Growth Patterns and Adult Diseases

Growth During Childhood and Determinants of Cardiovascular and Metabolic Profile in Young Adults

Ralph Wilhelmus Jean Leunissen
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Growth During Childhood and Determinants of Cardiovascular and Metabolic Profile in Young Adults

Groepatronen en ziekten op de volwassen leeftijd
Groei tijdens de kindertijd en determinanten van het cardiovasculaire en metabole profiel in jong volwassenen

Proefschrift

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Chapter 1

Introduction
This doctoral thesis describes the influence of different growth patterns during childhood on determinants of adult disease, like cardiovascular disease, type 2 diabetes mellitus and osteoporosis, in healthy young adults. Additionally, differences in these determinants are described between four clinically relevant subgroups: young adults born small for gestational age (SGA), with and without catch-up growth and young adults born appropriate for gestational age with short and normal adult height. Finally, the relation between early growth and determinants of adult disease, and the relation between nutrient intakes and body size in infancy are discussed. This chapter describes several hypotheses with regard to the influence of birth size and childhood growth on adult diseases and their determinants. In addition, the definitions, prevalence and etiologies of SGA and idiopathic short stature (ISS) are discussed together with the study design. Finally, the aims and outline of the thesis are presented.

1. Relations between low birth weight, childhood growth and adult disease

Hypotheses

In the early 1990s, several epidemiological studies showed an inverse relationship between birth weight and the risk for cardiovascular events, type 2 diabetes, hypertension, insulin resistance, adverse lipid profile and reduced bone mass density in elderly subjects (1-11). The exact mechanism underlying these associations are yet unknown, but several hypotheses have been postulated over time.

**Fetal origin hypothesis:** Barker et al. were the first to formulate a hypothesis based on the inverse association found between birth weight and adult disease. They suggested that events during pregnancy leading to fetal malnutrition could result in permanent endocrine and metabolic changes in the fetus, called re-programming (Figure 1) (6,12). At first, the fetus would benefit from the adaptations, as it would remain alive during fetal life, but in the long-term, this re-programming would result in diseases in later adulthood.

**Fetal insulin hypothesis:** This hypothesis was generated in 1999 and states that the inverse association between birth weight and adult insulin resistance is principally genetically mediated (Figure 2) (13). Parental genes involved in insulin resistance which are passed to the fetus could result in low-insulin-mediated fetal growth and in insulin resistance leading to type 2 diabetes in later life.
Introduction

Maternal glucose concentration (in the third trimester) 
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<tr>
<th>Glucose sensing by fetal pancreas</th>
<th>Fetal genetics</th>
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<td>Insulin secretion by fetal pancreas</td>
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<td>Insulin mediated fetal growth</td>
<td>Birth weight</td>
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<td>Insulin resistance in later life</td>
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Figure 2. Simplified representation of the fetal insulin hypothesis. Adapted from Hattersley et al. (13).

Growth acceleration hypothesis: Singhal and Lucas postulated in 2004 the hypothesis that not low birth weight per se, but growth acceleration during childhood is responsible for the increased risk for adult diseases in later life (14). Almost every child is genetically determined to grow to their growth potential. Thus, children born after fetal growth restriction, thus below their genetic growth potential, will experience postnatal catch-up growth. According to the hypothesis this postnatal catch-up growth, which might be stimulated by nutrient-enriched diets, will lead to the development of adult diseases in later life (Figure 3).

Figure 3. Representation of the growth acceleration hypothesis. Adapted from Singhal et al. (14).

2. Adult diseases and their determinants

The studies described in this thesis investigated determinants of 3 adult diseases, cardiovascular disease, type 2 diabetes and osteoporosis, in young adults.

Cardiovascular disease and type 2 diabetes

Although mortality of coronary heart disease has declined since the 1970s, the decline in mortality becomes less and might even reverse in the upcoming years (15,16). Cardiovascular disease (CVD) is still the primary cause of death. Obesity is a leading factor in the development of CVD and type 2 diabetes. The prevalence of obesity is rising in both children and adults, especially in developing countries (17-19). The exact factors involved in the development of CVD and type 2 diabetes are still unknown. Several studies have indicated that the development of CVD might start in childhood or even in infancy (20-22). It is therefore relevant to investigate whether different growth patterns during childhood influence determinants for CVD and type 2 diabetes in early adulthood.
Insulin sensitivity
To maintain a healthy glucose homeostasis, insulin sensitivity and insulin secretion should be balanced. A decline in insulin sensitivity is normally compensated by an increase in insulin secretion to maintain glucose tolerance. When insulin secretion does not change appropriately, impaired glucose tolerance and eventually type 2 diabetes will develop (23). Glucose homeostasis can be measured by Frequent Sampling Intravenous Glucose Tolerance test (FSIGT), which is explained in Appendix A. Low birth weight has consistently been associated with a higher prevalence of type 2 diabetes in later life, and some calculations indicated that low birth weight might be responsible for 35% of the type 2 diabetes cases (3,24,25). However, as low birth weight subjects show catch-up growth during childhood, it remains difficult to determine whether birth size is directly linked to the prevalence of type 2 diabetes, or that catch-up growth in early life determines the increased risk for type 2 diabetes. Our research group previously reported that a group of prepubertal short small for gestational (SGA) children had a reduced insulin sensitivity compared to age and height matched AGA born controls (26). Glucose homeostasis was still balanced by the compensatory increase of insulin secretion. This suggests that birth size might influence the development of type 2 diabetes in later life, but prior to the start of our studies no data in young adults were available.

Body composition
Body composition can be measured by Dual Energy X-ray Absorptiometry (DXA), which is explained in Appendix A, and gives more insight in the total amount of lean body mass (LBM) and fat mass (FM) of the body. It is well-known that a higher fat percentage results in an increased risk for type 2 diabetes and cardiovascular events. Studies investigating whether low birth size results in a different body composition were inconsistent and difficult to compare, mainly due to different ways of measuring LBM and FM as well as a wide variation in age of the various study groups (27-30). Short children born SGA have a typical lean appearance, which was confirmed by one study showing a low body mass index (BMI) and low sum of skin folds SDS (31). However, in children born SGA with catch-up growth in weight, early development of adiposity has been reported (32). Whether these differences in body composition would persist into early adulthood was unknown. Population-based studies showed that obesity during infancy tracked into adulthood (33,34). Understanding the mechanisms underlying the development of adult diseases in later life could lead to specific preventive interventions during childhood (33,35).

Lipid metabolism and Acylation Stimulating Protein (ASP)
Raised serum levels of total cholesterol (TC), low-density lipoprotein (LDLc), apolipoprotein B (apoB) and lipoprotein a (Lp(a)) together with reduced levels of high-density lipoprotein (HDLc) and apolipoprotein A-1 (apoA-I) increase the risk for cardiovascular disease (36-39). Although the association between birth weight and serum lipid levels had been investigated in several studies, no consistency was found (40,41). If low birth weight would affect lipid levels in later life, this should be present in SGA subjects. Our research group has investigated lipid levels in prepubertal SGA children and found no abnormalities (26). This was consistent with other literature, but no study differentiated between SGA children with or without catch-up growth during childhood (42-44).

Apolipoprotein E (apoE) is an important regulator of serum lipid levels because it affects hepatic binding and uptake of several lipoproteins (45). There are six apoE genotypes which all have specific affinity for the LDLc receptor. ApoE genotype ε3/ε3 is most common and subjects with an ε2 allele have a reduced risk for CVD, while subjects with an ε4 allele have a higher risk for CVD (46,47). No study evaluated the prevalence of the different ApoE genotypes in subjects born SGA and if it has an effect on their serum lipid levels.

Acylation stimulating protein (ASP) is a relatively new hormone generated through the activation of the alternative complement pathway proteins C3, B and adipin and produced by adipocytes (48). The function of ASP is to stimulate uptake of glucose and free fatty acid (FFA) in adipocytes (49,50). By increasing chylomicron derived FFA incorporation into triglycerides (TG) in adipocytes, ASP prevents inhibition of lipoprotein lipase (LPL) by FFA and therefore helps to maintain normal serum FFA levels (51). Additionally, ASP inhibits TG lipolysis in adipocytes by reducing the activity of hormone-sensitive lipase (HSL), resulting in lower serum FFA levels. By controlling the storage of TGs, ASP helps to keep lipid levels in the normal range. Because it is a relatively new hormone, clinical data on ASP are limited. High fasting levels of TG and ASP have been associated with obesity, insulin resistance, cardiovascular disease and dyslipidemia. This resulted in the hypothesis that higher ASP levels in the presence of higher TG might indicate ASP resistance (48,52). Gianflone et al. showed that ASP as a predictor of delayed TG clearance might predict postprandial hypertriglyceridemia, which is an early stage of dyslipidemia (52,53). Thus, the ASP level might actually predict a very early stage of CVD. It might therefore be interesting to investigate which factors are related to fasting ASP levels in early adulthood and whether different growth patterns during childhood are related to a different fasting ASP level in early adulthood.
Blood pressure and carotid intima media thickness (carotid IMT)

Blood pressure is an important determinant of cardiovascular events and is the most investigated determinant regarding the relationship between birth size and the risk for development of CVD in later life (2,5,7,54,55). Despite this extensive research, several reviews concluded that clarity was lacking because the results remained inconclusive (56,57). Other reports stated that subjects on nutrient enriched diets had rapid weight gain in early life and that this was related with increased blood pressure in later life (58). For that reason, it was relevant to investigate whether prenatal or postnatal growth influences blood pressure in later life.

Eventually, cardiovascular events are caused by occlusion of arteries at the site of atherosclerotic plaques. The presence of atherosclerotic plaques in the carotid arteries can be determined by investigating the intima media thickness in the vessel wall of the carotid arteries by non-invasive ultrasound measurements (Appendix A) (59). A greater thickness is associated with the development to atherosclerotic plaques and is positively correlated with cardiovascular events (60, 61). As development of atherosclerosis already starts in childhood, determining carotid IMT in early adulthood might give more insight in the risk of cardiovascular events later in life and early factors influencing carotid IMT might be found. No study had investigated carotid IMT in SGA subjects, either with or without catch-up growth.

Osteoporosis

Birth size, childhood growth and bone mineral density

Osteoporosis is a condition characterized by reduced bone mineralization, resulting in an increased fracture risk. Bone mineral density (g/cm²) (BMD) can be measured by Dual Energy X-ray Absorptiometry (DXA) (Appendix A). In young adults, total body BMD and BMD of the lumbar spine were measured. To evaluate BMD, it is important to take body size into account as BMD is a measure for aerial bone density instead of volumetric bone density, otherwise BMD will be underestimated in short subjects (62,63). For bone density of the vertebra, apparent BMD (BMAD) is calculated to take body size into account (64). The prevalence of osteoporosis varies in developed countries between 8-18% in elderly women and 3-6% in elderly men; the prevalence in elderly women is higher due to their lower estrogen levels after the menopause (65,66).

Several studies found a positive association between birth size and BMD in adults, indicating that subjects with a low birth weight had an increased risk for development of osteoporosis in later life (1,11,67,68). However, adult size had hardly been taken into account and most studies were performed in elderly subjects. Therefore, additional data regarding the association between birth size and BMD in later life were required.

Growth in early life

Timing and tempo of early growth and determinants of cardiovascular disease

Some studies reported that rapid weight gain in early life might have adverse effect on determinants of CVD (35,69-71). Especially first year growth seemed to be an important period and it was suggested that in this period a special 'time window' might be applicable (72). However, whether slow or rapid growth is deleterious for later outcome of CVD was unclear (73). Studies from the Helsinki Birth Cohort concluded that growth retardation in the first year followed by rapid growth between 2 and 10 years is related to an increase in cardiovascular events (74). In contrast, several other studies reported that rapid weight gain in the first few months of life are related to increased fat mass in childhood and a disadvantageous metabolic profile in early adulthood (70,75). As most low birth weight subjects will show catch-up growth in early life, which is to a certain extend wished for, it is relevant to investigate if tempo of postnatal weight gain has an influence on determinants of CVD in early adulthood.

Nutrition in early life and the relationship with body size and body composition at 12 months

As catch-up growth during childhood seems to result in an increased fat mass which is being tracked into adulthood (70,76), factors influencing catch-up growth need to be established. Nutrition is one of these influencing factors (77). For instance, breast-fed children grow at a slower rate, and accumulate less fat during this period (78,79). One explanation might be the lower content of protein in breast milk compared to formula milk (80). Rolland-Cachera et al formulated an early protein hypothesis, suggesting that high protein intake early in life leads to increased secretion of insulin-like-growth factor I and insulin (81). These hormones trigger multiplication of adipocytes and therefore stimulate fat accumulation. Reports of the DONALD study support this hypothesis while several other studies found no relation between protein intake and adiposity in early life (82-84). A possible explanation might be that proteins of different nutritional sources have a different influence on body composition in early life.
3. Small for gestational age (SGA) and idiopathic short stature (ISS)

To investigate the influence of different growth patterns during childhood on determinants of adult disease, we included subjects with extreme variants of normal growth, like subjects born small for gestational age (with and without catch-up growth) and subjects with unknown growth retardation during childhood (idiopathic short stature). This design created greater contrast in the study population, which contributed to a better statistical model in which relationships between various factors could be detected with more statistical power.

Definition of SGA

In 2001, the International SGA Advisory Board Panel formulated a consensus statement on the definition of SGA, by defining SGA as a birth length and/or birth weight below −2 standard deviation score, adjusted for gestational age and gender (85). Data from an appropriate reference population are necessary to take differences in race and ethnicity into account.

SGA is a term used for size at birth and does not refer to intra-uterine growth. Intra-uterine growth is used to describe growth velocity in fetal life, which is determined by at least two ultrasound measurements. A child born SGA might have been small from the beginning of fetal life, or could have experienced intra-uterine growth retardation later in gestation, resulting in a small size at birth. However, children with intra-uterine growth retardation late in gestation can be born with a normal birth size. Figure 4 shows these different growth patterns in fetal life.

Prevalence and etiology of SGA

When SGA is defined as a birth length and/or birth weight below −2 standard deviation score, 2.3% of all live-born children are born SGA. For the Netherlands this means that in 2005, of all 187,910 live-born children, 4322 were born SGA (Central Bureau of Statistics, Voorburg, the Netherlands).

Several factors influence intra-uterine growth and may therefore cause SGA, including fetal, maternal, placental and demographic factors (86-89). Although many factors are known, in 40% of the cases, no cause can be found. Identification of the cause of SGA is important as underlying mechanisms may influence the prognosis and treatment. Table 1 lists factors associated with intrauterine growth retardation.

Short stature and SGA

Approximately 85% of the SGA children show catch-up growth in the first two years of life to a height above −1.88 SDS (91-93). Recently, de Riddler et al showed that 91% of SGA children reached a normal height, 6% achieved this between 2 and 8 years of age (94). Catch-up growth is most pronounced in the first 6 months of life, but this might be prolonged in prematurely born SGA children. Although subjects born SGA with catch-up growth (SGA-CU) attain an adult height which is within the normal ranges of the population, they remain significantly smaller than AGA born subjects (91).
Table 1. Factors associated with intrauterine growth retardation.

<table>
<thead>
<tr>
<th>Fetal factors</th>
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<td>Multiple births</td>
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<td>Congenital malformations</td>
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<tr>
<td>Chromosomal anomalies</td>
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<tr>
<td>– Turner syndrome</td>
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<td>– Down syndrome</td>
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<td>Inborn errors of metabolism</td>
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<td>–</td>
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<tr>
<td>Intrauterine infections</td>
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<tr>
<td>– TORCHES (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex, Syphilis)</td>
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<th>Maternal factors</th>
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<td>Medical conditions</td>
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<td>– Hypertension</td>
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<td>– Pre-eclampsia</td>
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<td>– Severe chronic disease or infections</td>
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<td>– Systemic lupus erythematosus</td>
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<tr>
<td>– Antiphospholipid syndrome</td>
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<tr>
<td>– Anemia</td>
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<tr>
<td>– Malignancies</td>
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<tr>
<td>– Abnormalities of the uterus</td>
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<tr>
<td>Social conditions</td>
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<tr>
<td>– Maternal nutrition</td>
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<tr>
<td>– Low prepregnancy BMI</td>
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<td>– Low maternal weight gain</td>
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<tr>
<td>– Delivery at &lt;16 or &gt;35 years</td>
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<tr>
<td>– Low social economic status</td>
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<td>– Use of drugs</td>
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<table>
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<th>Placental factors</th>
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<td>Reduced blood flow</td>
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<td>Reduced area for exchange of nutrients and oxygen</td>
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<td>– Infarcts</td>
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<td>– Hematomas</td>
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<td>– Partial abruption</td>
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<th>Environmental factors</th>
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<td>High altitude</td>
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<td>Toxic substances</td>
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Adapted from Bryan and Hindmarch (90).

