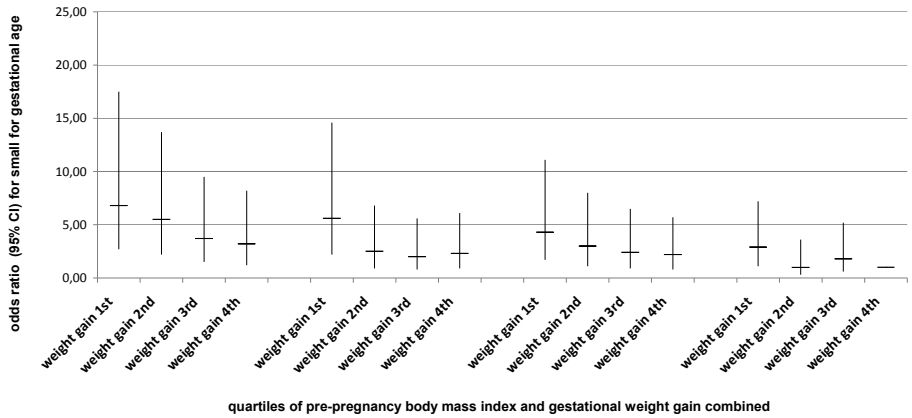

Figure 4. Pre-pregnancy Body Mass Index and Gestational Weight Gain until Late Pregnancy and the Risk of Small for Gestational Age at Birth
late pregnancy= gestational age >25 weeks, Small for gestational age = birth weight < 5th percentile adjusted for gestational age
Values presented are results of logistic regression with reference group weight gain 4th quintile and pre-pregnancy body mass index 4th quintile. Models were adjusted for infant gender, maternal age, educational level, ethnicity, parity, smoking, alcohol consumption, maternal complications (diabetes, pre-eclampsia, hypertension), and mode of delivery
P-value for trend for weight gain per BMI quartile: 1st quartile <0.01, 2nd quartile 0.03, 3rd quartile 0.02, 4th quartile <0.01

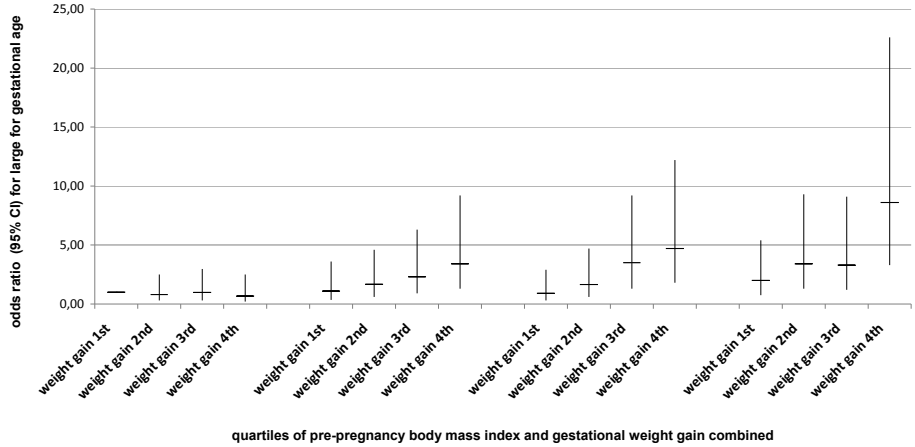

Figure 5. Pre-pregnancy Body Mass Index and Gestational Weight Gain until Late Pregnancy and the Risk of Large for Gestational Age at Birth
late pregnancy= gestational age >25 weeks, Large for gestational age = birth weight > 95th percentile adjusted for gestational age
Values presented are results of logistic regression with reference group weight gain 1st quintile and pre-pregnancy body mass index 1st quintile. Models were adjusted for infant gender, maternal age, educational level, ethnicity, parity, smoking, alcohol consumption, maternal complications (diabetes, pre-eclampsia, hypertension), and mode of delivery
P-value for trend for weight gain per BMI quartile: 1st quartile 0.70, 2nd quartile <0.01, 3rd quartile <0.01, 4th quartile <0.01

DISCUSSION

We found that maternal body mass index affects fetal growth from mid-pregnancy onwards. The effects on fetal growth become larger with increasing gestational age. We also found that maternal height, pre-pregnancy body mass index and gestational weight gain are strongly associated with the risks of delivering small and large size for gestational age infants. The effect of pre-pregnancy body mass index on birth outcomes was strongly modified by gestational weight gain.

Strengths and limitations

One strength of this study was the large population-based cohort from early pregnancy onwards and information about a large number of potential confounders being available. To our knowledge, this is the first study that examined the associations of maternal anthropometrics with fetal growth during different periods of pregnancy. Furthermore, this study is one of the largest cohort studies that explored the associations of maternal anthropometrics before and during pregnancy with birth outcomes.

Birth weight was 42 g (95% CI: 12, 72) lower in the offspring of mothers without information about pre-pregnancy body mass index than those with this information. Response rate for this questionnaire was lower among non-Dutch, lower educated and younger mothers. These selective missing might lead to biased results. However, body mass index at enrolment was based on measured weight and height and was available in 99% of the participants and was strongly correlated with pre-pregnancy body mass index. The associations of body mass index with birth weight were similar when we used body mass index at enrolment instead of pre-pregnancy body mass index. Follow up rates in our study were high: birth outcomes were available in 97% of all pregnancies. Recently, it has been shown that bias in large cohort studies primarily arises from loss to follow up rather than from non-response at baseline. (35) We therefore do not expect that our results are biased due missing pre-pregnancy weight data. Weight gain was partly based on self-reported weights. Women in this age group may systematically underestimate their weights. (36) Since we were interested in differences between subjects and the effects on birth outcomes, systematic underestimation of pre-pregnancy weight and body mass index does not bias our results. Maternal gestational weight gain was defined as the difference between weight in late pregnancy 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. In a sub analysis in 42% of the participants (n=3,106) with highest weight in pregnancy available from questionnaires, we found similar results. Since the largest differences in gestational weight gain can be expected at delivery, our effect estimates may be underestimated.

Gestational age was established by fetal ultrasound examination, which method assumes that the growth variation of the fetal characteristics used for pregnancy dating is zero. In our study, crown-rump length and biparietal diameter were used for pregnancy dating but not for assessing fetal growth. (29) Since pregnancy dating characteristics and growth characteristics are correlated

throughout pregnancy, growth variation in head circumference, abdominal circumference, and femur length may be somewhat reduced by dating pregnancy on the other fetal characteristics. As correlations between pregnancy dating and fetal growth characteristics are strongest in early pregnancy, we only used mid- and late pregnancy ultrasounds for fetal weight estimations.

Due to missing questionnaire data, information was limited for some covariates. Shorter height and larger body mass index and lower birth weight were found in subjects with missing data on ethnicity and education. Since we performed a complete case analysis in the adjusted regression models, our effect estimates may be underestimated by leaving out relatively more subjects with shorter height and higher weight.

Fetal growth and adverse birth outcomes

We showed for the first time that maternal body mass index influences fetal growth from mid-pregnancy onwards. Previous studies on this topic have consistently used birth weight as measure of fetal growth. (37) Some studies suggested that both pre-pregnancy weight and weight gain during pregnancy affect fetal growth. (14-18) Also, several studies have looked at the timing of this influence during pregnancy, however the evidence on which is the most sensitive trimester remains inconclusive (9, 38-41). These studies were focused on birth weight as end result of fetal growth. Our results suggest that differences in fetal growth due to maternal anthropometrics occur already from mid-pregnancy and the relative effects seem to become larger with increasing gestational age. These results were independent of maternal lifestyle and socioeconomic status related variables. Positive associations were found for maternal height, pre-pregnancy body mass index and gestational weight gain with continuous measured birth weight. In line with that, the risks of small size for gestational age in the offspring were highest in short mothers with lowest pre-pregnancy body mass index and gestational weight gain. The risks of large size for gestational age in the offspring were highest for the tallest mothers with the highest pre-pregnancy body mass index and gestational weight gain. Similar associations between maternal weight gain and birth weight have been reported previously. (10-13) The effect of pre-pregnancy body mass index on birth outcomes was influenced by gestational weight gain. We found trends for associations between weight gain during pregnancy with the risks for small and large size for gestational age within each quartile of pre-pregnancy body mass index. These findings suggest that the effect of gestational weight gain is to an extent dependent on pre-pregnancy body mass index.

Pre-pregnancy body mass index reflects nutritional status, whereas gestational weight gain reflects both nutritional status and tissue expansion. (21) Gestational weight gain comprises placental and fetal growth, amniotic fluid, uterine and mammary tissue expansion, increased blood and extracellular fluid volumes and fat stores. Anthropometrics in pregnancy reflect maternal nutritional and health status and may be measures of the fetal environment. (22, 23) However, it is not known which dietary pattern in pregnancy affects maternal weight gain and fetal growth. Increased gestational weight gain is associated with pregnancy complications including gestational diabetes and hypertension. (20) In our study we adjusted for these complications. The underlying

pathways leading from maternal anthropometrics before and during pregnancy to adverse birth outcomes need to be studied in further detail. Higher maternal pre-pregnancy body mass index and weight gain during pregnancy are both associated with insulin resistance. Higher maternal glucose levels in pregnancy may lead to increased fetal glucose and insulin levels. Since insulin is the single most important fetal growth factor, increased levels might subsequently lead to higher fetal growth rates and eventually higher birth weight. Further studies focused on mechanisms underlying the associations between maternal anthropometrics and fetal growth are needed and should be focused on both environmental and genetic variants related to weight gain and insulin resistance. Also, since maternal body mass index and weight gain reflect maternal nutritional status and may be at least partly modifiable, further studies are needed to identify dietary factors that influence maternal weight gain during pregnancy, and examine the feasibility and effect of optimizing maternal anthropometrics before and during pregnancy.

We found that maternal body mass index affects fetal growth from mid-pregnancy onwards and that maternal height, pre-pregnancy body mass index and gestational weight gain are associated with the risks of small and large size for gestational age. The mechanisms by which maternal anthropometrics affect fetal growth remain to be studied. Additionally, whether and to what extent these effects on fetal size persist in childhood and in adulthood needs to be further studied.

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Chapter 3

Fetal and postnatal growth and body composition at 6 months of age

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ABSTRACT

Objectives

To examine which parental, fetal and postnatal characteristics are associated with fat and lean mass at the age of 6 months and to examine the effect of growth (catch-down, catch-up) in fetal life and early infancy on fat and lean mass.

Design

This study was embedded in the Generation R Study, a prospective cohort study from early fetal life onwards. Body composition was measured by Dual energy X-ray Absorptiometry (DXA) in 252 infants at 6 months. Parental, fetal and postnatal data were collected by physical and fetal ultrasound examinations and questionnaires.

Results

Children with fetal catch-up in weight (gain in weight-SD-scores>0.67) in the second trimester tended to have a higher fat mass percentage (FM(%)) at 6 months of age whereas children with fetal catch-down in weight had a lower FM(%) compared to non changers. In the third trimester, both catch-up and catch-down in weight were associated with an increase in FM(%) at 6 months. Children with catch-down in the third trimester had a greater risk for postnatal catch-up in weight >0.67 SDS. Birth weight and weight at 6 weeks were positively associated with fat mass at 6 months. Postnatal catch-up in weight within 6 weeks after birth had the highest association with total and truncal FM(%) at 6 months. Total and truncal FM were higher in girls.

Conclusion

Catch-down in weight in the third trimester was strongly associated with postnatal catch-up within 6 weeks after birth and both were associated with an increase in fat mass at the age of 6 months. Our study shows that fetal as well as postnatal growth patterns are associated with body composition in early childhood.

INTRODUCTION

The prevalence of childhood overweight and obesity has dramatically increased in developed countries over the past two decades. (1, 2) Obesity in childhood is not only associated with short-term morbidity such as asthma and psychological problems but also with an increased risk for chronic morbidity and mortality in adulthood, as childhood obesity tends to track into adulthood. (3-5)

Previous studies have shown that both high and low birth weight are associated with obesity in childhood. (6) Birth weight has been related to the expectant mother's weight and weight gain during pregnancy. (7, 8) This may imply that mother's nutrition during pregnancy has an effect on birth weight whereas insulin levels during pregnancy may also play an intermediate role. Low birth weight has been related to obesity in later life. (9, 10) The fetal origins hypothesis poses that an adverse fetal environment leads to adaptations which program the fetus' metabolism. These adaptations predispose the individual to increased fat mass and insulin resistance postnatally. Other studies have posed that genetic factors or epigenetic phenomena may explain the relation between low birth weight and cardiovascular diseases. (11, 12) To our knowledge, all of these studies have used birth weight as a proxy for fetal growth as they had no fetal growth parameters available.

On the other hand, it has been postulated that not birth weight, but postnatal catch-up in weight is related to overweight. Children with catch-up in weight within the first 2 years of life had more fat mass measured by skin folds at the age of 5 years. Also, adults with rapid catch-up in weight in childhood had the greatest risk for cardiovascular disease. (13-15) However, the exact timing of the rapid weight gain that contributes to these long-term risks is controversial. It is also unclear whether this unfavorable body composition is solely due to an excess of fat mass or due to a combination of higher fat mass and diminished lean mass.

Truncal fat is the major component of body fat associated with disease. (16) Body mass index (BMI) does not reflect body composition. Dual-energy X-ray Absorptiometry (DXA) is one of the most reliable and practical methods for measuring body composition in adults and children. To our knowledge most studies were performed in older children or had limited access to potential confounders. (17, 18)

Based on previous literature, we hypothesized that maternal pre-pregnancy BMI, height, blood pressure and smoking during pregnancy were related to fetal growth restraint, which in turn will lead to a diminished fat mass percentage in early infancy. On the other hand, we expected that catch-up in weight during early infancy will lead to a higher fat mass percentage at the age of 6 months.

We therefore examined in a prospective cohort of infants from early fetal life onwards whether parental, fetal and postnatal growth characteristics were associated with fat and lean mass measured by DXA at the age of 6 months. Additionally, we examined whether growth patterns in fetal life and infancy were associated with distribution of fat and lean mass at the age of 6 months.

MATERIAL AND METHODS

Design

The present study was embedded in the Generation R Study, a prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood, and has been previously described in depth. (19, 20) Detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch mothers and their children from 30 weeks of gestation. This subgroup is referred to as the Generation R Focus cohort and is ethnically homogeneous to exclude possible confounding or effect modification by ethnicity. Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands. (19) No other exclusion criteria were used. Of all approached women, 80% agreed to participate in the subgroup study. At the age of 6 months, DXA measurements were performed in 270 of 298 infants who were randomly selected from this subgroup. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

Parental and pregnancy characteristics:

Information about maternal weight before pregnancy was collected by questionnaire. Maternal height (cm) and weight (kg) during pregnancy were measured at a median gestational age (Inter Quartile Range (IQR)) of 12 weeks (11.9-13.6), 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9), in one of the research centers. Body mass index (kg/m^2) was calculated for each pregnancy period. Paternal height (cm) and weight (kg) were measured at intake and body mass index (kg/m^2) was calculated. Maternal maximum weight during pregnancy was available in 42% of participating mothers (n=114; mean SD-score) 81.4 kg (13)). Because of the number of missings of maximum weight, we defined weight gain as the difference between weight before pregnancy and weight at 30 weeks of gestation. This is actually weight gain during the first two trimesters, but was strongly correlated with weight gain during the entire pregnancy in mothers with both measures available ($r = 0.80$, $P < 0.01$).

Fetal growth:

In this study we used the new reference charts, which were based on 8313 pregnancies in the Generation R study. (21) Multilevel modeling according to Royston and Altman was used to produce growth centiles. The curves were fitted using repeated measurement analysis and these curves were plotted on the data. Fetal growth reference curves for BPD, HC and AC were calculated for a gestational age from 10 to 40 weeks. (21) In the present study, SD-scores for all fetal growth measures were based on these reference data. Estimated fetal weight (EFW) was

calculated using the formula by Hadlock: ($\log_{10}$ EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 (HC)^2 – 0.003685 (AC * FL)). (21)

Fetal ultrasound examinations were carried out at the research centers at a median gestational age (IQR) of 12 weeks (11.9-13.6), 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9). (20, 21) These fetal ultrasound examinations were used for either establishing gestational age or for assessing fetal growth characteristics. Crown-rump length was used for pregnancy dating in early pregnancy (up to a gestational age of 12 weeks and 5 days), and biparietal diameter was used for pregnancy dating thereafter. Fetal measurements in early pregnancy were not included as growth characteristics since these ultrasound examinations were primarily performed to establish gestational age. Fetal growth measurements at 20 and 30 weeks of gestation included head circumference (HC), abdominal circumference (AC), and femur length (FL), which were measured to the nearest mm using standardized ultrasound procedures.

Birth characteristics:

Date of birth, birth weight and gender were obtained from midwife and hospital registries.

Breastfeeding information:

Information on breastfeeding was collected by questionnaires at 2 and 6 months of age. This information was used as a categorical variable on ever having been breastfed (yes/no) and as a continuous variable on the duration of breastfeeding.

Anthropometrics of the child:

Weight was measured in naked infants at the age of 6 weeks and 6 months to the nearest grams by using an electronic infant scale (SECA®). Length was measured in supine position to the nearest 0.1 cm by a neonatometer (Holtain Limited®). Body mass index was calculated (kg/m^2).

Body composition:

Fat and lean mass were measured by Lunar Prodigy DXA scanner® (General Electrics). Previous studies have shown that this method is valid for measurement of body composition in adolescents and children. (22, 23) All DXA scans were performed with the same device and software and by the same technician. After the exclusion of scans with anomalies such as movement artifacts, complete scans were available for 252 infants. The DXA scans were used to derive total, truncal and peripheral body fat and total lean mass.

Statistical analysis

Differences between boys and girls were examined with Student's *t* tests, chi square tests or ANOVA analysis.

The associations of maternal and paternal anthropometrics with fat mass at the age of 6 months were assessed using linear regression models. For this purpose we calculated fat mass

percentage (FM(%)) as percentage of weight at 6 months. Similar models were used to examine the associations of FM(%) at 6 months with estimated fetal weight at a median gestational age (IQR) of 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9), birth weight and weight at 6 weeks, breastfeeding (ever (yes/no) and as continuous variable (months)). These regression models were adjusted for current age, gender, gestational age at birth and length at 6 months in order to adjust for current size.

Additionally, we examined the effect of catch-up in weight on FM(%) at the age of 6 months. For this purpose we used the change in SD-scores of (estimated fetal) weight at several ages. We defined catch-up as a gain in weight-SD-scores>0.67, catch-down as loss in weight-SD-scores>0.67 and no change as gain or loss in weight-SD-scores<0.67 as was previously done by Ong et al. (13) This was done as a change in SD-score for weight of 0.67 SD-scores represents the width of each percentile band on standard growth charts, meaning; second to ninth percentile, ninth to 25th, 25th to 50th,and so on. This indicates clinically significant catch-up or catch-down growth. For the prenatal assessments, second trimester was defined as the period between 20 and 30 weeks of gestation and the third trimester was defined as the period between 30 weeks of gestation and birth.

Subsequently, we investigated whether maternal characteristics (pre-pregnancy BMI, height and blood pressure and smoking during pregnancy) were significantly different for children with fetal catch-down in weight compared to children with postnatal catch-up in weight and which growth pattern had the greatest effect on body composition at 6 months. We also examined whether the association between fetal catch-down and postnatal catch-up in weight remained after adjustment for maternal pre-pregnancy BMI and systolic blood pressure and smoking during pregnancy and additionally for breastfeeding.

Finally, in order to determine whether the effect of change in weight-SD-scores was different for absolute fat mass compared to lean mass, we examined whether changes in weight-SD-scores during fetal life and infancy were associated with absolute fat and lean mass (grams) at the age of 6 months using comparable regression models. We used the SD-score of (estimated fetal) weight at several ages (gestational age 20 weeks, gestational age 30 weeks, birth, 6 weeks, 6 months) to calculate the change in weight-SD-scores in different periods.

Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA). A p-value of < 0.05 was regarded as significant.

RESULTS

Table 1 presents the clinical characteristics of the infants and their parents. Boys were heavier and had more lean mass (grams) than girls (6648 vs. 6112, P<0.01). However, girls had more total and truncal FM(%) at the age of 6 months (25.0% vs. 23.7%, P<0.05, and 8.9% vs. 8.0%, P<0.01, respectively).

Table 1. Parental, fetal and child characteristics

	Boys (n=145)	Girls (n=107)	P value
Parents			
Maternal characteristics			
Age (years)	31.4 (4.0)	32.5 (3.8)	<0.05
Pre-pregnancy weight (kg)	69.2 (12.1)	68.6 (11.3)	0.71
Height (cm)	171 (5.8)	170 (6.3)	0.68
Pre-pregnancy body mass index (kg/m2)	24.4 (4.3)	24.7 (4.1)	0.59
Weight gain during pregnancy (kg)	9.5 (4.6)	9.8 (4.1)	0.69
Paternal characteristics			
Age (years)	33.7 (4.7)	35.1 (6.2)	0.06
Weight (kg)	85.3 (14.3)	85.2 (12.6)	0.94
Height (cm)	184 (7.5)	184 (7.1)	0.73
Body mass index (kg/m2)	25.3 (3.6)	25.2 (3.3)	0.81
Fetal period			
20 weeks of gestation			
Gestational age (weeks)	20.5 (0.8)	20.3 (0.9)	<0.05
Estimated fetal weight (grams)	375 (71.5)	361 (71.1)	0.13
30 weeks of gestation			
Gestational age (weeks)	30.2 (1.1)	30.4 (1.1)	0.24
Estimated fetal weight (grams)	1606 (284)	1641 (279)	0.34
Birth			
Gestational age (weeks)	39.9 (1.8)	40.1 (1.2)	0.18
Weight (grams)	3494 (557)	3503 (477)	0.89
Weight-SD-score	-0.1 (0.9)	0.2 (0.9)	0.09
Infancy			
6 weeks			
Age (months)	1.6 (0.4)	1.6 (0.4)	0.83
Weight (grams)	5067 (761)	4741 (638)	<0.01
Weight-SD-score	0.3 (1.2)	0.4 (1.0)	0.72
Height (cm)	57.4 (2.6)	56.5 (2.4)	<0.01
Height SD-score	0.3 (1.0)	0.5 (0.9)	0.34
Body mass index (kg/m2)	15.3 (1.5)	14.8 (1.3)	<0.01
Body mass index SD-score	0.1 (1.1)	0.1 (1.0)	0.67
6 months			
Age (months)	6.4 (0.8)	6.3 (0.7)	0.24
Weight (grams)	8168 (923)	7564 (743)	<0.01
Weight-SD-score	0.1 (1.0)	0.1 (0.8)	0.78
Height (cm)	69.6 (2.4)	68.0 (2.3)	< 0.01
Height SD-score	0.4 (1.0)	0.4 (0.9)	0.67
Body mass index (kg/m2)	16.8 (1.4)	16.4 (1.3)	< 0.01
Body mass index SD-score	-0.3 (1.0)	-0.3 (1.0)	0.96

Table 1. continued

Body composition			
Total fat mass (grams)	1962 (508)	1904 (431)	0.35
Fat mass (%)			
Truncal	8.0 (1.8)	8.9 (2.0)	<0.01
Peripheral	11.6 (2.4)	12.1 (2.1)	0.06
Total	23.7 (4.1)	25.0 (3.9)	<0.05
Total lean mass (grams)	6648 (568)	6112 (478)	<0.01
Lean mass (%)			
Truncal	37.7 (3.3)	37.6 (2.9)	0.78
Peripheral	23.9 (2.8)	23.8 (2.0)	0.77
Total	82.0 (4.9)	81.1 (4.7)	0.15
Breastfeeding			
Ever breastfed (yes/no) (%)	87.4	91.6	0.29
At 2 months (yes/no) (%)	64.0	64.1	0.99
At 6 months (yes/no) (%)	33.3	28.6	0.42
Duration (months)	4.2	4.3	0.77

Weight gain during pregnancy= weight at 30 weeks of gestation - pre-pregnancy weight. Fat mass (%) =fat mass (grams) / weight at 6 months (grams) x100, Lean mass (%) = lean mass (grams)/ weight at 6 months (grams) x100

Values are means (SDS). Differences were tested using independent sample t-test for continuous variables and chi square test for dichotomous variables

Parents

Maternal pre-pregnancy BMI was positively associated with peripheral FM(%) of the offspring at 6 months, whereas height was inversely associated with total FM(%). However, the associations were borderline significant (Table 2). Maternal pre-pregnancy BMI and systolic blood pressure at 30 weeks of gestation were weakly but significantly correlated with fetal catch-down growth during the third trimester (from 30 weeks of gestation to birth) ($r = 0.17$, $P<0.01$ for BMI and $r = 0.17$, $P<0.01$ for blood pressure). The same maternal variables were not correlated with fetal catch-down growth during the second trimester (20 weeks to 30 weeks of gestation). Also smoking was not correlated with fetal catch-down growth. Paternal anthropometrics were not associated with infant body composition at 6 months (data not shown).

Fetal period

Fetal weight at a gestational age of 20 weeks and 30 weeks was not significantly associated with FM(%) at the age of 6 months (Table 2).

Children with fetal catch-up in weight (gain in weight-SD-scores>0.67) in the second trimester tended to have a higher FM(%) at 6 months whereas children with fetal catch-down in weight had a lower FM(%) compared to non changers. However, in the third trimester, both catch-up and catch-down in weight were associated with a similar increase in FM(%) at 6 months compared to non changers (Figure 1).

We also assessed the effect of change in fetal weight-SD-scores on absolute fat mass compared to absolute lean mass. The change in fetal weight-SD-scores from 20 weeks of gestation to birth

Table 2. Associations of fat mass percentage at 6 months with fetal and postnatal characteristics

	Fat mass (%)								
	Truncal			***Peripheral***			***Total***		
	B	95% CI	P value	B	95% CI	P value	B	95% CI	P value
Parents (mother)									
Height *(cm)*	-0.03	0.07, 0.02	0.22	-0.04	-0.09, 0.01	0.15	-0.08	-0.17, 0.11	0.09
BMI before pregnancy *(kg/m2)*	0.01	-0.05, 0.07	0.79	0.07	-0.00, 0.15	0.05	0.10	-0.03, 0.24	0.14
Weight gain during pregnancy *(kg)*	-0.01	-0.07, 0.04	0.62	-0.04	-0.11, 0.03	0.27	-0.06	-0.19, 0.06	0.34
Fetal period									
EFW at 20 weeks of gestation *(kg)*	2.33	-1.02, 5.68	0.17	2.60	-1.44, 6.64	0.21	5.95	-1.26, 13.17	0.10
EFW at 30 weeks of gestation *(kg)*	0.57	-0.28, 1.43	0.18	0.14	-0.91, 1.20	0.26	1.22	-0.64, 3.09	0.13
Birth and Infancy									
Birth weight *(kg)*	0.45	-0.13, 1.04	0.13	1.33	0.64, 2.03	**<0.01**	1.92	0.66, 3.18	**<0.01**
Weight at 6 weeks *(kg)*	0.69	0.31, 1.08	<0.01	0.94	0.47, 1.42	**<0.01**	1.92	1.10, 2.73	**<0.01**
Breastfeeding									
Ever been breastfed (yes/no)	0.10	-0.71, 0.91	0.81	0.18	-0.79, 1.16	0.71	0.51	-1.23, 2.24	0.56
At 2 months (yes/no)	0.26	-0.26, 0.78	0.31	-0.12	-0.74, 0.50	0.71	0.16	-0.94, 1.27	0.77
At 6 months (yes/no)	0.69	0.19, 1.20	<0.01	0.43	-0.19, 1.04	0.17	1.15	0.08, 2.24	**<0.05**
Duration (months)	0.17	0.02, 0.33	<0.05	0.01	-0.17, 0.19	0.97	0.15	-0.19, 0.49	0.38

EFW= estimated fetal weight, BMI= Body mass index, Weight gain during pregnancy= difference between weight at 30 weeks of gestation - pre-pregnancy weight, Fat mass (%) = fat mass (grams) / weight at 6 months (grams) x100

Values are regression coefficients and reflect the difference in percentage fat mass for the maternal, fetal and postnatal anthropometrics. Models are adjusted for gender, gestational age and age and length at 6 months.

was positively related to both absolute fat mass (grams) and lean mass (grams) at 6 months (Table 3). However, the change in fetal weight-SD-scores from 20 weeks of gestation to birth was positively related to FM(%) at 6 months (B (95% CI): 0.48, (0.02, 0.93)).