SGA children without catch-up growth to a height above –2 SDS during childhood will attain an average adult height of –2.1 SDS for boys and –2.8 SDS for girls (95). If catch-up growth in height to >–2 SDS is not achieved at 2 years, these children have a 7-fold increase risk for short stature (93). Therefore, SGA born children with short stature at the age of 3 years should be referred to a pediatrician with expertise in endocrinology (85). The pediatrician should investigate whether short adult stature could be prevented by growth hormone treatment.

Growth hormone treatment has shown to be effective and safe for preventing short adult stature (96-99).

Definition of idiopathic short stature (ISS)

ISS is defined as a condition in which postnatal height of an individual is < ~2 SDS of the corresponding mean height for a given age, sex and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities (100). These subjects have a normal size at birth and do not have growth hormone deficiency. A subcategorization can be made based on the expectancy of achieving target height range based on final height of the parents. A second criterion for subcategorization is the presence of delayed bone maturation, which suggests delayed growth and puberty.

Prevalence and etiology of idiopathic short stature

The group of ISS subjects is a heterogeneous group of subjects with an unknown cause of short stature. Familial short stature is a common feature in this group. It is estimated that approximately 60-80% of all short children at or below –2 SDS do fit the definition of ISS (100). The frequency of referral of these children is dependent on the socioeconomic environment and boys have a greater perceived disability of short stature compared with girls.

4. The PROGRAM-study

To investigate the relationship between birth size, childhood growth and determinants of adult disease in early adulthood, the PROgramming factors for GRowth And Metabolism (PROGRAM) study was started. The inclusion and exclusion criteria are described in Appendix B. The study consists of a cohort of 322 healthy young adults with different growth patterns during fetal life and childhood. The association between birth size, childhood growth and determinants of adult disease were analysed in two ways.

First, multiple regression analyses were used to investigate the association between birth size, childhood growth and determinants of adult disease. The wide range in birth size and childhood growth patterns increases the contrast in the study population, which increase the statistical power to detect relationships between various factors.

Secondly, the total study population was divided into four clinically relevant subgroups based on birth length and adult height. The criteria for being included into one of the subgroups
are described in Appendix C. Two subgroups consisted of small for gestational age (SGA) born adults, one without catch-up growth (SGA-short) and another one with catch-up growth (SGA-CU). The last two subgroups consisted of young adults born appropriate for gestational age. The first subgroup experienced growth retardation during childhood without a known reason (idiopathic short stature (ISS)). The second one had normal growth during childhood and attained a normal adult height (controls). The growth patterns during childhood of each of the subgroups are displayed in Figure 5.

5. Aims of the study

This thesis describes results of seven studies performed in 322 young adults, aged 18-24 years, who participated in the PROGRAM-study and of one study performed in 396 infants from the Cambridge Baby Growth Study. The PROGRAM-study was started to investigate if size at birth and different growth patterns during childhood influence determinants of adult disease in early adulthood. Also, efforts were made to investigate if there were other factors influencing these determinants. Finally, this thesis describes the influence of various nutritional factors on first year growth in 396 infants participating in the Cambridge Baby Growth Study.

Determinants of metabolic and cardiovascular disease

We investigated if size at birth and growth patterns during childhood were related to insulin sensitivity (measured by Frequent Sampling Intravenous Glucose Tolerance test), body composition (measured by Dual Energy X-ray absorptiometry), serum lipid and ASP levels, blood pressure and carotid intima media thickness (IMT), in young adults. In addition, we also investigated whether other factors influenced these determinants in early adulthood. With regard to all these determinants, subgroup analyses were performed to evaluate whether determinants differed between the subgroups.

Determinants of bone mineral density (BMD)

Bone mineral density of the total body and lumbar spine was measured by DXA to investigate the relation between size at birth and growth patterns and BMD. Additionally, subgroup analyses were performed to investigate whether there were differences in bone mass density between the subgroups.

Growth in early life

In 217 young adults, first year growth data was collected to investigate if growth during a specific time period in the first year had an influence on determinants of cardiovascular disease in early adulthood. Additionally, we investigated if fast catch-up in weight during the first year of life had a more adverse effect on these determinants than slow first year catch-up in weight. Finally, the relation between nutrient intake and first year growth was investigated in infants of the Baby Growth Study from Cambridge, United Kingdom. Fat mass was determined by skinfold thickness and extensive nutritional data at 12 months of age were collected.
6. Outline of the thesis

Chapter 1 gives an introduction in the topics described in this thesis.

Chapter 2 presents which growth patterns and factors influence insulin sensitivity in young adults and based on the findings, a new hypothesis could be formulated.

Chapter 3 reports the relation between size at birth and lean body mass as well as fat mass in early adulthood. Additionally, differences in lean body mass and fat mass between the four subgroups are described.

Chapter 4 describes the influence of environmental and genetic factors on serum lipid profiles in young adults. In addition, subgroup analyses have been performed to investigate differences in lipid profile with regard to different growth patterns.

Chapter 5 reports the relation between growth during childhood and fasting serum ASP levels in young adults and the four subgroups.

Chapter 6 depicts the relation between size at birth and blood pressure and carotid IMT in early adulthood. Additionally, differences between the four subgroups are described with regard to blood pressure and carotid IMT.

Chapter 7 depicts the relation between size at birth and bone mineral density in young adult and the four subgroups.

Chapter 8 reports which period of postnatal growth has an influence on determinants of cardiovascular disease in early adulthood. In addition, it describes whether differences in tempo of postnatal weight gain have different influences on determinants of cardiovascular disease.

Chapter 9 describes the influence of various nutrient intakes on body size and body composition in infants at 12 months of age. This study was performed in the Cambridge Baby Growth Study.

Chapter 10 discusses the results of the studies in relation to the current literature and gives conclusions and clinical implications of the study results.

Chapter 11 summarizes the findings of the study in English.

Chapter 12 provides a Dutch summary.

Appendix A

Frequent Sampling Intravenous Glucose Tolerance test (FSIGT-test)

Several values regarding glucose homeostasis can be measured by FSIGT; insulin sensitivity index, which is the ability of insulin to increase net glucose disposal; glucose effectiveness, which is the ability of glucose to increase its own disposal and reduce its own production; acute insulin response to glucose (AIRg), which is the integrated insulin release during the first 10 minutes after the glucose infusion; and the disposition index (DI), the product of insulin sensitivity and acute insulin response indicating the degree of glucose homeostasis. These indicators of glucose regulation were determined by the Bergman’s minimal model (MINMOD 6.01 copyright RN Bergman) calculating paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test (FSIGT) (101-103) with Tolbutamide (104).

When insulin sensitivity varies in healthy subjects, these changes are compensated proportionally by insulin secretion; reduced insulin sensitivity leads to increased insulin secretion by the beta cells (Figure 6) (105). If insulin secretion does not change appropriately, impaired glucose tolerance and eventually type 2 diabetes mellitus will develop (23).

Figure 6. Hyperbolic association between insulin secretion and insulin sensitivity. Adapted from Kahn et al. (106). IGT: impaired glucose tolerance, T2DM: type 2 diabetes mellitus.
Dual Energy X-ray Absorptiometry (DXA)

Dual Energy X-ray Absorptiometry (DXA) is a machine used to measure bone mineral density and body composition (fat mass and lean body mass). The person being assessed lies still for about 15 minutes while a scanner slides over the participant. DXA uses X-rays to assess these measures, but the radiation dose is about 1/10 of a chest X-ray.

Carotid intima media thickness (IMT)

Intima media thickness is the thickness of the two inner layers of an arterial wall. The thickness of the intima media of the carotid artery is related to cardiovascular events in later life (60). Carotid IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using the same 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Tech. Laboratories, Bethel Washington, USA). On the R wave of the electrocardiogram, three longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These frozen images were digitalized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). The common carotid IMT was determined as the mean of the mean near and far wall measurements of both the left and right side common carotid artery.

Appendix B

Inclusion criteria of the PROGRAM-study:
- Chronological age at inclusion: 18.00-23.99 years,
- Neonatal period without signs of severe asphyxia (defined as Apgar score < 3 after 5 minutes), no other serious diseases such as long-term artificial ventilation and oxygen supply, broncho-pulmonary dysplasia or other chronic lung disease,
- Gestational age of 36 weeks or more,
- Well documented growth data,
- Caucasian,
- Born singleton,
- Signed informed consent.

Exclusion criteria of the PROGRAM-study:
- Chromosomal disorders, known syndromes and serious dysmorphic symptoms suggestive for a yet unknown syndrome, except Silver-Russell Syndrome,
- Any disease, endocrine or metabolic disorder that could interfere with growth during childhood (like diabetes mellitus, growth hormone deficiency, malignancies, severe chronic disease, emotional deprivation),
- Treatment that could have interfered with growth (like radiotherapy or growth hormone treatment),
- Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood.

Appendix C

Additional criteria for inclusion into one of the four subgroups:
- Normal birth length and adult height were set at an SDS> –1 (± 0.1 SDS),
- Born small for gestational age (<–2 SDS) with a short adult height (<-2 SDS) (SGA-short, SGA-S),
- Born small for gestational age (<–2 SDS) with catch-up growth resulting in a normal adult height (>–1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length >–1 SDS) with growth retardation resulting in a short adult height (<–2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length >–1 SDS) and a normal adult height (>–1 SDS) (controls).
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Part 1

Birth Size, Adult Size and Determinants of Adult Diseases
Fat Mass Accumulation During Childhood Determines Insulin Sensitivity in Early Adulthood

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Abstract

Background/Objectives: Low birth weight and postnatal catch-up growth has been associated with an increased risk for diabetes mellitus type II (DMII). We evaluated the contribution of birth- and adult size, body composition and waist to hip ratio to DMII risk factors in young adulthood.

Methods: In a group of 136 young adults, aged 18-24 years, insulin sensitivity and disposition index were determined by Frequent Sampling Intravenous Glucose Tolerance test (FSIGT). The association of clinical parameters with these variables was analyzed with multiple regression modeling. In addition, differences in insulin sensitivity and disposition index, a measure for beta cell function, were analyzed in 4 subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) (n= 25) or with catch-up growth (SGA-CU) (n= 23) or, born Appropriate for Gestational Age (AGA) with Idiopathic Short Stature (ISS) (n= 23) or with normal stature (controls) (n= 26).

Results: Fat mass (FM) was the only significant predictor of insulin sensitivity, whereas birth length and birth weight were not significant. After correction for age, gender and adult body size, insulin sensitivity was significantly lower in SGA-CU subjects compared to controls. None of the variables had a significant influence on disposition index and there was no significant difference in disposition index between the subgroups.

Conclusions: Our data show that a higher body fat mass at 21 years is associated with reduced insulin sensitivity, independent of birth size. These findings have important implications for public health practice.

Introduction

Size at birth has been inversely associated with adult diseases, such as diabetes mellitus type II (DMII) and cardiovascular diseases (1-3). A meta-analysis estimated that low birth weight was responsible for 35% of cases of DMII(4). Reduced insulin sensitivity is a well recognised, early determinant of DMII and cardiovascular disease, and it usually precedes clinical symptoms (5,6). Several studies found an association between low birth weight and reduced insulin sensitivity (1,7), but these studies did not correct for adult weight.

Unfortunately, many study populations were heterogeneous with regard to gestational age, parity, ethnicity, used tests and age at evaluation. Also, many studies did not differentiate between height and weight, neither at birth nor in adulthood. Often insulin sensitivity is described, whereas actually the disposition index (insulin sensitivity x insulin secretion) is a better determinant of DMII (8). The disposition index reflects how well the beta cells can compensate for a reduction in insulin sensitivity by increasing their insulin secretion (9).

At this moment it is not clear who is most at risk for DMII, the subject born small for gestational age (SGA) with persistent short stature or the one born SGA with a normal stature after postnatal catch-up growth. We recently reported lower insulin sensitivity in 8 year olds born SGA with persistent short stature (SGA-S) compared to age-matched short children who were born appropriate for gestational age (AGA-Idiopathic Short Stature (ISS)) (10). Comparable results were found in another group of short prepubertal SGA children (11). Postnatal catch-up growth in weight and height is found in approximately 85% of SGA subjects (12,13). Catch-up in height has been associated with increased insulin secretion, whereas catch-up in weight with reduced insulin sensitivity (14). In young adulthood, comparison of insulin sensitivity and secretion between SGA subjects with short stature and subjects with catch-up growth has not been performed.

We hypothesized that both pre- and postnatal factors associate with insulin sensitivity. We therefore evaluated the relative contribution of birth length, birth weight, adult height, adult weight, fat mass (FM), lean body mass (LBM) and waist to hip ratio to insulin sensitivity and disposition index in young adulthood. In addition, we evaluated if there were differences with regard to insulin sensitivity and disposition index between 4 clinically recognizable subgroups of young adults, born SGA with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).
Methods

Subjects
The total population consisted of 136 healthy subjects with an age between 18 and 24 years. They were randomly selected from hospitals in the Netherlands, where they had been registered because of their being small at birth (SGA with a birth length <-2SD) (15) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height <-2SD) (16) or having a minor accidental health problem, but otherwise being normal. Only those born at 36 weeks or more of gestation, being singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Birth data were taken from hospital records, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam The Netherlands, approved this study. Written informed consent was obtained from all the participants.

Based on SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. In order to increase the statistical power for subgroup comparison, the cut-off value was set at -1 SDS (± 0.1 SDS).
- Born small for gestational age (<-2 SDS) with a short adult height (<-2 SDS) (short-SGA, SGA-S),
- Born small for gestational age (<-2 SDS) with catch-up growth resulting in a normal adult height (>1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length >-1 SDS) with growth retardation resulting in a short adult height (<-2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length >-1 SDS) and a normal adult height (>1 SDS) (controls).

The number of males and females per subgroup are shown in Table 1. Of the 136 subjects, 97 were included in one of the subgroups.

Assessments
All subjects were invited to the Erasmus University Medical Centre after a 12-h overnight fast and had abstained from smoking and alcohol for 16 hours. At this visit, in all subjects anthropometry was recorded, blood was drawn for fasting lipids, body composition was determined using Dual Energy X-ray Absorptiometry (DXA) and a Frequent Sampling Intravenous Glucose Tolerance test (FSIGT) with Tolbutamide was performed (17). All women were tested between 3-5 days before their expected menstrual period. A questionnaire concerning subject’s medical history and that of their relatives, use of contraceptives, physical activity and their substance use including smoking was completed. Adult height was measured using a Harpenden stadiometer, whereas weight in kilograms was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S). Waist and hip circumference were measured at the level of the umbilicus and greater trochanters with a non-extendable measuring tape. One trained investigator performed all measurements according to standardised methods. Body composition was assessed on the same DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) and quality assurance was performed daily. Indicators of glucose regulation were determined by the Bergman’s minimal model (MINMOD 6.01 copyright RN Bergman) calculating paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test (FSIGT) (17-19) with Tolbutamide (9). Values derived were insulin sensitivity index (SI), which is the ability of insulin to increase net glucose disposal, glucose effectiveness (Sg), which is the ability of glucose to increase its own disposal and reduce its own production, acute insulin response to glucose (AIRg), which is the integrated insulin release during the first 10 minutes after the glucose infusion, and the disposition index (DI), the product of insulin sensitivity and acute insulin response indicating the degree of glucose homeostasis. Blood samples were centrifuged directly after withdrawal and serum and plasma were immediately frozen at –80 °C for later analyses.

Assays
All plasma glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson&Johnson Company, Beerse, Belgium). All plasma insulin levels were measured by IRMA (Medgenix; Biosource Europe, Nivelles, Belgium). The intra-assay coefficient of variation (CV) was 2% to 4.7% (19-405 pmol/l) and the inter-assay CV was 4.2% to 11.3% (32-375 pmol/l). All assays were performed in one central laboratory.
Statistical analysis
SD-scores for birth length, birth weight, adult height and adult weight were calculated in order to correct for gestational age, gender and age (15,16). Multiple linear regression (MR) analysis was performed to determine which variables contribute to insulin sensitivity and disposition index. First, we entered age, gender, height SDS, birth length SDS and birth weight SDS to the model (model A). The interaction term birth length SDS * adult height SDS was added to all MR models because the study group had been selected on birth length and adult height, in order to ensure that the effect of these variables was modeled correctly. Secondly, weight SDS and waist-to-hip ratio were added (model B). Thirdly, lean body mass (LBM) and fat mass (FM) were added as independent variables instead of adult weight SDS and waist-to-hip ratio was removed (model C). Finally, we added waist-to-hip ratio again to the model (model D).

Prior to the study, a power analysis with a level of significance (α) of 0.05 and a chosen power of 80% estimated that there should be at least 17 subjects in each subgroup to enable detection of relevant differences in insulin sensitivity (10). ANOVA was used to determine if there were differences between the subgroups with regard to the group characteristics. Bonferroni correction was used for pair wise group comparisons. To determine differences between the groups after correction for age, gender, adult size and adult fat mass, an ANCOVA model was used, with controls as reference group and SGA-S, SGA-CU and ISS as dummy variables. Statistical package SPSS version 11.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.

Results
The clinical characteristics of the study population are shown in Table 1. The total population consisted of 136 subjects with a mean (SD) age of 21 (1.5) years. We evaluated the relative contribution of several variables to insulin sensitivity in a multiple linear regression analysis. The initial analysis with insulin sensitivity as dependent variable included age, gender, adult height SDS, birth length SDS, birth weight SDS and the interaction term birth length SDS x adult height SDS (Table 2). Only gender and birth weight were significant determinants of insulin sensitivity (Model A, adjusted R²= 0.06, p= 0.03). However, when we added adult weight SDS and waist-to-hip ratio to the regression model, gender, adult height SDS, adult weight SDS and waist-to-hip ratio were significant determinants of insulin sensitivity, whereas birth weight was no longer significant (Model B, adjusted R²= 0.17). After specification of adult weight in fat mass and lean body mass, measured by DXA, fat mass appeared to be the only significant determinant of insulin sensitivity (Model C, adjusted R²= 0.23), even after adding waist-to-hip ratio to the model (Model D, adjusted R²= 0.24). There were no significant interactions between birth weight or -length and fat mass or lean body mass with regard to insulin sensitivity.

For disposition index as dependent variable, no significant determinants were found.

Table 1. Clinical characteristics of the total study group and the 4 subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Total group (n= 136)</th>
<th>SGA-S (n=25)</th>
<th>SGA-CU (n=23)</th>
<th>ISS (n=23)</th>
<th>Controls (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>68/68</td>
<td>11/14</td>
<td>12/11</td>
<td>11/12</td>
<td>14/12</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.1 (1.5)</td>
<td>39.4 (1.3)</td>
<td>38.1 (1.3)‡</td>
<td>39.4 (1.6)</td>
<td>39.0 (1.6)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.5 (2.78)</td>
<td>45.6 (1.31)</td>
<td>44.7 (1.68)*</td>
<td>49.7 (1.43)</td>
<td>50.6 (1.58)</td>
</tr>
<tr>
<td>Birth length (SDS)</td>
<td>-1.58 (1.36)</td>
<td>-2.73 (0.57)*</td>
<td>-2.75 (0.76)*</td>
<td>-0.46 (0.46)</td>
<td>0.08 (0.70) $</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.76 (0.63)</td>
<td>2.50 (0.37)*</td>
<td>2.16 (0.28)*</td>
<td>3.29 (0.63)</td>
<td>3.27 (0.53)</td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>-1.28 (1.34)</td>
<td>-1.96 (0.72)*</td>
<td>-2.39 (0.63)*</td>
<td>-0.04 (1.38)</td>
<td>-0.12 (1.12)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21.0 (1.5)</td>
<td>20.9 (1.6)</td>
<td>21.1 (1.4)</td>
<td>21.0 (1.5)</td>
<td>20.9 (1.6)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 (0.11)</td>
<td>1.59 (0.07)</td>
<td>1.75 (0.07)</td>
<td>1.60 (0.06)</td>
<td>1.78 (0.08)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-1.39 (1.32)</td>
<td>-2.57 (0.56)**</td>
<td>-0.28 (0.77)</td>
<td>-2.50 (0.41)</td>
<td>0.13 (0.91)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.1 (13.8)</td>
<td>56.6 (9.1)**</td>
<td>73.7 (14.0)</td>
<td>56.6 (13.3)**</td>
<td>71.5 (14.4)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-0.80 (1.56)</td>
<td>-1.68 (1.42)**</td>
<td>0.31 (1.20)</td>
<td>-1.88 (1.64)**</td>
<td>0.11 (1.22)</td>
</tr>
<tr>
<td>Adult Fat%</td>
<td>24.6 (10.0)</td>
<td>24.9 (8.6)</td>
<td>25.9 (10.9)</td>
<td>24.6 (9.8)</td>
<td>23.2 (9.9)</td>
</tr>
<tr>
<td>Adult Fat (kg)</td>
<td>16.0 (8.4)</td>
<td>14.1 (5.7)</td>
<td>19.5 (10.2)</td>
<td>14.5 (8.8)</td>
<td>16.8 (9.5)</td>
</tr>
<tr>
<td>Adult LBM (kg)</td>
<td>45.2 (10.3)</td>
<td>40.0 (7.7)**</td>
<td>51.0 (9.7)</td>
<td>39.8 (7.9)**</td>
<td>51.3 (10.8)</td>
</tr>
<tr>
<td>Waist/Hip ratio</td>
<td>0.84 (0.07)</td>
<td>0.84 (0.08)</td>
<td>0.87 (0.06)</td>
<td>0.83 (0.08)</td>
<td>0.85 (0.06)</td>
</tr>
</tbody>
</table>

Values are given as means (sd). M: Male; F: Female.
$: p< 0.001 compared with the other subgroups, #: p=0.05 compared with the other subgroups.
*: p=0.02 compared with SGA-S and ISS, #: p<0.001 compared with controls, #: p<0.001 compared with ISS.
&: p=0.001 compared with SGA-CU.

Comparison of the subgroups
Ninety-seven subjects were eligible for the analysis of differences between the four clinically relevant groups. There was a significant difference in gestational age between the SGA-CU group and the SGA-S and ISS group (p< 0.02) and a significant difference in birth length SDS between ISS and AGA subjects (p< 0.05) (Table 1). Other differences were due to the selection criteria. There was a tendency for a higher weight and FM in SGA-CU subjects but weight and
absolute or relative FM were not significant between groups. Physical activity, alcohol use and smoking were not significantly different between the subgroups.

Table 2. Multiple regression for Si in young adulthood

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A β</th>
<th>p-value</th>
<th>Model B β</th>
<th>p-value</th>
<th>Model C β</th>
<th>p-value</th>
<th>Model D β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>-0.21</td>
<td>0.389</td>
<td>-0.15</td>
<td>0.516</td>
<td>-0.13</td>
<td>0.543</td>
<td>-0.11</td>
<td>0.620</td>
</tr>
<tr>
<td>Gender</td>
<td>-1.84</td>
<td>0.013</td>
<td>-2.75</td>
<td>0.001</td>
<td>-0.13</td>
<td>0.927</td>
<td>-0.79</td>
<td>0.614</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.34</td>
<td>0.381</td>
<td>0.87</td>
<td>0.038</td>
<td>0.47</td>
<td>0.314</td>
<td>0.49</td>
<td>0.305</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>0.01</td>
<td>0.987</td>
<td>0.001</td>
<td>0.998</td>
<td>0.12</td>
<td>0.801</td>
<td>0.12</td>
<td>0.791</td>
</tr>
<tr>
<td>BL*AH (SDS)</td>
<td>0.19</td>
<td>0.369</td>
<td>0.16</td>
<td>0.413</td>
<td>0.14</td>
<td>0.464</td>
<td>0.14</td>
<td>0.464</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>0.89</td>
<td>0.040</td>
<td>0.66</td>
<td>0.116</td>
<td>0.55</td>
<td>0.169</td>
<td>0.51</td>
<td>0.209</td>
</tr>
<tr>
<td>Weight SDS</td>
<td></td>
<td></td>
<td>-0.74</td>
<td>0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W/H ratio</td>
<td>-13.8</td>
<td>0.026</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-7.70</td>
<td>0.210</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td></td>
<td></td>
<td>-0.24</td>
<td>&lt;0.0005</td>
<td>-0.21</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM (kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.02</td>
<td>0.758</td>
<td>0.02</td>
<td>0.799</td>
</tr>
<tr>
<td>Overall</td>
<td>0.026</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td>&lt;0.0005</td>
<td></td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.10</td>
<td>0.22</td>
<td>0.28</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.06</td>
<td>0.17</td>
<td>0.23</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows the results of the FSIGT tests. The SGA-CU group had the lowest insulin sensitivity and highest insulin secretion of the four groups, but this difference was not statistically significant before correction for possible confounders. In order to correct for age, gender, adult height and adult weight, we additionally performed multiple linear regression analyses. After correction, the SGA-CU group appeared to have a significantly lower insulin sensitivity compared to the controls (p= 0.01). Since fat mass is an important factor in determining insulin sensitivity, we also corrected for adult fat mass (Table 4, model 2). The explained variance rose from 0.17 to 0.30, indicating the relevance of FM. The difference in insulin sensitivity between the three subgroups and controls tended to become less significant, but the difference between SGA-CU and controls remained significant (p= 0.02).

**Table 3. FSIGT results of the total study group and the 4 subgroups**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n= 136)</th>
<th>SGA-S (n= 25)</th>
<th>SGA-CU (n= 23)</th>
<th>ISS (n= 23)</th>
<th>Controls (n= 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si *10⁴/min (µU/ml)</td>
<td>6.9 (4.3)</td>
<td>6.3 (3.9)</td>
<td>4.9 (3.1)</td>
<td>6.7 (4.1)</td>
<td>8.0 (4.0)</td>
</tr>
<tr>
<td>Sg *10⁻²/min (mg/d)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.01)</td>
<td>0.02 (0.00)</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>AIRg (mU/l)</td>
<td>540 (509)</td>
<td>615 (703)</td>
<td>782 (777)</td>
<td>401 (261)</td>
<td>456 (283)</td>
</tr>
<tr>
<td>DI (Sg/AIRg)</td>
<td>2833 (1818)</td>
<td>2698 (1618)</td>
<td>2716 (1908)</td>
<td>2264 (1306)</td>
<td>3208 (2242)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.9 (0.51)</td>
<td>5.0 (0.45)</td>
<td>4.9 (0.50)</td>
<td>4.8 (0.5)</td>
<td>4.9 (0.4)</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>10.2 (4.7)</td>
<td>10.2 (5.1)</td>
<td>11.5 (4.1)</td>
<td>9.1 (3.9)</td>
<td>10.1 (5.0)</td>
</tr>
</tbody>
</table>

*Not corrected for age, gender and adult size.

Values are given as means (sd).

Si: insulin sensitivity; Sg: sensitivity of glucose disposal by glucose, AIRg: acute insulin response to glucose; DI: disposition index.

**Table 4. Difference in insulin sensitivity of the subgroups versus controls**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1 β</th>
<th>p-value</th>
<th>Model 2 β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>-0.33</td>
<td>0.177</td>
<td>-0.23</td>
<td>0.316</td>
</tr>
<tr>
<td>Gender</td>
<td>-1.88</td>
<td>0.13</td>
<td>0.01</td>
<td>0.988</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.32</td>
<td>0.570</td>
<td>-0.19</td>
<td>0.723</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>-0.94</td>
<td>0.001</td>
<td>0.89</td>
<td>0.077</td>
</tr>
<tr>
<td>FM (kg)</td>
<td></td>
<td></td>
<td>-0.33</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>SGA-S*</td>
<td>-2.25</td>
<td>0.214</td>
<td>-1.34</td>
<td>0.421</td>
</tr>
<tr>
<td>SGA-CU*</td>
<td>-2.66</td>
<td>0.013</td>
<td>-2.28</td>
<td>0.021</td>
</tr>
<tr>
<td>ISS*</td>
<td>-2.13</td>
<td>0.237</td>
<td>-0.60</td>
<td>0.720</td>
</tr>
<tr>
<td>Overall</td>
<td>0.001</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.23</td>
<td>0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.17</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*After correction for age, gender, adult height and adult weight.

1: The control group is the reference group.

After correction for age, gender, adult height and adult weight, the acute insulin response (AIR) was significantly higher in all three subgroups compared to controls (Table 5). After an additional correction for fat mass, the AIR was only significantly higher in the SGA-S and SGA-CU groups, compared to controls.
Multiple regression analysis showed, however, no significant difference in disposition index between the four groups after correction for age, gender, adult height and adult weight.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p-value</td>
<td>β</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>-60.0</td>
<td>0.077</td>
<td>-65.2</td>
<td>0.055</td>
</tr>
<tr>
<td>Gender</td>
<td>167.3</td>
<td>0.104</td>
<td>71.0</td>
<td>0.556</td>
</tr>
<tr>
<td>Height SDS</td>
<td>93.5</td>
<td>0.222</td>
<td>120.0</td>
<td>0.125</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>169.7</td>
<td>&lt; 0.0005</td>
<td>75.3</td>
<td>0.309</td>
</tr>
<tr>
<td>FM (kg)</td>
<td></td>
<td></td>
<td>17.5</td>
<td>0.138</td>
</tr>
<tr>
<td>SGA-S³</td>
<td>702.4</td>
<td>0.005</td>
<td>659.0</td>
<td>0.009</td>
</tr>
<tr>
<td>SGA-CU³</td>
<td>345.4</td>
<td>0.019</td>
<td>328.9</td>
<td>0.025</td>
</tr>
<tr>
<td>ISS³</td>
<td>526.8</td>
<td>0.034</td>
<td>451.3</td>
<td>0.073</td>
</tr>
<tr>
<td>Overall</td>
<td>&lt; 0.0005</td>
<td></td>
<td>&lt; 0.0005</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.32</td>
<td></td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.26</td>
<td></td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

³: The control group is the reference group.

Discussion

Our study in 136 young adults shows that adult fat mass is the main predictor of insulin sensitivity in young adulthood, whereas birth length and birth weight were not. When data on body composition are not available, gender, adult height, adult weight and waist-to-hip ratio appear to be significant determinants. None of these variables had a significant influence on the disposition index. Analysis in four clinically relevant subgroups showed that, after correction for age, gender, adult height and adult weight, SGA-CU subjects had significantly lower insulin sensitivity compared to controls. This difference remained significant even after an additional correction for fat mass. There were no significant differences between the subgroups with regard to disposition index.

Our data, measured by FSIGT and DXA, show a strong influence of fat mass on insulin sensitivity in young adults whereas birth size had no influence on insulin sensitivity. These findings are in line with a previous study in 25 years old adults born SGA, which showed that lower insulin sensitivity coincided with weight gain rather than with birth weight(20). Due to the DXA measurements, we could specify weight gain as accumulation of fat. Our data imply that all individuals, regardless of their size at birth, should try to achieve or maintain a normal fat mass for their body size.

Our first model showed that birth weight and gender were significant determinants of insulin sensitivity. This is consistent with studies of Barker, who showed an inverse association between birth size and adult diseases, like DM II and cardiovascular diseases (1,3). However, when we added adult weight and waist-to-hip ratio to the model, birth weight was no longer a significant determinant of insulin sensitivity, whereas adult size (height, weight and waist-to-hip ratio) was significant (Model B). This is in line with other studies demonstrating weight gain or growth acceleration during childhood as a significant factor (14,21-23). When we specified adult weight into FM and LBM, FM was the only significant determinant of insulin sensitivity (Model C). The regression model shows that, given a similar birth length or birth weight, the fat mass in young adults, accumulated from birth to 21 years, determines insulin sensitivity. Thus, an additional accumulation of 10 kilograms of fat will reduce insulin sensitivity by 2.42*10⁻⁶/min (μU/ml). Our last model showed that waist-to-hip ratio had no additional value in determining insulin sensitivity (Model D). This is probably due to the prior correction for FM, because in Model B, W/H ratio is a significant determinant. Another reason might be the small variation of W/H ratio at this age.

The ’growth acceleration hypothesis’, as suggested by Singhal and Lucas, in which fetal growth restriction relative to genetic growth potential could result in deleterious growth acceleration postnatally (24), can be taken a step further. In our study, we had the opportunity to correct for birth-, adult size and adult body composition. As fat accumulation appeared to be the only significant factor in the determination of insulin sensitivity, we therefore propose to specify growth acceleration by increased accumulation of fat mass. One may call this the ”fat accumulation hypothesis”, indicating that an increased accumulation of fat during childhood, independent of birth size, will result in reduced insulin sensitivity. Increased fat mass is associated with changes in levels of free fatty acids and adipocytokines (e.g. adiponectin, leptin) (25), which affect insulin sensitivity and action. These changes might, in time, lead to adult diseases like DMII and cardiovascular diseases. Growth acceleration in height and weight as such is not a problem as long as a normal amount of fat is accumulated.