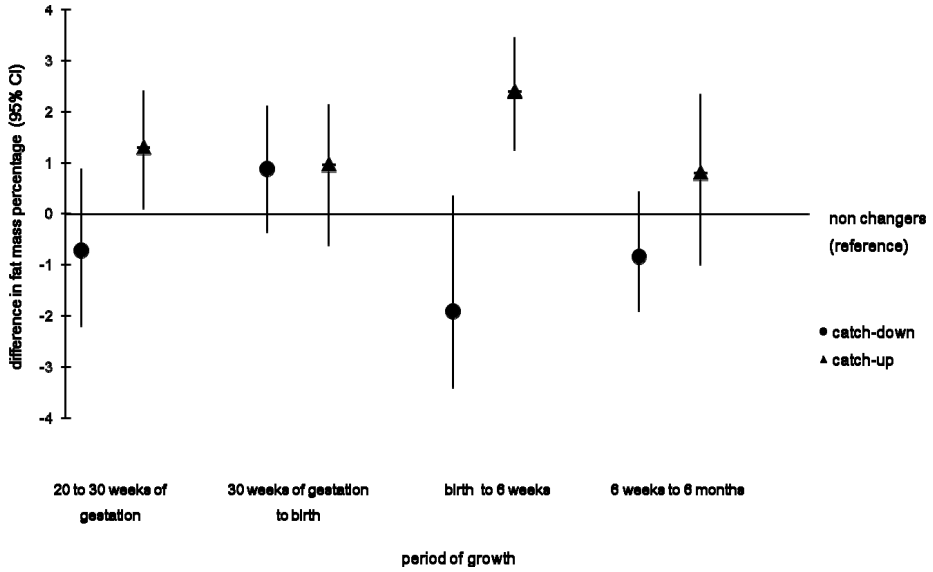

Figure 1. Catch-up and catch-down growth in weight and the difference in fat mass percentages

Table 3. Associations between fetal and postnatal change in SD-score for weight and absolute fat mass and absolute lean mass (grams) and fat mass percentage at 6 months

Change in fat mass or lean mass per change in weight-SD-scores						
Period	***Fat mass (grams)***		***Fat mass percentage***		***Lean mass (grams)***	
	B (95% CI)	**P value**	**B (95% CI)**	**P value**	**B (95% CI)**	**P value**
Fetal period						
20 weeks to 30 weeks of gestation	79 (18, 140)	**<0.05**	0.59 (0.04, 1.14)	**<0.05**	48 (-2, 97)	0.06
30 weeks of gestation to birth	28 (-35, 91)	0.39	0.13 (-0.43, 0.69)	0.66	40 (-11, 91)	0.12
20 weeks of gestation to birth	73 (21, 123)	**<0.01**	0.48 (0.02, 0.93)	**<0.05**	65 (25, 106)	**<0.01**
Postnatal period						
Birth to 6 weeks	186 (108, 264)	**<0.01**	1.61 (0.91, 2.30)	**<0.01**	46 (-18, 109)	0.16
6 weeks to 6 months	108 (32, 185)	**<0.01**	0.72 (0.04, 1.40)	**<0.05**	50 (-11, 111)	0.11
Birth to 6 months	214 (158, 271)	**<0.01**	1.67 (1.14, 2.18)	**<0.01**	89 (40, 138)	**<0.01**

Values are regression coefficients (95% Confidence Interval) and reflect the difference in fat and lean mass for change in SD-score for weight per period. Model adjusted for gender, age and gestational age and length at 6 months

Adjustment for maternal pre-pregnancy BMI and systolic blood pressure and smoking during pregnancy and additionally for breastfeeding did not materially change the effects of the associations (data not shown).

Birth and infancy

Birth weight and weight at 6 weeks were positively associated with FM(%) (Table 2). Infants with postnatal catch-up in weight (gain in weight-SD-scores >0.67) had more FM(%) at 6 months (Figure 2). These effects were strongest when catch-up occurred within 6 weeks after birth. Children with postnatal catch-up in weight had not only more total FM(%) compared to infants with catch-down or non changers, but this fat was relatively more located in the truncal area. These effects were also strongest when catch-up occurred in the first 6 weeks of life.

Postnatal change in weight-SD-scores was positively related to absolute fat mass (grams) at 6 months (Table 3). The association was also positive for absolute lean mass (grams), however, the effects were much smaller than for fat mass (B (95%CI): 89 (40, 138) vs. 214 (158, 271), respectively). These children had also a higher FM(%): B (95% CI): 0.48, (0.02, 0.93). The effects were already present within 6 weeks after birth. Adjustment for maternal pre-pregnancy BMI and systolic blood pressure and smoking during pregnancy and additionally for breastfeeding did not change the effects of the associations (data not shown).

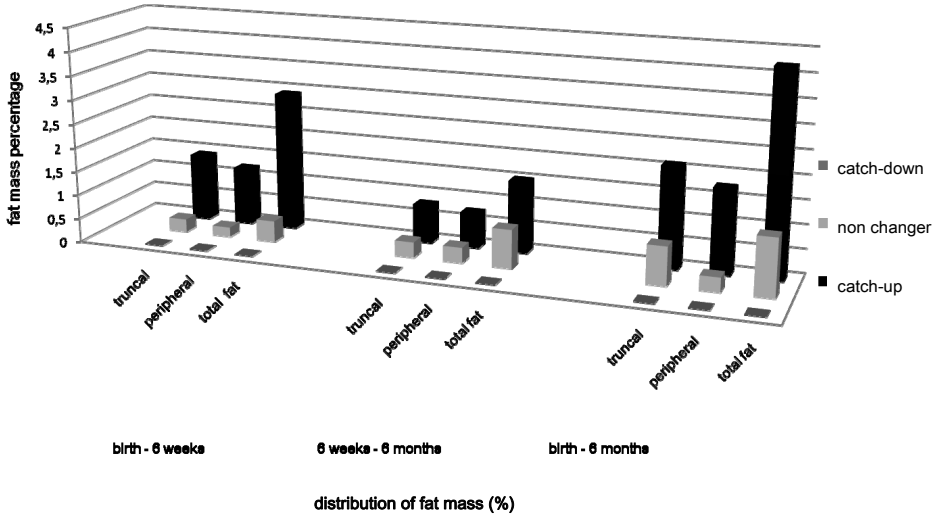

Figure 2. Truncal and peripheral fat mass at the age of 6 months compared to growth in different periods

Breastfeeding

Breastfeeding was positively associated with FM(%) at 6 months and the effect was significant for truncal and total FM(%) (Table 2). Still breastfeeding at 6 months was also positively associated and the effect was significant for truncal and total FM(%). The later the breastfeeding was discontinued, the more truncal FM(%) was observed at 6 months. Breastfeeding at 2 months (yes/no) had no significant effect.

Association between fetal life and infancy

Children with fetal catch-down in weight (loss in weight-SD-scores>0.67) showed more catch-up in weight (gain in weight-SD-scores>0.67) in the first 6 months of postnatal life (Table 4). Children with catch-down growth in the second trimester had higher odds for catch-up in weight in the first 6 months of life compared to non changers (Odds Ratio (OR) 95%(CI): 2.8 (1.38, 5.75)). Children with catch-down growth in the third trimester, however, had even higher odds for catch-up in weight in the first 6 months, in fact already in the first 6 weeks of life (OR(95%CI): 5.0 (2.47, 9.99) and 2.5 (1.26, 5.04), respectively). Adjustment for maternal pre-pregnancy BMI and systolic blood pressure and smoking during pregnancy and additionally for breastfeeding did not materially change the effects of the associations (data not shown).

Table 4. Odds ratios for postnatal catch-up in weight

	Postnatal catch-up[1] in weight		
Fetal growth	Birth to 6 weeks (n=82) OR (95%)	6 weeks to 6 months (n= 30) OR (95%)	Birth to 6 months (n=62) OR (95%)
20 weeks to 30 weeks of gestation			
Catch-down[2] (n=32)	2.2 (0.94, 5.14)	1.1 (0.33, 3.77)	2.2 (0.97, 5.18)
Catch-up (n=72)	1.1 (0.60, 2.01)	0.9 (0.31, 2.28)	0.8 (0.41, 1.69)
30 weeks of gestation to birth			
Catch-down (n=59)	2.5 (1.26, 5.04)**	1.6 (0.66, 4.03)	5.0 (2.47, 9.99)**
Catch-up (n=45)	0.8 (0.38, 1.81)	0.4 (0.08, 1.94)	0.8 (0.34, 2.07)
20 weeks of gestation to birth			
Catch-down (n=64)	1.5 (0.76, 3.15)	1.8 (0.64, 4.73)	2.8 (1.38, 5.75)**
Catch-up (n=82)	0.5 (0.27, 1.11)	1.4 (0.48, 4.03)	0.5 (0.21, 1.06)

* P<0.05, ** P<0.01

[1]catch-up = gain in weight-SD-scores >0.67, [2]catch-down = loss in weight-SD-scores >0.67, non changers = gain or loss in weight-SD-scores < 0.67

Values are odds ratios (95% CI) estimated by logistic regression for postnatal catch-up growth compared to non changers. Models are adjusted for gender, age and gestational age and length at 6 months

DISCUSSION

This prospective cohort study shows that birth weight and weight at 6 weeks have a highly significant positive association with FM(%) at the age of 6 months. Catch-up in weight during the third trimester and after birth were both positively associated with more absolute fat and lean mass at 6 months, but particularly to a relatively higher FM(%). Infants with catch-down in weight in the third trimester had more postnatal catch-up in weight and more FM(%) at 6 months. Postnatal catch-up in weight during the first 6 weeks after birth had the strongest association with a higher FM(%) at 6 months. Additionally, we found that girls had relatively more total and truncal fat than boys at 6 months.

To our knowledge, our study demonstrates for the first time that fat mass at 6 months is related to both fetal and postnatal growth patterns and we were also able to pinpoint a more exact timing. Children with catch-up growth as early as in the first 6 weeks after birth had a higher FM(%) at 6 months. Additionally, this study shows for the first time that there is an association between fetal growth retardation and postnatal catch-up growth.

We found that girls had more total and truncal fat mass at 6 months. Gender differences have been shown in several other studies. Females are shorter and weigh less at birth and throughout infancy but have more fat and less lean mass than males. (24, 25) In 4-16 year-olds the prevalence of overweight was higher in girls and in 5-12 years-olds, girls had more subcutaneous fat and higher subscapular to triceps ratios. (26, 27) Girls also have their adiposity rebound, which is associated with development of obesity, at a younger age than boys. (28, 29) However, in adulthood truncal fat was found to be increased in males and it has been related to a greater risk of developing cardiovascular disease. (16) Changes occurring in puberty and under the influence of hormones may alter fat patterning and lead to a disproportionately high fat mass in relation to lean mass in males. (17, 30-32) Further studies are needed to determine the timing and determinants of these changes.

Children with catch-up in weight had higher FM(%) at 6 months. Previously, catch-up growth within 2 years of life was associated with an unfavorable body composition in childhood. (13) We have now shown that catch-up growth as early as within 6 weeks of life is related to an increased FM(%) at 6 months. Also children with catch-up in the third trimester tended to have more FM(%) at 6 months. Further studies are needed to examine which factors influence this catch-up growth as hormones like insulin and nutrition might play a significant role in this process.

Catch-up in the second trimester was associated with increased fat mass and catch-down with decreased fat mass at 6 months. It may be that at this stage of the pregnancy, the fetus will develop towards its genetic potential without reference to environmental factors. These environmental factors (e.g. placental insufficiency due to hypertension or smoking etc) may be detrimental to fetal growth later in pregnancy (from 30 weeks of gestation onwards). We showed that maternal pre-pregnancy BMI and systolic blood pressure had the highest impact on fetal growth during the third trimester. Smoking was not correlated with fetal catch-down growth. However, this effect might be underestimated due the small numbers of participants in our study that (ever) smoked during pregnancy (17.5%).

We found that children with catch-down growth in the third trimester had more FM(%) at 6 months. These associations may support the *developmental origins of health and disease* hypothesis, which suggests that an adverse fetal environment leads to adaptations that program the fetus' metabolism. (10) This programming may have beneficial effects on short term but predisposes the individual to diseases in adulthood, including obesity and insulin resistance. (9)

Children with fetal catch-down in weight showed more catch-up already during the first 6 weeks of life. We also found that maternal characteristics before and during pregnancy were associated with fetal growth. This may support the hypothesis that infants with intra uterine

growth-restriction, resulting in low birth weight, have catch-up in weight in early infancy in order to reach their genetic growth trajectory. In later life these prenatally growth-restricted children who experience a greater postnatal catch-up may be at greater risk for developing cardiovascular disease. Further studies are needed to establish which genetic and environmental factors influence these growth patterns and whether fetal growth retardation or postnatal catch-up growth is the most important factor for subsequent obesity in later life.

Our findings are in line with the ALSPAC study, which found a positive association of birth weight with both lean mass and total body fat in 9-10 year-olds. (17) It was also reported that programming might be different in males and females. A study conducted in adolescents found an inverse association between birth weight and central fat distribution in boys, and a positive association with lean mass in girls. (11) In our study we did not find a gender difference with regard to the associations with fat and lean mass. Additionally, the association was stronger in the presence of certain genetic profiles. In adolescents carrying the Ala12 allele in the PPARγ-2 gene lower birth weight was associated with lower lean mass, while the associations in the Pro12Pro group disappeared after adjustment for potential confounders.. (33)

We adjusted our regression models with regard to fat and lean mass at the age of 6 months for current length. Previous studies have adjusted for various current body size measures (e.g. current weight, BMI etc). (12, 34) Recent studies have reported that adjusting for current measures as weight and BMI might be erroneous. (35) We decided to adjust for current length, as it is important to account for current body size. This adjustment did not materially change the associations with fat mass; however it did have a significant effect on the associations with lean mass.

Breastfeeding was related to an increase in FM(%) at 6 months. In previous studies, it was reported that breastfeeding had a protective effect against obesity in adulthood. (36) Other studies found no association of exclusive breast feeding with adiposity at 6.5 years. (37) The positive association in early infancy may be biased due to the practice that parents will be discouraged from breastfeeding when the infant does not seem to thrive in the first weeks of life. Further studies are needed to examine the effects of breastfeeding in early and later life.

In conclusion, our study on fat and lean mass strongly suggests that the risk of development of obesity and its main health consequences are at least partly established in fetal and early postnatal life. Follow up studies are needed to assess in more detail whether and to what extent maternal anthropometrics, fetal and postnatal growth patterns and nutrition have an effect on body composition in later life.

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Chapter 4

Tracking and determinants of subcutaneous fat mass in early childhood

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ABSTRACT

Objectives

To examine the development and tracking of subcutaneous fat mass in the first 2 years of life and to examine which parental, fetal and postnatal characteristics are associated with subcutaneous fat mass.

Design

This study was embedded in the Generation R Study, a prospective cohort study from early fetal life onwards. Subcutaneous fat mass was measured by skinfold thickness (biceps, triceps, suprailiacal, subscapular) at the ages of 1.5 months, 6 months and 24 months in 1012 children. Information about parental, fetal and postnatal growth characteristics was collected by physical and fetal ultrasound examinations and questionnaires.

Results

Normal values of subcutaneous fat mass are presented. Total subcutaneous fat mass was higher in girls than in boys at the age of 24 months (P =0.01). Subjects in the lowest and highest quartiles at the age of 6 months tended to keep their position in the same quartile at the age of 24 months (odds ratio's 1.86 (95% confidence interval (CI: 1.3, 2.7)) and 1.84 (95% CI: 1.3, 2.6), respectively). Maternal height and weight, paternal weight, fetal weight at 30 weeks, birth weight and weight at the age of 6 weeks were each inversely associated with subcutaneous fat mass at the age of 24 months after adjustment for current weight at 24 months.

Conclusion

This study shows for the first time that subcutaneous fat mass tends to track in the first 2 years of life. Furthermore, the results suggest that an adverse fetal environment and growth are associated with increased subcutaneous fat mass at the age of 24 months. Further studies are needed to examine whether these associations persist in later life.

INTRODUCTION

The prevalence of childhood overweight and obesity has increased dramatically in developed countries over the past two decades. (1, 2) Obesity in childhood is associated with short-term morbidity such as asthma and psychological problems, and with an increased risk for chronic morbidity and mortality in adulthood. (3, 4) Childhood obesity tends to track into adulthood, meaning that subjects keep their ranking position in body mass index distribution over time. (5)

Obesity in childhood is defined by body mass index. However, body mass index is not an appropriate measure for fat mass, which is the actual harmful factor of obesity. (6) Body mass index can remain the same while body composition can change. (7) Subcutaneous fat mass can be measured by skinfold thicknesses, which are valid measurements for use in epidemiological studies. (7, 8) With the use of skinfold thickness measurements, development of fat mass or adiposity in early childhood can be studied in more detail than only by height and weight.

Previous studies have shown that both parental anthropometrics and anthropometrics at birth are associated with obesity in childhood. (9, 10) The fetal origins hypothesis poses that an adverse fetal environment leads to adaptations that program the fetus' metabolism. These adaptations predispose the individual to increased fat mass and insulin resistance postnatally. (11, 12) However, studies relating these early life factors with more detailed measures of fat mass are scarce. (13)

We examined the development of subcutaneous fat mass, measured by skinfold thickness, in the first 2 years of life in a population-based, prospective cohort study from early fetal life onwards. We also examined whether subcutaneous fat mass tracks in early childhood and whether parental, fetal and postnatal growth characteristics are associated with subcutaneous fat mass at the age of 2 years.

PATIENTS AND METHODS

Design

The present study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. In total the cohort includes 9,778 mothers and their children living in Rotterdam, the Netherlands. (14) All children were born between April 2002 and January 2006. We have previously shown that of all eligible children in the study area, 61% participates in the study. (15) No exclusion criteria were used. Response analyses showed that the study has a somewhat higher level of education than the general population. Additional detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch mothers and their children from late pregnancy. This subgroup is referred to as the Generation R Focus cohort and is ethnically homogeneous. Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands. (15) Between February

2003 and April 2005, all mothers participating in the Generation R Study and pregnant of children who met this criterion, were approached for additional measurements in late pregnancy (gestational age 30 weeks). (14, 15) No other exclusion criteria were used. Of all approached women, 80% agreed to participate in the subgroup study. In total, 1039 children participated in at least one of the postnatal assessments at the ages of 1.5 months, 6 months and 24 months. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants or their parents.

Population for analysis

From the total of 1039 children, 87% (n = 900), 87% (n = 902) and 82% (n=857) participated in the assessments at the ages of 1.5 months, 6 months and 24 months, respectively. Skinfold measurements were performed in 88% (n = 791) at 1.5 months, in 87% (n = 785) at 6 months and in 87% (n=747) at 24 months of age of these children. From the total of 1039 children, 87% (n = 900), 87% (n = 902) and 82% (n=857) participated in the assessments at the ages of 1.5 months, 6 months and 24 months, respectively. Skinfold measurements were performed in 88% (n = 791) at 1.5 months, in 87% (n = 785) at 6 months and in 87% (n=747) at 24 months of age of these children. Twins (n = 22) were not excluded from the analysis, as they did not differ in the outcome measure from the singletons (sum of skinfolds at 24 months (mm) 27.1 versus 24.6, P=0.13) and no differences in results were observed after excluding one or both of them. Missing skinfold measurements were mainly due to crying behavior. In total, 1012 children had skinfold measurements for at least one visit.

Data collection and measurements

Maternal and pregnancy characteristics:

Information about maternal weight before pregnancy was collected by a questionnaire. Paternal height (cm) and weight (kg) were measured at intake and body mass index (kg/m^2) was calculated. Maternal height (cm) and weight (kg) during pregnancy were measured in early (gestational age <18 weeks), mid- (gestational age 18-25 weeks) and late pregnancy (gestational age > 25 weeks) in one of the research centers at the visits. Body mass index (kg/m^2) was calculated for each pregnancy period. Since enrolment in our study was in pregnancy, we were not able to measure maternal weight before pregnancy. However, in our population for analysis, the median gestational age at enrolment was 13.1 weeks. Correlation of pre-pregnancy weight obtained by questionnaire and weight measured at enrolment was 0.97 (p <0.001). No differences in results were found when we used weight measured at enrolment instead of pre-pregnancy weight obtained by questionnaire.

Fetal growth:

Fetal ultrasound examinations were carried out at the research centers in early, mid-, and late pregnancy. (15, 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 up to a gestational age of 12 weeks and 5 days (crown-rump length <65 mm), and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter >23 mm). Fetal growth measurements used in the present study included head circumference (HC), abdominal circumference (AC), and femur length (FL) measured in mid- and late pregnancy, measured to the nearest mm using standardized ultrasound procedures. Estimated fetal weight (EFW) was calculated using the formula by Hadlock: ($\log_{10}$ EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 $(HC)^2$ – 0.003685 (AC * FL)). (17) Fetal measurements in early pregnancy were not included as growth characteristics since these ultrasound examinations were primarily performed to establish gestational age.

Birth characteristics:

Date of birth, birth weight and gender were obtained from midwife and hospital registries.

Information on breastfeeding and solid foods introduction:

Information on breastfeeding and introduction solid foods was collected by questionnaires at 2, 6 and 12 months of age. This information was used first to make a categorical variable on ever having been breastfed (yes/no) breastfeeding at the ages of 2 and 6 months (yes/no) and finally, to create a continuous variable on the duration of breastfeeding (months). Also we looked at the age of starting solid foods. This was defined as the age that a fruit snack was given for the first time as is recommended in the Netherlands. For this purpose we created a variable on starting solid foods before the age of 5 months (yes/no).

Anthropometrics of the child:

Weight was measured in naked infants at the age of 1.5 and 6 months to the nearest grams by using an electronic infant scale (SECA®) and in 24 month-olds by a mechanical personal scale (SECA®). Height was measured in infants in supine position to the nearest 0.1 cm by a neonanometer (Holtain Limited®) and in 24 month-olds by a Harpenden stadiometer (Holtain Limited®) in standing position. Body mass index was calculated (kg/m^2). Waist circumference and hip circumference were measured to the nearest 0.1 cm with a measuring tape (SECA®) without clothing. Waist circumference was measured at the minimum circumference between the iliac crest and the rib cage and HC was measured at the maximum width over the greater trochanters. (18) Waist-hip ratio was then calculated as waist circumference divided by hip circumference.

Subcutaneous fat mass:

Skinfold thicknesses (SFT) were measured 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®). (19) Four well-trained medical assistants performed all measurements. (20) The consensus between and among observers for the medical assistants was analyzed using the Intraclass Correlation Coefficient (ICC). (21, 22) 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 + suprailliacal SFT + subscapular SFT. Central subcutaneous fat mass was calculated from the sum of suprailliacal SFT + subscapular SFT. Peripheral subcutaneous fat mass was calculated from the sum of triceps SFT + biceps SFT.

Covariates

Maternal age was established at enrolment in the study. Information about smoking during pregnancy (yes/no) was obtained from questionnaires during pregnancy. (15, 23) Educational level was defined in 4 categories: lower than secondary school, secondary school, higher education 1st phase, higher education 2nd phase according to the classification of Statistics Netherlands. (24) Information about pregnancy and delivery complications was obtained by midwife and hospital registries

Statistical analysis

Differences between boys and girls in parental, fetal and child characteristics were examined with Student's *t* tests or chi square test. The same strategy was used for the differences in subcutaneous fat mass between boys and girls at each visit.

We used Pearson's correlation coefficients to assess the relationships between continuously measured subcutaneous fat mass between the different visits. To examine whether subjects remain in the same percentile of skinfold thickness (tracking), we created quartiles of the total, peripheral and central sum of subcutaneous fat mass at the ages of 1.5, 6 and 24 months. We used logistic regression models to calculate the risks (odds ratios) of remaining in the same quartile from 1.5 and 6 months to 24 months.

Subsequently, the associations of paternal and maternal weight, height and body mass index before pregnancy with subcutaneous fat mass at the age of 24 months, defined as the total sum of skinfolds, were assessed using linear regression models. Similar models were used to examine the associations of estimated fetal weight in mid- and late pregnancy, birth weight, and weight, length and body mass index at the ages of 1.5, 6, 14 and 24 months and waist-hip ratio at the age of 24 months with subcutaneous fat mass. At the age of 1,5 months, we used the ponderal index instead of body mass index. These regression models were adjusted for current age, gender, gestational age (continuous) at birth and observer (Model I) and additionally for current weight (Model II) and socio-economic status, defined as maternal age and educational level, and smok-

ing during pregnancy (Model III). To take account for differences between observers, we have adjusted all the analyses for the specific observer as covariate. Variables were included in these models when they changed the effects estimates of interest on subcutaneous fat mass substantially (> 10%), or when they were strongly associated with fat mass in our or previous studies. We also used the central to peripheral skinfold ratio at the age of 24 months and the subscapular to triceps ratio at the age of 24 months as other measures of subcutaneous fat mass and examined the associations using the same regression models. Finally, we constructed tertiles of estimated fetal weight at 30 weeks, birth weight and postnatal weight at 6 months and 24 months of age and used linear regression models to examine the difference in skinfold thickness at 24 months between the tertiles using the first tertile as reference. These models were adjusted for the same possible confounders as the previous models.

All measures of association are presented with their 95% confidence intervals (CI). Statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Of all participating children, 51.8% was male. Birth weight was higher in boys than in girls. No differences were found between boys and girls in breastfeeding and introduction of solid foods (Table 1). At the ages of 1.5 months and 6 months, there was no difference between boys and girls in measured skinfold thicknesses. At the age of 24 months, girls had more central and total subcutaneous fat mass (Table 2).

No correlations of total, central and peripheral skinfold thicknesses were found at the age of 1.5 with the age of 24 months. Low but significant correlations were found for 6 months to 24 months ($r = 0.19$, $p<0.01$, for total, $r = 0.22$, $p<0.01$, for central, $r = 0.09$, $p=0.02$, for peripheral skinfold thickness) (Figures 1 and 2). We found that children tend to remain in the lowest and highest quartiles of total sum of skinfold thickness from 6 months to 24 months; OR 1.86 (95% confidence interval (CI) 1.3, 2.7) and OR 1.84 (95% CI (1.3, 2.6), respectively). No trend was found for tracking from the age of 1.5 months to 24 months (data not shown). We also found that the central but not peripheral sum of skinfold thickness tracks from the age of 6 months (OR for staying in the lowest quartile 2.28 (1.6, 3.2), OR for staying in the highest quartile 1.50 (1.0, 2.1)) (Table 3).

We found that breastfeeding has an inverse relation with fat mass, however this was not significant. Waist-hip ratio had a positive relation, which became significant after adjustment. Introduction of solid foods before the age of 5 months was not associated with subcutaneous fat mass (data not shown). Weight at the ages of 6 months, 14 months and 24 months were positively associated with skinfold thickness at the age of 24 months. However, maternal height and weight, paternal weight, fetal weight at 30 weeks, birth weight and weight at 1.5 months were inversely

Table 1. Parental, fetal and child characteristics

	Boys (n=524)	Girls (n=488)	P value
Maternal characteristics			
Pre-pregnancy			
Age (years)	31.5 (4.2)	32.0 (3.9)	0.05
Weight before pregnancy (kg)	68.0 (12.8)	69.4 (12.5)	0.11
Height (cm)	170.9 (6.0)	171.0 (6.6)	0.81
Body mass index (kg/m^2)	23.2 (4.0)	23.7 (4.1)	0.11
Late pregnancy			
Gestational age (weeks)	30.4 (1.0)	30.3 (1.0)	0.30
Weight (kg)	79.2 (13.1)	79.9 (13.0)	0.45
Body mass index (kg/m^2)	27.1 (4.2)	27.2 (4.3)	0.57
Paternal characteristics			
Age (years)	33.6 (4.8)	34.1(5.1)	0.11
Weight (kg)	85.8 (12.1)	85.7 (12.5)	0.93
Height (cm)	184.6 (7.0)	184.5 (7.0)	0.76
Body mass index (kg/m^2)	25.2 (3.3)	25.2 (3.3)	0.96
Fetal characteristics			
Mid-pregnancy			
Gestational age (weeks)	20.5 (0.9)	20.4 (1.0)	0.19
Estimated fetal weight (grams)	378 (83.6)	370 (78.5)	0.13
Late pregnancy			
Gestational age (weeks)	30.4 (1.0)	30.3 (1.0)	0.30
Estimated fetal weight (grams)	1642 (259.8)	1624 (260.9)	0.33
Birth characteristics			
Weight (grams)	3530 (555.7)	3436 (574.1)	0.01
Gestational age (weeks)	39.9 (1.8)	39.9 (1.9)	0.85
Gender (%)	51.8	48.2	
Postnatal characteristics			
1.5 months	***n=462***	***n=429***	
Age at visit (months)	1.6 (0.5)	1.6 (0.5)	0.83
Weight (grams)	5082 (744)	4748 (631)	< 0.01
Length (cm)	57.5 (2.6)	56.3 (2.5)	< 0.01
Body mass index (kg/m^2)	15.3 (1.5)	14.9 (1.3)	< 0.01
6 months	***n=466***	***n=431***	
Age at visit (months)	6.5 (0.7)	6.5 (0.7)	0.86
Weight (grams)	8193 (858)	7638 (811)	< 0.01
Length (cm)	69.5 (2.5)	67.8 (2.5)	< 0.01
Body mass index (kg/m^2)	16.9 (1.3)	16.6 (1.3)	< 0.01
14 months	***n=438***	***n=418***	
Age at visit (months)	14.6 (0.8)	14.7 (1.0)	0.66
Weight (grams)	10839 (1051)	10217 (1024)	< 0.01
Length (cm)	79.9 (2.7)	78.4 (3.0)	< 0.01
Body mass index (kg/m^2)	17.0 (1.2)	16.6 (1.2)	< 0.01
24 months	***n=444***	***n=411***	
Age at visit (months)	25.3 (1.2)	25.3 (1.2)	0.68
Weight (grams)	12844(1389)	12415 (1325)	< 0.01

Table 1. continued

Length (cm)	89.5 (3.3)	88.5 (3.1)	< 0.01
Body mass index (kg/m^2))	16.0 (1.3)	15.9 (1.3)	0.13
Waist-hip ratio	1.00 (0.05)	0.99 (0.05)	0.16
Breastfeeding			
Ever (%)	89.6	90.7	0.55
Duration (months)	4.8 (3.6)	5.1 (3.9)	0.30
At the age of 2 months (%)	59.7	63.1	0.67
At the age of 6 months (%)	26.1	28.7	0.50
Introduction of solid foods before the age of 5 months	61.6	63.3	0.58

Values are means (SDS). Differences were tested using independent sample t-test for continuous variables and chi square test for dichotomous variables. Of the total group, data were missing on maternal weight before pregnancy (n=159), maternal BMI (n=159), paternal age (n=61) paternal weight (n=60), paternal height (n=60), paternal BMI (n=60), estimated fetal weight at 20 weeks (n=49), estimated fetal weight at 30 weeks (n=42), waist-hip ratio (n=296), breastfeeding ever (n=38), breastfeeding duration (n=298, introduction of solid foods (n=380).