We analysed if there were differences in insulin sensitivity and disposition index between four clinically relevant groups of young adults (SGA-S, SGA-CU, ISS and controls) because some investigators, including ourselves, described reduced insulin sensitivity in prepubertal
short SGA children (10,11,26). We wanted to know what the insulin sensitivity would be in subjects born SGA when they had reached young adulthood. Our study revealed no difference between the SGA-S subjects compared to the other subgroups. The difference with the studies in prepubertal children might be due to puberty, when insulin sensitivity decreases in all children (27). The explanation might be that the decline in insulin sensitivity in SGA-S children is less during puberty, which would result in almost the same values as the ISS and controls in young adulthood.

After correction for age, gender, adult height and adult weight, we found significantly lower insulin sensitivity in SGA-CU compared to controls. As fat mass is a significant determinant of insulin sensitivity, a difference in fat mass might explain the lower insulin sensitivity in SGA-CU. This would be in line with data in 4 year old SGA-CU subjects who already had a higher fat mass and a lower insulin sensitivity compared to age-matched AGA subjects (23). We, therefore, performed an additional correction for fat mass. The difference in insulin sensitivity became less, but remained significant, indicating that also other factors contribute to the reduced insulin sensitivity in SGA-CU subjects compared to controls. Differences in IGF-I have been described but are controversial (23,28). Another factor that has been suggested is the amount of LBM. Decreased muscle mass might lead to more insulin resistance (29,30). However, in our total study population, LBM had no significant association with insulin sensitivity (Table 2). Other relevant factors might be genetic factors (31), such as a higher allele frequency of polymorphism in the glucocorticoid receptor gene (32), leading to increased glucocorticoid sensitivity, which might contribute to the lower insulin sensitivity in SGA-CU subjects.

Several studies concluded that intra-uterine growth retardation (IUGR) has an independent effect on insulin sensitivity, but they did not take adult size or weight gain into account (1,22,33). We have studied two SGA subgroups, with one group having lower insulin sensitivity and the other group having similar insulin sensitivity compared to controls. In our opinion, this supports our conclusion that birth size as such is not a relevant factor in determining insulin sensitivity.

ISS subjects had the same insulin sensitivity compared to controls. After correction for adult height and adult weight, their insulin sensitivity was non-significantly lower compared to controls, but this disappeared after correction of fat mass, indicating a relatively higher fat mass percentage in ISS subjects than controls. Insulin sensitivity of young adults with ISS has never been described.

After correction for age, gender, adult height, adult weight and fat mass, SGA-S subjects had a significantly higher AIR compared to controls, whereas their insulin sensitivity was comparable. The reason for this is unknown, but an increased sensitivity of the beta cells for glucose might be a reason, or a reduced insulin clearance by the liver. SGA-CU subjects also had a significantly higher AIR compared to controls after the corrections, but their insulin sensitivity was significantly lower. This is a known compensation mechanism to maintain glucose tolerance (9). The significantly higher AIR in ISS subjects might be due to a difference in fat mass, because after the additional correction for fat mass, the significance disappeared.

We determined which factors influence disposition index, because the most important determinant of DM II is beta cell function (8). We applied the MR models on disposition index, but none of the variables appeared to be a significant factor. Also our subgroups comparison showed no difference in disposition index. It has been shown that a reduction in disposition index and not reduced insulin sensitivity relates to an increased risk of DM II (34). Normally, the beta cells will increase their insulin secretion when insulin sensitivity declines. When the beta cells start to fail, the disposition index, which stands for insulin sensitivity x insulin secretion, will decline. Two studies in SGA-S and SGA subjects showed a reduced insulin sensitivity, but recalculations show that the disposition index was normal, indicating an increased insulin secretion (10,35). Our present study shows that all four groups had a normal disposition index indicating that all groups had normal functioning beta cells at the age of 21 years. Thus the fear that SGA subjects might be at a higher risk of DM II at the age of 21 years compared to those born AGA, could not be substantiated by our study.

We have chosen for a study population, which consisted for a relative large percentage of subjects born small for gestational age and of short adults, compared to the normal population. This results in a better statistical model because there is more contrast in the study population, so relationships between various factors can be detected with more statistical power. Another advantage of this study population is that it allows comparison between clinically relevant subgroups. Better insight could be obtained in the differences between the subgroups with regard to insulin sensitivity and disposition index, after correction for age, gender, adult size and fat mass.

In conclusion, our study in 136 young adults shows that adult fat mass is the main predictor of insulin sensitivity in young adulthood, whereas birth length and birth weight had no significant influence on insulin sensitivity. None of these variables had a significant influence on the disposition index. SGA-CU subjects had significantly lower insulin sensitivity compared to controls, after correction for age, gender, adult height, adult weight and fat mass. No differences in disposition index were found between the subgroups. As a result of our findings, we propose to introduce the "fat accumulation hypothesis". For public health practice this means, that...
parents of all children, independent of birth size or growth during childhood, should be aware of the risks of fat accumulation in their children. Children at risk for fat accumulation, like SGA-CU subjects, need to be controlled on a regular basis by primary health workers.

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Potential Financial Conflicts of Interest: none

References:
Chapter 3

Influence of Birth Size on Body Composition in Early Adulthood: the Programming Factors for Growth and Metabolism (PROGRAM)-study

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Abstract

Background / Objectives: Several studies have investigated the relationship of birth size with fat mass (FM) and lean body mass (LBM), but the findings differed greatly due to different ways of measuring FM and LBM, different study populations and age groups. We hypothesized that birth size has no influence on adult body composition, whereas weight gain during childhood has.

Methods: In the PROGRAM-study, a cohort of 312 young adults, aged 18-24 years, FM and LBM were determined by Dual Energy X-ray Absorptiometry (DXA). Subsequently, differences in FM and LBM were analyzed in 4 subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) or with catch-up growth (SGA-CU), or born Appropriate for Gestational Age (AGA) with Idiopathic Short Stature (ISS) or with normal stature (controls).

Results: Age, gender, adult height SDS and adult weight SDS were significant positive determinants of FM and LBM, whereas weight gain during childhood was positively significant for FM and negatively for LBM. Birth weight SDS tended to be significant and birth length SDS was not. Weight gain during childhood was positively correlated with waist/hip ratio and trunk fat/total fat ratio. SGA-CU subjects had significantly higher FM and significantly lower LBM than controls.

Conclusion: Weight gain during childhood is an important determinant of body composition in young adulthood, whereas birth size is less important. In clinical practice, too much weight gain in childhood should be prevented as it results in a relatively high fat mass, especially in children with catch-up growth in weight, like SGA-CU subjects.

Introduction

Birth size, especially birth weight, has been associated with adult diseases like diabetes mellitus type II (DMII) and cardiovascular diseases (1). Some studies showed a significant association between low birth weight and an increased risk for cardiovascular diseases (2,3), but other studies found controversial results with regard to the relationship between birth weight and BMI (4-6), whereas BMI was recognized as an important predictor for cardiovascular disease (7,8). As a higher BMI might be due to either a higher fat mass (FM) or more lean body mass (LBM) or both, BMI is an inappropriate measure for explaining the risk of cardiovascular disease in adulthood.

Some studies have investigated the relationship of birth weight with FM and LBM separately (9-16). These studies are inconsistent with each other due to differences in measuring FM and LBM. Various methods have been used like skinfold-thickness measurements, bioelectrical-impedance analysis, hydrostatic weight measurements and Dual Energy X-ray Absorptiometry (DXA) measurements. These studies have been performed in different age groups. Additionally, not all studies corrected for adult size (height and weight). For this reason, it is difficult to compare these studies and to draw conclusions with regard to the relationship between birth size and body composition in adulthood. Based on recent literature, we hypothesized that birth size has no influence on adult body composition, whereas weight gain during childhood has (6,17).

We therefore investigated in a cohort of 312 young adults (PROGRAM-study), the influence of birth length and birth weight on FM and LBM measured by DXA, with and without correction for adult size. Additionally, we investigated if there were differences in FM and LBM between four clinically recognizable subgroups of young adults, born SGA with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).
at birth (SGA with a birth length <−2SD) (18) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height <−2SD) (19). In addition, healthy subjects of different schools were randomly asked to participate as controls. Only those born at 36 weeks or more of gestation, being born singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Birth data were also assigned to one of four subgroups. Normal birth length and adult height were set at an SDS>−1 (± 0.1 SDS), in order to increase the statistical power to identify differences between analyses in the total group. Based on SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. Normal birth length and adult height were set at an SDS>−1 (± 0.1 SDS), in order to increase the statistical power to identify differences between subgroups. Of the 312 subjects, 217 were included in one of the subgroups:

- Born small for gestational age (<−2 SDS) with a short adult height (<−2 SDS) (SGA-short, SGA-S),
- Born small for gestational age (<−2 SDS) with catch-up growth resulting in a normal adult height (>−1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length >−1 SDS) with growth retardation resulting in a short adult height (<−2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length >−1 SDS) and a normal adult height (>−1 SDS) (controls).

Methods

All participants were invited to visit the Erasmus Medical Centre in Rotterdam. They had been fasting for 12-hours and had abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer and weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice and the mean value was used for analysis.

All participants had FM and LBM measured by one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was daily performed. The intra-essay coefficient of variation for lean tissue and fat tissue has been reported to be 1.57–4.49% and 0.41–0.88%, respectively (20).

Statistical analysis

In order to correct for gestational age, gender and age, SD-scores for birth length, birth weight, adult height and adult weight were calculated (18,19). Because of a skewed distribution, FM was log transformed. Multiple linear regression (MR) analyses, as suggested by Lucas et al (21), were performed to determine the association between birth size and FM and LBM, correcting for age, gender, and adult size. Firstly, we entered age, gender, adult height SDS, birth length SDS and birth weight SDS to the model, to investigate the influence of birth size on the outcomes (model A). The interaction term birth length SDS * adult height SDS was added to all MR models because the study group had been selected on birth length and adult height in order to ensure that the effect of these variables was modeled correctly. Secondly, birth weight SDS was replaced by adult weight SDS, to relate adult size to FM and LBM (model B). Thirdly, birth weight SDS was added to the model. When birth size and adult size are shown in one model, it is possible to investigate if the change in weight (centile crossing) between birth and adulthood is related to the outcomes (model C). Finally, we added the interaction term birth weight SDS * adult weight SDS to investigate whether birth weight modifies the effect of adult weight on outcome. This means that we can answer the question whether being growth retarded at birth and having weight gain during childhood is more serious in terms of outcome than having a normal birth size and an equivalent weight gain during childhood (model D). To analyze associations between fat distribution and weight gain SDS after correction for age and gender, partial correlations were used.

For the subgroup comparisons, FM and LBM were expressed as percentage of bodyweight to correct for weight and subsequently as SD-scores with controls as reference group. ANOVA was used to determine if there were differences between the subgroups with regard to the group characteristics. Bonferroni correction was used for pair wise group comparisons. To determine differences between the groups corrected for age, gender and adult size, an ANCOVA model was used, with controls as reference group and SGA-S, SGA-CU and ISS as dummy variables.
Statistical package SPSS version 11.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.

**Results**

The clinical characteristics of our study group are shown in Table 1. Mean (SD) age was 20.9 (1.67) years.

Table 1. Clinical characteristics of the total study group and the 4 subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Total group (n=312)</th>
<th>SGA-S (n=44)</th>
<th>SGA-CU (n=56)</th>
<th>ISS (n=38)</th>
<th>Controls (n=79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n) (%)</td>
<td>122 (39.1)</td>
<td>15 (34.1)</td>
<td>23 (41.1)</td>
<td>16 (42.1)</td>
<td>29 (36.7)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.6 (3.08)</td>
<td>45.2 (1.98)*</td>
<td>44.6 (1.9)*</td>
<td>49.6 (1.4)*</td>
<td>50.8 (1.7)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.78 (0.67)</td>
<td>2.50 (0.48)$</td>
<td>2.19 (0.33)$</td>
<td>3.25 (0.56)</td>
<td>3.30 (0.62)</td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>–1.2 (1.39)</td>
<td>–1.94 (0.94)**</td>
<td>–2.36 (0.70)*</td>
<td>–0.17 (1.14)</td>
<td>–0.09 (1.27)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.9 (1.68)</td>
<td>20.7 (1.76)</td>
<td>21.0 (1.5)</td>
<td>21.0 (1.7)</td>
<td>20.8 (1.71)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.2 (10.7)</td>
<td>157.3 (6.7)^</td>
<td>174.7 (7.8)</td>
<td>159.3 (6.7)^</td>
<td>176.8 (7.7)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>–1.1 (1.37)</td>
<td>–2.63 (0.55)**</td>
<td>–0.14 (0.77)$</td>
<td>–2.48 (0.40)**</td>
<td>0.24 (0.90)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.8 (12.5)</td>
<td>56.7 (11.4)**</td>
<td>70.5 (14.4)</td>
<td>56.5 (12.2)**</td>
<td>68.8 (11.0)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>–0.46 (1.42)</td>
<td>–1.55 (1.54)**</td>
<td>0.12 (1.17)</td>
<td>–1.77 (1.67)**</td>
<td>0.07 (0.98)</td>
</tr>
<tr>
<td>Delta weight SDS</td>
<td>0.57 (1.99)</td>
<td>0.39 (1.76)</td>
<td>2.48 (1.33)$</td>
<td>–1.60 (2.22)$</td>
<td>0.16 (1.43)</td>
</tr>
<tr>
<td>BMI</td>
<td>22.5 (3.9)</td>
<td>22.9 (4.6)</td>
<td>23.1 (4.5)</td>
<td>22.4 (4.7)</td>
<td>21.9 (2.9)</td>
</tr>
<tr>
<td>Waist/Hip Ratio</td>
<td>0.88 (0.07)</td>
<td>0.87 (0.08)</td>
<td>0.90 (0.07)</td>
<td>0.87 (0.08)</td>
<td>0.89 (0.06)</td>
</tr>
<tr>
<td>FM (kg)**</td>
<td>15.7 (8.73)</td>
<td>15.7 (8.87)</td>
<td>18.7 (10.7)</td>
<td>14.7 (10.1)</td>
<td>15.6 (8.71)</td>
</tr>
<tr>
<td>FM (SDS)**</td>
<td>0.01 (1.02)</td>
<td>0.35 (1.23)</td>
<td>–0.10 (1.05)</td>
<td>0.00 (1.00)</td>
<td></td>
</tr>
<tr>
<td>Trunk fat/total fat ratio</td>
<td>0.48 (0.06)</td>
<td>0.47 (0.06)</td>
<td>0.49 (0.06)</td>
<td>0.47 (0.07)</td>
<td>0.48 (0.05)</td>
</tr>
<tr>
<td>LBM (kg)**</td>
<td>45.1 (9.85)</td>
<td>38.3 (7.28)**</td>
<td>48.5 (9.86)</td>
<td>39.1 (7.17)**</td>
<td>50.0 (9.78)</td>
</tr>
<tr>
<td>LBM (SDS)**</td>
<td>–1.20 (0.74)**</td>
<td>–1.16 (1.01)</td>
<td>–1.11 (0.73)**</td>
<td>0.00 (1.00)</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means (sd). **Not corrected for age, gender, adult height and adult weight.

Fat mass

Table 2 shows the results of the multiple regressions for FM. Model A shows the influence of birth length and birth weight on FM after correction for age, gender and adult height SDS.

In this model, birth length SDS was not a significant determinant for adult FM, whereas birth weight SDS was borderline significant (β= -0.057, p= 0.082) and age and gender were strong significant determinants (adjusted R²= 0.23). The second model shows the influence of adult weight SDS on FM. Adult weight SDS was a strong significant determinant of FM, next to age, gender and adult height SDS (Model B, adjusted R²= 0.71). Model C demonstrates that both birth weight and adult weight were significant factors, but the beta and the level of significance were reasonably higher for adult weight than for birth weight. In addition, when birth weight is related to FM after adjustment for adult weight, this indirectly demonstrates change in weight during childhood (see Methods). This model shows that the subjects with the highest weight gain had relatively more FM (adjusted R²= 0.72). The final model shows no significant influence of the interaction term BW*AW, meaning that having weight gain from a lower birth weight has no extra effect on FM in young adulthood than having weight gain from a higher birth weight. The last three models showed that age, gender and adult height were also significant determinants of FM.