Table 2. Subcutaneous fat mass (mm)

	Boys	Girls	*P value*
1.5 months	***n=462***	***n=429***	
Age (months)	1.6 (1.0-2.9)	1.6 (1.0-2.7)	0.83
Triceps	6.6 (3.5-12.0)	6.5 (3.5-12.5)	0.65
Biceps	5.5 (3.0-11.0)	5.3 (3.0-11.0)	0.08
Suprailliacal	5.7 (3.0-10.9)	5.8 (3.0-11.0)	0.50
Subscapular	6.1 (3.0-10.6)	6.2 (3.0-10.7)	0.87
Peripheral fat mass	12.0 (7.0-23.0)	11.7(6.5-22.9)	0.20
Central fat mass	11.8 (6.5-20.0)	11.9 (6.5-20.0)	0.67
Total fat mass	23.7 (14.0-41.3)	23.4 (13.9-40.1)	0.50
6 months	***n=466***	***n=431***	
Age (months)	6.5 (5.5-8.1)	6.5 (5.5-8.4)	0.86
Triceps	8.0 (4.2-13.5)	7.8 (4.0-13.0)	0.08
Biceps	6.6 (4.0-11.0)	6.4 (3.3-11.0)	0.05
Suprailliacal	6.3 (3.0-11.0)	6.4 (3.0-11.0)	0.41
Subscapular	6.3 (4.0-11.0)	6.4 (3.5-11.0)	0.93
Peripheral fat mass	14.6 (9.0-23.8)	14.1 (8.5-22.0)	0.03
Central fat mass	12.6 (8.0-21.0)	12.7 (7.3-20.0)	0.60
Total fat mass	27.2 (18.0-42.0)	26.8 (17.0-41.5)	0.29
24 months	***n=444***	***n=411***	
Age (months)	25.3 (23.7-28.1)	25.3 (23.5-28.6)	0.68
Triceps	8.9 (4.0-17.0)	8.8 (4.7-15.0)	0.05
Biceps	6.6 (3.0-12.0)	6.9 (4.0-12.0)	0.85
Suprailliacal	5.3 (3.0-9.0)	5.8 (3.0-11.0)	<0.01
Subscapular	5.8 (3.0-9.2)	6.3 (3.0-11.0)	<0.01
Peripheral fat mass	15.4 (8.5-29.0)	15.6 ((9.0-26.0)	0.49
Central fat mass	11.1 (6.0-18.0)	12.1 (7.0-20.0)	<0.01
Total fat mass	26.4 (15.0-42.3)	27.7 (17.0-42.0)	0.01

Values are means (mid 95% range). Differences were tested using independent sample t-test.

Table 3. Subcutaneous fat mass tracking from 6 months to 24 months

	Quartiles 24 months			
Total sum of skin folds				
Quartiles 6 months	**1st**	**2nd**	**3rd**	**4th**
1st	1.86 (1.3, 2.7)**	0.89 (0.6, 1.3)	0.77 (0.5, 1.2)	0.65 (0.4, 1.0)*
2nd	1.11 (0.8, 1.6)	1.23 (0.9, 1.8)	1.17 (0.8, 1.7)	0.98 (0.7, 1.4)
3rd	0.71 (0.5, 1.1)	1.04 (0.7, 1.5)	1.42 (1.0, 2.1)	1.10 (0.8, 1.6)
4th	0.59 (0.4, 0.9)*	0.86 (0.6, 1.3)	0.90 (0.6, 1.4)	1.84 (1.3, 2.6)**
	Central sum of skin folds			
Quartiles 6 months	**1st**	**2nd**	**3rd**	**4th**
1st	2.28 (1.6, 3.2)**	1.14 (0.8, 1.7)	0.79 (0.5, 1.2)	0.32 (0.2, 0.5)**
2nd	1.01 (0.7, 1.4)	0.95 (0.6, 1.4)	1.25 (0.8, 1.8)	1.34 (0.9, 1.9)
3rd	0.72 (0.5, 1.1)	0.80 (0.5, 1.2)	1.14 (0.8, 1.7)	1.45 (1.0, 2.1)
4th	0.67 (0.4, 1.0)*	0.86 (0.6, 1.3)	1.23 (0.8, 1.8)	1.50 (1.0, 2.1)*
	Peripheral sum of skin folds			
Quartiles 6 months	**1st**	**2nd**	**3rd**	**4th**
1st	1.55 (1.1, 2.2)*	0.77 (0.5, 1.2)	0.87 (0.6, 1.3)	0.99 (0.7, 1.4)
2nd	1.06 (0.7, 1.6)	1.17 (0.8, 1.7)	0.98 (0.7, 1.5)	0.94 (0.6, 1.4)
3rd	0.77 (0.5, 1.1)	1.50 (1.1, 2.1)*	0.86 (0.6, 1.3)	1.12 (0.8, 1.6)
4th	0.73 (0.5, 1.1)	0.73 (0.5, 1.1)	1.34 (0.9, 2.0)	1.33 (0.9, 1.9)

*Values are unadjusted odds ratios (95% CI) estimated by logistic regression for quartiles of total, central and peripheral sum of skin folds at the ages of 6 months and 24 months. Total sum of skin folds= biceps + triceps+subscapular+suprailliacal, Central sum of skinfolds = subscapular+suprailliacal, Peripheral sum of skinfolds= biceps + triceps. *p=< 0.05, ** p=< 0.01*

associated with skinfold thickness at the age of 24 months after adjustment for current weight at 24 months. Adjustment for maternal age and educational level did not materially change these effect estimates (Table 4). In the models using the central to peripheral skinfold ratio at the age of 24 months and the subscapular to triceps ratio at the age of 24 months as outcome, the results were similar (data not shown). In late pregnancy there is an inverse relation with skin fold thickness at 24 months of age. The differences of tertiles of birth weight with skin fold thickness at 24 months of age are less clear, but postnatal weight from the age of 6 months on is positively associated with skinfold thickness at the age of 24 months (Figure 3).

DISCUSSION

This population based prospective cohort study suggests that girls have more central subcutaneous fat mass than boys at the age of 24 months. We showed that subcutaneous fat mass tends to track from the age of 6 months to the age of 24 months. Postnatal weight is strongly associated with subcutaneous fat mass. However, after adjustment for current weight, inverse associations were found for fetal weight measured in late pregnancy, birth weight and weight at 1.5 months and parental anthropometrics with subcutaneous fat mass at the age of 24 months.

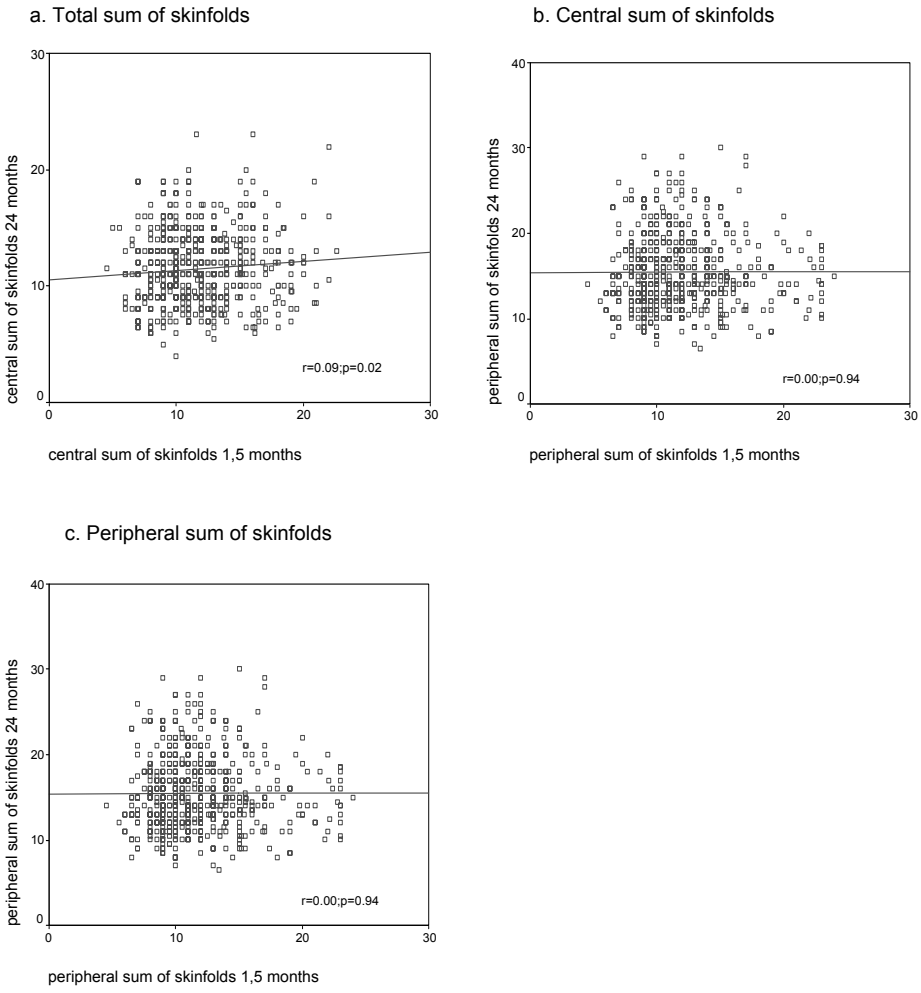

Figure 1. Correlation of sum of skinfolds between 1.5 months and 24 months
Total sum of skinfolds= biceps + triceps + subscapular + suprailliacal, Central sum of skinfolds = subscapular + suprailliacal, Peripheral sum of skinfolds= biceps + triceps

Of all postnatal participants (1039 children), skinfold measurements were performed in at least 80%. Missing skinfold measurements were mainly due to crying behavior. No differences were found between children with and without skinfold measurements: birth weight (grams) 3454 versus 3483 (P = 0.5), gestational age (weeks): 39.8 versus 39.9 (P= 0.3), gender (% male): 53.7 versus 51.8 (P = 0.6). Also, maternal anthropometrics in pregnancy were similar between infants with and without skinfold thickness measurements. The effect estimates would be biased if the associations of maternal anthropometrics with skinfold thickness differ between those included and not included in the present analyses. This seems unlikely.

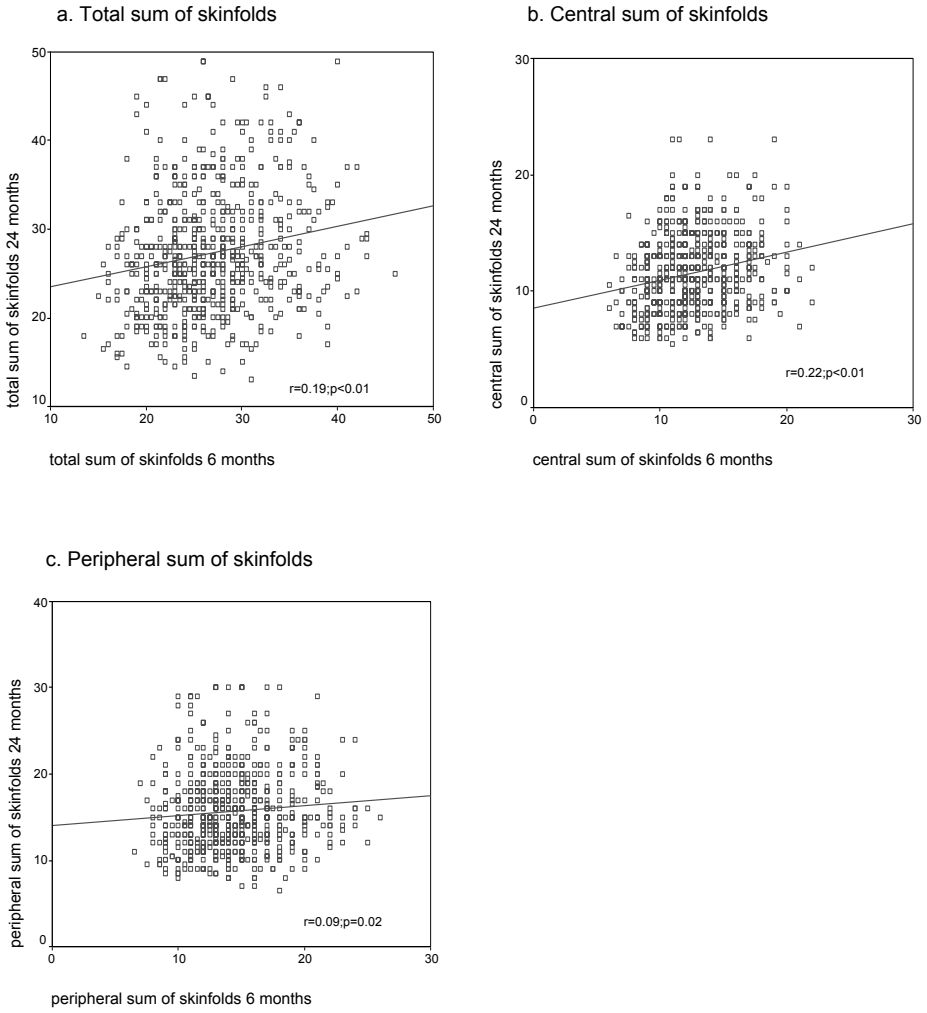

Figure 2. Correlation of sum of skinfolds between 6 months and 24 months
Total sum of skinfolds= biceps + triceps + subscapular + suprailliacal, Central sum of skinfolds = subscapular + suprailliacal, Peripheral sum of skinfolds= biceps + triceps

Measurement of skinfolds at birth would be of great value in our study. However, due to logistical and financial constraints we were not able to measure skinfolds at birth in this study. The first study specific measurements were planned at the age of 1.5 months

We used skinfold thickness as a measure of subcutaneous fat mass. This method is easy to perform and can be used in large-scale epidemiological studies. Previous studies have shown that this method is valid for measurement of fat mass in adolescents and children from 5 to 18 years; however in extremely overweight children the measurement error is larger. (20, 25)

Table 4. Associations between parental, fetal and child characteristics and total sum of skinfolds at the age of 24 months

		Sum of skinfolds at the age of 24 months (mm)					
		Model I		*Model II*		*Model III*	
		Beta (95%CI)	*P value*	*Beta (95%CI)*	*P value*	*Beta (95%CI)*	*P value*
Parents							
Mother	weight (kg)	0.01 (-0.03, 0.04)	0.76	-0.05 (-0.09,-0.01)	0.02	-0.03 (-0.07, 0.02)	0.21
	height (cm)	-0.07 (-0.15, 0.01)	0.07	-0.18 (-0.26,-0.11)	< 0.01	-0.19 (-0.27,-0.11)	< 0.01
	BMI (kg/m2)	0.08 (-0.04, 0.20)	0.19	-0.03 (-0.14, 0.09)	0.66	0.03 (-0.11, 0.16)	0.67
Father	weight (kg)	0.00 (-0.04, 0.04)	0.98	-0.04 (-0.08, 0.00)	0.06	-0.04 (-0.08, 0.01)	0.11
	height (cm)	0.01 (-0.06, 0.08)	0.84	-0.05 (-0.11, 0.02)	0.18	-0.02 (-0.10, 0.06)	0.64
	BMI (kg/m2)	0.00 (-0.15, 0.15)	1.00	-0.09 (-0.23, 0.06)	0.24	-0.12 (-0.30, 0.05)	0.17
Child							
Estimated fetal weight							
	20 weeks (kg)	3.46 (-2.61, 9.54)	0.26	-0.44 (-6.22, 5.33)	0.88	-3.19 (-9.62, 3.24)	0.33
	30 weeks (kg)	-0.46 (-2.30, 1.38)	0.62	-2.12 (-3.89,-0.35)	0.02	-4.11 (-6.22,-2.01)	< 0.01
At birth	Weight (kg)	0.74 (0.36, 1.84)	0.18	-1.44 (-2.56,-0.32)	0.01	-2.00 (-3.27,-0.74)	0.02
1.5 months	Weight (kg)	0.22 (-0.58, 1.02)	0.59	-1.00 (-1.81,-0.20)	0.01	-1.15 (-2.08,-0.22)	0.02
	Length (cm)	-0.12 (-0.34, 0.10)	0.29	-0.47 (-0.69,-0.25)	< 0.01	-0.50 (-0.76,-0.25)	< 0.01
	PI (kg/m3)	0.21 (0.01, 0.40)	0.04	0.17 (-0.01, 0.36)	0.07	0.15 (-0.07, 0.36)	0.18
6 months	Weight (kg)	1.53 (0.93, 2.13)	< 0.01	-0.20 (-0.91, 0.51)	0.59	-0.32 (-1.16, 0.53)	0.46
	Length (cm)	0.07 (-0.14, 0.28)	0.52	-0.46 (-0.68,-0.24)	< 0.01	-0.45 (0.71,-0.19)	< 0.01
	BMI (kg/m2)	1.12 (0.76, 1.57)	< 0.01	0.56 (0.17, 0.94)	< 0.01	0.47 (0.01, 0.93)	< 0.05
14 months	Weight (kg)	1.81 (1.33, 2.29)	< 0.01	-0.10 (-0.92, 0.73)	0.81	-0.14 (-1.11, 0.84)	0.79
	Height (cm)	0.05 (-0.13, 0.23)	0.56	-0.54 (-0.73,-0.33)	< 0.01	-0.56 (-0.79,-0.32)	< 0.01
	BMI (kg/m2)	1.95 (1.55, 2.35)	< 0.01	1.27 (0.80, 1.75)	< 0.01	1.25 (0.67, 1.83)	< 0.01
24 months	Weight (kg)	1.71 (1.37, 2.05)	< 0.01			1.74 (1.38, 2.15)	< 0.01
	Height (cm)	0.10 (-0.06, 0.26)	0.24	-0.74 (-0.93,-0.54)	< 0.01	-0.72 (-0.95,-0.50)	< 0.01
	BMI (kg/m2)	2.29 (1.94, 2.64)	< 0.01	.2.07 (1.54, 2.61)	< 0.01	2.01 (1.38, 2.65)	< 0.01
	Waist-hip ratio	5.00 (-5.84, 15.83)	0.71	11.58 (1.35, 21.80)	0.03	12.14 (1,88, 22.40)	0.02
Breastfeeding							
Ever breastfed (reference =yes)		-1.19 (-1.21, 0.09)	0.15	-0.91 (-2.45, 0.63)	0.25	-0.93 (-2.72, 0.87)	0.31
Age of stopping (months)		-0.10 (-0.25, 0.04)	0.17	-0.10 (-0.24, 0.04)	0.02	-0.19 (-0.34,-0.03)	0.03

PI= ponderal index, BMI= Body mass index

Values are regression coefficients (95% confidence interval) and reflect the difference in skinfold thickness for the parental, fetal and child anthropometrics. Model I: adjusted for gender, age, gestational age and observer; Model II: Model I additionally adjusted for current weight; Model III: Model II additionally adjusted for maternal age and educational level and smoking in pregnancy

The inter- and intraobserver measurement error is known to be small. (26) From these skinfold thickness measurements, we calculated total, central and peripheral subcutaneous fat mass. All anthropometric measurements were done at the research centers except for maternal pre-pregnancy weight. Since self-reported weight just before pregnancy was highly correlated (r = 0.97) with measured weight at intake, we do not think that because of using this variable, our results are biased.

We showed that skinfold thickness is not strongly age dependent in the first 2 years. This is consistent with other studies. In adolescents aged 13-18 years it was also shown that skinfold thickness is independent of age. (27) Moreover, a previous study in India suggested that skinfold

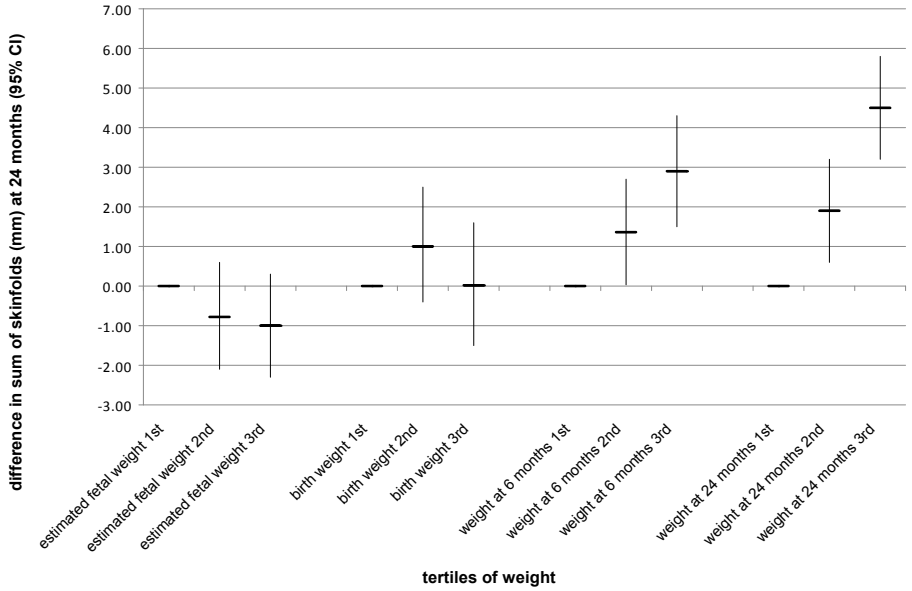

Figure 3. The associations of fetal and postnatal weight in tertiles with sum of skinfolds at 24 months
sum of skinfolds= biceps + triceps + subscapular + suprailliacal
estimated fetal weight = estimated fetal weight in late pregnancy (gestational age > 24 weeks)

thickness in adults tends to decrease with older age. (28) Our and these previous studies suggest that skinfold thickness may be a rather stable measure of fat mass from infancy to young adulthood.

We found that girls have more total and central subcutaneous fat mass at the age of 24 months. No differences were found in peripheral subcutaneous fat mass or subcutaneous fat mass at younger ages than 24 months. Although both sexes have a rise in fat mass from 1,5 months to 6 months, in boys we found a decline in fat mass after the age of 6 months but in girls the increase continued. Gender differences have been shown in several other studies. Females are shorter and weigh less than males at birth and throughout infancy but have more fat mass and less lean body mass than males, even in the neonatal period. (29-31) In 11-12 year-olds the prevalence of overweight was higher in girls. (32, 33) In Shantal children between 5-12 years old, fat patterning was different between girls and boys. Girls had more subcutaneous fat mass and higher subscapular to triceps ratios. (34) Girls also tend to have their adiposity rebound, which is associated with future development of obesity and reflect the turning point of decreasing to increasing body mass index, at younger age than boys. (35, 36) This growth acceleration in childhood can lead to a disproportionately high fat mass in relation to lean body mass. (37) These effects on body composition may favor the development of cardiovascular disease later in life. (38, 39) The underlying mechanisms as well as the critical periods of weight gain that contribute to later disease are still debated. (13, 40)

Some studies suggest that these gender differences in adiposity measured at younger age are partly explained by differences in food preferences. At preschool age, boys have been reported to dislike more food items than girls. (41) Also, it has been reported that girls are less physically active than boys. In a study among 2185 children aged, 9 to 13-years, it was shown that boys are much more active than girls. (42) In the first 6 months of life weight increases more than length for both sexes. (43) This could lead to an increase of fat mass in both sexes. It was also shown that BMI between the ages of 6 months and 2 years decreases in both sexes, but girls end up with a lower BMI. (43) BMI consists of lean body mass and fat mass. A higher level of physical activity in boys may lead to a relative higher lean body mass and combined with food preferences; this may explain the decline in fat mass compared to girls.

To our knowledge, our study demonstrates for the first time that subcutaneous fat mass has a tendency for tracking from the age of 6 months to the age of 24 months. Also, we have shown that this tracking is stronger for central than for peripheral subcutaneous fat mass. We are not aware of any other studies examining tracking of skinfold thickness in childhood. However, tracking of obesity, defined by body mass index, has previously been shown from the age of 2 years into adulthood. (44, 45) In a cohort of 474 boys and 448 girls in New Zealand, the correlation between body mass index at the age of 7 years and body mass index at the age of 21 years were 0.61 for boys and 0.52 for girls. (46) In China 1455, 6-13 year-olds were followed for 2 years. Of all children with overweight at enrolment 36.8% remained overweight 2 years later. (47) Also, in children from the age of 6-9 years old followed up for 6 years, the body mass index of thin and fat children were more likely to track: 51% and 46% remained in the bottom and upper quartiles, respectively. Overweight children were 2.8 times as likely as other children to become overweight as adolescents. Underweight children were 3.6 times as likely to remain underweight as adolescents. (48) These studies strongly suggest that the risk of development of obesity and its main health consequences are at least partly established in fetal and early postnatal life.

In the fully adjusted model, we found that maternal height and weight and paternal weight were inversely associated with subcutaneous fat mass. Also, fetal weight at 30 weeks, birth weight and weight at 1.5 months were inversely associated with subcutaneous fat mass at the age of 24 months. These findings suggest that of all children with the same weight at the age of 24 months, those with fetal growth retardation or low birth weight tend to have increased subcutaneous fat mass. These associations support the *developmental origins of health and disease* hypothesis, which suggests that an adverse fetal environment leads to adaptations that program the fetus' metabolism. (12) This programming may have beneficial effects on short term but predispose the individual to diseases in adulthood, including obesity and insulin resistance. (11) Our findings are in line with previous studies showing associations of both low and high birth weight with an increased risk of developing overweight. (49-51) Recent studies have reported that this might be dependent on programming of lean body mass as well as programming of fat mass. The ALSPAC study found a positive association of birth weight with both lean body mass and total body fat. (52) It was also reported that this programming might be different in males and females. A study

conducted in 234 adolescents in Spain found an inverse association of birth weight with central fat distribution in boys, but a positive association with lean body mass in girls. (53) Additionally, it seems that not only gender could modify this association, but the association was also shown to be stronger in certain genetic predispositions. In adolescents carrying the Ala12 allele in the PPARγ-2 gene lower birth weight was associated with lower lean body mass, while the associations in the Pro12Pro group disappeared after adjustment for potential confounders. (54)

In summary, our findings suggest that subcutaneous fat mass in early childhood is at least partly established in fetal life and may have consequences for adiposity in later life. Follow up studies are needed to assess whether subcutaneous fat mass in early childhood tracks to adulthood, and whether and to what extent the effects of parental, fetal anthropometrics and postnatal growth patterns on subcutaneous fat mass persist.

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Chapter 5

Glucocorticoid receptor gene polymorphisms and body composition in infancy

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Submitted

ABSTRACT

Objective

To examine whether Glucorticoid Receptor (GCR)-gene-haplotypes are associated with fat mass (FM) at the age of 6 months and to examine the interaction of the haplotypes with catch-up in weight.

Design

This study was embedded in the Generation R Study, a prospective cohort from early fetal life onwards.

Methods

Body composition was measured by Dual energy X-ray Absorptiometry (DXA) in 214 infants at 6 months. Fetal and postnatal growth data were collected by physical and fetal ultrasound examinations and questionnaires. DNA was collected from cord blood samples for genotyping five GR gene polymorphisms *Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I.

Results

In boys, haplotype 1(characterized by *Bcl*I) tended to be associated with more FM at 6 months (2 alleles: + 650 grams, P=0.007). In boys with postnatal catch-up in weight (gain in weight-SD-score>0.67) within 6 weeks after birth, haplotype 1 carriers tended to have more total, central and peripheral FM at 6 months. Carriers of haplotype 5 (characterized by *TthIII*I) had less FM at 6 months (-510 grams, P=0.004). Haplotype 3 (characterized by ER22/23EK), haplotype 2 (characterized by N363S) and haplotype 4 (characterized by GR-9β) were not associated with FM at 6 months. In girls no associations were found for any of the haplotypes.

Conclusion

Our results suggest that in boys, the GCR haplotype 1 is associated with an increased FM, whereas haplotype 5 is associated with less FM in infancy. The first 6 weeks of life seems a critical period in which catch-up in weight can modulate genetic susceptibility. Replication studies in larger study populations are needed before definitive conclusions can be drawn.

INTRODUCTION

Small size at birth and also postnatal catch-up in weight are associated with an increased risk of common diseases in adult life, such as hypertension, cardiovascular disease (CVD), insulin resistance and obesity. (1-6) This association might be explained by altered fetal programming of the hypothalamic-pituitary-adrenal(HPA)-axis. (7) Exogenous glucocorticoids (GC) lead to fetal growth retardation and lower birth weight (3, 7). Also, the organ systems affected by fetal life programming, including blood pressure and insulin resistance, are GC sensitive. (3) Programming may also occur after birth. Catch-up in weight within a few weeks after birth has been associated with an increased risk of unhealthy metabolic profile in later life (8, 9). Glucocorticoids are important regulators of growth, development and metabolism. Their effects are mediated by glucocorticoid receptors. Polymorphisms in the glucocorticoid receptor (GCR) gene may contribute to a difference in sensitivity and thereby to associations between growth characteristics in early life and disease in adult life (10, 11).

The GCR is a member of the nuclear receptor family and is expressed in most fetal tissues from the early embryonic stages (3, 7). Five different variants in the glucocorticoid receptor gene have been described to be associated with cortisol sensitivity in adults (12, 13) (Figure 1). The *Bcl*1 and the N363S polymorphisms are associated with increased sensitivity to GCs, visceral obesity and type 2 diabetes. (13) GR-9β has been associated with decreased GC transrepressive activity. (14) decreased microbial colonization, (15) and with increased inflammatory mediators leading to an increased risk of cardiovascular disease. (16-19) The ER22/23EK polymorphism consists of two linked single nucleotide polymorphisms in codons 22 and 23 in exon 2. (20) This polymorphism is associated with a relative GC resistance, a healthier metabolic profile and increased insulin sensitivity (21, 22). The *TthIII*I polymorphism was associated with elevated diurnal cortisol levels, but not with any anthropometric or glucose related phenotype in adults (19, 23). On the other hand, no associations were found between any of the GCR haplotypes and fetal and early postnatal growth patterns (24).

Haplotype	Polymorphism						Allele frequency, %
0		T	GG	A	C	A	42.4
1	*Bcl*I	T	GG	A	**G**	A	22.7
2	N363S	T	GG	**G**	C	A	4.1
3	ER22/23EK + GR9B+ *TthIII* I	**C**	**AA**	A	C	**G**	3.1
4	GR9B+ *TthIII* I	**C**	GG	A	C	**G**	13.4
5	*TthIII* I+ *Bcl*I	**C**	GG	A	**G**	A	14.3

Figure 1. Schematic overview of the Glucocorticoid Receptor gene polymorphisms

Previous studies suggest that variants of the glucocorticoid receptor gene may affect body composition. (21, 25-28) It was also described that the effects of some GCR gene polymorphisms on risk factors of CVD were different for men and women. (29) Thus far no studies were performed on the effects of the GCR gene polymorphisms on body composition in young children. Dual-energy X-ray Absorptiometry (DXA) is one of the most reliable and practical methods for measuring body composition in adults and children. Based on previous literature, we hypothesized that the *Bcl*1, N363S and the GR-9β polymorphisms would be associated with increased fat mass (FM) in early infancy and the ER22/23EK polymorphism with lower FM and that some of these effects might be different for boys and girls. In addition, we hypothesized that catch-up in weight in early infancy modifies the effect of the polymorphism on body composition.

We therefore examined in a prospective cohort of infants the effects of the *Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I polymorphisms on body composition from birth until the age of 6 months.

MATERIAL AND METHODS

Design

The present study was embedded in the Generation R Study, a prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood, and has been previously described in depth (30, 31). Detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch mothers and their children from 30 weeks of gestation. This subgroup is ethnically homogeneous to exclude possible confounding or effect modification by ethnicity. Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands. (30) No other exclusion criteria were used. Of all approached women, 80% agreed to participate in the subgroup study. In total, 1039 children participated in at least one of the postnatal assessments at the ages of 6 weeks, 6 months and 24 months. At the age of 6 months, DXA measurements were performed in 270 of 298 infants who were randomly selected from this subgroup. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

In this study we used the new reference charts, which were based on 8,313 pregnancies in the Generation R study. (32)

Date of birth, birth weight and gender were obtained from midwife and hospital registries. Weight was measured in naked infants at the age of 1.5 and 6 months to the nearest grams by using an electronic infant scale (SECA®). Length was measured in infants in supine position

to the nearest 0.1 cm by a neonanometer (Holtain Limited®). Body mass index was calculated (kg/m^2). Fat and lean mass were measured by Lunar Prodigy DXA scanner® (General Electrics). Previous studies have shown that this method is valid for measurement of body composition in adolescents and children (33, 34). All DXA scans were performed with the same device and software and by the same technician. After the exclusion of scans with anomalies such as movement artifacts, complete scans were available for 252 infants. The DXA scans were used to derive total, central and peripheral body FM. From the children with genotype information available, DXA scans were performed in 214 children.

Genotyping

DNA was collected from cord blood samples at birth. All participants were genotyped for five known glucocorticoid receptor gene polymorphisms which are known to be associated with changes in glucocorticoid sensitivity: *Bcl*I (rs41423247), N363S (rs6195), ER22/23EK (rs6189 and 6190), GR-9β (rs6198) and *TthIII*I (rs10052957) (12, 13). Figure 1 schematically shows the specific nucleotide variations and allele frequencies of these polymorphisms. Genotyping of the five glucocorticoid receptor gene polymorphisms 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 minutes), then 40 cycles of 94° C (15 seconds) and 60° C (1 minute)). 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). Genotyping was successful in 97-99% of the samples for the five genotypes. To confirm the accuracy of the genotyping results 276 randomly selected samples were genotyped for a second time with the same method. The error rate was less than 1% for all genotypes. We used the genotype data for each of the 5 polymorphisms to infer the haplotypes present in the population using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. (35) For each haplotype, 3 genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele. Haplotype 0 carries the major alleles of the polymorphisms; therefore, the reference allele is defined as carrying 2 copies of haplotype 0. Genotype and allele frequencies were in Hardy Weinberg equilibrium ($p>0.01$).

Statistical analysis

Differences in baseline characteristics between boys and girls were examined with Student's *t* tests, chi square tests or ANOVA analysis. Differences in FM (total, central and peripheral), measured by DXA, between carriers and non-carriers of the haplotypes were examined by using linear regression models. Because of the absence of homozygous subjects for haplotype 2 and 3, these haplotypes were analyzed as carriers (1 copy) and non-carriers (0 copies). The regression models were adjusted for gestational age at birth. We examined separately in boys and girls, whether these associations were different using similar models. Additionally, we examined the

effect of catch-up in weight on the associations of the haplotypes with FM at 6 months. For this purpose we used the change in standard deviation (SD) scores of weight from birth to 6 weeks and from 6 weeks to 6 months. We defined catch-up in weight as gain in weight SD-score >0.67, catch-down as loss in weight SD-score >0.67 and no change as gain or loss in weight SD-score <0.67 as was previously done by Ong et al.. (36) Finally, comparable regression models were used to examine whether these associations were different for boys and girls. Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA). Since we tested five haplotypes in relation with three outcome parameters separately in boys and girls, we also corrected for multiple testing. A significance level of $p=0.05/30 = p<0.0015$ was considered statistically significant after multiple testing adjustment.

RESULTS

Table 1 presents the clinical characteristics of the infants. Anthropometrics at birth and at postnatal ages of 6 weeks and 6 months were larger in boys than in girls.

Haplotypes 0, 1, 4 and 5 were most frequent with allele frequencies of 42.4%, 22.7%, 13.4% and 14.3%, respectively (Figure 1). Haplotypes 2 and 3 had allele frequencies of only 4.1% and 3.1%, respectively. The distribution of the different GCR haplotypes in our study population is presented in Table 2.

Table 1. Fetal and child characteristics

	Boys (n=124)	**Girls** (n=90)	**P value**
Birth characteristics			
Weight (grams)	3530 (555.7)	3436 (574.1)	0.01
Gestational age (weeks)	39.9 (1.8)	39.9 (1.9)	0.85
Gender (%)	57.9	42.1	
Postnatal characteristics			
1.5 months			
Age at visit (months)	1.6 (0.5)	1.6 (0.5)	0.83
Weight (grams)	5082 (744)	4748 (631)	< 0.01
Length (cm)	57.5 (2.6)	56.3 (2.5)	< 0.01
Body mass index (kg/m^2)	15.3 (1.5)	14.9 (1.3)	< 0.01
6 months			
Age at visit (months)	6.5 (0.7)	6.5 (0.7)	0.86
Weight (grams)	8193 (858)	7638 (811)	< 0.01
Length (cm)	69.5 (2.5)	67.8 (2.5)	< 0.01
Body mass index (kg/m^2)	16.9 (1.3)	16.6 (1.3)	< 0.01

Values are means (SDS). Differences were tested using independent sample t-test for continuous variables and chi square test for dichotomous variables

Table 2. Distribution of the different haplotype alleles of the glucocorticoid receptor gene

Glucocorticoid receptor haplotype (copies)	N (%)
Haplotype 0	
0	77(36)
1	105(49.1)
2	32 (15.0)
Haplotype 1	
0	122 (57.0)
1	83 (38.8)
2	9 (4.2)
Haplotype 2	
0	195 (91.1)
1	19 (8.9)
2	0 (0)
Haplotype 3	
0	789 (93.5)
1	14 (6.5)
2	0 (0)
Haplotype 4	
0	162 (75.7)
1	47 (22.0)
2	5 (2.3)
Haplotype 5	
0	152 (71)
1	60 (28)
2	2 (0.9)

Values are number of persons (%).

The associations between the haplotypes and DXA measurements at the age of 6 months were not significant, however a trend was found towards a positive association between haplotype 1 (characterized by *Bcl*I polymorphism) and total and peripheral fat mass (FM) (data not shown).

Some of the associations between haplotypes and FM at 6 months were different in boys and girls. After adjustment for multiple testing, in boys, haplotype 1 (characterized by *Bcl*I polymorphism) tended to be associated with higher total FM at 6 months (2 alleles +650 grams, P <0.007) (Table 3). No associations were found in girls for any of the haplotypes (data not shown).

In infants with postnatal catch-up in weight (gain in weight SD-score > 0.67) in the first 6 weeks after birth, carriers of haplotype 1 tended to have more total, central and peripheral FM at 6 months (Figure 2). However, after adjustment for multiple testing the results were no longer significant. The effect of catch-up in weight on the associations between the GCR haplotypes and FM at 6 months were also different for boys and girls (Table 4). Haplotype 1 (characterized by *Bcl*I polymorphism) tended to be positively associated with FM in boys with catch-up in weight in the first 6 weeks, however after multiple testing this did not reach significance. Haplotype 5 (characterized by *TthIII*I polymorphism) tended to be associated with less FM only in boys with catch-up in weight in the first 6 weeks of life (-510 grams, P0.004). No associations were found in girls for any of the haplotypes.

Table 3. Associations of glucocorticoid receptor haplotypes with fat mass at 6 months in boys

	Total	P value	Central	P value	Peripheral	P value
GCR haplotype (copies)						
Haplotype 0						
0	154	0.29	63	0.24	37	0.64
1	26	0.86	10	0.85	-1	0.99
2	Reference		Reference		Reference	
Haplotype 1						
0	Reference		Reference		Reference	
1	164	0.09	53	0.15	90	0.09
2	650	0.007	175	0.04	227	0.11
Haplotype 2						
0	Reference		Reference		Reference	
1 or 2	-55	0.76	14	0.22	-29	0.77
Haplotype 3						
0	Reference		Reference		Reference	
1 or 2	-83	0.70	-18	0.82	-20	0.87
Haplotype 4						
0	Reference		Reference		Reference	
1	-19	0.86	7	0.86	-1	0.99
2	54	0.84	20	0.84	-223	0.20
Haplotype 5						
0	Reference		Reference		Reference	
1 or 2	-137	0.19	-41	0.29	-51	0.37

Values are regression coeffecients and reflect the difference in fat mass (gram) for the different glucocorticoid haplotypes. Models are adjusted for gestational age at birth.

DISCUSSION

In our population-based prospective cohort study we showed that glucocorticoid receptor gene polymorphisms are not significantly associated with FM in early infancy. However, the results suggest that boys carrying haplotype 1 (characterized by *Bcl*I polymorphism), tend to have more total FM at 6 months and the effect tended to be augmented when catch-up in weight occurred in the first 6 weeks after birth. In contrast, boys carrying haplotype 5 (characterized by *TthIII*I polymorphism) tend to have less FM at 6 months even after catch-up in weight during 6 weeks after birth.

We found that male carriers of haplotype 1 (characterized by *Bcl*I polymorphism) tended to have more FM at 6 months. The effect of the haplotype increased with the number of alleles. In previous studies, the *Bcl*I polymorphism was associated with hypersensitivity to glucocorticoids (26-28).The effect tended to be enhanced when catch-up in weight occurred within 6 weeks after birth. In boys with catch-up in weight between 6 weeks and 6 months no associations were found for any of the haplotypes. This finding may show that the first 6 weeks of life might be a critical period in which environmental factors modulate susceptibility for this GCR haplotype in

boys. In girls no effects were found for this haplotype. This could not be explained by a gender difference in allele frequency of the haplotypes. Also, no difference was found for catch-up in weight between boys and girls. Different effects of GCR haplotypes in females and males were also described by Di Blasio et al.. (29) It is well known that men have more central adiposity than women and that both central FM and male gender are risk factors for developing CVD (37, 38). Our findings that the unfavourable effects of this common haplotype in males are modified by environmental factors already in the first 6 weeks after birth, may be important as this haplotype is common in the general population and obesity an ever growing problem for society. However, for every genetic association study there is always a possibility that associations have arisen by chance, especially if they are novel and the study population relatively small. For these reasons, replication studies in larger study populations are needed before definitive conclusions can be drawn.

Our study suggests that in young children, haplotype 5 (characterized by *TthIII*I polymorphism) leads to lower FM at 6 months. The effect was largest in boys. This finding suggests that the fattening effect of catch-up in weight is far less in carriers of this haplotype thus leading to a metabolically healthier profile in later life. This is remarkable as this haplotype also contains the *Bcl*I polymorphism which leads to increased FM. The protective effects of the *TthIII*I polymorphism must be very strong as it occurs in spite of the presence of the *Bcl*I polymorphism within this haplotype. It is the first time to our knowledge, that an association is found between this haplotype and FM. Previously, no associations were found with the *TthIII*I polymorphism, however these studies were performed in older subjects (12, 23).

We found no association between haplotype 4 (characterized by GR-9β) and FM at 6 months. This haplotype has been associated with decreased GC transrepressive activity and with increased inflammatory mediators leading to an increased risk to cardiovascular disease (14, 19). It may be that the effect of the GR-9β polymorphism on body composition appears later in life when inflammatory factors have been expressed over a longer a period of time. Also, no associations were found for haplotypes N363S and ER22/23EK. This may partly be due to the low number of subjects within the normal population and absence of homozygotes.

The major strength of our study is its prospective design from early fetal life. To our knowledge most studies on GCR haplotypes were performed in adults (39, 40). A possible limitation is that the current study was performed in a healthy, population-based cohort study. Also, the effects might not have reached significance for the uncommon haplotype 2 (characterized by N363S) and haplotype 3 (characterized by ER22/23EK) due to the low number of subjects.

We hypothesized that genetic variants leading to increased or decreased glucocorticoid sensitivity are associated with body composition in early infancy. We have not found any significant associations of GCR polymorphisms with fat mass in early infancy. However, haplotype 1 (characterized by *Bcl*I polymorphism) tended to be associated with higher FM at 6 months in boys, possibly leading to an unfavourable metabolic profile in later life. As this effect was found only in boys, it is important to examine haplotypes in males and females separately. On the other

hand, we found a trend towards an inverse association of haplotype 5 (characterized by *TthIII*I polymorphism) with fat mass at 6 months, possibly leading to a healthier metabolic profile already in infancy. The first 6 weeks of life appears a critical period in which catch-up in weight can modulate genetic susceptibility. Further systematic searches for common genetic variants will enable us to obtain a more complete understanding of what genes and polymorphisms are involved in development of fat mass and the effects of growth patterns.

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Chapter 6

Associations of glucocorticoid receptor gene polymorphisms with subcutaneous fat mass in infancy and overweight in preschool children

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ABSTRACT

Objectives

To examine whether Glucorticoid Receptor (GCR)-gene-haplotypes are associated with skinfold thickness (SFT) in early infancy and the risk of overweight and obesity in preschool children.

Design

This study was embedded in the Generation R Study, a prospective cohort study from early fetal life onward. DNA from 3990 children was collected from cord blood samples and used for genotyping of five GR gene polymorphisms (*Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I). Body composition was measured using skinfold thickness in a subgroup of 746 children at the age of 2 years. Information on overweight (SDS BMI 1.10 - 2.30) and obesity (SDS BMI > 2.30) at age 4 years was available in all children.

Results

Glucocorticoid receptor gene polymorphisms were not associated with peripheral, central or total sum of SFT at the age of 2 years. Also, no associations were found with the risks of overweight or obesity at the age of 4 years.

Conclusions

We found in a large population-based cohort no evidence for an effect of known glucocorticoid receptor gene polymorphisms on subcutaneous fat mass in infancy and on overweight or obesity in preschool children. Further systematic searches for common genetic variants by means of genome-wide association studies will enable us to obtain a more complete understanding of what genes and polymorphisms are involved in development of subcutaneous fat mass and overweight and obesity in preschool children.

INTRODUCTION

Small size at birth has been associated with increased risks of diseases in adult life, such as hypertension, cardiovascular disease (CVD), insulin resistance and obesity. (1-7) It has been hypothesized that these associations might be explained by altered fetal programming of the hypothalamic-pituitary-adrenal(HPA)-axis. (8) This hypothesis is supported by studies showing that exogenous glucocorticoids (GC) lead to fetal growth retardation and lower birth weight (1, 8). Also, blood pressure and insulin resistance are GC sensitive. (1) Glucocorticoids are important regulators of growth, development and metabolism. Their effects are mediated by glucocorticoid receptors. Polymorphisms in the glucocorticoid receptor (GCR) gene may contribute to a genetically determined difference in sensitivity and thereby to associations between growth characteristics in early life and diseases in adult life (9, 10).

The GCR is a member of the nuclear receptor family and is expressed in most fetal tissues from the early embryonic stages (1, 8). Five different variants in the glucocorticoid receptor gene have been described to be associated with cortisol sensitivity in adults (11, 12) (Figure 1). The *Bcl*1 and the N363S polymorphisms are associated with increased sensitivity to GCs, visceral obesity and type 2 diabetes. (12) GR-9β has been associated with decreased GC transrepressive activity, (13) decreased microbial colonization, (14) and with increased inflammatory mediators leading to an increased risk of cardiovascular disease. (15-18) The ER22/23EK polymorphism consists of two linked single nucleotide polymorphisms in codons 22 and 23 in exon 2. (19) This polymorphism is associated with a relative GC resistance, a healthier metabolic profile and increased insulin sensitivity (20, 21). The *TthIII*I polymorphism was associated with elevated diurnal cortisol levels, but not with any anthropometric or glucose related phenotype in adults (18, 22). Previous studies suggest that variants of the glucocorticoid receptor gene may affect body composition (21, 23-26). It was also described that the effects of some GCR gene polymorphisms on risk factors of CVD were different for men and women. (27) We have previously shown that these GCR gene polymorphisms are not associated with growth in fetal life and infancy (28).

However, thus far no studies were performed on the effects of the GCR gene polymorphisms on body composition and the risk of obesity in preschool children. The effect of these GCR gene polymorphisms might be stronger on body composition in early life because of the limited life style influences. Based on previous studies, we hypothesized that the *Bcl*1, N363S and the GR-9β polymorphisms are associated with increased subcutaneous fat mass measured as skinfold thickness (SFT) and risk of overweight in early childhood and the ER22/23EK polymorphism with lower subcutaneous fat mass and risk of overweight (12, 15, 18, 20, 21). We therefore examined in a prospective cohort study from early fetal life onwards, the associations of the *Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I polymorphisms with body composition measured as subcutaneous fat mass by skinfold thickness and the risks of overweight and obesity in preschool children.

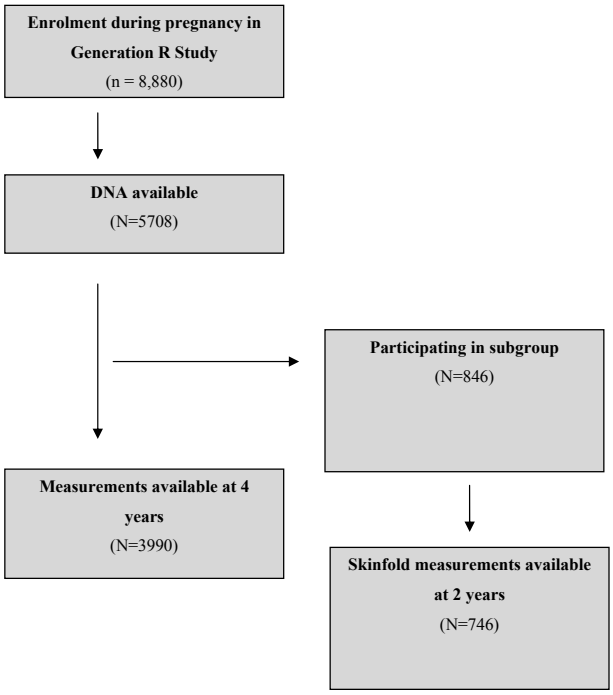

Figure 1. Study population

MATERIAL AND METHODS

Design

The present study was embedded in the Generation R Study, a prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood, and has been previously described in depth. (29, 30) Additional detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of Dutch mothers and their children from 30 weeks of gestation. This subgroup is ethnically homogeneous to exclude possible confounding or effect modification by ethnicity. (29) No other exclusion criteria were used. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Population for analysis

Analyses were restricted to children of whom DNA was available for genotyping (n = 5708). Reasons for non-availability of DNA were mainly due to logistical constraints at birth. A total of 70% (n = 3990) participated in the postnatal assessments at the age of 4 years. From the children with genotyping performed, 846 children participated in the subgroup. Skinfold measurements

were performed in 746 (=88%) children at the age of 2 years. Missing skinfold measurements were mainly due to crying behaviour (Figure 1).

Genotyping

DNA was collected from cord blood samples at birth. All participants were genotyped for five known glucocorticoid receptor gene polymorphisms which are known to be associated with changes in glucocorticoid sensitivity: *Bcl*I (rs41423247), *TthIII*I (rs10052957), GR-9β (rs6198), N363S (rs6195) and ER22/23EK (rs6189 and 6190) (11, 12). Figure 2 schematically shows the specific nucleotide variations and allele frequencies of these polymorphisms. Genotyping of the five glucocorticoid receptor gene polymorphisms 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 minutes), then 40 cycles of 94° C (15 seconds) and 60° C (1 minute)). 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). Genotyping was successful in 97-99% of the samples for the five genotypes. To confirm the accuracy of the genotyping results 276 randomly selected samples were genotyped for a second time with the same method. The error rate was less than 1% for all genotypes. We used the genotype data for each of the 5 polymorphisms to infer the haplotypes present in the population using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes from population genotype data. (31) For each haplotype, 3 genotype combinations were distinguished as carrying 0, 1, or 2 copies of the haplotype allele. Because of the low prevalence of homozygous subjects for haplotype 2and 3, these haplotypes were analyzed as carriers (1 or 2 copies) and non-carriers (0 copies). Haplotype 0 carries the major alleles of the polymorphisms; therefore, the reference allele is defined as carrying 2 copies of haplotype 0. Genotype and allele frequencies were in Hardy Weinberg equilibrium (p>0.01).

Haplotype	Polymorphism						Allele frequency, %
0		T	GG	A	C	A	42.4
1	*Bcl*I	T	GG	A	**G**	A	22.7
2	N363S	T	GG	**G**	C	A	4.1
3	ER22/23EK + GR9B+ *TthIII* I	**C**	**AA**	A	C	**G**	3.1
4	GR9B+ *TthIII* I	**C**	GG	A	C	**G**	13.4
5	*TthIII* I+ *Bcl*I	**C**	GG	A	**G**	A	14.3

Figure 2. Schematic overview of the Glucocorticoid Receptor gene polymorphisms

Data collection and measurements

Subcutaneous fat mass

Skinfold thicknesses (SFT) were measured at the age of 2 years 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®). (32) Four well-trained medical assistants performed all measurements. (33) The consensus between and among observers for the medical assistants was analyzed using the Intraclass Correlation Coefficient (ICC). (34, 35) Intraobserver ICC was 0.88 and Interobserver ICC was 0.76. Peripheral subcutaneous fat mass was calculated from the sum of triceps SFT + biceps SFT. Central subcutaneous fat mass was calculated from the sum of suprailliacal SFT + subscapular SFT. Total subcutaneous fat mass was calculated from the sum of biceps SFT + triceps SFT + suprailliacal SFT + subscapular SFT.

Anthropometrics of the child

Well-trained staff in community health centers obtained growth characteristics at the age of four years using standardized procedures based on the routine health care program. Weight was measured in naked children by a mechanical personal scale (SECA®). Height was measured by a Harpenden stadiometer (Holtain Limited®) in standing position. Body mass index (BMI) was calculated (kg/m^2). We defined 3 groups: overweight (SDS BMI 1.10 - 2.30), obesity (SDS BMI > 2.30) and overweight or obesity (SDS BMI > 1.10). (36)

Covariates

Gestational age was established by ultrasound in early pregnancy. Date of birth, birth weight and sex were obtained from midwife and hospital registries. Information on breastfeeding was collected by questionnaires at 2 and 6 months of age. This information was used as a categorical variable on ever having been breastfed (yes/no) and as breastfeeding at the age of 6 months (yes/no).

Statistical analysis

Differences between boys and girls were examined with Student's *t* tests, chi square tests. In the subgroup, we examined the differences between carriers and non carriers of the haplotypes in subcutaneous fat mass (peripheral, central and total) measured by skinfolds, by using linear regression models. The regression models were adjusted for sex, gestational age at birth and breastfeeding. Next, in the entire study population, we performed multiple logistic regression models to analyze the associations of the different glucocorticoid receptor haplotypes with risks of overweight and obesity at the age of 4 years. These models were also adjusted for sex, gestational age at birth and breastfeeding. Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA). Since we tested five haplotypes in relation with three outcome parameters, we also corrected for multiple testing. A significance level of $p=0.05/15 = p<0.003$ was considered statistically significant after multiple testing adjustment.

RESULTS

Table 1 presents the baseline characteristics of infants who participated in the postnatal visits. Anthropometrics at birth and at the postnatal ages of 2 and 4 years were larger in boys than in girls. However, at 4 years, the percentage of overweight was higher in girls. At the age of 2 years, girls had more central and total subcutaneous fat mass.

Table 1. Baseline characteristics

	Boys (n=2001)	**Girls** (n=1967)	**P value**
Birth characteristics			
Weight (grams)	3515 (523)	3404 (482)	<0.01
Gestational age (weeks)	40.0 (1.5)	39.9 (1.5)	0.06
Sex (%)	50.4	49.6	
Postnatal characteristics			
2 years			
Age at visit (months)	25.3 (1.2)	25.3 (1.2)	0.68
Weight (grams)	12844(1389)	12415 (1325)	< 0.01
Length (cm)	89.5 (3.3)	88.5 (3.1)	< 0.01
Body mass index (kg/m^2))	16.0 (1.3)	15.9 (1.3)	0.13
Subcutaneous fat mass			
Triceps	8.9 (4.1-15.0)	8.7 (5.0-13.0)	0.34
Biceps	6.5 (4.0-11.0)	6.9 (4.0-11.0)	<0.05
Suprailliacal	5.3 (3.0-9.0)	6.0 (3.0-10.1)	<0.01
Subscapular	5.9 (4.0-8.0)	6.4 (4.0-10.0)	<0.01
Peripheral fat mass	15.4 (9.0-24.0)	15.6 ((9.5-24.0)	0.59
Central fat mass	11.2 (7.0-16.0)	12.2 (7.1-18.4)	<0.01
Total fat mass	26.6 (17.0-40.0)	28.0 (18.4-41.0)	<0.05
4 years			
Age at visit (years)	3.5 (0.5)	3.5 (0.6)	0.57
Weight (kg)	16.5(2.4)	16.2 (2.5)	< 0.01
Length (cm)	101 (6.0)	100 (6.1)	< 0.01
Body mass index (kg/m^2))	16.1 (1.4)	15.9 (1.5)	<0.05
Overweight			
Overweight (SDS BMI 1.10 - 2.30) (%)	11.4	14.0	<0.05
Obesity (SDS BMI > 2.30) (%)	2.1	2.3	0.67
Overweight or obesity (SDS BMI > 1.10) (%)	13.6	16.3	<0.05
Breastfeeding			
Ever (%)	89.6	90.7	0.55
At the age of 6 months (%)	26.1	28.7	0.50

Values are means (SDS). Differences were tested using independent sample t-test for continuous variables and chi square test for dichotomous variables.