Table 2. Multiple regression for log transformed FM.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>4.36</td>
<td>3.40</td>
<td>3.50</td>
<td>3.53</td>
</tr>
<tr>
<td>Gender</td>
<td>52.1</td>
<td>&lt;0.0005</td>
<td>52.1</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Height SDS</td>
<td>5.00</td>
<td>&lt;0.0005</td>
<td>4.28</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>1.11</td>
<td>0.397</td>
<td>0.32</td>
<td>0.436</td>
</tr>
<tr>
<td>BMI</td>
<td>0.88</td>
<td>0.631</td>
<td>0.082</td>
<td>0.048</td>
</tr>
<tr>
<td>Waist/Hip Ratio</td>
<td>0.48</td>
<td>0.048</td>
<td>0.23</td>
<td>0.846</td>
</tr>
<tr>
<td>FM (kg)**</td>
<td>15.7</td>
<td>18.7</td>
<td>14.7</td>
<td>15.6</td>
</tr>
<tr>
<td>FM (SDS)**</td>
<td>0.01</td>
<td>0.35</td>
<td>–0.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Trunk fat/total fat ratio</td>
<td>0.48</td>
<td>0.048</td>
<td>–0.23</td>
<td>0.846</td>
</tr>
<tr>
<td>LBM (kg)**</td>
<td>45.1</td>
<td>38.3</td>
<td>39.1</td>
<td>50.0</td>
</tr>
<tr>
<td>LBM (SDS)**</td>
<td>–1.20</td>
<td>–1.16</td>
<td>–1.11</td>
<td>0.00</td>
</tr>
</tbody>
</table>

| Overall        | <0.0005 | <0.0005 | 33.3    | <0.0005 |
|                | 0.23    | 0.71    | 0.72    | 0.72    |

1: refers to the unstandardized regression coefficients (1/100).

Lean body mass

The results of the MR analysis are shown in Table 3. Model A showed that birth length SDS was not significant, after correction for age, gender and adult height. Birth weight SDS and age were
Influence of Birth Size on Body Composition

borderline significant (β = 0.55, p = 0.06 and β = 0.33, p = 0.03, respectively), whereas gender and adult height SDS were strong significant determinants of LBM (adjusted R² = 0.79). Model B shows that adult weight was a strong significant determinant of LBM (Model B, adjusted R² = 0.86). Model C demonstrates that both birth weight and adult weight were significant factors, but the beta and the level of significance were reasonably higher for adult weight than for birth weight. In addition, when birth weight is related to LBM after adjustment for adult weight, this indirectly demonstrates change in weight during childhood. This model shows that subjects with the highest weight gain had relatively less LBM (adjusted R² = 0.86). The final model shows that the interaction term was not significant, which indicates that weight gain from a lower birth weight has no extra effect on LBM in young adulthood than having weight gain from a higher birth weight. All models show that age, gender and adult height SDS were also significant determinants of LBM.

Table 3. Multiple regression for LBM (kg)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A β</th>
<th>p-value</th>
<th>Model B β</th>
<th>p-value</th>
<th>Model C β</th>
<th>p-value</th>
<th>Model D β</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>0.33</td>
<td>0.031</td>
<td>0.29</td>
<td>0.020</td>
<td>0.28</td>
<td>0.030</td>
<td>0.27</td>
<td>0.032</td>
</tr>
<tr>
<td>Gender</td>
<td>–15.6</td>
<td>&lt; 0.0005</td>
<td>–15.5</td>
<td>&lt; 0.0005</td>
<td>–15.5</td>
<td>&lt; 0.0005</td>
<td>–15.6</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Height SDS</td>
<td>3.69</td>
<td>&lt; 0.0005</td>
<td>2.35</td>
<td>&lt; 0.0005</td>
<td>2.44</td>
<td>&lt; 0.0005</td>
<td>2.45</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>–0.39</td>
<td>0.223</td>
<td>0.19</td>
<td>0.287</td>
<td>–0.35</td>
<td>0.180</td>
<td>–0.29</td>
<td>0.303</td>
</tr>
<tr>
<td>BL*AH (SDS)</td>
<td>–0.04</td>
<td>0.723</td>
<td>0.04</td>
<td>0.737</td>
<td>0.02</td>
<td>0.833</td>
<td>0.05</td>
<td>0.691</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>0.55</td>
<td>0.061</td>
<td>0.66</td>
<td>0.006</td>
<td>0.60</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight SDS</td>
<td>2.09</td>
<td>&lt; 0.0005</td>
<td>2.12</td>
<td>&lt; 0.0005</td>
<td>1.99</td>
<td>&lt; 0.0005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW*AW (SDS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>–0.084</td>
<td>0.456</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.79</td>
<td>0.86</td>
<td>0.86</td>
<td>0.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Fat distribution

As weight gain appeared to be an important determinant of adult body composition, we additionally investigated if weight gain during childhood was associated with waist / hip ratio and trunk fat / total fat ratio, after correction for age and gender. Weight gain during childhood was strongly correlated to waist / hip ratio (r = 0.20, p < 0.0005), but especially to trunk fat / total fat ratio (r = 0.42, p < 0.0005).

Comparison of the subgroups

The clinical characteristics of the subgroups are shown in Table 1. ISS subjects had a significantly lower birth length SDS than controls (p < 0.001) and SGA-CU subjects had significantly shorter adult height than controls (p < 0.02). SGA-CU subjects had the highest FM (kg) of the 4 subgroups, but this difference did not reach significance. Further, LBM (in kg and SDS) was significantly lower in SGA-S and ISS subjects than in SGA-CU subjects and controls (p < 0.001). However, these data were not corrected for confounders like age, gender, adult height and adult weight. Other differences were due to the selection criteria.

To determine possible differences between the subgroups with regard to FM and LBM, corrections for age, gender and adult height were made. We used percentage of FM (%FM) and LBM (%LBM) as outcome parameters. Figure 1 shows the corrected differences in %FM between the subgroups. SGA-CU subjects had significantly higher %FM than controls, after correction for age, gender and adult height SDS (p = 0.026). The other subgroups did not significantly differ from controls.

Figure 1. Difference in %FM SD-score between the 3 subgroups compared to controls (with 95% CI), after correction for age, gender and adult height. # p = 0.026
Corrected differences in %LBM are shown in Figure 2. SGA-CU subjects had a significantly lower %LBM than controls (p= 0.018). The other subgroups did not significantly differ from controls.

Figure 2. Difference in %LBM SD-score between the 3 subgroups compared to controls (with 95% CI), after correction for age, gender and adult height. * p= 0.018

Fat distribution was not significantly different between the subgroups after correction for age and gender, although the trunk fat / total fat ratio was highest in SGA-CU subjects (difference with controls was 0.012 (p= 0.187)). Weight gain during childhood was not significantly correlated with parameters of fat distribution in the subgroups (data not shown).

Discussion

Our study in 312 young adults shows that age, gender, adult height SDS and adult weight SDS as well as weight gain during childhood are significant determinants of FM and LBM, whereas birth weight SDS tends to be. Comparison of the subgroups showed that SGA-CU subjects had a significantly higher %FM and a significantly lower %LBM than controls, after correction for age, gender and adult height.

The first MR models showed that birth weight was borderline significant for adult FM as well as adult LBM, whereas adult weight was a strong significant determinant of both FM and LBM, next to age, gender and adult height. The third model showed that adult weight was a reasonably stronger determinant of adult FM and LBM than birth weight. The model indirectly showed that the gain in weight during childhood was a significant positive determinant of FM and a significant negative determinant of LBM, indicating that of two subjects, the one with the greatest change in weight during childhood will gain relatively more FM than LBM. The final model answered the question whether a certain weight gain after low birth weight gives a different body composition than a similar weight gain after normal birth weight. Because the interaction term BW*AW was not significant, our data show that birth size has no additional effect on body composition when subjects have the same weight gain. We therefore conclude that adult weight and weight gain during childhood are determinants of body composition and that subjects with more catch-up growth in weight during childhood gain relatively more FM than LBM.

Our results are in line with other reports showing a positive association between birth weight and LBM (9,10,12-16,22,23). With regard to FM, there was no conclusive answer in recent studies (12-16,22,23). Our study shows that birth weight has opposite effects on FM and LBM. One explanation might be that subjects who were born after fetal growth retardation use their energy for their vital organs and do not use their spare energy for accumulation of LBM, but rather for FM as a buffer for difficult periods later in life.

It remains difficult to determine whether prenatal or postnatal growth is the most important determinant of long-term outcome parameters. Catch-up growth in weight can only be measured indirectly by subtracting early weight SDS of later weight SDS. Nevertheless, with the MR models suggested by Lucas et al (21), we were able to assess the influence of weight gain during childhood on FM and LBM. Because the time span between birth and adulthood is large and we have no growth data within this period, we could not define in which period this catch-up in weight took place. It would be interesting to collect data on growth in infancy and during childhood as it might specify the time period wherein catch-up in weight takes place. Some reports showed that infancy is an important period for catch-up in weight, especially the first few months after birth (24-26). However, other investigators reported that the association between catch-up in weight and later blood pressure becomes only detectable after a longer period of time (27). We were able to demonstrate that catch-up in weight, somewhere during childhood, had an effect on FM and LBM at the age of 18-24 years.
Fat distribution had a strong correlation with weight gain during childhood. This indicates that persons with higher weight gain during childhood accumulate relatively more central fat than peripheral fat. Too much weight gain will result in a relatively large amount of central fat. This has serious consequences for later life as central fat distribution has been associated with cardiovascular risk factors, like dyslipidemia and hyperglycemia. (28,29) On the other hand, specification in visceral or subcutaneous fat is necessary (30).

Short SGA subjects (SGA-S) had similar %LBM and %FM as controls, after correction for age, gender and adult height. Previous findings reported that prepubertal SGA-S subjects had a lower fat percentage and a lower BMI compared to controls, but no corrections for height and weight were made (31,32). Another explanation might be that SGA-S subjects accumulate more fat during puberty, which results in a similar BMI and %FM as controls.

SGA-CU subjects had a significantly higher %FM and a significantly lower %LBM than controls, which is in line with the literature (11,33,34). While their low birth weight might explain some of this difference, their weight gain during childhood explained most of this difference, because SGA-S subjects had a similar size at birth but less catch-up growth. Thus, SGA-CU subjects accumulated more FM. They also increased their LBM, but to a lower extend than FM.

Högler et al showed that growth retardation during childhood resulted in a reduced LBM when no correction for weight was made (35). We corrected for adult size and our results in ISS subjects show that their body composition in young adulthood is equal to controls. Their reduced weight gain during childhood will lead to a reduced amount of LBM and FM, but not to a different body composition (%LBM and %FM).

Our study population consisted of a relative high percentage of subjects born small for gestational age and of short adults. This created greater contrast in the study population, which contributed to a better statistical model in which relationships between various factors could be detected with more statistical power. In addition, this study population allowed comparison between clinically relevant subgroups.

In conclusion, our hypothesis that birth size has no association with adult body composition, whereas weight gain during childhood has, is only partially correct. Our study in 312 young adults showed that adult weight SDS as well as weight gain during childhood are strong significant determinants, next to age, gender and adult height. In contrast, birth length SDS is not a significant factor and birth weight SDS was borderline significant. Thus, weight gain during childhood is a strong determinant of body composition in young adulthood, whereas birth size is less important. For clinical practice it means that too much weight gain in childhood should be prevented as it results in a relatively high fat mass, especially in children with catch-up growth in weight, like SGA-CU subjects.

Acknowledgements

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Potential Financial Conflicts of Interest: none
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Chapter 4

Fat Mass and Apolipoprotein E Genotype Influence Serum Lipoprotein Levels in Early Adulthood, whereas Birth Size does not

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Abstract

Background / Objectives: An association between an unfavourable lipid profile and low birth weight has been reported, although this association remains controversial. We hypothesized that birth size does not have any influence on serum lipid levels but fat accumulation during childhood has.

Methods: In the PROGRAM-study, a cohort of 297 young adults, aged 18-24, the influence of clinical parameters on total cholesterol (TC), triglycerides, low-density lipoprotein, high-density lipoprotein, lipoprotein a, apolipoprotein A-1 and B (apoA-1 and apoB) were analyzed with multiple regression modeling. In addition, differences in these lipid levels and apolipoprotein E (ApoE) genotype prevalence were analyzed in 4 subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) or with catch-up growth or, born Appropriate for Gestational Age with Idiopathic Short Stature or with normal stature (controls).

Results: Birth length SDS and birth weight SDS were no significant determinants of the serum lipid levels, while gender, ApoE genotype, adult height SDS, adult weight SDS and fat mass were. Comparison of the subgroups showed that SGA-S subjects had a significantly higher apoB than controls. There were no other significant differences in lipid levels or ApoE genotype prevalence between the four subgroups.

Conclusion: ApoE genotype is an important genetic determinant of lipid levels in young adulthood. Furthermore, fat accumulation during childhood significantly determines serum lipid levels, whereas birth size has no significant contribution. For public health practice this means, that parents and their children need to be informed about the risks of fat accumulation during childhood.

Introduction

Raised levels of total cholesterol (TC), low-density lipoprotein (LDLc), apolipoprotein B (apoB) and lipoprotein a (Lp(a)) together with reduced levels of high-density lipoprotein (HDLc) and apolipoprotein A-I (apoA-I) increase the risk for cardiovascular disease (1-4). In the literature, there is an ongoing discussion whether low birth weight has a significant influence on various lipid levels, like TC, LDLc, HDLc, apoA-I and apoB. Some reports found an association between low birth weight and high TC levels (5-8), indicating that subjects born with a low birth weight would have an increased risk for cardiovascular disease due to an unfavourable lipid profile. Conversely, systematic reviews showed no associations between serum birth weight and lipid levels (9,10). Also in subjects born small for gestational age (SGA), normal lipid profiles were found, although a distinction between SGA subjects with or without catch-up growth was seldom made (11-16).

Apolipoprotein E (ApoE) is an important regulator of serum lipid levels because it affects hepatic binding and uptake of several lipoproteins (17). The affinity for the LDLc receptor is different for the six ApoE genotypes. ApoE genotype ε2/ε3 is most common. Subjects carrying the ε2 allele have a lower affinity for the LDLc receptor, which results in an up-regulation of the LDLc receptor. This leads to more uptake of lipoproteins and thus lower serum lipid levels. In contrast, subjects carrying the ε4 allele have a higher affinity for the LDLc receptor, which results in higher lipid levels (17,18). The ε4 genotype is associated with higher coronary disease risk (18,19). So far, no study has investigated the influence of the ApoE genotype in subjects born SGA and subjects with idiopathic short stature (ISS).

Recently, we demonstrated in young adults of the PROGRAM study, that birth size had no influence on insulin sensitivity in young adulthood, whereas fat accumulation during childhood had (20). Therefore, we hypothesized that prenatal growth, reflected by size at birth, has no influence on serum lipid levels in young adulthood, whereas postnatal growth and genetic factors have. To test our hypothesis, we investigated the influence of birth length, birth weight, adult size, body composition and ApoE genotype on TC, triglycerides (TG), HDLc, LDLc, apoA-I, apoB and Lp(a) in 297 young adults. In addition, we investigated if there were differences in the prevalence of ApoE genotype and serum lipid levels between four clinically relevant subgroups of young adults: born SGA with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).
Subjects and Methods

Subjects
The PROgramming factors for GRowth And Metabolism (PROGRAM) study cohort consists of 323 healthy subjects with an age between 18 and 24 years. They were randomly selected from hospitals in the Netherlands, were they had been registered because of their being small at birth (SGA with a birth length <−2SD) (21) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height <−2SD) (22). In addition, healthy subjects of different schools were randomly asked to participate as controls. Only those born at 36 weeks or more of gestation, born singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Birth data were taken from records of hospitals, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam The Netherlands, approved this study. Written informed consent was obtained from all participants.

Of the 323 participants who entered the study, complete data on lipid levels, body composition and anthropometry were obtained in 297 subjects. Based on the SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. Normal birth length and adult height were set at an SDS >−1 (± 0.1 SDS), in order to increase the contrast between the subgroups. This resulted in an increased statistical power to identify differences between subgroups. Of the 297 subjects, 208 fulfilled the inclusion criteria for the subgroup analyses:

- Born small for gestational age (<−2 SD) with a short adult height (<−2 SDS) (SGA-S),
- Born small for gestational age (<−2 SDS) with catch-up growth resulting in a normal adult height (>−1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length >−1 SDS) with growth retardation resulting in a short adult height (<−2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length >−1 SDS) and a normal adult height (>−1 SDS) (Controls).

Measurements
All participants were invited to visit Erasmus Medical Centre in Rotterdam. They had been fasting for 12-hours and had abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer and weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S, Katwijk, Netherlands). All anthropometric measurements were performed twice and the mean value was used for analysis.

In all participants, lean body mass (LBM) and fat mass (FM) were measured on one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was daily performed. The intra-assay coefficient of variation for lean tissue and fat tissue was 1.57–4.49% and 0.41–0.88%, respectively (23).

Laboratory methods
Fasting levels of TC, TG, HDLc, apoA-1, apoB and Lp(a) were measured. Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = total cholesterol – HDLc – 0.45 x triglycerides.