The distribution of the different glucocorticoid receptor haplotypes within our study population is presented in Table 2. Haplotypes 0, 1, 4 and 5 were most frequent with allele frequencies of 42.4%, 22.7%, 13.4% and 14.3%, respectively (Figure 2). Haplotypes 2 and 3 had allele frequencies of only 4.1% and 3.1%, respectively.

Table 2. Distribution of the different haplotype alleles of the glucocorticoid receptor gene

Glucocorticoid receptor haplotype (copies)	N (%)
Haplotype 0	
0	1590 (27.9)
1	2780 (48.7)
2	1338 (23.4)
Haplotype 1	
0	3684 (64.5)
1	1801 (31.6)
2	223 (3.9)
Haplotype 2	
0	5400 (94.6)
1	298 (5.2)
2	10 (0.2)
Haplotype 3	
0	5463 (95.7)
1	243 (4.3)
2	2 (0.0)
Haplotype 4	
0	4260 (74.6)
1	1328 (23.3)
2	120 (2.1)
Haplotype 5	
0	4394 (77.0)
1	1232 (21.6)
2	82 (1.4)

Values are number of persons (%).

Table 3 shows the associations of glucocorticoid receptor haplotypes with subcutaneous fat mass measured as skinfolds at the age of 2 years. No associations were found between the different haplotypes and peripheral, central or total sum of SFT at the age of 2 years. Adjustment for breastfeeding did not change the effects (data not shown).

Associations of the different haplotypes with the risks of overweight at the age of four years are presented in Table 4. No significant differences were found in risk of overweight for the different haplotypes. Also, no differences were found in risk of obesity for the haplotypes. Again, adjustment for breastfeeding did not materially change the effects (data not shown).

Table 3 Associations of glucocorticoid receptor haplotype with subcutaneous fat mass measured by skinfolds at the age of 2 years

	Peripheral SFT (95% CI)	P value	Central SFT (95% CI)	P value	Total SFT (95% CI)	P value
Glucocorticoid receptor haplotype (copies)						
Haplotype 0						
0	0.64 (-0.38, 1.66)	0.22	0.55 (-0.21, 1.30)	0.16	0.75 (-0.95, 2.46)	0.39
1	0.02 (-0.96, 0.99)	0.97	0.26 (-0.47, 0.98)	0.49	-0.30 (-1.93, 1.33)	0.72
2			Reference			
Haplotype 1						
0			Reference			
1	0.55 (-0.20, 1.28)	0.15	0.11 (-0.43, 0.64)	0.70	0.07 (-1.16, 1.30)	0.92
2	0.08 (-1.75, 1.91)	0.94	-0.38 (-1.74, 0.98)	0.58	-0.87 (-3.97, 2.23)	0.58
Haplotype 2						
0			Reference			
1	-0.63 (-1.87, 0.59)	0.31	0.39 (-0.51, 1.30)	0.39	-0.23 (-2.28, 1.82)	0.82
2	-5.82 (-10.25, -1.38)	0.01	-1.92 (-5.15, 1.32)	0.25	-7.93 (-15.18, -0.69)	0.03
Haplotype 3						
0			Reference			
1	-0.64(-2.34, 0.76)	0.37	0.34 (-0.71, 1.38)	0.52	-1.25 (-3.65, 1.14)	0.30
Haplotype 4						
0			Reference			
1	-0.06(- 0.90, 0.78)	0.89	-0.03 (-0.64, 0.59)	0.94	-0.11(-1.49, 1.28)	0.88
2	-0.03 (-3.20, 3.13)	0.98	-0.74 (-3.20, 1.71)	0.55	-0.72 (-7.22, 5.79)	0.83
Haplotype 5						
0			Reference			
1	1.07 (0.28, 1.87)	0.008	0.75 (0.17, 1.33)	0.01	2.36 (1.06, 3.67)	0.0004
2	1.26 (-1.72, 4.23)	0.41	-0.23 (-2.39, 1.94)	0.84	1.01 (-3.81, 5.83)	0.68

SFT= skinfold thickness, Peripheral = biceps + triceps, central = subscapular + supraillical, Sum of skinfolds= biceps + triceps+ subscapular + supraillical. CI: confidence interval

Values are regression coefficients and reflect the difference in skinfold thickness (mm) for the different glucocorticoid haplotypes. Models are adjusted for gestational age at birth and sex.

DISCUSSION

In our population-based prospective cohort study we showed that glucocorticoid receptor gene polymorphisms are not associated with subcutaneous fat mass measured as skinfold thickness in infancy. Furthermore, we demonstrated that these polymorphisms were not related to higher risk of overweight or obesity at the age of 4 years.

The strengths of our study are the size of the population-based cohort and its prospective design. Furthermore, the relative effect of variants of the glucocorticoid receptor gene on growth measurements might be larger in childhood, when the effect of various environmental factors, such as life style habits, is limited. A possible limitation is that the current study was performed in a healthy, population-based cohort study. DNA for genotyping was available in 59% of all subjects and was isolated from cord-blood. Missing cord-blood was mainly caused by logistical restraints at delivery.

Table 4. Glucocorticoid receptor haplotype and the risk of overweight at the age of 4 years

	overweight (SDS BMI 1.10 - 2.30) (95% CI)	P value	obesity (SDS BMI > 2.30) (95% CI)	P value	overweight or obesity (SDS BMI > 1.10) (95% CI)	P value
Glucocorticoid receptor haplotype (copies)						
Haplotype 0						
0	1.17 (0.86, 1.54)	0.27	0.54 (0.29, 0.98)	0.04	1.02 (0.79, 1.31)	0.90
1	1.32 (1.03, 1.69)	0.03	0.71 (0.44, 1.15)	0.17	1.17 (0.94, 1.48)	0.16
2	Reference		Reference		Reference	
Haplotype 1						
0	Reference		Reference		Reference	
1	1.07 (0.87, 1.30)	0.52	0.89 (0.56, 1.41)	0.63	1.04 (0.86, 1.25)	0.86
2	0.97 (0.59, 1.59)	0.90	0.54 (0.13, 1.22)	0.39	0.89 (0.55, 1.43)	0.63
Haplotype 2						
0	Reference		Reference		Reference	
1	0.72 (0.46, 1.13)	0.16	0.57 (0.18, 1.82)	0.34	0.69 (0.45, 1.06)	0.09
2	0.00 (0.00, 0.00)	0.99	0.00 (0.00, 0.00)	0.99	0.00 (0.00, 0.00)	0.99
Haplotype 3						
0	Reference		Reference		Reference	
1 or 2	1.22 (0.80, 1.86)	0.35	0.73 (0.23, 2.31)	0.59	1.14 (0.76, 1.71)	0.52
Haplotype 4						
0	Reference		Reference		Reference	
1	1.25 (1.01, 1.54)	0.04	1.08 (0.66, 1.76)	0.76	1.23 (1.01, 1.51)	0.04
2	1.53 (0.85, 2.73)	0.16	1.11 (0.27, 4.64)	0.88	1.48 (0.85, 2.58)	0.17
Haplotype 5						
0	Reference		Reference		Reference	
1	1.06 (0.62, 1.81)	0.84	0.59 (0.32, 1.06)	0.08	0.81 (0.66, 1.02)	0.07
2	0.72 (0.31, 1.67)	0.45	0.66 (0.09, 4.82)	0.68	0.70 (0.32, 1.55)	0.38

SDS: standard deviation score, BMI: body mass index, CI: confidence interval

Values are odds ratios (95% confidence interval) and reflect the difference in risk of overweight. Models are adjusted for gestational age at birth and sex.

Of all postnatal participants in the subgroup (n=846), skinfold measurements were performed in 88% (n=746) at the age of 2 years. Missing skinfold measurements were mainly due to crying behaviour. No differences were found between children with and without skinfold measurements: birth weight (grams) 3454 versus 3483 (P = 0.5), gestational age (weeks): 39.8 versus 39.9 (P= 0.3), gender (% male): 53.7 versus 51.8 (P = 0.6). The effect estimates would be biased if the associations of maternal anthropometrics with skinfold thickness differ between those included and not included in the present analyses, but this seems unlikely.

We used skinfold thickness as a measure of subcutaneous fat mass. This method is easy to perform and can be used in large-scale epidemiological studies. Previous studies have shown that this method is valid for measurement of subcutaneous fat mass in adolescents and children from 5 to 18 years; however in extremely overweight children the measurement error is larger. (33, 37)

The inter- and intraobserver measurement error is known to be small. (38) From these skinfold thickness measurements, we calculated total, central and peripheral subcutaneous fat mass.

We defined overweight and obesity at the age of 4 years using BMI-SDS. BMI is an expression of weight and height and not fat mass, and the accuracy of these reference values in classifying adiposity in children has not yet been validated in most countries. (39) However, measurement of BMI is a practical and reproducible method for classifying overweight in adults. (40, 41) Recently, the use of BMI-SDS is increasingly recommended for screening overweight in children and adolescents (42, 43). Growth charts from the WHO include age- and sex-specific BMI reference values for children and adolescents. (44) In our study we used the cut-off points of BMI-SDS to define overweight and obesity as previously described by Cole et al. (36)

Glucocorticoid receptor gene polymorphisms have been identified as contributors to the variability in glucocorticoid sensitivity. This sensitivity to glucocorticoids is known to show a large interindividual variation. (24) It is likely that these polymorphisms are to some extent responsible for the variability in the sensitivity to glucocorticoids. Glucocorticoids are important regulators of the immune system, inflammatory processes and many other processes involved in fat and glucose metabolism. Previous studies examined the potential role of glucocorticoids in the development of adult disease. (45, 46) Increased exposure to cortisol in adults leads to increased risks of cardiovascular disease, type 2 diabetes and obesity. (9, 10) Therefore, these polymorphisms in the glucocorticoid receptor gene could, by increasing glucocorticoid sensitivity in the fetus for maternal glucocorticoids, lead to intrauterine growth retardation and metabolic and cardiovascular diseases in adulthood. Genetically established differences between individuals in glucocortcoid sensitivity may also be associated with these diseases.

The effect of glucocorticoids is mediated by the glucocorticoid receptor, which is thought to be the connection between HPA axis function and early life conditions. (2) Previous studies have examined the associations of different polymorphisms in the glucocorticoid receptor gene and sensitivity to glucocorticoids. The results of these studies are conflicting. A few studies report positive associations between the N363S and *Bcl*I polymorphisms and hypersensitivity to glucocorticoids, (24-26) while other studies found the opposite effect. (47, 48) The ER22/23EK polymorphism was associated with relative resistance to glucocorticoids. (21, 23) No associations were found yet with the *TthIII*I polymorphism. (11, 22) These studies suggest that genetically established differences in glucocortcoid sensitivity are important for various health related outcomes. In addition, it is known that environmental, dietary, and socioeconomic factors also play an important role in body composition and metabolic factors. Associations with polymorphisms depend on many additional factors, for example differences in characteristics between populations, prevalence of the polymorphism, and interactions with other genetic polymorphism. All these factors may play a role in the discrepancies found between studies so far.

We hypothezised that genetic variants leading to increased glucocorticoid sensitivity are associated with subcutaneous fat mass in infancy and with overweight in childhood. This hypothesis is based on previous observations showing associations of the glucocorticoid receptor gene with

body composition in adulthood. However, we did not find any effect on peripheral, central or total sum of SFT between the different glucocorticoid receptor haplotypes in our population-based study. Neither did we find significant differences in risk of overweight in preschool children for the different haplotypes. Also, no differences were found in risk of obesity for the haplotypes. Therefore, we may conclude that our results do not support our hypothesis. Further systematic searches for common genetic variants by means of genome-wide association studies will enable us to obtain a more complete understanding of what genes and polymorphisms are involved in development of subcutaneous fat mass and overweight and obesity in preschool children.

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Chapter 7

Fetal and postnatal growth and bone mass at 6 months

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ABSTRACT

Objective

To examine whether parental, fetal and postnatal characteristics and growth patterns in fetal life and infancy are associated with bone mass at 6 months, as bone acquisition seems to be associated with genetic and environmental factors.

Design

This study was embedded in the Generation R Study, a prospective cohort from early fetal life onward.

Patients and Measurements

Bone mineral density (BMD) and bone mineral content (BMC) total body (TB) and BMD lumbar spine (LS) were measured by dual-energy X-ray absorptiometry (DXA) in 252 infants at 6 months. Parental, fetal, and postnatal data were collected by physical and fetal ultrasound examinations and questionnaires.

Results

Maternal, fetal and postnatal anthropometrics were positively associated with BMD_{TB} and BMC_{TB} at 6 months but only postnatal anthropometrics were associated with $BMD_{LS.}$ A gain in weight-SD-score during fetal life and prenatal catch-up in weight were positively associated with BMD_{TB}. After birth, a gain in weight-SD-score was positively associated with BMD_{LS} and $BMAD_{LS.}$ The effect was strongest between 6 weeks and 6 months. Catch-up in weight was associated with a lower probability of low (lowest quartile of) BMD_{TB} and $BMD_{LS.}$ Children remaining in the first tertile of weight from birth to 6 months had a much higher probability of low BMD_{TB} at 6 months (OR(95%CI): 15(2, 88)).

Conclusions

Our findings suggest that growth patterns in fetal and as postnatal life are associated with bone mass in infancy and may have consequences for bone mass in later life. Follow up studies are needed to assess whether and to what extent maternal anthropometrics, fetal and postnatal growth patterns have an effect on bone status in adulthood.

INTRODUCTION

Low bone mineral density (BMD) and low bone mineral content (BMC) are associated with a higher probability of fractures. Bone mass is the result of the equilibrium between bone formation and bone resorption. Bone mineral acquisition is thought to be associated with genetic and environmental factors. (1, 2)

Most studies were, however, performed in an elderly population. Accrual of bone mineral density during the first years of life is a major determinant of bone mineral density in later life. (3, 4) Bone mineral density increases rapidly during childhood and adolescence. (5-7) Among adolescents, childhood weight and height was found to be associated with BMD. (8, 9)

It was suggested that poor growth during fetal life and infancy is associated with decreased BMD and BMC in adulthood. (4, 10, 11) Also, reduced growth during intrauterine and early postnatal life was directly linked with an increased risk of hip fracture 6 to 7 decades later. (4, 12-14) These associations may be explained by an adverse uterine environment, which may affect both early skeletal development and the acquisition of bone mineral density in childhood. (14) Consistent with the programming hypothesis, maternal diet in pregnancy was found to be associated with 'areal' BMD in 9-year-old children in the ALSPAC study. (14) However, this was based on relatively small numbers of subjects as studies in young children are limited.

Bone mineral density is different for total body and for lumbar spine. Lumbar spine (LS) mainly consists of trabecular bone, and BMD_{LS} is mostly affected by weight-bearing. (15-17) In young adults, many factors unrelated to weight bearing also influence spine BMD (e.g. smoking, calcium intake).

The bone of the total body (TB) consists of 80% cortical bone and BMD_{TB} is mostly affected by nutrition and physical activity. (5, 17-20) Based on previous literature, we hypothesized that parental anthropometrics and fetal growth patterns are related to BMD_{TB}, BMD_{LS} and BMC_{TB}, but that the associations will be different for the bone types. On the other hand, we expected that growth in weight and height during early infancy will lead to a higher BMD_{TB} and BMD_{LS} at the age of 6 months.

We therefore examined in a prospective cohort of infants from early fetal life onwards whether parental, fetal and postnatal characteristics were associated with bone mass measured by dual-energy X-ray absorptiometry (DXA) at the age of 6 months. Additionally, we examined whether growth patterns in fetal life and infancy were associated with bone mineral density at the age of 6 months.

PATIENTS AND METHODS

Design

The present study was embedded in the Generation R Study, a prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood, and has been previously described in depth. (21, 22)

Detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of 1,232 Dutch mothers and their children from 30 weeks of gestation. This subgroup is referred to as the Generation R Focus cohort and is ethnically homogeneous to exclude possible confounding or effect modification by ethnicity. Dutch ethnicity was defined as having two parents and four grandparents born in the Netherlands. (22) No other exclusion criteria were used. Of all approached women, 80% agreed to participate in the subgroup study. At the age of 6 months, DXA measurements were performed in 270 of 298 infants who were randomly selected from this subgroup. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents.

Data collection and measurements

Parental and pregnancy characteristics

Information about maternal weight before pregnancy was collected by questionnaire. Maternal height (cm) and weight (kg) during pregnancy were measured at a median gestational age (Inter Quartile Range (IQR)) of 12 weeks (11.9-13.6), 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9), in one of the research centers. Body mass index (kg/m^2) was calculated for each pregnancy period. Paternal height (cm) and weight (kg) were measured at intake and body mass index (kg/m^2) was calculated. Maternal maximum weight during pregnancy was available in 42% of participating mothers (n=114; mean SD-score) 81.4 kg (13)). Because of the number of missings of maximum weight, we defined weight gain as the difference between weight before pregnancy and weight at 30 weeks of gestation. This is actually weight gain during the first two trimesters, but was strongly correlated with weight gain during the entire pregnancy in mothers with both measures available ($r = 0.80$, $P < 0.01$).

Fetal growth

In this study we used the new reference charts, which were based on 8313 pregnancies in the Generation R study. (23) Ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL Philips® Model HDI 5000 (Seattle, WA, USA). Standard ultrasound planes for fetal measurements were used as described previously. (24-26) The ultrasounds were performed by multiple operators. The intraclass correlation coefficient (ICC) was higher than 0.98 and coefficient of variation (CV) lower than 6% for all fetal biometry parameters. (27)

Bland and Altman plots to test agreement of measurements for fetal biometry demonstrated normal distributions; the mean difference was around zero and 95% of measurements fell within 2SD of the mean. The 95% limits of agreement for differences in fetal biometry measurements between and among operators in proportions fell within 10% of the mean of the measurements indicating good reproducibility. (27)

Fetal ultrasound examinations were carried out at the research centers at a median gestational age (interquartile range (IQR)) of 12 weeks (11.9-13.6), 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9). (21, 22) All participating mothers underwent ultrasound examinations at 20 and 30 weeks of gestation. However, in a subgroup of these mothers, additional, more detailed examinations were performed at 30 weeks of gestation.

These fetal ultrasound examinations were used for either establishing gestational age or for assessing fetal growth characteristics. Crown-rump length was used for pregnancy dating in early pregnancy (up to a gestational age of 12 weeks and 5 days), and biparietal diameter was used for pregnancy dating thereafter. Fetal measurements in early pregnancy were not included as growth characteristics since these ultrasound examinations were primarily performed to establish gestational age.

Fetal growth measurements at 20 and 30 weeks of gestation included head circumference (HC), abdominal circumference (AC), and femur length (FL), which were measured to the nearest mm using standardized ultrasound procedures.

Multilevel modeling according to Royston and Altman was used to produce growth centiles. This method applies a particular type of statistical model to longitudinal data to produce growth centiles and the same model may also be used to calculate valid size centiles. (28, 29) The best fitting fractional polynomial curves were chosen by comparing the deviances and by visually checking the goodness of fit. The curves were fitted using repeated measurement analysis. Next, regression lines were fitted for the dependency of the residual SD on gestational age. (30) Subsequently, plotting the SD-scores against gestational age was used to assess correctness of the model.

The curves were fitted using repeated measurement analysis and these curves were plotted on the data. Fetal growth reference curves for biparietal diameter (BPD), HC and AC were calculated for a gestational age from 10 to 40 weeks. (23) In the present study, SD-scores for all fetal growth measures were based on these reference data. Estimated fetal weight (EFW) was calculated using the formula by Hadlock: ($\log_{10}$ EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL)) + 0.00034 $(HC)^2$ – 0.003685 (AC * FL)). (31)

Birth characteristics

Date of birth, birth weight and gender were obtained from midwife and hospital registries.

Breastfeeding information

Information on breastfeeding was collected by questionnaires at 2 and 6 months of age. This information was used as a categorical variable on ever having been breastfed (yes/no) and as a continuous variable on the duration of breastfeeding.

Anthropometrics of the child

Weight was measured in naked infants at the age of 6 weeks and 6 months to the nearest grams by using an electronic infant scale (SECA®). Length was measured in supine position to the nearest 0.1 cm by a neonatometer (Holtain Limited®). Body mass index was calculated (kg/m^2).

Body composition and bone mineral density

In all participants, bone mineral density (BMD) of total body (TB) and lumbar spine (LS), bone mineral content (BMC), lean mass (LM) and fat mass (FM) was measured by DXA (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was performed daily. The children were wrapped in vacuum blankets to ensure minimal movements. All DXA scans were performed with the same device and software and by the same technician. The operator decided what movement was excessive. Clinical guidelines for performing DXA scans combined with recommendations of the manufacturer were followed in order to obtain reliable and clinically comparable results. After the exclusion of scans with anomalies such as movement artifacts, complete scans were available for 252 infants.

Due to short stature in children, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$). (32, 33)

To account for differences in bone size we calculated apparent BMD (BMAD) of lumbar spine with the model

$BMAD_{LS} = BMD_{LS} \times [4/(\pi \times width)]$

Width is the mean width of the second to fourth lumbar vertebral body. This model was validated by in vivo volumetric data obtained from magnetic resonance imaging of lumbar vertebrae. (34)

Statistical analysis

Differences between boys and girls were examined with Student's *t* tests, chi square tests or ANOVA analysis.

The associations of maternal and paternal anthropometrics with bone mass (BMD_{TB}, $BMD_{LS,}$ $BMAD_{LS}$ and BMC_{TB}) at the age of 6 months were assessed using multiple linear regression models. Similar models were used to examine the associations of bone mass at 6 months with estimated fetal weight at a median gestational age (IQR) of 20 weeks (19.9-20.9) and 30 weeks (29.5-30.9), birth weight and weight at 6 weeks and 6 months and breastfeeding (ever (yes/no) and as continuous variable (months)). Next, we performed a multivariate analysis to investigate which of these parental, fetal and postnatal factors contribute most to the development of bone mass.

In addition, we examined whether changes in weight-SD-scores during fetal life and infancy were associated with bone mineral density (BMD_{TB}, BMD_{LS} and $BMAD_{LS}$) at the age of 6 months using comparable regression models. We used the SD-score of (estimated fetal) weight at several ages (gestational age 20 weeks, gestational age 30 weeks, birth, 6 weeks, 6 months) to calculate the change in weight-SD-scores in different periods.

Additionally, we examined the probability of catch-up in weight and height for low bone mineral density at the age of 6 months using logistic regression models. For this purpose we used the change in SD-scores of (estimated fetal) weight at several ages. We defined catch-up as a gain in weight-SD-scores>0.67, catch-down as loss in weight-SD-scores>0.67 and no change as gain or loss in weight-SD-scores<0.67 as was previously reported by Ong et al. (35) For the prenatal assessments, second trimester was defined as the period between 20 and 30 weeks of gestation and the third trimester was defined as the period between 30 weeks of gestation and birth. For catch-up in height we used the same cut off points, however only height at 6 weeks and 6 months were available. In order to define low bone mineral density, we created tertiles of bone mineral density and the lowest tertile was considered as low bone mineral density. All the regression models were adjusted for current age, gender and gestational age at birth.

Finally, we examined the probability of remaining in the lowest tertile of weight from birth to 6 months on low bone mineral density at the age of 6 months using comparable logistic regression models. For this purpose we created tertiles of birth weight and of weight at 6 months. Low bone density in this analysis was defined as the lowest quartile of BMD_{TB}. These models were adjusted for current age, gender and gestational age at birth and length at 6 months.

Statistical analyses were performed using the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA). A p-value of < 0.05 was regarded as significant.

RESULTS

Characteristics of infants who participated in the bone mineral density measurement study and their parents are presented in Table 1. No large gender differences were found except that girls had higher BMD_{LS} than boys at the age of 6 months, but boys had higher BMC_{TB}.

Bone mass

BMD_{TB}

In linear correlation analyses we found that weight, length and BMI at the age of 6 months were all highly correlated with BMD_{TB} at 6 months (P value <0.001 for all correlations) (Figure 1).

Multiple regression analysis models showed that maternal weight gain in pregnancy, fetal weight at 20 and 30 weeks of gestation had a positive association with BMD_{TB} at the age of 6 months (P< 0.05 and P 0.08) (Table 2). Also, birth weight, weight at 6 weeks and BMI at 6 weeks

Table 1. Parental, fetal and child characteristics

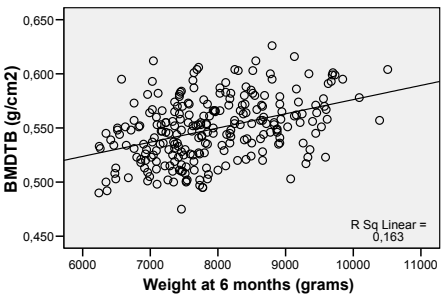
	Boys (n=145)	Girls (n=107)	P value
Maternal characteristics			
Age (years)	31.4 (4.0)	32.5 (3.8)	<0.05
Weight (kg)	69.2 (12.1)	68.6 (11.3)	0.71
Height (cm)	171 (5.8)	170 (6.3)	0.68
Body mass index (kg/m2)	24.4 (4.3)	24.7 (4.1)	0.59
Weight gain in pregnancy (kg)	9.5 (4.6)	9.8 (4.1)	0.69
Paternal characteristics			
Age (years)	33.7 (4.7)	35.1 (6.2)	<0.05
Weight (kg)	85.3 (14.3)	85.2 (12.6)	0.94
Height (cm)	184 (7.5)	184 (7.1)	0.73
Body mass index (kg/m2)	25.3 (3.6)	25.2 (3.3)	0.81
Fetal characteristics			
Mid-pregnancy			
Gestational age (weeks)	20.5 (0.8)	20.3 (0.9)	<0.05
Estimated fetal weight (grams)	375 (71.5)	361 (71.1)	0.13
Late pregnancy			
Gestational age (weeks)	30.2 (1.1)	30.4 (1.1)	0.24
Estimated fetal weight (grams)	1606 (284)	1641 (279)	0.34
Birth			
Gestational age (weeks)	39.9 (1.8)	40.1 (1.2)	0.18
Weight (grams)	3494 (557)	3503 (477)	0.89
Postnatal characteristics			
6 weeks			
Age (months)	1.6 (0.4)	1.6 (0.4)	0.83
Weight (grams)	5067 (761)	4741 (638)	<0.01
Length (cm)	57.4 (2.6)	56.5 (2.4)	<0.01
Head circumference	38.9 (1.5)	38.1 (1.5)	< 0.01
Body mass index (kg/m2)	15.3 (1.5)	14.8 (1.3)	<0.01
6 months			
Age (months)	6.4 (0.8)	6.3 (0.7)	0.24
Weight (grams)	8168 (923)	7564 (743)	<0.01
Length (cm)	69.6 (2.4)	68.0 (2.3)	< 0.01
Head circumference (cm)	44.3 (1.3)	43.2 (1.3)	< 0.01
Body mass index (kg/m2)	16.8 (1.4)	16.4 (1.3)	< 0.01
Breastfeeding			
Ever breastfed (yes/no) (%)	87.4	91.6	0.29
At 2 months (yes/no) (%)	64.0	64.1	0.99
At 6 months (yes/no) (%)	33.3	28.6	0.42
Duration (months)	4.2	4.3	0.77
DXA-measurements at 6 months			
Body composition			
Fat mass (grams)	1962 (508)	1904 (431)	0.35
Fat mass percentage (%)	23.7 (4.1)	25.0 (3.9)	<0.05
Lean mass (grams)	6648 (568)	6112 (478)	<0.01

Table 1. continued

Bone mass			
BMD total body (TB) *(grams/cm2)*	0.55 (0.03)	0.55 (0.03)	0.40
BMD lumbar spine (LS) *(grams/cm2)*	0.31 (0.04)	0.33 (0.04)	<0.01
BMAD lumbar spine (LS) *(grams/cm2)*	0.20 (0.02)	0.21 (0.03)	<0.01
BMC total body (grams)	120.9 (23.5)	110.5 (20.4)	<0.01
BMC L2-L4 (grams)	2.7 (0.5)	2.6 (0.4)	0.35

Weight gain in pregnancy= weight in late pregnancy- pre-pregnancy weight. BMD= Bone mineral density, BMAD= Bone mineral apparent density, BMC= Bone mineral content

DXA= dual-energy X-ray absorptiometry. *Values are means (SDS). Differences were tested using independent sample t-test for continuous variables and chi square test for dichotomous variables*



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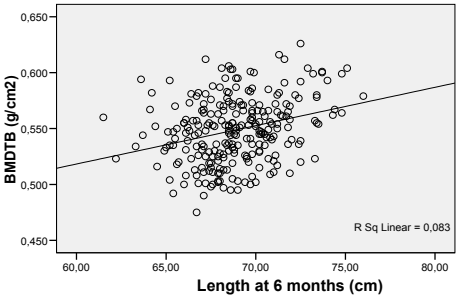