Total cholesterol and TG were measured using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). HDLc was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics). ApoA-1, apoB and Lp(a) were determined by rate nephelometry on the Image Immunochemistry System, according to the manufacturers instructions (Beckman Coulter, Mijdrecht, the Netherlands). The intra-assay variations of measurements of TC, TG and HDLc were 2.9, 3.3 and 3.9%. Between-run coefficients of variation for apoA-1, apoB and Lp(a) were 4.2%, 2.8% and 6.9% at levels of 0.94, 0.53 and 0.35 g/l, respectively.

ApoE genotype was analyzed in 246 of the 297 subjects. There were no differences in clinical characteristics between the 246 with ApoE genotyping and the 51 without. The ApoE genotype was determined on DNA samples using a polymerase chain reaction followed by enzymatic digestion using methods previously described (24).

Statistical analysis
SD-scores for birth length, birth weight, adult height and adult weight were calculated, in order to correct for gestational age, gender and age (21,22). Percentage of body fat was calculated as [body fat (kg) / weight (kg)] x 100%. Due to a skewed distribution, TG, LDLc, apoB and Lp(a) were log transformed. Multiple linear regression (MR) analysis was performed to determine the association between birth size and the lipid variables, correcting for age, gender and adult size. First, we entered age, gender, birth length SDS, birth weight SDS and adult height SDS.
**Table 1. Clinical characteristics and lipid concentrations of the total study group, the 4 subgroups and the unclassified group.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n = 297)</th>
<th>SGA–S (n = 42)</th>
<th>SGA–CU (n = 54)</th>
<th>ISS (n = 36)</th>
<th>Controls (n = 76)</th>
<th>Unclassified (n = 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>116/181</td>
<td>14/28</td>
<td>21/33</td>
<td>15/21</td>
<td>28/48</td>
<td>38/51</td>
</tr>
<tr>
<td>Age (years)</td>
<td>20.9 (1.7)</td>
<td>20.6 (1.7)</td>
<td>21.0 (1.5)</td>
<td>20.9 (1.7)</td>
<td>20.8 (1.7)</td>
<td>20.8 (1.7)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.6 (3.0)</td>
<td>45.2 (2.00) a</td>
<td>44.6 (1.87) a</td>
<td>49.5 (1.38) b</td>
<td>50.8 (1.75)</td>
<td>47.1 (2.27)</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>–1.50 (1.44)</td>
<td>–2.91 (0.80) a</td>
<td>–2.82 (0.77) a</td>
<td>–0.52 (0.46) b</td>
<td>0.11 (0.75)</td>
<td>–1.80 (1.02)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.78 (0.7)</td>
<td>2.50 (0.49) b</td>
<td>2.19 (0.33) b</td>
<td>3.22 (0.56) c</td>
<td>3.29 (0.63) c</td>
<td>2.65 (0.55)</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>–1.21 (1.38)</td>
<td>–1.94 (0.96) a</td>
<td>–2.35 (0.70) a</td>
<td>–0.20 (1.12) c</td>
<td>–0.09 (1.27) c</td>
<td>–1.54 (1.06)</td>
</tr>
<tr>
<td>Adult height (m)</td>
<td>1.68 (0.11)</td>
<td>1.57 (0.07) d,e</td>
<td>1.75 (0.08)</td>
<td>1.59 (0.07) d,e</td>
<td>1.77 (0.08)</td>
<td>1.66 (0.09)</td>
</tr>
<tr>
<td>Adult height SDS</td>
<td>–1.09 (1.36)</td>
<td>–2.61 (0.56)  d,e</td>
<td>–0.16 (0.72) b</td>
<td>–2.48 (0.41) d,e</td>
<td>0.24 (0.86) b</td>
<td>–1.52 (0.97)</td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>63.7 (12.6)</td>
<td>57.1 (11.5) d,e</td>
<td>70.2 (14.6)</td>
<td>56.4 (12.4) d,e</td>
<td>69.0 (11.0)</td>
<td>61.5 (9.2)</td>
</tr>
<tr>
<td>Adult weight SDS</td>
<td>–0.64 (1.41)</td>
<td>–1.47 (1.53)  d,e</td>
<td>0.09 (1.17)</td>
<td>–1.78 (1.71) de</td>
<td>0.08 (0.97)</td>
<td>–0.86 (1.08)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>15.7 (8.87)</td>
<td>16.0 (8.93)</td>
<td>18.8 (10.86)</td>
<td>14.6 (9.34)</td>
<td>15.6 (8.78)</td>
<td>14.1 (8.89)</td>
</tr>
<tr>
<td>Fat mass (SDS)</td>
<td>0.04 (1.02)</td>
<td>0.36 (1.24)</td>
<td>–0.12 (1.06)</td>
<td>0.00 (1.00)</td>
<td>–0.17 (0.78)</td>
<td>–0.17 (0.78)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>45.1 (9.89)</td>
<td>38.4 (7.37) de</td>
<td>48.1 (9.88)</td>
<td>39.2 (7.17) de</td>
<td>50.2 (9.89)</td>
<td>44.6 (9.77)</td>
</tr>
<tr>
<td>Lean body mass (SDS)</td>
<td>–1.42 (0.25) de</td>
<td>–2.10 (0.75) de</td>
<td>–1.12 (0.72) de</td>
<td>0.00 (1.00)</td>
<td>–0.57 (0.89)</td>
<td>–0.57 (0.89)</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>27.3</td>
<td>26.3</td>
<td>30.4</td>
<td>45.2</td>
<td>23.6</td>
<td>237</td>
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</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total group (n= 297)</th>
<th>SGA–S (n= 42)</th>
<th>SGA–CU (n= 54)</th>
<th>ISS (n= 36)</th>
<th>Controls (n= 76)</th>
<th>Unclassified (n= 89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.57 (0.95)</td>
<td>4.75 (0.93)</td>
<td>4.68 (0.96)</td>
<td>4.61 (0.93)</td>
<td>4.55 (0.95)</td>
<td>4.55 (0.95)</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.03 (0.52)</td>
<td>2.89 (0.81)</td>
<td>2.74 (0.46)</td>
<td>2.70 (0.80)</td>
<td>2.80 (0.71)</td>
<td>2.80 (0.71)</td>
</tr>
<tr>
<td>LDLc (mmol/l)</td>
<td>0.92 (0.34)</td>
<td>0.80 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>1.38 (0.35)</td>
<td>1.39 (0.35)</td>
<td>1.34 (0.34)</td>
<td>1.36 (0.34)</td>
<td>1.36 (0.34)</td>
<td>1.36 (0.34)</td>
</tr>
<tr>
<td>HDLc (mmol/l)</td>
<td>1.21 (0.25)</td>
<td>0.25 (0.24)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
</tr>
<tr>
<td>HDLc</td>
<td>0.92 (0.34)</td>
<td>0.80 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
<td>0.84 (0.34)</td>
</tr>
<tr>
<td>ApoA–1 (g/l)</td>
<td>1.30 (0.23)</td>
<td>1.29 (0.23)</td>
<td>1.33 (0.24)</td>
<td>1.34 (0.24)</td>
<td>1.34 (0.24)</td>
<td>1.34 (0.24)</td>
</tr>
<tr>
<td>Lp(a) (g/l)</td>
<td>0.21 (0.25)</td>
<td>0.25 (0.24)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
<td>0.16 (0.29)</td>
</tr>
</tbody>
</table>


1Not corrected for age, gender and adult size, 2Lp(a) was measured in 276 subjects of the total study group, 36 SGA-S, 57 SGA-CU, 31 ISS and 73 controls.

a: p< 0.01 compared with controls, b: p< 0.001 compared with SGA–S and SGA–CU.

The first model showed that gender had a significant influence on all serum lipid levels, whereas birth length and birth weight had not. The second model showed that shorter adult height SDS and higher adult weight SDS resulted in a significantly higher TC, TG, LDLc and apoB. For HDLc the relation was inversely, a higher adult height SDS and a lower adult weight SDS resulted in a significantly higher level of HDLc. For apoA-1 this was not significant. Birth length SDS and birth weight SDS remained not significant.

After replacement of adult weight SDS by FM and LBM, FM was a significant determinant for all lipid variables, except for HDLc and apoA-1 (Model 3). The significance of gender disappeared for most lipid variables, but adult height SDS remained a significant determinant of lipid levels after correction for FM and LBM, except for apoA-1. LBM, birth length SDS and birth weight SDS were no significant determinants for any lipid variable.

Finally, several ApoE genotype were significant determinants for total cholesterol, LDLc and apoB. Subjects carrying a ε2 genotype have lower levels, while subjects carrying a ε4 genotype have higher levels. In these models, the explained variance rose by 6%, 10% and 7%, respectively. The rise in explained variance established by fat mass was less, 1%, 2% and 2%, respectively. For Lp(a), none of the variables had a significant influence (data not shown). An additional correction for smoking did not change the results in the models of Table 4 (data not shown).
Table 3. Multiple linear regression of the lipid variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model</th>
<th>Gender</th>
<th>Birth length SDS</th>
<th>Birth weight SDS</th>
<th>Adult height SDS</th>
<th>Adult weight SDS</th>
<th>Fat mass (kg)</th>
<th>LBM (kg)</th>
<th>ApoE genotype ε2/ε2</th>
<th>ε2/ε3</th>
<th>ε2/ε4</th>
<th>ε3/ε4</th>
<th>ε4/ε4</th>
<th>Adj R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/l)</td>
<td>1</td>
<td>0.68</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.06</td>
<td>n.s.</td>
<td>0.02</td>
<td>0.14</td>
<td>0.001</td>
<td>0.003</td>
<td>0.11</td>
<td>0.008</td>
<td>0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.68</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.13</td>
<td>0.023</td>
<td>0.11</td>
<td>0.008</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.07</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.48</td>
<td>0.051</td>
<td>n.s.</td>
<td>-0.10</td>
<td>0.049</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.10</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.15</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-0.07</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.10</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td>TG (log)</td>
<td>1</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.04</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.08</td>
<td>0.024</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.49</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-0.08</td>
<td>0.024</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.70</td>
<td>0.092</td>
<td>n.s.</td>
<td>-0.06</td>
<td>0.093</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td>LDLc (log)</td>
<td>1</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.02</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.12</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.04</td>
<td>0.020</td>
<td>0.04</td>
<td>0.008</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.05</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.33</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-0.04</td>
<td>0.056</td>
<td>0.00</td>
<td>0.044</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.06</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.11</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-0.03</td>
<td>n.s.</td>
<td>0.01</td>
<td>0.083</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.07</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td>ApoB (log)</td>
<td>1</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.04</td>
<td>0.014</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.07</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.06</td>
<td>0.001</td>
<td>0.04</td>
<td>0.002</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.42</td>
<td>0.056</td>
<td>n.s.</td>
<td>-0.05</td>
<td>0.007</td>
<td>0.00</td>
<td>0.018</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.06</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.27</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-0.04</td>
<td>0.043</td>
<td>0.01</td>
<td>0.073</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.07</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td>HDLc (mmol/l)</td>
<td>1</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>0.03</td>
<td>n.s.</td>
<td>0.03</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.22</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>0.05</td>
<td>0.021</td>
<td>-0.04</td>
<td>0.026</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.05</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.43</td>
<td>n.s.</td>
<td>0.069</td>
<td>0.10</td>
<td>0.001</td>
<td>-0.00</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.10</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.49</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.11</td>
<td>0.001</td>
<td>-0.01</td>
<td>n.s.</td>
<td>-0.01</td>
<td>n.s.</td>
<td>0.07</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td>ApoA-1 (g/l)</td>
<td>1</td>
<td>0.20</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>-0.02</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.20</td>
<td>&lt;0.001</td>
<td>n.s.</td>
<td>0.01</td>
<td>-0.01</td>
<td>n.s.</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.39</td>
<td>0.026</td>
<td>n.s.</td>
<td>0.02</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.03</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.39</td>
<td>n.s.</td>
<td>0.070</td>
<td>0.01</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.00</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>0.019</td>
<td>0.044</td>
</tr>
</tbody>
</table>

Significant p-values are given in bold, n.s.: not significant (p > 0.10). β: regression coefficient, P: p-value.
All models include a correction for age (n.s.). ApoE genotype ε3/ε3 is the reference group in model 4.
Table 4. Differences in LDLc and apoB levels between subgroups and controls after correction for age, gender, %body fat and ApoE genotype.

<table>
<thead>
<tr>
<th>Variables</th>
<th>LDLc^2 (n= 206)</th>
<th>LDLc^2 (n= 171)</th>
<th>ApoB (log) (n= 208)</th>
<th>ApoB (log) (n= 173)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model A</td>
<td>Model B</td>
<td>Model A</td>
<td>Model B</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.015 0.661</td>
<td>-0.034 0.354</td>
<td>0.006 0.594</td>
<td>-0.008 0.468</td>
</tr>
<tr>
<td>Gender</td>
<td>0.327 0.005</td>
<td>0.238 0.104</td>
<td>0.164 &lt; 0.0005</td>
<td>0.132 0.004</td>
</tr>
<tr>
<td>%FM</td>
<td>0.009 0.180</td>
<td>0.004 0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoE genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA–S^1</td>
<td>0.389 0.014 [0.076-0.702]</td>
<td>0.322 0.055 [-0.008-0.652]</td>
<td>0.141 0.006 [0.019-0.225]</td>
<td>0.111 0.034 [0.009-0.213]</td>
</tr>
<tr>
<td>SGA–CU^1</td>
<td>0.190 0.183 [-0.078-0.497]</td>
<td>0.183 0.229 [-0.116-0.482]</td>
<td>0.091 0.051 [-0.003-0.171]</td>
<td>0.070 0.141 [-0.024-0.163]</td>
</tr>
<tr>
<td>ISS^1</td>
<td>0.49 0.363 [-0.148-0.505]</td>
<td>0.098 0.582 [-0.253-0.449]</td>
<td>0.091 0.091 [-0.002-0.194]</td>
<td>0.046 0.407 [-0.063-0.156]</td>
</tr>
<tr>
<td>Overall</td>
<td>0.014 &lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

R^2 adjusted 0.04
0.36
0.10
0.23

1: The control group is the reference group, ^: expressed as mmol/l.
CI: confidence interval, FM: fat mass, ApoE genotype tested as dummy’s.

Conclusion on ApoE Genotype

Table 2 and Figure 1 show the prevalence of ApoE genotype was higher and the favourable ε2/ε3 genotype was lower than in controls, while the SGA-CU subjects had a higher frequency of the unfavourable ε3/ε4 genotype than controls, but these differences were not significant.

We also analyzed if there were differences in LDLc and apoB levels between the subgroups, after correction for age and gender. These analyses showed that SGA-S subjects had significantly higher LDLc and apoB than controls (p= 0.014 and p= 0.006, respectively). There were no other significant differences in LDLc between the subgroups. However, the prevalence of the ε3/ε3 genotype was higher and the favourable ε2/ε3 genotype was lower than in controls, while the SGA-CU subjects had a higher prevalence of the unfavourable ε3/ε4 genotype than controls, but these differences were not significant.
Discussion

Our study in 297 young adults shows that birth length SDS and birth weight SDS were no significant determinants of serum lipid levels, whereas gender, adult height SDS, adult weight SDS, fat mass and ApoE genotype were. ApoE genotype was the most important determinant. There was no difference in ApoE genotype prevalence between the subgroups, although the SGA-S group had a lower prevalence of the favourable ε2 allele. After correction for age, gender, % body fat and ApoE genotype, comparison of the subgroups showed that only apoB was significantly higher in SGA-S subjects than in controls.

Birth weight SDS was not a significant determinant in any of the models. Some studies found a negative correlation between birth weight and cholesterol after correction for adult weight, but this might have occurred because they did not adjust birth weight for gestational age (25-27). Two reviews concluded that there was no relationship between birth weight and adult lipid profile (9,10). Birth length SDS was also not a significant determinant in any of the models. In other reports, the relationship between birth length and lipid levels remained unclear (28-30). Our study results showed no significant relation between birth weight as well as birth length and adult serum lipid levels, after correction for age, gender, ApoE genotype and adult size.

All serum lipid levels were higher in young women than in men after correction for birth size and adult size, as shown by model 1 and 2. However, after correction for fat mass this significant difference between women and men disappeared. This indicates that for serum lipid levels, gender related body composition is an important factor in young adulthood.

The first model showed that for all lipid variables, except HDLc and apoA-1, a shorter adult height and a higher adult weight resulted in significantly higher serum lipid levels. It is likely that this reflexes fat mass as subjects with a similar adult weight, but a shorter adult height have a higher fat mass. The same accounts for HDLc, but inversely.

Several reports have indicated that weight gain during life might result in an unfavourable lipid profile (31,32). While it has been suggested that this is due to an increase in fat mass, none of the studies could substantiate this theory because fat mass had not been measured. Our third model showed that an increase in fat mass resulted in higher serum levels of TC, TG, LDLc and apoB. Only HDLc and apoA-1 were not related to fat mass. The latter might indicate that HDLc and apoA-1 are not directly influenced by fat or fat accumulation, but more by other mechanisms like an impaired lipolysis of triglyceride-rich lipoproteins. This would lead to a decreased transfer of apolipoproteins and phospholipids from triglyceride-rich lipoproteins to HDLc (33).