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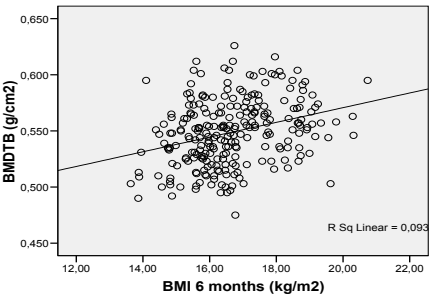

r= 0.31 p <0.001

Figure 1. Correlations of current anthropometrics and BMD_{TB}

BMD= Bone Mineral Density, TB =total body

Values are Pearson's correlation coefficients between current anthropometrics and bone mineral density.

were all positively associated with BMD_{TB} at the age of 6 months (P< 0.05 for all) (Table 2). The association of BMD_{TB} with absolute fat mass and absolute lean mass, but also fat mass percentage was positive (Table 2). The multivariate analysis on which of the parental, fetal and postnatal variables contribute most to predicting bone mass did not provide additional insights into the mechanisms as all factors sem to contribute equally. (data not shown)

Table 2. Associations between parental, fetal and child characteristics and bone mass at the age of 6 months

		Bone mass at the age of 6 months											
		BMD_{TB}			BMD_{LS}			$BMAD_{LS}$			BMC_{TB}		
		Beta (95%CI)		P	Beta (95%CI)		P	Beta (95%CI)		P	Beta (95%CI)		P
Parents													
Mother	weight (kg)	0.001	(-0.001, 0.003)	0.26	0.001	(-0.001, 0.003)	0.25	6.22 E-005	(0.00,0.00)	0.69	0.17	(-0.06, 0.40)	0.14
	height (cm)	0.001	(-0.001, 0.003)	0.24	-0.001	(-0.001, 0.000)	0.15	-0.001	(-0.001, 0.00)	< 0.01	0.09	(-0.34, 0.52)	0.69
	BMI (kg/m2)	0.001	(-0.001, 0.003)	0.14	0.001	(0.000, 0.003)	<0.05	0.001	(0.001, 0.002)	0.17	0.46	(-0.20, 1.13)	0.17
	weight gain (kg)	0.002	(0.001, 0.003)	<0.05	-0.001	(-0.002, 0.000)	0.10	0.001	(-0.001, 0.002)	0.33	0.40	(-0.22, 1.02)	0.21
Father	weight (kg)	6.5 E-005	(0.00, 0.00)	0.64	0.001	(-0.001, 0.000)	0.33	-0.001	(-0.002, 0.000)	< 0.05	0.04	(-0.17, 0.24)	0.72
	height (cm)	0.001	(-0.001, 0.000)	0.37	-0.001	(-0.001, 0.000)	0.06	-0.001	(-0.002,-0.001)	< 0.01	0.17	(-0.21, 0.52)	0.37
	BMI (kg/m2)	-3.0 E-005	(-0.001, 0.000)	0.96	0.001	(-0.002, 0.001)	0.57	0.001	(-0.001, 0.001)	0.37	-0.07	(-0.86, 0.72)	0.87
Fetal													
	EFW 20 weeks (kg)	0.040	(-0.010, 0.090)	0.08	0.090	(0.020, 0.150)	< 0.05	0.040	(-0.010, 0.090)	0.09	0.04	(0.01, 0.08)	< 0.05
	30 weeks (kg)	0.020	(0.001, 0.003)	< 0.05	-0.001	(-0.020, 0.020)	0.95	-0.001	(-0.020, 0.010)	0.83	0.01	(0.01, 0.02)	< 0.01
Postnatal													
Birth	weight (kg)	0.002	(0.001, 0.003)	< 0.01	0.010	(-0.010, 0.020)	0.18	-0.001	(-0.010, 0.010)	0.71	0.02	(0.01, 0.02)	< 0.01
6 weeks	weight (kg)	0.001	(0.001, 0.002)	< 0.01	2.080	(0.880, 3.270)	< 0.01	0.010	(0.004, 0.016)	< 0.01	0.01	(0.01, 0.02)	< 0.01
	BMI (kg/m2)	0.001	(0.001, 0.002)	< 0.01	0.730	(0.150, 1.300)	< 0.05	0.002	(0.000, 0.004)	0.11	4.32	(2.46, 6.17)	< 0.01
	length (cm)	0.001	(0.001, 0.003)	0.35	0.001	(-0.001, 0.002)	0.84	-0.001	(-0.003, 0.000)	0.15	1.44	(0.26, 2.61)	< 0.05
6 months	weight (kg)	0.010	(0.001, 0.002)	< 0.01	0.020	(0.010, 0.025)	< 0.01	0.007	(0.003, 0.110)	< 0.01	14	(12, 17)	< 0.01
	length (cm)	0.010	(0.001, 0.002)	< 0.01	0.005	(0.003, 0.007)	< 0.01		NA		4.64	(3.60, 5.71)	< 0.01
	BMI (kg/m2)	0.010	(0.001, 0.002)	< 0.01	0.007	(0.003, 0.010)	< 0.01	0.004	(0.002, 0.007)	< 0.01	4.57	(2.86, 6.78)	< 0.01
Fat mass (kg)		0.014	(0.007, 0.021)	< 0.01	0.031	(0.021, 0.040)	< 0.01	0.017	(0.011, 0.024)	< 0.01	8.87	(3.95, 13.78)	< 0.01
Fat mass percentage (%)		0.001	(0.000, 0.002)	< 0.05	0.003	(0.002, 0.004)	< 0.01	0.002	(0.001, 0.003)	< 0.01	0.56	(0.01, 1.12)	< 0.05
Lean mass (kg)		0.018	(0.011, 0.240)	< 0.01	0.017	(0.007, 0.027)	< 0.01		NA		18.37	(12.6, 24.1)	< 0.01
Breastfeeding													
Ever been breastfed (yes/no)		0.000	(-0.010, 0.010)	0.94	-0.008	(-0.020, 0.010)	0.31	-0.00	(-0.020, 0.010)	0.40	0.9	(-6.89, 8.76)	0.81
At the age of 2 months (yes/no)		-0.000	(-0.010, 0.011)	0.43	-0.020	(-0.030, -0.010)	< 0.01	-0.009	(-0.016, -0.002)	< 0.05	-4.7	(-9.76, 3.36)	0.07
At the age of 6 months (yes/no)		-0.001	(-0.010, 0.010)	0.13	-0.010	(-0.020, 0.001)	0.08	-0.010	(-0.010, 0.000)	0.08	-6.5	(- 11.44, -1.63)	< 0.05
Duration (months)		-0.001	(-0.010, 0.000)	0.09	-0.005	(-0.010, -0.002)	< 0.01	-0.010	(-0.010, -0.001)	< 0.05	-2.2	(- 3.76, -0.72)	< 0.05

BMI= Body mass index, BMD= Bone mineral density (g/cm2), BMAD= bone mineral apparent density, TB =total body, LS= lumbar spine, BMC = Bone mineral content (grams), EFW: estimated fetal weight, fat mass percentage= fat mass (kg)/ total weight (kg) at 6 months.

Values are regression coefficients (95% confidence interval) and reflect the difference in bone mineral density for the parental, fetal and child anthropometrics. Model adjusted for adjusted for gender, age and gestational age at birth.

Breastfeeding (ever) and at 2 and 6 months (yes/no) were not associated with BMD_{TB} at 6 months (Table 2). The duration of breastfeeding showed a trend towards an inverse association BMD_{TB} at 6 months (P 0.09). Adjustment for weight at 6 months did not significantly alter the effects.

A change in weight-SD-score in fetal life was positively associated with total BMD_{TB} (P value <0.01) (Table 3). Both prenatal catch-up in weight from 20 weeks of gestation to birth and catch-up in weight within 6 weeks after birth were associated with a lower probability of low BMD for BMD_{TB} (Odds Ratio (OR) (95% CI) respectively: 0.4 (0.20, 0.94) and 0.2 (0.08, 0.52)) (Table 4).

We also examined the effect of remaining thin on BMD. Children remaining in the first tertile of weight from birth to 6 months had a much higher probability of low BMD_{TB} at 6 months compared to the reference group (children remaining in the highest tertile of weight from birth to 6 months) (OR (95%CI): 15 (2, 88)) (Figure 2).

Table 3. SD change and bone mineral density

Change in bone mineral density per change in weight-SD-scores						
Period	***BMD$_{TB}$***		***BMD$_{LS}$***		***BMAD$_{LS}$***	
	B (95% CI)	**P value**	**B (95% CI)**	**P value**	**B (95% CI)**	**P value**
Fetal period						
20 weeks to 30 weeks of gestation	0.005 (0.001, 0.008)	<0.01	0.002 (-0.003, 0.008)	0.34	-0.001 (-0.005, 0.003)	0.56
30 weeks of gestation to birth	0.001 (-0.001, 0.001)	0.70	0.002 (-0.001, 0.007)	0.41	0.001 (-0.002, 0.005)	0.48
20 weeks of gestation to birth	0.004 (0.001, 0.007)	<0.01	0.003 (-0.001, 0.007)	0.19	0.001 (-0.003, 0.003)	0.99
Postnatal period						
Birth to 6 weeks	0.003 (-0.001, 0.008)	0.17	0.005 (-0.001, 0.012)	0.10	0.004 (-0.001, 0.08)	0.09
6 weeks to 6 months	-0.003 (-0.008, 0.001)	0.17	0.010 (0.003, 0.016)	<0.01	0.005 (0.001, 0.010)	<0.05
Birth to 6 months	-0.001 (-0.004, 0.003)	0.89	0.009 (0.004, 0.014)	<0.01	0.006 (0.002, 0.009)	<0.01

BMD= Bone Mineral Density, BMAD= Bone Mineral Apparent Density, TB =total body, LS= lumbar spine BMC= Bone mineral content
Values are regression coefficients (95% Confidence Interval) and reflect the difference in bone mineral density for change in SD-score in weight per period. Models adjusted for gender, age and gestational age

Table 4. Catch-up in weight and probability of low bone mineral density

	Probability on lowest tertile of bone mineral density		
	BMD_{TB} OR (95%)	**BMD_{LS}** OR (95%)	**$BMAD_{LS}$** OR (95%)
Catch-up [1] growth in weight			
Prenatal			
20 weeks to 30 weeks of gestation	0.6 (0.26, 1.47)	1.7 (0.69, 4.29)	2.5 (0.96, 6.32)
30 weeks of gestation to birth	0.4 (0.17, 1.11)	0.8 (0.31, 1.85)	0.9 (0.35, 2.10)
20 weeks of gestation to birth	0.4 (0.20, 0.94)*	1.2 (0.57, 2.61)	2.1 (0.97, 4.52)
Postnatal			
Birth to 6 weeks	0.2 (0.08, 0.52)**	0.4 (0.16, 0.92)*	0.6 (0.26, 1.48)
6 weeks to 6 months	0.6 (0.23, 1.81)	0.6 (0.23, 1.75)	0.4 (0.16, 1.25)
Birth to 6 months	0.5 (0.23, 1.21)	0.3 (0.15, 0.77)*	0.6 (0.26, 1.24)
Catch-up in height			
6 weeks to 6 months	0.7 (0.25, 1.76)	0.5 (0.19, 1.32)	0.7 (0.26, 1.81)

*P<0.05, ** P<0.01

BMD= Bone Mineral Density, BMAD= Bone Mineral Apparent Density, TB =total body, LS= lumbar spine

[1]catch-up = gain in SD-score > 0.67

Values are odds ratios (95% CI) estimated by logistic regression for postnatal catch-up compared to catch down (catch-down =loss in SD-score > 0.67). Models are adjusted for gender, age and gestational age

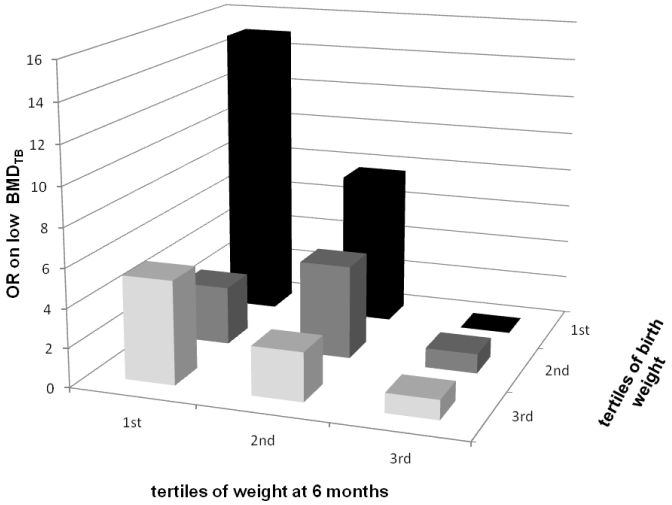

Figure 2. Birth weight and current weight and the probability for low BMD at 6 months
BMD= Bone Mineral Density, TB =total body, low BMD_{TB}= lowest quartile of BMD_{TB}
Values are odds ratios (95% CI) estimated by logistic regression for remaining in the lowest tertile of weight from birth to 6 months and the probability for low bone mineral density. Models are adjusted for gender, gestational age and length and age at 6 months.

BMD_{LS} and $BMAD_{LS}$

Maternal and paternal height were inversely associated with $BMAD_{LS}$ at the age of 6 months (Table 2). Only fetal weight at 20 weeks of gestation was associated with BMD_{LS} and $BMAD_{LS}$ (P <0.05 and P 0.09). Weight and BMI at 6 weeks were also positively associated with BMD_{LS} and $BMAD_{LS}$.

Also, length and BMI at the age of 6 months, and absolute fat mass and lean mass at the age of 6 months were positively associated with BMD_{LS} at the age of 6 months (Table 2). However, after correction for length ($BMAD_{LS}$), absolute lean mass was no longer associated with BMD_{LS}. The association of BMD_{LS} and $BMAD_{LS}$ with fat mass percentage was positive (Table 2).

Breastfeeding at the age of 2 months and duration of breastfeeding (months) were inversely associated with BMD_{LS} and $BMAD_{LS}$ at the age of 6 months (Table 2). Breastfeeding at 6 months was also inversely associated with BMD_{LS} and $BMAD_{LS}$ at the age of 6 months, but this effect was not significant. Adjustment for weight at 6 months did not significantly alter the effects.

A change in weight-SD-score in fetal life was not associated with BMD_{LS} or $BMAD_{LS}$ (Table 3). However, after birth, a change in weight-SD-score was positively associated with BMD_{LS} and $BMAD_{LS.}$ The effect was strongest between 6 weeks and 6 months. Prenatal catch-up in weight from 20 weeks of gestation to birth was not associated with low BMD_{LS} but postnatal catch-up in weight during the first 6 weeks after birth was associated with a decreased probability of low BMD_{LS} at the age of 6 months (OR (95% CI) 0.4 (0.16, 0.92)) (Table 4).

BMC_{TB}

Parental anthropometrics were not associated with BMC_{TB} at the age of 6 months (Table 2). Fetal weight at 20 and 30 weeks of gestation, birth weight, weight, BMI and length at 6 weeks and weight, BMI and length at 6 months were all positively associated with BMC_{TB} at the age of 6 months ($P< 0.05$ for all) (Table 2). The association of BMC_{TB} with absolute fat mass and absolute lean mass, but also fat mass percentage was positive (Table 2).

Breastfeeding (ever) and at 2 months (yes/no) were not associated with BMC_{TB} at 6 months (Table 2). Breastfeeding at the age of 6 months and the duration of breastfeeding were inversely associated with BMC_{TB} at 6 months. ($P <0.05$) Adjustment for weight at 6 months did not significantly alter the effects.

DISCUSSION

Our prospective cohort study shows for the first time that maternal, fetal and birth parameters are associated with BMD and BMC in early infancy. Also postnatal growth in weight was correlated to BMD and BMC at 6 months. Additionally, our study shows that not only low birth weight is associated with a lower BMD but that remaining in the lowest weight tertile at the age of 6 months considerably increases the probability of a low BMD at 6 months. We showed that catch-up in weight during the first 6 weeks of life decreases this probability of low BMD. All associations persisted after adjustment for current age, gender and gestational age at birth. Most importantly, we found that BMD_{TB} and BMC_{TB} were mostly associated with prenatal characteristics whereas BMD_{LS} is associated with postnatal characteristics. Additionally, we found that girls have a higher BMD_{LS} than boys at the age of 6 months.

We found that growth in fetal life is associated with BMD_{TB} and BMC_{TB} at 6 months. Previously, it was shown that maternal birth weight, height, parity, triceps skinfold thickness and lower walking speed in late pregnancy were not only associated with body composition but also with neonatal bone mass. On the other hand maternal smoking was associated with lower neonatal bone mass. (36, 37) These associations may support the *developmental origins of health and disease* hypothesis, which suggests that an adverse fetal environment leads to adaptations that program the fetus' metabolism. (38) This programming may have beneficial effects on short term but predispose the individual to diseases in adulthood. (39) Children who were exposed to malnutrition in fetal life will be born with a lower BMD_{TB} and BMC_{TB}. High energy nutrition after birth might have a positive effect on the development of bone mass, however this will lead to unfavorable effects on body composition. (40) An interesting issue is the possibility of preventing long-term effects of poor intrauterine growth on bone mass by aiming at normal postnatal weight gain of these babies.

We found that weight gain between 6 weeks and 6 months was associated with BMD_{LS} and not with BMD_{TB}. These associations may support earlier studies showing that weight bearing

positions (e.g. sitting up straight from the age of 3 months) is related to $BMD_{LS.}$ (15-17) Catch-up in weight was also associated with a lower probability for low BMD, which might lead to a decreased probability on fractures in later life. Further studies are needed to examine whether this beneficial effect of weight persists on the long term.

Our findings are in line with previous studies showing associations of current body size measures and BMD_{TB}. (15, 41) However, these studies were primarily performed in adults and older children, not in infancy. (8, 9, 42) It is well known that body weight is positively associated with BMD_{TB} in adults, possibly as a consequence of enhanced mechanical strain. (43, 44) Due to short stature in children, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$). (32, 33) We have now shown that the influence of body weight on BMD accrual in childhood is already present in early infancy.

In our study, we found that breastfeeding had no beneficial effect on bone mass at the age of 6 months. This is in line with other studies. No correlation was found between the duration of breastfeeding after discharge from hospital and lumbar BMD at the age of 6–7 y in a study on prematurely born children who after birth all received breast-milk until discharge from hospital and 71% were breastfed for a median age of 5–7 months. (45) Also, it was shown that, although longer duration of breastfeeding was associated with lower fat mass at 4 years, no association was found between duration of breast-feeding in the first year of life and 4-year bone size or density. This might be explained by the fact that breastfed children have less fat mass leading to lower mechanical strain. (46) In addition, bottle fed children receive higher nutritional intake as standard prescription, which will lead to higher BMD_{TB}. However, another study showed that term born children who had received breastfeeding longer than 3 months had higher BMD at the age of 8 years. (47) Variations in infant feeding patterns were also not associated with differences in childhood bone mass at age 4 years. (48, 49) Follow up studies are needed to examine the effects of breastfeeding in later life.

We found that girls have a higher BMD_{LS} at the age of 6 months. Gender differences have been shown in several other studies. Females are shorter and weigh less at birth and throughout infancy. (50, 51) It was previously shown that girls have more fat mass percentage than boys at the age of 6 months. (46) This difference in fat mass might lead to more mechanical strain resulting in higher BMD_{LS} in girls at 6 months. Further studies are needed to determine the clinical relevance of this gender difference.

Strengths and limitations

Our study demonstrates that bone mass is related to both fetal and postnatal growth patterns. Fetal growth parameters were measured several times during pregnancy whereas other studies have used birth weight as a proxy for fetal growth as they had no access to fetal growth parameters. We also had detailed examinations in early infancy. To our knowledge this is the largest study

examining bone mass in early infancy after the neonatal period with DXA scans. Additionally, we had extensive information on parental characteristics and potential confounders.

The ultrasounds in our study were performed by multiple operators. However, the ICC was higher than 0.98 and the corresponding CV lower than 6% for all fetal biometry parameters. Bland and Altman plots to test agreement of measurements for fetal biometry showed normal distributions. The 95% limits of agreement for differences in fetal biometry measurements between and among operators indicated good reproducibility. Thus we could reliably construct reference curves for fetal size from early pregnancy onwards for clinical purposes.

In our study we used the same DXA scanner for all measurements and the scans were performed by the same technician. Quality assurance was performed daily. The coefficient of variation (CV) was 0·64% for bone mineral content (BMC), 1·04% for spine BMD and 0·64% for total body BMD. (52) The CV for lean tissue and fat tissue has been reported to be 1·57–4·49% and 0·41–0·88%, respectively.(53) Due to short stature in children, true BMD is underestimated by the standard areal measurement and we corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$). (32, 33)

We used 0.67SD as a cut off point to define catch-up in weight. It would have been informative to use continuous gain or loss in weight. However in this cohort of healthy children the differences were too small and this did not provide any useful information. A change in SD-score for weight of 0.67 SD-scores represents the width of each percentile band on standard growth charts, meaning; second to ninth percentile, ninth to 25th, 25th to 50th, and so on. This indicates clinically significant catch-up or catch-down growth, not only postnatally but also prenatally.(35)

Of the subgroup of participants (n= 1247) with more detailed investigations, 298 participants were randomly selected at the age of 6 months, and were approached for DXA measurements. Of the 298 children, 24 parents refused consent mostly because of time issues and a few because of concerns about safety. Of the remaining 274 participants, 4 children failed to be scanned due to crying. In total 270 scans were performed, of which 252 were reliable to be used in the study. The children with DXA measurements available were not different form the children without DXA scans in maternal anthropometrics and most child anthropometrics. However, the children with DXA scans available were taller at the age of 6 months than the children without DXA scans (68.9 vs. 68.5 cm, $P < 0.05$). We do not think this biases our results as we correct for length at the age of 6 months.

We used validated questionnaires to obtain information on breastfeeding. It is not possible to measure intake of breastfeeding vs. formula feeding by any other method. Use of questionnaires is a valid and reliable method to obtain information on feeding practices. The response rates of the questionnaires were high (69% at the age of 2 months and 89 % at the age of 6 months).

In conclusion, our findings suggest that growth patterns in fetal as well as postnatal life are associated with BMD in early childhood and may have consequences for bone mineral density in later life. Catch-up in weight seems to be a major contributor to higher bone mass in infancy. It would be interesting to see whether this effect persists after puberty and during adolescence.

As the major risks on osteoporotic fractures are in adulthood, more specifically after 50 years of age, it would be of interest to see whether this protective effect is still present at that age. Another very interesting issue is whether catch-up later in childhood (e.g. until 2 years of age) would also lead to higher bone mass in later life. Follow up studies are needed to assess whether and to what extent maternal anthropometrics, fetal and postnatal growth patterns have an effect on bone status in adulthood.

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Chapter 8

General discussion

GENERAL DISCUSSION

This present thesis describes results of studies performed to investigate the associations of parental anthropometrics as well as genetic determinants of fetal growth, with body composition and bone mineral density (BMD) in early infancy. Also, the effect of catch-up in weight during early infancy on fat mass (FM) and BMD in infancy was investigated, as well as tracking of fat mass from infancy to early childhood.

The associations of maternal anthropometrics before and during pregnancy with fetal growth measured in different periods of pregnancy and the risks of small and large size for gestational age at birth are described (Chapter 2). Also, associations between pre-pregnancy BMI, height, blood pressure and smoking during pregnancy and fetal growth restraint and fat mass percentage in early infancy are reported as well as the effect of catch-up in weight during early infancy on fat mass percentage at the age of 6 months (Chapter 3). Additionally, the development of subcutaneous fat mass, measured by skinfold thickness, in the first 2 years of life is presented as well as associations between parental, fetal and postnatal growth characteristics and subcutaneous fat mass at the age of 2 years. Also, tracking of subcutaneous fat mass from infancy to early childhood is described (Chapter 4). Two studies report the associations between GCR polymorphisms (*Bcl*1, N363S, ER22/23EK, GR-9β and *TthIII*I polymorphisms) and fat mass in early infancy and overweight in childhood, and the influence of catch-up in weight on the effect of the polymorphisms on body composition (Chapter 5.1 and 5.2) . Finally, this thesis describes the associations between parental anthropometrics and fetal growth patterns with bone mass and the effect of higher gain in weight and height during early infancy on BMD_{TB} and BMD_{LS} at the age of 6 months (Chapter 6).

Maternal and fetal determinants of adult disease

The prevalence of childhood obesity increases and its health implications are becoming more evident. Obesity is associated with significant health problems in childhood and is an important early risk factor for much of adult morbidity and mortality. Childhood obesity frequently persists into adulthood, with up to 80%of obese children reported to become obese adults. (1) Many of the metabolic and cardiovascular complications of obesity are already present during childhood and are closely related to the presence of insulin resistance/hyperinsulinemia, the most common abnormality of obesity. (2-6) Low birth weight might be related to diseases in adulthood and has been related to obesity in later life. (7, 8)

Our study shows for the first time that maternal body mass index influences fetal growth from mid- pregnancy onwards. Previous studies suggested that both pre-pregnancy weight and weight gain during pregnancy affect fetal growth; however these studies consistently used birth weight as measure of fetal growth. (9-14) Also, several studies have looked at the timing of this influence during pregnancy, however the evidence on which is the most sensitive trimester remained inconclusive. (15-19) Our results suggest that differences in fetal growth due

to maternal anthropometrics occur already from mid-pregnancy and the relative effects become larger with increasing gestational age. These results were independent of maternal lifestyle and socioeconomic status related variables. Positive associations were found for maternal height, pre-pregnancy body mass index and maternal weight gain during pregnancy with birth weight. In line with that, the risks of small size for gestational age in the offspring were highest in short mothers with lowest pre-pregnancy body mass index and gestational weight gain. The risks of large size for gestational age in the offspring were highest for the tallest mothers with the highest pre-pregnancy body mass index and gestational weight gain. The effect of pre-pregnancy body mass index on birth outcomes was influenced by maternal weight gain during pregnancy. We found trends for associations between maternal weight gain during pregnancy with the risks for small and large size for gestational age within each quartile of pre-pregnancy body mass index. These findings suggest that the effect of gestational weight gain is partly dependent on pre-pregnancy body mass index.

Anthropometrics during pregnancy reflect maternal nutritional and health status and may be measures of the fetal environment. (20-22) Increased gestational weight gain might be associated with pregnancy complications including gestational diabetes and hypertension. (23) In our study we adjusted for these complications.

Conclusion

We found that maternal body mass index during pregnancy is positively associated with fetal growth from mid-pregnancy onwards and that high maternal height, pre-pregnancy body mass index and gestational weight gain are associated with increased risk of large size for gestational age whereas low maternal anthropometrics increase the risk of small for gestational age.

Influence of postnatal growth acceleration on determinants of adult disease

Our study demonstrates for the first time that fat mass at 6 months is related to both fetal and postnatal growth patterns and we were also able to pinpoint a more exact timing. Children with catch-up in weight whithin the first 6 weeks of life had a higher FM(%) at 6 months. Additionally, this study shows for the first time that there is an association between fetal growth retardation and postnatal catch-up growth.

We found that girls had more total and truncal fat mass at 6 months. Gender differences have been shown in several other studies. (24, 25) In 4-16 year-olds the prevalence of overweight was higher in girls and in 5-12 years-olds, girls had more subcutaneous fat and higher subscapular to triceps ratios. (26, 27) Girls also have their adiposity rebound, which is associated with development of obesity, at a younger age than boys. (28, 29)

Children with catch-up in weight had higher FM(%) at 6 months. Previously, catch-up growth within 2 years of life was associated with an unfavorable body composition in childhood. Recently it was shown that catch up in weight within 3 months leads to an unhealthy metabolic profile. (30, 31) We have now shown that very early catch-up growth in the first 6 weeks of life is related

to an increased FM(%) at 6 months. In addition, children with catch-up in the third trimester of pregnancy tended to have more FM(%) at 6 months, whereas catch-up in the second trimester was also associated with increased fat mass and catch-down with decreased fat mass at 6 months. It may be that at this stage of pregnancy, the fetus will develop towards its genetic potential without reference to environmental factors. These environmental factors (e.g. placental insufficiency due to hypertension or smoking etc) may be detrimental to fetal growth later in pregnancy (from 30 weeks of gestation onwards). We showed that maternal pre-pregnancy BMI and systolic blood pressure had the highest impact on fetal growth during the third trimester. Smoking was not correlated with fetal catch-down growth. However, this effect might be underestimated due the small number of participants in our study that (ever) smoked during pregnancy. (17.5%)

These associations may support the *developmental origins of health and disease* hypothesis, which suggests that an adverse fetal environment leads to adaptations that program the fetus' metabolism. (8) This programming may have beneficial effects on short term but predisposes the individual to diseases in adulthood, including obesity and insulin resistance. (7)

Children with fetal catch-down in weight in the third trimester showed more catch-up already during the first 6 weeks of life and had more FM(%) at 6 months. This may support the hypothesis that infants with intra uterine growth-restriction, resulting in low birth weight, have catch-up in weight in early infancy in order to reach their genetic growth trajectory. In later life these prenatally growth-restricted children who experience a greater postnatal catch-up may be at greater risk for developing cardiovascular disease.