Next to fat mass, other factors determine serum lipid levels, like genetic factors (34,35). In our total study population, the prevalence of ApoE genotypes was comparable with that of 86,000 disease-free adults (18). ApoE genotype is an important gene in the determination of especially TC, LDLc and apoB as shown by the last MR model. Not all ApoE genotypes were significant, but this might be due to the relatively small number of subjects in these subgroups. The explained variance rose from 6-16% to 14-21% when only ApoE genotype was added to the model. This may suggest that ApoE genotype is more important than body composition in determining lipid levels in young adulthood.

Comparison of the subgroups showed a significant difference in LDLc and apoB between SGA-S subjects and controls, after correction for age, gender and % body fat. After an additional correction for ApoE genotype the difference in LDLc disappeared. Various studies investigated lipid profiles in SGA subjects because it is thought that subjects born SGA have an increased risk for an unfavourable lipid profile, due to their low birth size. One study found a less favourable lipid profile in 3 days old SGA infants (36), but most studies showed no increased lipid levels in children born SGA, although they did not differentiate between SGA children with or without catch-up growth (11-15). Several studies Only Arends et al investigated various lipid variables in prepubertal SGA-S subjects but did not find any differences compared to age and height matched controls (11).

Only one study was performed in young adults. They found significantly lower levels of HDLc and higher levels of TG in a mixed group of SGA-S and SGA-CU subjects (16). Our present study specified the SGA population and in contrast to our expectations, not SGA-CU but SGA-S subjects had higher apoB in early adulthood, although all lipid levels were still in the normal range. The reason for these higher levels remains unknown, but a reduced amount of LDLc receptors might be an explanation, because this leads to increased levels of apoB. In SGA-CU subjects, apoB was borderline significantly higher than controls, but this difference disappeared after an additional correction for % body fat and ApoE genotype. The reason that SGA-CU subjects did not have a significant difference in lipid levels might be due to the fact that the difference in FM SDS between the SGA-CU and other subgroups is not large enough. Only one study reported lipid levels in ISS children, which were normal (37). Our results in the adult ISS group are in line with that study.

ApoE genotype did not differ between the subgroups, although SGA-S subjects had a lower prevalence for the advantageous ε2 allele (ε2/ε2 and ε2/ε3). Infante-Rivard et al concluded that the ε2 allele might protect from IUGR (38). Our results showed that SGA-CU subjects had the same prevalence of the ε2 allele, so we could not confirm their results.
Our study population consisted of a relative high percentage of subjects born small for gestational age and of short adults. This created greater contrast in the study population, which contributed to a better statistical model in which relationships between various factors could be detected with more statistical power. In addition, this study population allowed comparison between clinically relevant subgroups. Better insight could be obtained in the differences between the subgroups, with regard to serum lipid levels after correction for age, gender, ApoE genotype and adult size. Although a larger sample size in our subgroup analyses might reveal smaller significant differences in lipid levels, this sample size revealed significant differences in two lipid variables between the subgroups and controls. Therefore we state that our negative results in the subgroup analyses are justified.

In conclusion, our study demonstrates that prenatal growth has no influence on serum lipid levels in young adulthood, whereas postnatal growth, specified as fat accumulation during childhood, and genetic factors have. For public health practice this means, that parents of all children, regardless of size at birth, should be aware of the risks of fat accumulation in their children. Therefore, health care workers need to inform parents about the risks of fat accumulation during childhood.

Acknowledgements

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84

FM and ApoE Genotype


Chapter 5
Growth Patterns During Childhood and the Relationship with Acylation Stimulating Protein


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Submitted
Growth Patterns During Childhood and the Relationship with Acylation Stimulating Protein

Abstract

Background / Objectives: Acylation stimulating protein (ASP) is an adipose tissue-derived hormone, which stimulates glucose and free fatty acid (FFA) uptake into adipocytes. Changes in ASP metabolism are associated with alterations in lipid metabolism. As postnatal catch-up growth has been associated with dyslipidemia in later life, we investigated the association between ASP and birth size, adult size and different growth patterns during childhood.

Methods: The associations were investigated by multiple regression analyses in 285 young adults, aged 18-24 years. Subsequently, differences in ASP were analyzed in 4 clinically relevant subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) or with catch-up growth (SGA-CU), or born Appropriate for Gestational Age (AGA) with Idiopathic Short Stature (ISS) or with normal stature (controls).

Results: Weight gain during childhood, particularly fat accumulation, was positively related to ASP levels in early adulthood, independent of birth size, age and gender. Fetal growth, reflected by birth size, was not related to ASP levels. Between the subgroups, no differences in ASP were found, but SGA-CU and ISS subjects had significantly higher levels of FFA.

Conclusion: Not fetal growth, but exaggerated weight gain during childhood contribute to alteration in ASP metabolism and might therefore lead to impairment of FFA uptake, which results in delayed TG clearance. Therefore, exaggerated weight gain during childhood should be prevented.

Introduction

Acylation stimulating protein (ASP), also known as C3adesArg, is a relatively new hormone generated through the activation of the alternative complement pathway proteins C3, B and adipsin, and produced by adipocytes (1). ASP stimulates glucose and FFA uptake into adipocytes by increasing membrane glucose transport (2) and by increasing triacylglycerol synthesis in the adipocytes (3). By increasing incorporation of chylomicron-derived FFA into triglycerides (TG) in adipocytes, ASP prevents inhibition of lipoprotein lipase (LPL) by FFA and therefore helps to maintain normal serum FFA levels (4). In addition, ASP inhibits TG lipolysis in adipocytes by reducing the activity of hormone-sensitive lipase (HSL), resulting in lower serum FFA levels (5). By controlling the storage of TG, ASP helps to keep lipid levels in the normal range.

Normally, higher ASP levels should lead to lower TG levels and more accumulation of fat. However, in obesity, insulin resistance, cardiovascular disease and dyslipidemia, plasma ASP and TG levels are both increased, indicating reduced ASP activity, also called ASP resistance (6-10). In vitro studies have shown that cells from subjects with high ASP levels have reduced specific binding and response to ASP (11,12), which is partially induced by FFA (13). Higher ASP levels may be indicative for ASP resistance in the same way as higher insulin levels are indicative for insulin resistance, even with normal or near normal glucose (14). These findings resulted in the postulated hypothesis that higher ASP levels are related to ASP resistance, even when TG levels are normal (1,15).

Previous research showed that high fasting ASP predicts delayed clearance of TG in healthy men and women (15). Subsequently, postprandial hypertriglyceridemia has been associated with early dyslipidemia (16), which is a risk factor for cardiovascular disease. Therefore, fasting ASP levels might predict dyslipidemia. Alterations in ASP metabolism might already begin at a young age as seen in 2-6 year-old obese children who had higher ASP levels and a small increase in plasma TG in absence of other lipid changes than controls (17).

Postnatal catch-up growth in weight is related to determinants of cardiovascular disease (CVD) and type 2 diabetes in later life, such as reduced insulin sensitivity, adverse lipid profiles and higher fat mass in early adulthood, even when later BMI and percentage of body fat remain in normal range (18-21). Ninety percent of subjects born small for gestational age (SGA) show catch-up growth in the first two years of life (22,23). These subjects have a higher percentage body fat and reduced insulin sensitivity in early adulthood and may be at risk of development of CVD or type 2 diabetes (18,19). Higher ASP levels, indicating ASP resistance, have been found in subjects with increased body fat and reduced insulin resistance (10). We therefore
hypothesized that subjects with catch-up growth in weight would have higher levels of ASP, and thus might be at risk for early dyslipidemia.

Our aims were to investigate whether different growth patterns during childhood influence ASP levels in early adulthood. In addition, we evaluated if there were differences in serum ASP levels between 4 clinically recognizable subgroups of young adults, born SGA with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).

Subjects and Methods

Subjects
The PROgramming factors for GRowth And Metabolism (PROGRAM) study cohort consists of 323 healthy subjects with an age between 18 and 24 years. They were randomly selected from hospitals in the Netherlands, where they had been registered because of their being small at birth (SGA with a birth length < –2SD) (24) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height < –2SD) (25). In addition, healthy subjects of different schools were randomly asked to participate as controls. Only those born at 36 weeks or more of gestation, born singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive of a yet unknown syndrome. Birth data and childhood growth data were obtained from records of hospitals, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam the Netherlands, approved this study. Written informed consent was obtained from all participants.

Of the 323 participants who entered the study, 38 were excluded from analysis because of incomplete data, resulting in a total number of 285 subjects eligible for the multiple regression analyses in the total group. Based on SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. Normal birth length and adult height were set at an SDS > –1 (± 0.1 SDS), in order to increase the statistical power to identify differences between subgroups. Of the 285 subjects, 198 were included in one of the subgroups:

- Born small for gestational age (< –2 SDS) with a short adult height (< –2 SDS) (SGA-short, SGA-S),
- Born small for gestational age (< –2 SDS) with catch-up growth resulting in a normal adult height (≥ –1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length > –1 SDS) with growth retardation resulting in a short adult height (< –2 SDS) (Idiopathic short stature (ISS))
- Born appropriate for gestational age (birth length ≥ –1 SDS) and a normal adult height (≥ –1 SDS) (controls).

Measurements
All participants were invited to visit Erasmus Medical Centre in Rotterdam. They had been fasting for 12-hours and had abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer and weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice and the mean value was used for analysis.

In all participants, lean body mass (LBM) and fat mass (FM) were measured on the same DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was performed daily. The intra-assay coefficient of variation for lean tissue and fat tissue was 1.57-4.49% and 0.41-0.88%, respectively (26). Blood samples were drawn to determine serum ASP and lipid levels. In 105 of the 285 subjects insulin sensitivity index (SI) and acute insulin response to glucose (AIRg) were available (18). The variables were determined by the Bergman’s minimal model (MINMOD 6.01 copyright RN Bergman) calculating paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test (FSIGT) (27-29) with Tolbutamide (30).

Laboratory methods
Plasma ASP concentration was measured using a sandwich ELISA immunoassay method as previously described in detail (7). Intra-assay coefficient of variations was <4% and interassay coefficient of variations was <8%. Free fatty acids, total cholesterol (TC) and TG were measured using an enzymatic colometric method (WAKO Chemicals, Germany), an automated enzymatic
method with the CHOD-PAP reagent kit, and with the GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). HDLc was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = total cholesterol – HDLc – 0.45 x triglycerides. ApoA-1 and ApoB were determined by rate nephelometry on the Image Immunochemistry System, according to the manufacturers instructions (Beckman Coulter, Mijdrecht, the Netherlands). The intra-assay variations of measurements of TC, TG and HDLc were 2.9, 3.3 and 3.9%. Between-run coefficients of variation for apoA-1 and apoB were 4.2% and 2.8% at levels of 0.94 and 0.53 g/l, respectively. Plasma glucose levels were determined on a VITROS analyzer 750 (Orthoclinical Diagnostics, Johnson&Johnson Company, Beerse, Belgium) and plasma insulin levels were measured by IRMA (Medgenix, Biosource Europe).

Statistical analysis

SD-scores for birth length, birth weight, adult height and adult weight were calculated, in order to correct for gestational age, gender and age (24,25). Because of a skewed distribution, ASP, correcting for age and gender. The first model consisted of age, gender, adult height SDS, birth length SDS and birth weight SDS to investigate the influence of birth size on the outcome (model A). The interaction term birth length SDS * adult height SDS was added to all MR models because the study group had been selected on birth length and adult height in order to ensure that the effect of these variables was modeled correctly. Secondly, adult weight SDS was added to the model. When birth size and adult size are shown in one model, it is possible to investigate if the change in weight (centile crossing) between birth and adulthood is related to the outcomes (model B). Finally, adult weight SDS was replaced by fat mass (FM) and lean body mass (LBM) to specify weight (model C). The interaction term gender * LBM was added to the model to correct for the extra effect of gender on LBM. Partial correlations were used to determine the relationship between ASP, lipid variables and insulin sensitivity, after adjustment for age and gender. For the subgroup comparisons, ANOVA was used to determine if there were differences between the subgroups with regard to the group characteristics. Bonferroni correction was used for pair wise group comparisons. To determine differences between the groups corrected for age and gender, an ANCOVA model was used, with controls as reference group and SGA–S, SGA–CU and ISS as dummy variables (model A). Additional adjustment for % body fat was made (model B). Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was <0.05.

Results

Clinical characteristics

The clinical characteristics of the total study population are shown in Table 1. The mean (sd) age of the study population was 20.9 (1.7) years.

Table 1. Clinical characteristics of the total study population and the 4 subgroups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n= 285)</th>
<th>SGA–S (n= 31)</th>
<th>SGA–CU (n= 58)</th>
<th>ISS (n= 30)</th>
<th>Controls (n= 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (%)</td>
<td>38</td>
<td>36</td>
<td>38</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>Age</td>
<td>20.9 (1.7)</td>
<td>20.6 (1.8)</td>
<td>21.1 (1.5)</td>
<td>20.8 (1.7)</td>
<td>20.8 (1.7)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.7 (3.1)</td>
<td>45.0 (2.2)</td>
<td>44.5 (2.0)</td>
<td>49.7 (1.1)</td>
<td>50.7 (1.7)</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>-1.45 (1.4)</td>
<td>-2.96 (0.85)</td>
<td>-2.85 (0.83)</td>
<td>-0.51 (0.5)</td>
<td>0.07 (0.7)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.78 (0.7)</td>
<td>2.44 (0.50)</td>
<td>2.17 (0.35)</td>
<td>3.28 (0.6)</td>
<td>3.29 (0.6)</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>-1.19 (1.4)</td>
<td>-2.04 (1.0)</td>
<td>-2.36 (0.72)</td>
<td>-0.15 (1.2)</td>
<td>-0.11 (1.2)</td>
</tr>
<tr>
<td>Adult height (m)</td>
<td>1.69 (10.4)</td>
<td>1.58 (6.8)</td>
<td>1.74 (7.7)</td>
<td>1.59 (6.4)</td>
<td>1.77 (7.8)</td>
</tr>
<tr>
<td>Adult height SDS</td>
<td>-0.95 (1.3)</td>
<td>-2.60 (0.6)</td>
<td>-0.18 (0.7)</td>
<td>-2.44 (0.4)</td>
<td>0.23 (0.9)</td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>64.2 (12.3)</td>
<td>57.0 (12.6)</td>
<td>69.8 (13.2)</td>
<td>55.0 (11.3)</td>
<td>66.7 (11.0)</td>
</tr>
<tr>
<td>Adult weight SDS</td>
<td>-0.56 (1.4)</td>
<td>-1.52 (1.5)</td>
<td>0.09 (1.1)</td>
<td>-1.90 (1.7)</td>
<td>0.05 (1.0)</td>
</tr>
<tr>
<td>BMI</td>
<td>22.4 (3.5)</td>
<td>23.0 (4.9)</td>
<td>22.9 (3.6)</td>
<td>21.8 (4.9)</td>
<td>21.9 (2.9)</td>
</tr>
<tr>
<td>ASP (nM)*</td>
<td>15.7 (11–21)</td>
<td>12.9 (9–21)</td>
<td>15.6 (11–21)</td>
<td>13.8 (10–18)</td>
<td>15.8 (11–21)</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.52 (0.93)</td>
<td>4.67 (0.95)</td>
<td>4.67 (1.05)</td>
<td>4.44 (0.98)</td>
<td>4.41 (0.85)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.03 (0.52)</td>
<td>0.99 (0.56)</td>
<td>1.11 (0.53)</td>
<td>1.03 (0.53)</td>
<td>0.99 (0.48)</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.61 (0.25)</td>
<td>0.60 (0.25)</td>
<td>0.67 (0.22)</td>
<td>0.69 (0.28)</td>
<td>0.53 (0.24)</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.66 (0.81)</td>
<td>2.83 (0.83)</td>
<td>2.73 (0.95)</td>
<td>2.59 (0.83)</td>
<td>2.53 (0.72)</td>
</tr>
<tr>
<td>ApoA (g/l)</td>
<td>0.82 (0.23)</td>
<td>0.87 (0.25)</td>
<td>0.84 (0.25)</td>
<td>0.83 (0.26)</td>
<td>0.76 (0.22)</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.39 (0.37)</td>
<td>1.34 (0.30)</td>
<td>1.43 (0.41)</td>
<td>1.39 (0.38)</td>
<td>1.42 (0.36)</td>
</tr>
<tr>
<td>Insulin sensitivity**</td>
<td>6.95 (4.40)</td>
<td>6.19 (3.97)</td>
<td>4.88 (3.11)</td>
<td>7.10 (4.49)</td>
<td>7.98 (4.05)</td>
</tr>
<tr>
<td>Acute insulin response**</td>
<td>556 (356)</td>
<td>684 (790)</td>
<td>782 (777)</td>
<td>415 (279)</td>
<td>456 (283)</td>
</tr>
</tbody>
</table>

Data given in mean (sd), * median (quartiles), ** Data of 105 subjects in the total study population, 16 SGA–S, 23 SGA–CU, 16 ISS and 25 controls. 1 p< 0.05 compared with all other subgroups, 2 p< 0.05 compared with SGA–S and SGA–CU, 3 p< 0.05 compared with SGA–S and ISS, 4 p< 0.05 compared with SGA–CU and ISS, 5 p< 0.05 compared with SGA–CU.
Relationship between ASP and growth patterns during childhood

Birth length as well as birth weight were no significant determinants of serum fasting ASP levels in early adulthood, after adjustment of age, gender and adult height SDS (Table 2, Model A). Adding adult weight SDS to the model indirectly shows whether weight gain (centile crossing) from birth to early adulthood is related to later ASP levels (Model B). Adult weight SDS was a determinant of ASP (p< 0.0005), indicating that the attained weight in early adulthood influenced ASP levels, but Model B indirectly showed that in fact weight gain during childhood resulted in a higher ASP level in early adulthood. To specify adult weight SDS, we replaced adult weight SDS by fat mass (FM) and lean body mass (LBM) (Model C). FM tended to be a positive determinant for ASP levels in early adulthood (p= 0.06). The significance of gender in the first two models disappeared after adjustment for body composition, indicating that the higher level of ASP in women was explained by the difference in fat mass.