Breastfeeding was related to higher FM(%) at 6 months. In previous studies, it was reported that breastfeeding had a protective effect against obesity in adulthood. (32) Other studies found no association of exclusive breast feeding with adiposity at 6.5 years. (33) The positive association in early infancy may be biased due to the practice that parents will be discouraged from breastfeeding when the infant does not seem to thrive in the first weeks of life.

Conclusion

Our study suggests that catch-up in weight during the third trimester and after birth were both positively associated with more absolute fat and lean mass at 6 months, but particularly to a relatively higher FM(%). Postnatal catch-up in weight during the first 6 weeks after birth has the strongest association with a higher FM(%) at 6 months. These findings strongly suggest that the risk of development of obesity and its main health consequences are at least partly established in fetal and early postnatal life.

Tracking of adiposity in childhood to obesity in adulthood

Our study showed that skinfold thickness is not strongly age dependent in the first 2 years. This is consistent with other studies. (34, 35) Together these studies suggest that skinfold thickness may be a rather stable measure of subcutaneous fat mass from infancy to young adulthood.

We found that girls have more total and central subcutaneous fat mass at the age of 24 months. No differences were found in peripheral subcutaneous fat mass, or subcutaneous fat mass at younger ages than 24 months. Although both sexes have a rise in fat mass from 1.5 months to 6 months, in boys we found a decline in fat mass after the age of 6 months but in girls the increase continued. As mentioned earlier, gender differences have been shown previously. (24, 25, 36) In schoolchildren prevalence of overweight was higher in girls and girls had more subcutaneous fat mass and girls tend to have their adiposity rebound at younger age than boys. (26-29, 37) This growth acceleration in childhood can lead to a disproportionately high fat mass in relation to lean body mass. (9, 38, 39) Some studies suggest that these gender differences in adiposity measured at younger age are partly explained by differences in food preferences or due to the fact that girls are less physically active than boys. (40, 41)

To our knowledge, our study demonstrates for the first time that subcutaneous fat mass has a tendency for tracking from the age of 6 months to the age of 24 months. Also, we have shown that this tracking is stronger for central than for peripheral subcutaneous fat mass. We are not aware of any other studies examining tracking of skinfold thickness in infancy. However, tracking of obesity, defined by body mass index, has previously been shown from the age of 6 years into adulthood. (42-46)

We found that maternal height and weight and paternal weight were inversely associated with subcutaneous fat mass. Postnatal weight is strongly associated with subcutaneous fat mass. However, after adjustment for current weight, inverse associations were found for fetal weight measured in late pregnancy, birth weight and weight at 1.5 months with subcutaneous fat mass at the age of 24 months.

These findings suggest that of all children with the same weight at the age of 24 months, those with fetal growth retardation or low birth weight tend to have increased subcutaneous fat mass. These associations again support the *developmental origins of health and disease* hypothesis. (7, 8) Our findings are in line with previous studies showing associations of both low and high birth weight with an increased risk of developing overweight. (47-49) Recent studies have reported that this might be dependent on programming of lean body mass as well as programming of fat mass. (50) It was also reported that this programming might be different in males and females. (51) Additionally, it seems that not only gender could modify this association, but the association was also shown to be stronger in certain genetic predispositions. (52)

Conclusion

Our findings suggest that subcutaneous fat mass tends to track from the age of 6 months to the age of 24 months. Postnatal weight is strongly associated with subcutaneous fat mass. Fetal weight in late pregnancy, birth weight and weight at 1.5 months and parental anthropometrics are inversely associated with subcutaneous fat mass at the age of 24 months after correction for current weight. These findings suggest that subcutaneous fat mass in early childhood is at least partly established in fetal life and may have consequences for adiposity in later life.

Role of Glucocorticoid Receptor (GCR) Gene polymorphisms on body composition

We showed that glucocorticoid receptor gene polymorphisms are not significantly associated with FM in early infancy. However, the results suggest that boys carrying haplotype 1 (characterized by *Bcl*I polymorphism), tend to have more total FM at 6 months and the effect tended to be augmented when catch-up in weight occurred in the first 6 weeks after birth. In contrast, boys carrying haplotype 5 (characterized by *TthIII*I polymorphism) tend to have less FM at 6 months even after catch-up in weight during 6 weeks after birth.

In previous studies, the *Bcl*I polymorphism was associated with hypersensitivity to glucocorticoids (26-28). The effect tended to be enhanced when catch-up in weight occurred within 6 weeks after birth. This finding may show that the first 6 weeks of life might be a critical period in which environmental factors modulate susceptibility for this GCR haplotype in boys. In girls no effects were found for this haplotype. It is well known that men have more central adiposity than women and that both central FM and male gender are risk factors for developing CVD (37, 38). Our findings that the unfavourable effects of this common haplotype in males are modified by environmental factors already in the first 6 weeks after birth, may be important as this haplotype is common in the general population and obesity an ever growing problem for society. However, for every genetic association study there is always a possibility that associations have arisen by chance, especially if they are novel and the study population relatively small. For these reasons, replication studies in larger study populations are needed before definitive conclusions can be drawn.

Our study suggests that in young children, haplotype 5 (characterized by *TthIII*I polymorphism) leads to lower FM at 6 months. The effect was largest in boys. This finding suggests that the fattening effect of catch-up in weight is far less in carriers of this haplotype thus leading to a metabolically healthier profile in later life. Previously, no associations were found with the *TthIII*I polymorphism, however these studies were performed in older subjects (12, 23).

We found no association between haplotype 4 (characterized by GR-9β) and FM at 6 months. This haplotype has been associated with decreased GC transrepressive activity and with increased inflammatory mediators leading to an increased risk to cardiovascular disease (14, 19). It may be that the effect of the GR-9β polymorphism on body composition appears later in life when inflammatory factors have been expressed over a longer a period of time. Also, no associations were found for haplotypes N363S and ER22/23EK. This may partly be due to the low number of subjects within the normal population and absence of homozygotes.

We hypothesized that genetic variants leading to increased or decreased glucocorticoid sensitivity are associated with body composition in early infancy. We have not found any significant associations of GCR polymorphisms with fat mass in early infancy. However, haplotype 1 (characterized by *Bcl*I polymorphism) tended to be associated with higher FM at 6 months in boys, possibly leading to an unfavourable metabolic profile in later life. As this effect was found only in boys, it is important to examine haplotypes in males and females separately. On the other hand, we found a trend towards an inverse association of haplotype 5 (characterized by *TthIII*I

polymorphism) with fat mass at 6 months, possibly leading to a healthier metabolic profile already in infancy. The first 6 weeks of life appears a critical period in which catch-up in weight can modulate genetic susceptibility. Further systematic searches for common genetic variants will enable us to obtain a more complete understanding of what genes and polymorphisms are involved in development of fat mass and the effects of growth patterns.

Conclusion

Our results suggest that in boys, the GCR haplotype 1 is associated with an increased FM, whereas haplotype 5 is associated with less FM in infancy. The first 6 weeks of life seems a critical period in which catch-up in weight can modulate genetic susceptibility. Replication studies in larger study populations are needed before definitive conclusions can be drawn.

Role of Glucocorticoid Receptor (GCR) Gene polymorphisms on subcutaneous fat mass and overweight

We demonstrated that glucocorticoid receptor gene polymorphisms are not associated with subcutaneous fat mass measured as skinfold thickness in infancy. Furthermore, we demonstrated that these polymorphisms were not related to higher risk of overweight or obesity at the age of 4 years.

Previous studies have examined the associations of different polymorphisms in the glucocorticoid receptor gene and sensitivity to glucocorticoids. The results of these studies are conflicting. A few studies report positive associations between the N363S and *Bcl*I polymorphisms and hypersensitivity to glucocorticoids, (53-55) while other studies found the opposite effect. (61, 62) The ER22/23EK polymorphism was associated with relative resistance to glucocorticoids. (63, 64) No associations were found yet with the *TthIII*I polymorphism. (57, 58) These studies suggest that genetically established differences in glucocortcoid sensitivity are important for various health related outcomes. In addition, it is known that environmental, dietary, and socioeconomic factors also play an important role in body composition and metabolic factors. Associations with polymorphisms depend on many additional factors, for example differences in characteristics between populations, prevalence of the polymorphism, and interactions with other genetic polymorphism. All these factors may play a role in the discrepancies found between studies so far.

We hypothesized that genetic variants leading to increased glucocorticoid sensitivity are associated with subcutaneous fat mass in infancy and with overweight in childhood. However, we did not find any effect on peripheral, central or total sum of SFT between the different glucocorticoid receptor haplotypes in our population-based study. Neither did we find significant differences in risk of overweight in preschool children for the different haplotypes. Also, no differences were found in risk of obesity for the haplotypes.

Conclusion

We did not find any association of glucocorticoid receptor haplotypes with subcutaneous fat mass or the risks of overweight and obesity in our population-based study. Further systematic searches for common genetic variants by means of genome-wide association studies will enable us to obtain a more complete understanding of what genes and polymorphisms are involved in development of subcutaneous fat mass and overweight in preschool children.

Pre- and postnatal determinants of bone development

Our study shows for the first time that maternal, fetal and birth parameters are associated with BMD and BMC in early infancy. Additionally, our study shows that not only low birth weight is associated with a lower BMD but that remaining in the lowest weight tertile at the age of 6 months considerably increases the risk of a low BMD at 6 months. We showed that catch-up in weight during the first 6 weeks of life decreases the probability of low BMD.

We found that growth in fetal life is associated with BMD_{TB} and BMC_{TB} at 6 months. These associations may support the *developmental origins of health and disease* hypothesis, which suggests that an adverse fetal environment leads to adaptations that program the fetus' metabolism. (8) This programming may have beneficial effects on short term but predispose the individual to diseases in adulthood. (7) Children who were exposed to malnutrition in fetal life will be born with a lower BMD_{TB} and BMC_{TB}. High energy nutrition after birth might have a positive effect on the development of bone mass, however this will lead to unfavorable effects on body composition. (30) An interesting issue is the possibility of preventing long-term effects of poor intrauterine growth on bone mass by aiming at normal postnatal weight gain of these babies.

We found that weight gain between 6 weeks and 6 months was associated with BMD_{LS} and not with BMD_{TB}. These associations may support earlier studies showing that weight bearing positions (e.g. sitting up straight from the age of 3 months) is related to $BMD_{LS.}$ (65-67) Catch-up in weight was also associated with a lower probability for low BMD, which might lead to a decreased probability on fractures in later life. Further studies are needed to examine whether this beneficial effect of weight persists on the long term.

Our findings are in line with previous studies showing associations of current body size measures and BMD_{TB}. (65, 68) However, these studies were primarily performed in adults and older children, not in infancy. (69-71) It is well known that body weight is positively associated with BMD_{TB} in adults, possibly as a consequence of enhanced mechanical strain. (72, 73) Due to short stature in children, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$). (74, 75) We have now shown that the influence of body weight on BMD accrual in childhood is already present in early infancy.

In our study, we found that breastfeeding had no beneficial effect on bone mass at the age of 6 months. This is in line with other studies. (76, 77) This might be explained by the fact that breastfed children have less fat mass leading to lower mechanical strain. In addition, bottle fed

children receive higher nutritional intake as standard prescription, which will lead to higher BMD_{TB}. Follow up studies are needed to examine the effects of breastfeeding in later life.

We found that girls have a higher BMD_{LS} at the age of 6 months. Gender differences have been shown in several other studies. Females are shorter and weigh less at birth and throughout infancy. (24, 25) It was previously shown that girls have more fat mass percentage than boys at the age of 6 months. (77) This difference in fat mass might lead to more mechanical strain resulting in higher BMD_{LS} in girls at 6 months. Further studies are needed to determine the clinical relevance of this gender difference.

Conclusion

BMD_{TB} is mainly associated with prenatal characteristics whereas BMD_{LS} is associated with postnatal characteristics. Additionally, our study shows that remaining in the lowest weight tertile at the age of 6 months considerably increases the risk of a low BMD at 6 months. Catch-up in weight during the first 6 weeks of life decreases this risk of low BMD. Our findings suggest that fetal growth patterns as well as postnatal patterns are associated with bone mineral density in early childhood and may have consequences for BMD in later life.

Strengths and limitations

One strength of the studies presented in this thesis was the large population-based cohort from early pregnancy onwards and information about a large number of potential confounders being available. Furthermore, this study is one of the largest cohort studies that explored the associations of maternal anthropometrics before and during pregnancy with birth outcomes. To our knowledge, this is the first study that examined the associations of maternal anthropometrics with fetal growth during different periods of pregnancy.

The ultrasounds in our study were performed by multiple operators. However, the ICC was higher than 0.98 and the corresponding CV lower than 6% for all fetal biometry parameters. Bland and Altman plots to test agreement of measurements for fetal biometry showed normal distributions. The 95% limits of agreement for differences in fetal biometry measurements between and among operators indicated good reproducibility. Thus we could reliably construct reference curves for fetal size from early pregnancy onwards for clinical purposes.

Gestational age was established by fetal ultrasound examination. In our study, crown-rump length and biparietal diameter were used for pregnancy dating but not for assessing fetal growth. (78) Since pregnancy dating characteristics and growth characteristics are correlated throughout pregnancy, growth variation in head circumference, abdominal circumference, and femur length may be somewhat reduced by dating pregnancy on the other fetal characteristics. As correlations between pregnancy dating and fetal growth characteristics are strongest in early pregnancy, we only used mid- and late pregnancy ultrasounds for fetal weight estimations.

In our study we used the same DXA scanner for all measurements and the scans were performed by the same technician. Quality assurance was performed daily. The coefficient of varia-

tion (CV) was 0·64% for bone mineral content (BMC), 1·04% for spine BMD and 0·64% for total body BMD. (79) The CV for lean tissue and fat tissue has been reported to be 1·57–4·49% and 0·41–0·88%, respectively.(80) Due to short stature in children, true BMD is underestimated by the standard areal measurement and we corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$). (74, 75)

Of the subgroup of participants (n= 1247) with more detailed investigations, 298 participants were randomly selected at the age of 6 months, and were approached for DXA measurements. Of the 298 children, 24 parents refused consent mostly because of time issues and a few because of concerns about safety. Of the remaining 274 participants, 4 children failed to be scanned due to crying. In total 270 scans were performed, of which 252 were reliable to be used in the study. The children with DXA measurements available were not different form the children without DXA scans in maternal anthropometrics and most child anthropometrics. However, the children with DXA scans available were taller at the age of 6 months than the children without DXA scans (68.9 vs. 68.5 cm, $P < 0.05$). We do not think this biases our results as we correct for length at the age of 6 months.

We adjusted our regression models with regard to fat and lean mass at the age of 6 months for current length as it is important to account for current body size. Previous studies have adjusted for various current body size measures (e.g. current weight, BMI etc). (81, 82) Recent studies have reported that adjusting for current measures as weight and BMI might be erroneous. (83) Our adjustment did not materially change the associations with fat mass; however it did have a significant effect on the associations with lean mass.

We used skinfold thickness to measure subcutaneous fat mass. This method is easy to perform and valid for measurement of fat mass in adolescents and children from 5 to 18 years. (84, 85) The inter- and intraobserver measurement error is known to be small. (86) From these skinfold thickness measurements, we calculated total, central and peripheral subcutaneous fat mass. Of all postnatal participants, skinfold measurements were performed in at least 80%. Missing skinfold measurements were mainly due to crying behavior. No differences were found between children with and without skinfold measurements. Also, maternal anthropometrics in pregnancy were similar between infants with and without skinfold thickness measurements. The effect estimates would be biased if the associations of maternal anthropometrics with skinfold thickness differ between those included and not included in the present analyses. This seems unlikely. Measurement of skinfolds at birth would be of great value in our study. However, due to logistical and financial constraints we were not able to measure skinfolds at birth in this study. The first study specific measurements were planned at the age of 1.5 months.

We defined overweight and obesity at the age of 4 years using BMI-SDS. BMI is an expression of weight and height and not fat mass, and the accuracy of these reference values in classifying adiposity in children has not yet been validated in most countries. (87) However, measurement of BMI is a practical and reproducible method for classifying overweight in adults. (88, 89) Recently, the use of BMI-SDS is increasingly recommended for screening overweight in children

and adolescents (90-92) In our study we used the cut-off points of BMI-SDS to define overweight and obesity as previously described by Cole et al. (93)

All anthropometric measurements were done at the research centers except for maternal pre-pregnancy weight. Since self-reported weight just before pregnancy was highly correlated with measured weight at intake, we do not think that because of using this variable has biased our results.

We used 0.67SD as a cut-off point to define catch-up in weight. It would have been informative to use continuous gain or loss in weight. However in this cohort of healthy children the differences were too small and this did not provide any useful information. A change in SD-score for weight of 0.67 SD-scores represents the width of each percentile band on standard growth charts, meaning; second to ninth percentile, ninth to 25th, 25th to 50th, and so on. This indicates clinically significant catch-up or catch-down growth, not only postnatally but also prenatally.(31)

A possible limitation of the studies on GCR haplotypes is that these were performed in a healthy, population-based cohort study. DNA for genotyping was available in 59% of all subjects and was isolated from cord-blood. Missing cord-blood was mainly caused by logistical restraints at delivery. Also, the effects might not have reached significance for the uncommon haplotype 2 (characterized by N363S) and haplotype 3 (characterized by ER22/23EK) due to the low number of subjects within the normal population.

Methodological considerations

The studies performed in this thesis have been conducted whithin the generation R study. A population based cohort study. The prospective design enables detailed data collection and provides the opportunity to examine the temporal relationships. Types of bias that could have affected our results are selection bias, information bias and confounding. (94)

Selection bias

When the relation between determinant and outcome is different for those who participate and those who were eligible but do not participate, selection bias may occur. Of all children 61% participate in the Generation R study. (95) Non response due to non participation is not likely to be random. The percentage of mothers from ethnic minorities and lower socio economic background and of mothers with children with medical complications are lower in the group participating than would be expected from the population figures in Rotterdam. (96) We do not think that this selection towards a more healthy and affluent study population substantially affected our etiological association studies, since these selection mechanisms are not related to both determinant and outcome and we do not expect these mechanisms to differ between study population and eligible population. However, this will affect frequency rate and therefore the statistical power and generalizability of our studies.

Selection bias may not only occur due to selective non response but also due to selective lost to follow up. Follow up rates in our study were high: birth outcomes were available in 97% of all pregnancies. Recently, it has been shown that bias in large cohort studies primarily arises from

loss to follow up rather than from non-response at baseline. (97) We therefore do not expect that our results are biased due to missing pre-pregnancy weight data.

Information bias

Information on the determinants and outcomes in the studies described in this thesis was mainly obtained by physical examinations, ultrasound examinations and questionnaires. Random misclassification might lead to bias towards the null. When misclassification of the determinant is related to the outcome, bias may occur. (98) Exposure data in our studies were collected before outcome measured. Therefore differential misclassification of the exposure seems unlikely.

Birth weight was lower in the offspring of mothers without information about pre-pregnancy body mass index than those with this information. Response rate for this questionnaire was lower among non-Dutch, lower educated and younger mothers. These selective missing might lead to biased results. However, body mass index at enrolment was based on measured weight and height and was available in 99% of the participants and was strongly correlated with pre-pregnancy body mass index. The associations of body mass index with birth weight were similar when we used body mass index at enrolment instead of pre-pregnancy body mass index.

Weight gain was partly based on self-reported weights. Women in this age group may systematically underestimate their weights. (99) Since we were interested in differences between subjects and the effects on birth outcomes, systematic underestimation of pre-pregnancy weight and body mass index does not bias our results. Maternal gestational weight gain was defined as the difference between weight in late pregnancy 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. In a sub analysis in 42% of the participants with highest weight in pregnancy available from questionnaires, we found similar results. Since the largest differences in gestational weight gain can be expected at delivery, our effect estimates may be underestimated.

Due to missing questionnaire data, information was limited for some covariates. Shorter height and larger body mass index and lower birth weight were found in subjects with missing data on ethnicity and education. Since we performed a complete case analysis in the adjusted regression models, our effect estimates may be underestimated by leaving out relatively more subjects with shorter height and higher weight.

Confounding

In the Generation R study many variables related to growth and development were collected. Therefore many potential confounders were available for analysis. Confounding may be considered as biased effects due to an extraneous factor, which leads to an effect which is mistaken for or mixed with the real effects. As a consequence, the effect of the exposure of interest is distorted. (100) A confounder must be related to both exposure and outcome and may not be in the causal pathway from exposure to outcome. Although we had information on many variables, we might have missed potential confounders. Residual confounding due to unmeasured effects might still be possible.

General conclusions, implications and directions for future research

We found that maternal body mass index during pregnancy is positively associated with fetal growth from mid-pregnancy onwards and that high maternal height, pre-pregnancy body mass index and gestational weight gain are associated with increased risk of large size for gestational age whereas low maternal anthropometrics lead to small for gestational age. Further studies focused on mechanisms underlying the associations between maternal anthropometrics and fetal growth are needed and should be focused on both environmental and genetic variants related to weight gain and insulin resistance. For instance, mother's nutrition before and during pregnancy may have an effect on fetal growth and birth weight whereas insulin levels during pregnancy may also play an intermediate role. Higher maternal glucose levels in pregnancy may lead to increased fetal glucose and insulin levels. Since insulin is the single most important fetal growth factor, increased levels might subsequently lead to higher fetal growth rates and eventually higher birth weight. Also, since maternal body mass index and weight gain reflect maternal nutritional status and may be at least partly modifiable, further studies are needed to identify dietary factors that influence maternal weight gain during pregnancy, and examine the feasibility and effect of optimizing maternal anthropometrics before and during pregnancy. Additionally, whether and to what extent these maternal effects on fetal size persist in childhood and in adulthood needs to be further studied.

We found that children with catch-down growth in the third trimester had more FM(%) at 6 months. Children with catch-up in weight had higher FM(%) at 6 months. Further studies are needed to examine which factors influence this catch-up growth as hormones like insulin and nutrition might play a significant role in this process. Children with fetal catch-down in weight showed more catch-up already during the first 6 weeks of life. This may support the hypothesis that infants with intra uterine growth-restriction, resulting in low birth weight, have catch-up in weight in early infancy in order to reach their genetic growth trajectory. In later life these prenatally growth-restricted children who experience a greater postnatal catch-up may be at greater risk for developing cardiovascular disease. Further studies are needed to establish which genetic and environmental factors influence these growth patterns and whether fetal growth retardation or postnatal catch-up growth is the most important factor for subsequent obesity in later life.

Breastfeeding was related to an increase in FM(%) at 6 months. The positive association in early infancy may be biased due to the practice that parents will be discouraged from breastfeeding when the infant does not seem to thrive in the first weeks of life. Further studies are needed to examine the effects of breastfeeding in childhood and in later life.

On the other hand, we found that breastfeeding had no beneficial effect on BMD at the age of 6 months. This is in line with other studies. (76, 77) However, one study showed that term born children who had received breastfeeding longer than 3 months had higher BMD at the age of 8 years. (101) Follow up studies are needed to examine the effects of breastfeeding in later life on bone density.

Our findings suggest that girls have more central subcutaneous fat mass than boys at the age of 24 months. Also, we found that girls had more total and truncal fat mass at 6 months. Gender differences have been shown in several other studies. (24-29) However, in adulthood truncal fat was found to be increased in males and it has been related to a greater risk of developing cardiovascular disease. (102) Changes occurring in puberty and under the influence of hormones may alter fat patterning and lead to a disproportionately high fat mass in relation to lean mass in males. (38, 50, 103, 104) Further studies are needed to determine the timing and determinants of these changes. Additionally, we found that girls have a higher BMD_{LS} at the age of 6 months. Differences in fat mass might lead to more mechanical strain resulting in higher BMD_{LS} in girls at 6 months. Further studies are required to determine the clinical relevance of this gender difference.

We showed that subcutaneous fat mass tends to track from the age of 6 months to the age of 24 months. Postnatal weight is strongly associated with subcutaneous fat mass. However, after adjustment for current weight, inverse associations were found for fetal weight measured in late pregnancy, birth weight and weight at 1.5 months and parental anthropometrics with subcutaneous fat mass at the age of 24 months.

This suggests that subcutaneous fat mass in early childhood is at least partly established in fetal life and may have consequences for adiposity in later life. Follow up studies are needed to assess whether subcutaneous fat mass in early childhood tracks to adulthood, and whether and to what extent the effects of parental, fetal anthropometrics and postnatal growth patterns on subcutaneous fat mass persist.

Figure 1. Tracking of fat mass from infancy to childhood and beyond

Our findings strongly suggest that haplotype 1 (characterized by BclI polymorphism) and haplotype 5 (characterized by TthIIII polymorphism) are associated with development of early adiposity in boys which might have health consequences in later life. In boys, the GCR haplotype 1 leads to more FM, whereas haplotype 5 is associated with less FM in infancy. The first 6 weeks of life appears a critical period in which catch-up in weight can modulate genetic susceptibility. Follow up studies are required to assess whether and to what extent these effects persist in later life.

On the other hand, we did not find consistent associations of glucocorticoid receptor haplotypes with subcutaneous fat mass or the risks of overweight and obesity in preschool children. Further

systematic searches for common genetic variants by means of genome-wide association studies might be useful to obtain a more complete understanding of which genes and polymorphisms are involved in development of subcutaneous fat mass and overweight in preschool children.

Our study demonstrates that bone mass is related to both fetal and postnatal growth patterns. Children who were exposed to malnutrition in fetal life will be born with a lower BMD_{TB}. High energy nutrition after birth might have a positive effect on the development of $BMD_{TB,}$ however this will lead to unfavorable effects on body composition. (30) An interesting issue is the possibility of preventing long-term effects of poor intrauterine growth on bone mineral density by aiming at normal postnatal weight gain of these babies.

Additionally, our study shows that not only low birth weight is associated with a lower BMD but that remaining in the lowest weight tertile at the age of 6 months considerably increases the risk of a low BMD at 6 months. We showed that catch-up in weight during the first 6 weeks of life decreases this risk of low BMD, which might lead to a decreased risk of fractures in later life. Further studies are needed to examine whether this beneficial effect of weight persists on the long term.

Catch-up in weight seems to be a major contributor to higher bone mass in infancy. It would be interesting to see whether this effect persists after puberty and during adolescence. As the major risks on osteoporotic fractures are in adulthood, more specifically after 50 years of age, it would be of interest to see whether this protective effect is still present at that age. Another very interesting issue is whether catch-up later in childhood (e.g. until 2 years of age) would also lead to higher bone mass in later life.

Our findings suggest that growth patterns in fetal as well as postnatal life are associated with bone mass in early childhood. It would be interesting to explore whether and to what extent maternal anthropometrics, fetal and postnatal growth patterns have an effect on bone status in adulthood.

In conclusion, our findings suggest that parental, fetal as well as postnatal determinants are associated with body composition and bone mass in early childhood. (Figure 2)

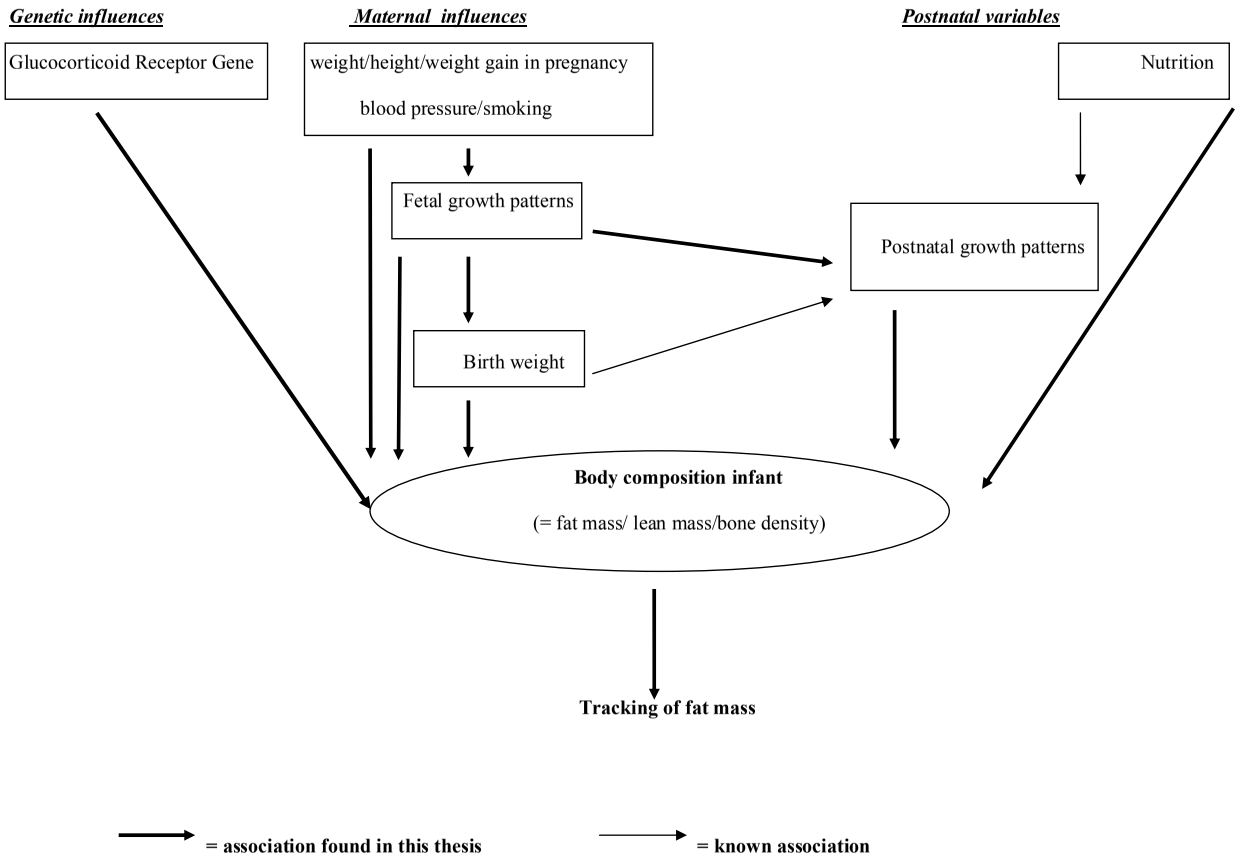

Figure 2. Associations investigated in this thesis

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Chapter 9

Summary in English

SUMMARY

Chapter 1

This chapter provides an introduction in the different hypotheses with regard to influences of fetal size, birth weight and childhood growth on adult diseases and their determinants. The relationships of fetal size and childhood growth patterns with body composition and bone mass are discussed. In addition, the study design is described. Finally, the aims and outline of the thesis are presented.

Chapter 2

In this chapter we describe the associations of maternal anthropometrics with fetal weight measured in different periods of pregnancy and with birth outcomes. Maternal height and weight have been related to offspring weight and length at birth. Previous studies have suggested that both pre-pregnancy body mass index (BMI) and gestational weight gain are positively associated with birth weight in the offspring and are related to risks of both low and high offspring birth weight. In 8541 mothers, height, prepregnancy body mass index and gestational weight gain were available. Fetal growth was measured by ultrasound in mid- and late pregnancy. Maternal BMI in pregnancy was positively associated with estimated fetal weight during pregnancy. The effect estimates increased with advancing gestational age. All maternal anthropometrics were positively associated with fetal size. Mothers with both their prepregnancy BMI and gestational weight gain quartile in the lowest and highest quartiles showed the highest risks of having a small and large size for gestational age child at birth, respectively. The effect of prepregnancy BMI was strongly modified by gestational weight gain.

In conclusion, fetal growth is positively affected by maternal BMI during pregnancy. Maternal height, prepregnancy BMI and gestational weight gain are all associated with increased risks of small and large size for gestational age at birth in the offspring, with an increased effect when combined.

Chapter 3

It has been postulated that not birth weight, but postnatal catch-up in weight (growth acceleration) is related to overweight and an unfavourable metabolic profile in adulthood. However, the exact timing of the rapid weight gain that contributes to these long-term risks was unknown. It was also unclear whether this unfavourable body composition is solely due to an excess of fat mass or due to a combination of higher fat mass and diminished lean mass. Therefore, we examined which parental, fetal, and postnatal characteristics are associated with fat and lean mass at the age of 6 months and examine the effect of growth (catch-down, catch-up) in fetal life and early infancy on fat and lean mass.

Body composition was measured by dual-energy X-ray absorptiometry in 252 infants at 6 months. Children with fetal catch-up in weight (gain in weight SD (standard deviation) score

>0.67) in the second trimester tended to have a higher fat mass percentage [FM(%)] at 6 months of age, whereas children with fetal catch-down in weight had a lower FM(%) compared with non changers. In the third trimester, both catch-up and catch-down in weight were associated with an increase in FM(%) at 6 months. Children with catch-down in the third trimester had a greater risk for postnatal catch-up in weight greater than 0.67 SD score. Birth weight and weight at 6 weeks were positively associated with fat mass at 6 months. Postnatal catch-up in weight within 6 weeks after birth had the highest association with total and truncal FM(%) at 6 months. Total and truncal FM were higher in girls.

In conclusion, catch-down in weight in the third trimester was strongly associated with postnatal catch-up within 6 weeks after birth, and both were associated with an increase in fat mass at the age of 6 months.

Chapter 4

Obesity in childhood is not only associated with short-term morbidity such as asthma and psychological problems but also with an increased risk for chronic morbidity and mortality in adulthood, as childhood obesity tends to track into adulthood, meaning that subjects keep their ranking position in body mass index distribution over time. We investigated the development and tracking of subcutaneous fat mass in the first 2 years of life and which parental, fetal and postnatal characteristics are associated with subcutaneous fat mass.

Subcutaneous fat mass was measured by skinfold thickness (biceps, triceps, suprailiacal, subscapular) at the ages of 1.5, 6 and 24 months in 1012 children. Normal values of subcutaneous fat mass were described. Total subcutaneous fat mass was higher in girls than in boys at the age of 24 months. Subjects in the lowest and highest quartiles at the age of 6 months tended to keep their position in the same quartile at the age of 24 months. Maternal height and weight, paternal weight, fetal weight at 30 weeks, birth weight and weight at the age of 6 weeks were each inversely associated with subcutaneous fat mass at the age of 24 months after adjustment for current weight at 24 months.

In conclusion: this study shows that subcutaneous fat mass tends to track in the first 2 years of life. Furthermore, the results suggest that an adverse fetal environment and growth are associated with increased subcutaneous fat mass at the age of 24 months.

Chapter 5

Studies have posed that genetic factors or epigenetic phenomena may explain the relation between low birth weight and cardiovascular diseases. As glucocorticoids are important regulators of growth, development and metabolism and their effects are mediated by glucocorticoid receptors, polymorphisms in the glucocorticoid receptor (GCR) gene may contribute to a difference in sensitivity and thereby to associations between growth characteristics in early life and disease in adult life. We examined whether GCR-gene-haplotypes are associated with fat mass (FM) at the age of 6 months and to examine the interaction of the haplotypes with catch-up in weight.

Body composition was measured by Dual energy X-ray Absorptiometry (DXA) in 214 infants at 6 months. DNA was collected from cord blood samples for genotyping five GR gene polymorphisms *Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I.

No significant associations of GCR polymorphisms with fat mass were found in early infancy. However, in boys, haplotype 1(characterized by *Bcl*I) tended to be associated with more FM at 6 months. In boys with postnatal catch-up in weight (gain in weight-SD-score>0.67) within 6 weeks after birth, haplotype 1 carriers tended to have more total, central and peripheral FM at 6 months. Carriers of haplotype 5 (characterized by *TthIII*I) tended to have less FM at 6 months. Haplotype 2, (characterized by N363S), haplotype 3 (characterized by ER22/23EK) and haplotype 4 (characterized by GR-9β) were not associated with FM at 6 months. In girls no associations were found for any of the haplotypes.

In conclusion, in boys the GCR haplotype 1 seems to increase FM, whereas haplotype 5 seems to be associated with less FM in infancy. The first 6 weeks of life appear a critical period in which catch-up in weight can modulate genetic susceptibility. However, replication studies in larger study populations are needed before definitive conclusions can be drawn.

Chapter 6

It is likely that the Glucocorticoid Receptor Gene polymorphisms are to some extent responsible for the variability in the sensitivity to glucocorticoids. As glucocorticoids are important regulators of many processes involved in fat and glucose metabolism, these polymorphisms in the glucocorticoid receptor gene could lead to intrauterine growth retardation and metabolic and cardiovascular diseases in adulthood. The results of previous studies are conflicting. In addition, it is known that environmental, dietary, and socioeconomic factors also play an important role in determinants of body composition and metabolic. As we hypothesized that the effect of these GCR gene polymorphisms might be stronger on body composition and overweight in early life because of the limited life style influences, we investigated whether GCR-gene-haplotypes are associated with skinfold thickness (SFT) in early infancy and the risk of overweight and obesity in preschool children.

DNA from 3990 children was collected from cord blood samples and used for genotyping of five GR gene polymorphisms (*Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I). Body composition was measured using skinfold thickness in a subgroup of 746 children at the age of 2 years. Information on overweight (SDS BMI 1.10 - 2.30) and obesity (SDS BMI > 2.30) at age 4 years was available in all children.

The glucocorticoid receptor gene polymorphisms were not associated with subcutaneous fat mass measured as peripheral, central or total sum of skinfold thickness at 2 years of age. At the age of 4 years, these polymorphisms were not related to higher risk of overweight. Also, no differences were found in risk of obesity for the haplotypes.

In conclusion, glucocorticoid receptor haplotypes were not associated with subcutaneous fat mass in infancy or the risks of overweight and obesity in preschool children.

Chapter 7

Bone mineral density (BMD) is the result of the equilibrium between bone formation and bone resorption. Bone mineral acquisition is thought to be associated with genetic and environmental factors. Low BMD is associated with a higher risk of fractures. Bone mineral density is different for total body and for lumbar spine. It was suggested that poor growth during fetal life and infancy is also associated with decreased bone mineral density in adulthood. These associations may be explained by an adverse uterine environment, which may affect both early skeletal development and the acquisition of bone mineral density in childhood. We investigated whether parental, fetal and postnatal characteristics and growth patterns in fetal life and infancy are associated with bone mass at 6 months. BMD and bone mineral content (BMC) total body (TB) and BMD lumbar spine (LS) were measured by dual-energy X-ray absorptiometry in 252 infants at 6 months.

Maternal, fetal and postnatal anthropometrics were positively associated with BMD_{TB} and BMC_{TB} at 6 months but only postnatal anthropometrics were associated with BMD_{LS}. A gain in weight-SD-score during fetal life and prenatal catch-up in weight were positively associated with BMD_{TB}. After birth, a gain in weight-SD-score was positively associated with BMD_{LS} and $BMAD_{LS}$. The effect was strongest between 6 weeks and 6 months. Catch-up in weight was associated with a lower probability of low BMD_{TB} and BMD_{LS}. Children remaining in the first tertile of weight from birth to 6 months had a much higher risk of low BMD_{TB} at 6 months.

In conclusion, BMD_{TB} is mainly associated with prenatal characteristics whereas BMD_{LS} is associated with postnatal characteristics. Additionally, our study shows that remaining in the lowest weight tertile at the age of 6 months considerably increases the risk of a low BMD at 6 months. Catch-up in weight during the first 6 weeks of life decreases this risk of low BMD. Our findings suggest that growth patterns in fetal and as postnatal life are associated with bone mass in infancy and may have consequences for bone mass in later life.

Chapter 8

This chapter provides a general discussion in which our findings are discussed in relation to current literature. Also, our conclusions are described together with general considerations and directions for future research.

Chapter 10

Summary in Dutch

SAMENVATTING

Hoofdstuk 1

Dit hoofdstuk geeft een inleiding in de verschillende hypotheses met betrekking tot de invloeden van foetale groei, geboortegewicht en groei op de kinderleeftijd, op ziekten in volwassenheid en determinanten daarvan. De relaties van de foetale groeipatronen en groeipatronen in de vroege jeugd met lichaamssamenstelling en botmassa worden besproken. Daarnaast wordt de opzet van de studie beschreven. Ten slotte worden de doelstellingen en opzet van het proefschrift gepresenteerd.

Hoofdstuk 2

In dit hoofdstuk beschrijven we de associaties van maternale antropometrie met foetaal gewicht, gemeten in verschillende perioden van de zwangerschap, en met geboorte uitkomsten. Maternale lengte en gewicht zijn gerelateerd aan het gewicht en lengte bij de geboorte van haar nakomelingen. Eerdere studies hebben gesuggereerd dat zowel de body mass index vòòr de zwangerschap en gewichtstoename tijdens de zwangerschap, geassocieerd zijn met het geboortegewicht van de kinderen en gerelateerd zijn aan de risico's van zowel hoge als lage geboortegewicht van het kind. In 8541 moeders waren lengte, body mass index (BMI) vòòr de zwangerschap en gewichtstoename in de zwangerschap beschikbaar. Foetale groei werd gemeten door middel van ultrageluid in het 2^{e} en 3^{e} trimester van de zwangerschap. Maternale BMI tijdens de zwangerschap was positief geassocieerd met foetale gewichtstoename tijdens de zwangerschap. Het effect werd grotere met de toename van de zwangerschapsduur. Alle antropometrie van moeder was positief geassocieerd met foetale groei. Moeders met zowel hun BMI vòòr de zwangerschap als gewichtstoename tijdens de zwangerschap in de laagste en hoogste kwartiel hadden de grootste risico's van het respectievelijk hebben van een klein en groot kind voor de zwangerschapsduur bij de geboorte. Het effect van BMI vòòr de zwangerschap werd sterk beinvloed door gewichtstoename in de zwangerschap.

Concluderend wordt groei van de foetus positief beïnvloed door maternale BMI tijdens de zwangerschap. Maternale lengte, BMI vòòr de zwangerschap en gewichtstoename in de zwangerschap zijn allemaal geassocieerd met een verhoogd risico's van klein en groot formaat voor de zwangerschapsduur bij de geboorte bij de nakomelingen, met een groter effect wanneer deze worden gecombineerd.

Hoofdstuk 3

Er is beschreven dat niet geboortegewicht, maar postnatale inhaalgroei in gewicht (groeiversnelling) is gerelateerd aan overgewicht en een ongunstig metabool profiel in de volwassenheid. Echter, de exacte timing van de snelle gewichtstoename dat bijdraagt aan deze lange-termijn risico's is onbekend. Het was ook onduidelijk of deze ongunstige lichaamssamenstelling uitsluitend is te wijten aan een overmaat aan vetmassa of als gevolg van een combinatie van hogere vetmassa

en verminderde vetvrije massa. Daarom hebben we onderzocht welke ouderlijke, foetale en postnatale kenmerken zijn geassocieerd met vet- en spiermassa op de leeftijd van 6 maanden en wat het effect is van de groei (catch-down, catch-up) in het foetale leven en de vroege jeugd, op vet- en spiermassa.

Lichaamssamenstelling werd gemeten door middel van dual-energy X-ray absorptiometrie in 252 zuigelingen op de leeftijd van 6 maanden. Kinderen met foetale catch-up in gewicht (gewichtstoename SD-score> 0,67) in het tweede trimester neigden naar een hogere vetmassa percentage [FM (%)] bij 6 maanden, terwijl kinderen met foetale catch-down in gewicht een lagere FM (%) hadden in vergelijking met kinderen die geen groeiversnelling of -vertraging lieten zien. In het derde trimester, waren zowel catch-up als catch-down in gewicht geassocieerd met een toename van de FM (%) op 6 maanden. Kinderen met een catch-down in het derde trimester hadden een groter risico op postnatale catch-up in gewicht van meer dan 0,67 SD-score. Geboortegewicht en gewicht op 6 weken waren positief geassocieerd met vetmassa op 6 maanden. Postnatale catch-up in het gewicht binnen 6 weken na de geboorte had de hoogste associatie met totaal en centrale FM (%) op 6 maanden. Totale en centrale FM waren hoger bij meisjes.

Samenvattend was de catch-down in gewicht in het derde trimester sterk geassocieerd met postnatale catch-up binnen 6 weken na de geboorte, en beiden waren geassocieerd met een toename van de vetmassa op de leeftijd van 6 maanden.

Hoofdstuk 4

Obesitas in de kindertijd is niet alleen gerelateerd aan korte-termijn morbiditeit, zoals astma en psychische problemen, maar ook aan een verhoogd risico op chronische morbiditeit en mortaliteit op volwassen leeftijd, omdat overgewicht bij kinderen de neiging heeft tot “tracking” tot in de volwassenheid. Dit betekent dat de proefpersonen hun positie in rang behouden in spreiding in de tijd. We onderzochten de ontwikkeling en het tracken van de onderhuidse vetmassa in de eerste 2 jaren van het leven, en welke ouderlijke, foetale en postnatale kenmerken zijn geassocieerd met onderhuidse vetmassa.

Onderhuidse vetmassa werd gemeten door dikte van de huidplooien (biceps, triceps, suprailiacaal, subscapulair) op de leeftijd van 1,5, 6 en 24 maanden in 1012 kinderen. Normale waarden van het onderhuidse vetmassa werden beschreven. Totaal onderhuidse vetmassa was hoger bij meisjes dan bij jongens op de leeftijd van 24 maanden. Kinderen in de laagste en hoogste kwartielen op de leeftijd van 6 maanden hadden de neiging om hun positie te behouden in hetzelfde kwartiel op de leeftijd van 24 maanden. Maternale lengte en gewicht, vaderlijke gewicht, gewicht van de foetus bij 30 weken, geboortegewicht en gewicht op de leeftijd van 6 weken waren elk omgekeerd geassocieerd met onderhuidse vetmassa op de leeftijd van 24 maanden na correctie voor huidig gewicht op 24 maanden.

Conclusie: deze studie toont aan dat onderhuidse vetmassa de neiging heeft om te tracken in de eerste 2 jaar van het leven. Verder suggereren de resultaten dat een ongunstige foetale omgeving en groei geassocieerd zijn met toegenomen onderhuids vetmassa op de leeftijd van 24 maanden.

Hoofdstuk 5

Studies hebben gesteld dat genetische factoren of epigenetische verschijnselen de relatie tussen een laag geboortegewicht en hart-en vaatziekten kunnen verklaren. Aangezien glucocorticoïden belangrijke regulatoren zijn van groei, ontwikkeling en metabolisme en hun effecten worden gemedieerd door glucocorticoïd receptoren, zouden polymorfismen in het glucocorticoid-receptor (GCR) gen kunnen bijdragen tot een verschil in gevoeligheid en daarmee tot associaties tussen groei-eigenschappen in het begin van het leven en ziekten bij volwassenen. Daarom hebben we onderzocht of GCR-gen-haplotypes geassocieerd zijn met vetmassa (FM) op de leeftijd van 6 maanden en of er een interactie is van de haplotypes met catch-up in gewicht.

Lichaamssamenstelling werd gemeten door Dual Energy X-ray absorptiometrie (DXA) in 214 zuigelingen op 6 maanden. DNA werd verzameld uit navelstrengbloed monsters voor genotypering van vijf GCR genpolymorfismen *Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I. Significante associaties van de GCR polymorfismen met vetmassa werden niet gevonden in de vroege jeugd. Echter, bij jongens was een trend bij haplotype 1 (gekenmerkt door *Bcl*I) richting een associatie met meer FM op 6 maanden. Bij jongens met postnatale catch-up in gewicht (gewichtstoename-SD-score> 0,67) binnen 6 weken na de geboorte, was er een neiging voor haplotype 1 carriers om meer totaal, centrale en perifere FM te hebben op 6 maanden. Dragers van het haplotype 5 (gekenmerkt door *TthIII*I) hadden een neiging tot minder FM op 6 maanden. Haplotype 2, (gekenmerkt door N363S), haplotype 3 (gekenmerkt door ER22/23EK) en haplotype 4 (gekenmerkt door GR-9β) waren niet geassocieerd met FM na 6 maanden. Bij meisjes werden geen associaties gevonden voor een van de haplotypes.

Concluderend lijkt het GCR haplotype 1 bij jongens FM te verhogen, terwijl haplotype 5 lijkt samen te hangen met minder FM op de kinderleeftijd. De eerste 6 weken van het leven lijkt een kritische periode waarin catch-up in het gewicht genetische gevoeligheid kan moduleren.

Er is echter replicatie onderzoek bij grotere populaties nodig voordat definitieve conclusies kunnen worden getrokken.

Hoofdstuk 6

Het is waarschijnlijk dat de glucocorticoid receptor gen polymorfismen tot op zekere hoogte verantwoordelijk zijn voor de variabiliteit in de gevoeligheid voor glucocorticoïden. Aangezien glucocorticoïden belangrijke regulatoren zijn van de vele processen die betrokken zijn in vet en glucose metabolisme, kunnen deze polymorfismen in het glucocorticoid receptor gen leiden tot intra-uteriene groeivertraging en metabole en cardiovasculaire aandoeningen op volwassen leeftijd. De resultaten van eerdere studies zijn tegenstrijdig. Daarnaast is het bekend dat milieu, het dieet, en sociaal-economische factoren ook een belangrijke rol in de determinanten van de lichaamssamenstelling en metabole aandoeningen spelen. Wij wilden de hypothese testen, dat het effect van deze GCR Gen polymorfismen misschien meer invloed uitoefenen op de lichaamssamenstelling en overgewicht in de vroege jeugd omdat dan de rol van omgeving en levensstijl beperkter is. Daarom hebben we onderzocht of Glucorticoid Receptor (GCR)-gen-haplotypes

geassocieerd zijn met dikte van de huidplooien (SFT) in de vroege kindertijd en met het risico van overgewicht en obesitas bij kleuters.

DNA van 3990 kinderen werd verzameld uit navelstrengbloed monsters en gebruikt voor genotypering van vijf GCR Genpolymorfismen (*Bcl*I, N363S, ER22/23EK, GR-9β and *TthIII*I). Lichaamssamenstelling werd gemeten met behulp huidplooi dikte in een subgroep van 746 kinderen op de leeftijd van 2 jaar. Informatie over overgewicht (BMI-SDS 1,10 tot 2,30) en obesitas (BMI -SDS> 2.30) op de leeftijd van 4 jaar was beschikbaar in alle kinderen.

De glucocorticoid receptor gen polymorfismen waren niet geassocieerd met onderhuids vetmassa gemeten als perifere, centrale of de totale som van de dikte van de huidplooien op 2-jarige leeftijd. Op de leeftijd van 4 jaar, waren deze polymorfismen niet gerelateerd aan een hoger risico op overgewicht. Ook werden geen verschillen gevonden in het risico van obesitas voor de haplotypes.

Concluderend waren glucocorticoïd receptor haplotypes niet geassocieerd met onderhuidse vetmassa in de kindertijd of met de risico's van overgewicht en obesitas bij kleuters.

Hoofdstuk 7

Botdichtheid (BMD) is het resultaat van het evenwicht tussen botvorming en botafbraak. Bot-ontwikkeling wordt geassocieerd met genetische en omgevingsfactoren. Lage BMD is geassocieerd met een hoger risico op fracturen. Botdichtheid is verschillend voor het gehele lichaam en voor de lumbale wervelkolom. Er werd gesuggereerd dat een slechte groei tijdens het foetale leven en kindertijd ook geassocieerd zijn met een verminderde botdichtheid op volwassen leeftijd. Deze associaties kunnen worden verklaard door een ongunstige intra-uteriene omgeving, die zowel in de vroege ontwikkeling van het skelet en de ontwikkeling van botdichtheid in de kindertijd van invloed kunnen zijn. We hebben onderzocht of ouderlijke, foetale en postnatale kenmerken en groeipatronen in foetale leven en kinderleeftijd zijn geassocieerd met de botmassa op 6 maanden. Bot dichtheid (BMD) en bot gehalte aan mineralen (BMC) van het gehele lichaam (TB) en lumbale wervelkolom BMD (LS) werden gemeten met dual-energy X-ray absorptiometrie (DXA) in 252 zuigelingen op 6 maanden.

Maternale, foetale en postnatale antropometrie waren positief geassocieerd met BMD_{TB} en BMC_{TB} op 6 maanden, maar alleen postnatale antropometrie was geassocieerd met BMD_{LS}. Een stijging in gewicht-SD-score tijdens het foetale leven en prenatale catch-up in gewicht waren positief geassocieerd met BMD_{TB}. Na de geboorte was een gewichtstoename in SD-score positief geassocieerd met BMD_{LS} en $BMAD_{LS}$. Het effect was het sterkst tussen 6 weken en 6 maanden. Catch-up in gewicht was geassocieerd met een lagere kans op lage BMD_{TB} en BMD_{LS}. Kinderen die in de eerste tertiel van het gewicht vanaf de geboorte tot 6 maanden bleven hadden een veel hoger risico op lage BMD_{TB} op 6 maanden.

Samenvattend was BMD_{TB} vooral geassocieerd met prenatale kenmerken, maar BMD_{LS} was geassocieerd met postnatale kenmerken. Bovendien toont onze studie aan dat bij persisteren in de laagste gewichtsklasse op de leeftijd van 6 maanden, het risico van een lage BMD op 6 maanden

aanzienlijk toeneemt. Catch-up in gewicht tijdens de eerste 6 weken van het leven vermindert dit risico van een lage BMD. Onze bevindingen suggereren dat groeipatronen bij de foetus en in het postnatale leven worden geassocieerd met botmassa in de kinderleeftijd en gevolgen kan hebben voor de botmassa in het latere leven.

Hoofdstuk 8

Dit hoofdstuk bevat een algemene discussie waarin onze bevindingen worden besproken in relatie tot de huidige literatuur. Ook zijn onze conclusies beschreven, samen met algemene overwegingen en aanbevelingen voor toekomstig onderzoek.

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LIST OF PUBLICATIONS

1. **Ay L, Hokken-Koelega AC, Hofman A, Steegers EA, Jaddoe VW** Associations of glucocorticoid receptor gene polymorfisms with subcutaneous fat mass in infancy and overweight in preschool children: The Generation R Study. Submitted
2. **Ay L, Hokken-Koelega AC, Mook-Kanamori DO, Hofman A, Moll HA, Mackenbach JP, Witteman JC, Steegers EA, Jaddoe VW** 2008 Tracking and determinants of subcutaneous fat mass in early childhood: the Generation R Study. Int J Obes (Lond) 32:1050-1059
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LIST OF ABBREVIATIONS

AC	abdominal circumference
BMC	bone mineral content
BMD	Bone mineral density
BMI	body mass index
BPD	biparietal diameter
CI	confidence interval
CV	coefficient of variation
CVD	cardiovascular disease
DXA	Dual-energy x-ray absorptiometry
EFW	estimated fetal weight
FL	femur length
FM	fat mass
FM(%)	fat mass percentage
GC	glucocorticoids
GCR	Glucocorticoid Receptor Gene
HC	head circumference
HPA	hypothalamic-pituitary-adrenal
ICC	intraclass correlation coefficient
IQR	interquartile range
LM	lean mass
LS	lumbar spine
OR	odds ratio
PI	ponderal index
SD	standard deviation
SFT	skin fold thickness
TB	total body

ABOUT THE AUTHOR

Lamise Ay was born on February 11th 1976 in Almelo, the Netherlands. She is the first child of Turkish immigrants Hatice and Muharrem Ay. She went to high school in Almelo and left home to study medicine in Utrecht in 1994. She graduated from Medical School in August 2000 and started working as a resident first for GAK and later at the pediatric department in the Elisabeth Hospital in Amersfoort. After this, she started working as a resident in Utrecht at the emergency room of the Ouderijn Hospital in 2002. She continued working as a pediatric resident at the Sophia Children's Hospital in January 2003. In September 2003 she started her AGIKO training, combining her pediatric training with her PhD training at the Generation R Study, and she will finish in April 2011.
She is married to Bülent. She is a fanatic collector of plastic and/or paper bags.

PHD PORTFOLIO

Summary of PhD training and teaching activities

Name of PhD student	Lamise Ay
Erasmus MC Department	Epidemiology and Pediatrics
Research school	Netherlands Institute for Health Sciences
PhD period	September 2003 – January 2011 (AGIKO)
Promotors	Prof.Dr. A.C.S. Hokken-Koelega and Prof.Dr. A. Hofman
Supervisor	Dr. V.W.V Jaddoe

PhD training

Erasmus Summer Programme
ESP01 Principles of Research in Medicine and Epidemiology
ESP04 Clinical Decision Analysis
ESP10 Methods of Clinical Research
ESP21 Pharmaco-epidemiology
ESP34 Topics in Evidence-based Medicine
ESP38 Conceptual Foundation of Epidemiologic Study Design
ESP44 Bayesian Analysis
ESP47 Spatial Epidemiology
ESP49 Decision Making in Medicine I

Core Curriculum
CC01 Study Design
CC02 Classical Methods for Data-analysis
CE02 Clinical Epidemiology
EP02 Methodologic Topics in Epidemiologic Research
EP03 Modern Statistical Methods

Advanced Short Courses
CE10 Advanced Diagnostic Research
CE11 Prognostic Research
DMR Discussion Meeting Research Proposal
GE03 Advances in Population-based Studies of Complex
Genetic Disorders
KEK Paediatric Clinical Epidemiology

Skills Courses

SC04 Working with SPSS for Windows

Presentation

nov 2004	presentation Generation R Congres "100 jaar schoolartsen"
jan 2005	Presentation congres kinderartsen in SKZ on multicultural relations
nov 2005	Posterpresentation Dohad congres Toronto, Canada
nov 2006	Presentation Interklinische Avond Sophia
nov 2007	Posterpresentation Dohad congres Perth, Australie

Teaching

Supervision co-assistenten and students

Managing skills

April 2004- July 2005 coördinator of Focus cohort

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DETAILS OF ETHICAL APPROVAL

Medical ethics committee approval (MEC 198.1782/2001/31) was obtained.