Table 2. Multiple regression of ASP in early adulthood

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
</tr>
</thead>
<tbody>
<tr>
<td>β p-value</td>
<td>β p-value</td>
<td>β p-value</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>-0.01</td>
<td>0.597</td>
<td>-0.01</td>
</tr>
<tr>
<td>Gender</td>
<td>0.26</td>
<td>&lt; 0.0005</td>
<td>0.26</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>-0.04</td>
<td>0.291</td>
<td>-0.04</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>0.02</td>
<td>0.591</td>
<td>0.03</td>
</tr>
<tr>
<td>Height SDS</td>
<td>0.02</td>
<td>0.668</td>
<td>-0.05</td>
</tr>
<tr>
<td>Weight SDS</td>
<td>0.11</td>
<td>&lt; 0.0005</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBM (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.012</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>R² adjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All models include the interaction term birth length x adult height. Model C includes the interaction term gender x LBM. *: log transformed

Relationship between ASP, lipid levels and insulin sensitivity

Correlations between ASP, serum lipid levels and insulin sensitivity are shown in Table 3. After adjustment for age and gender, positive but weak correlations were found between ASP and TG (p= 0.009), total cholesterol (p= 0.039), ApoB (p= 0.014), and acute insulin response (p= 0.048).

ASP was negatively related to insulin sensitivity (r= –0.28, p = 0.007). FFA levels were also related to insulin sensitivity (r= –0.31, p= 0.002).

Table 3. Partial correlations between ASP, lipid levels and insulin sensitivity.

<table>
<thead>
<tr>
<th>Variables</th>
<th>ASP*</th>
<th>FFA</th>
<th>TG*</th>
<th>TC</th>
<th>LDLc*</th>
<th>ApoB*</th>
<th>HDLc</th>
<th>ApoA1</th>
<th>Si*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG*</td>
<td>0.16</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>0.13</td>
<td>0.02</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDLc*</td>
<td>0.10</td>
<td>-0.01</td>
<td>0.18</td>
<td>0.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoB*</td>
<td>0.15</td>
<td>0.02</td>
<td>0.41</td>
<td>0.87</td>
<td>0.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDLc</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.13</td>
<td>-0.27</td>
<td>-0.11</td>
<td>-0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoA1</td>
<td>0.11</td>
<td>-0.13</td>
<td>0.10</td>
<td>0.27</td>
<td>-0.07</td>
<td>0.03</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Si*</td>
<td>-0.28</td>
<td>-0.31</td>
<td>-0.25</td>
<td>-0.25</td>
<td>-0.24</td>
<td>-0.26</td>
<td>0.14</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>AIRg*</td>
<td>0.19</td>
<td>-0.16</td>
<td>0.20</td>
<td>0.19</td>
<td>0.17</td>
<td>0.17</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.41</td>
</tr>
</tbody>
</table>

Values given are regression coefficients.
All correlations are corrected for age and gender. *: log transformed. 1: p <0.05, 2: p <0.005.

Comparison of subgroups

Of the total study population, 198 subjects were assigned to one of four subgroups and the clinical characteristics of the subgroups are shown in Table 1. ISS subjects had a significantly shorter birth length SDS than controls and SGA-CU subjects had significantly shorter adult height SDS than controls. SGA-CU and ISS subjects had a significantly lower FFA level than controls. However, these data were not corrected for confounders like age, gender and fat mass. Other differences between the subgroups were due to the selection criteria.

Comparison of the subgroups after adjustment for age and gender showed that fasting ASP levels did not significantly differ between the subgroups (Table 4). Even after additional adjustment for % body fat, SGA-CU and ISS subjects still had a significantly lower FFA level than controls (model B). SGA-S subjects had a significantly higher serum ApoB level, but this became non-significant after adjustment for % body fat. There were no other significant differences between the subgroups.
Our study shows that adult weight is associated with fasting ASP levels in early adulthood, independent of birth size, age, gender and adult height. Weight gain from birth to early adulthood was positively associated with ASP levels in early adulthood and this might be due to the amount of fat that was accumulated during childhood. Our subgroup analyses showed that there were no significant differences in ASP levels between the four subgroups, but FFA was significantly higher in SGA-CU and ISS subjects than in controls.

Obesity or high % body fat has been related to higher ASP levels in children, adolescents and adults (1,6,7). This might suggest a state of ASP resistance which is clinically characterized by increased levels of both ASP and TG (1,15). Our study shows that ASP levels were higher in subjects who attained a higher adult weight in early adulthood. Specification of adult weight childhood was related to higher TG levels in early adulthood (21). ASP was positively related to the amount of fat that was accumulated during childhood. Our subgroup analyses showed that the association was mainly based on % body fat, indicating that subjects with more fat mass, had higher ASP levels.

An explanation for the positive relationships between ASP and plasma lipids (total cholesterol, TG and ApoB) is twofold. Firstly, chylomicrons (dietary lipoproteins) increase adipocytes C3 and ASP production (8) and secondly, reduced ASP activity will lead to higher FFA levels. This results in increased FFA flux to the liver, leading to increased lipoprotein production by the liver.

To investigate whether different growth patterns during childhood have an influence on fasting ASP levels in early adulthood, we analyzed four different clinically relevant subgroups, based on size at birth and adult height. After adjustment for age and gender, fasting ASP levels were similar between the four subgroups, even after an additional adjustment for % body fat. We had expected that SGA-CU subjects would have higher ASP levels because they have reduced insulin sensitivity and relatively more body fat than controls (18,19). Nevertheless, their ASP levels were comparable with the other subgroups. However, SGA-CU subjects had a significantly higher FFA levels than controls, which might be explained by a reduced activity of HSL as SGA-CU are less insulin sensitive (18). Higher levels of FFA may result in an increased flux to the liver, causing increased production of ApoB by the liver. In early adulthood, this was not yet observed in SGA-CU subjects, but might develop later in adulthood. Serum TG levels were also similar between the subgroups and as serum TG levels influence ASP levels (15), this might explain why ASP levels were comparable between the subgroups. Also, ISS subjects had significantly higher FFA levels while all other serum lipid levels were similar to controls. Insulin sensitivity was also similar to controls, so the reason for these higher FFA levels cannot be explained by the activity of HSL (18). ASP levels in ISS subjects were non-significantly lower, so FFA trapping might be reduced in these subjects, what might have led to the higher serum FFA levels. SGA-S subjects had a significantly higher ApoB level, which disappeared after adjustment for % body fat, while all other serum lipid levels were similar to controls. Therefore, % body fat explained the difference in ApoB level between SGA-S subjects and controls.

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Although weight gain during childhood was a significant determinant of ASP in the MR analyses, subjects with weight gain during childhood did not have higher ASP levels. This difference might be explained by two reasons; the MR analyses had a higher number of subjects leading to a higher statistical power than the subgroup analyses, and the explained variance.

Table 4. Differences in ASP and lipid levels between the 4 subgroups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SGA-S</th>
<th>SGA-CU</th>
<th>ISS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASP</td>
<td>Model A</td>
<td>Model B</td>
<td>Model A</td>
</tr>
<tr>
<td>FFA</td>
<td>0.07 (0.16)</td>
<td>0.07 (0.19)</td>
<td>0.15 (&lt; 0.001)</td>
</tr>
<tr>
<td>TG*</td>
<td>-0.03 (0.73)</td>
<td>-0.07 (0.50)</td>
<td>0.12 (0.15)</td>
</tr>
<tr>
<td>TC</td>
<td>0.26 (0.18)</td>
<td>0.23 (0.24)</td>
<td>0.28 (0.09)</td>
</tr>
<tr>
<td>LDLc*</td>
<td>0.11 (0.09)</td>
<td>0.10 (0.13)</td>
<td>0.07 (0.22)</td>
</tr>
<tr>
<td>ApoB*</td>
<td>0.13 (0.03)</td>
<td>0.11 (0.06)</td>
<td>0.09 (0.07)</td>
</tr>
</tbody>
</table>

Data are given as mean difference (p-value) with controls. *: log transformed.

Model A: After adjustment for age and gender.
Model B: After adjustment for age and gender and % body fat.
of ASP by weight gain was relatively small (4%). Studies showing differences in ASP had subgroups with large differences in BMI, while our subgroups had a relatively small variance in BMI (6.17).

In conclusion, this study showed that ASP levels in early adulthood were positively associated with weight gain during childhood, specified as fat accumulation. Fetal growth, reflected by birth size, was not related to ASP levels. For clinical practice, weight gain during childhood should be in proportion to height gain, otherwise ASP levels might rise and FFA trapping will be impaired, which might result in dyslipidemia in later life.

Acknowledgements

We greatly thank Mrs. J. Dunk and Mrs. J. van Slobbe, research nurses, for their technical assistance and support with data collection. We acknowledge Mrs. J. Sluimer for checking the DXA results. We greatly thank Dr. Y.B. de Rijke and Dr. A.W. van Toorenbergen for analyzing the lipid values.

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Potential Financial Conflicts of Interest: none

References

Chapter 6
Blood Pressure and Carotid Intima Media Thickness in Early Adulthood are Related with Catch-up in Weight and not with Birth Size

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² Department of Epidemiology and Biostatistics, Leiden University Medical Centre, Leiden, the Netherlands

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31. Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K 2008 Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertility and Sterility...
Abstract

Background / Objectives: It remains unclear whether prenatal or postnatal growth influences determinants of cardiovascular disease. We investigated whether birth size or weight gain during childhood influenced blood pressure and carotid intima-media thickness (cIMT) in early adulthood.

Methods: The relationships between birth size and systolic blood pressure (SBP), diastolic blood pressure (DBP) and cIMT were investigated in 243 young adults, aged 18-24 years. Additionally, differences in SBP, DBP and cIMT were analyzed in 4 subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) or with catch-up growth, or born Appropriate for Gestational Age with Idiopathic Short Stature or with normal stature (controls).

Results: Birth size had no significant association with blood pressure and cIMT in early adulthood. Fat mass was positively related to both SBP and DBP, next to male gender, alcohol use and heart rate, while social economic status was negatively related. Carotid IMT was positively related to SBP, artery diameter and female gender, after correction for age, birth size, adult size and lifestyle factors. Subgroup analyses showed no difference in blood pressure between the subgroups, but cIMT was significantly greater in SGA-CU subjects than in controls, even after correction for age, gender, artery diameter, smoking and SBP.

Conclusion: Not birth size, but fat accumulation during childhood determines blood pressure in early adulthood. SGA-CU subjects had a greater cIMT than controls, while SGA-S subjects had similar cIMT as controls. Therefore, postnatal catch-up growth is more important than prenatal growth in the development of atherosclerosis.

Introduction

Vascular health deteriorates during life as cardiovascular risk factors induce changes in arteries leading to atherosclerosis, and this process starts already in childhood (1-3). Atherosclerosis can lead to cardiovascular events like myocardial infarction and stroke. Some studies reported that low birth weight is associated with an increased risk for cardiovascular diseases (CVD) (4). Recently, the role of postnatal catch-up in weight as a determinant of cardiovascular risk has been acknowledged (5-9). As 90% of subjects born small for gestational (SGA) show catch-up in weight and length during childhood (10-12), these subjects might be at risk for CVD later in life.

The relationship between birth weight and adult blood pressure is probably the most investigated association based on the ‘fetal origin’ hypothesis, but clarification is still needed as several studies and reviews showed conflicting results (13-19). The most recent review concluded that the association might reflect the impact of random error, selective emphasis of particular results and inappropriate adjustment for current weight and confounding factors (20). On the other hand, this has been opposed as well (21,22).

We hypothesized that prenatal growth is not related with adult blood pressure and carotid intima media thickness (cIMT), while postnatal growth is an important determinant for these factors, especially weight gain during childhood. Both blood pressure and cIMT are accurate predictors of cardiovascular events later in life (23-26), and cIMT may be regarded as a valid marker for generalized atherosclerosis (27). We, therefore, investigated the influence of birth length, birth weight, adult size and body composition on systolic (SBP), diastolic blood pressure (DBP) and cIMT in 243 young adults. Additionally, we investigated if there were differences in SBP, DBP and cIMT between four clinically recognizable subgroups of young adults, born SGA with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).

Subjects and Methods

Subjects

The PRogramming factors for GRowth And Metabolism (PROGRAM) study investigates a cohort of 323 healthy subjects with an age between 18 and 24 years. They were randomly selected from hospitals in the Netherlands, where they had been registered because of their
being small at birth (SGA with a birth length <-2SD) (28) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height <-2SD) (29). In addition, a group of healthy controls was invited to participate. Only those born at 36 weeks or more of gestation, born singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroids, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Birth data were taken from records of hospitals, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam the Netherlands, approved this study. Written informed consent was obtained from all participants.

Of the 323 participants who entered the study, 80 had incomplete blood pressure and cIMT data, resulting in a total number of 243 eligible subjects for analyses. There were no differences in anthropometric and DXA measurements between the included and excluded participants. Based on SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. In order to increase the statistical power for subgroup comparison, the cut-off values were set at –2 SDS and –1 SDS (± 0.1 SDS). Of the 243 subjects, 170 fulfilled the inclusion criteria for the subgroup analyses:

- Born small for gestational age (<–2 SDS) with a short adult height (<–2 SDS) (SGA-S),
- Born small for gestational age (<–2 SDS) with catch-up growth resulting in a normal adult height (>–1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length >–1 SDS) with growth retardation resulting in a short adult height (<–2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length >–1 SDS) and a normal adult height (>–1 SDS) (Controls).

Measurements

All participants were invited to visit Erasmus Medical Centre in Rotterdam. They had been fasting for 12-hours and had abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer and weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S, Servo Berkel Prior, Katwijk, Netherlands). All anthropometric measurements were performed twice and the mean value was used for analysis. All subjects filled out a questionnaire about their alcohol use, smoking habits and their social economic status (SES).

In all participants, lean body mass (LBM) and fat mass (FM) were measured by one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was daily performed. The intra-essay coefficient of variation for lean tissue and fat tissue has been reported to be 1.57-4.49% and 0.41-0.88%, respectively (30).

Blood pressure was measured in sitting position after 5 minutes rest with an automatic device (Accutorr Plus, Datascpe Corp., Montvale NJ, USA). Blood pressure was measured every 5 minutes for an hour and the average value of 13 measurements was taken to reflect the 24-hours blood pressure.

IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using the same 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Tech. Laboratories, Bethel Washington, USA). On the R wave of the electrocardiogram, three longitudinal images of the near and far wall of the common carotid artery were frozen and stored on videotape. These frozen images were digitalized and displayed on the screen of a computer using a frame grabber (VP 1400-KIT-512-E-AT, Imaging Technology). The common carotid IMT was determined as the mean of the mean near and far wall measurements of both the left and right side common carotid artery.

Statistical analysis

In order to correct for gestational age, gender and age, SD-scores for birth length, birth weight, adult height and adult weight were calculated (28,29). Multiple linear regression (MR) analysis was performed to determine the association between birth size and SBP, DBP and cIMT. Firstly, we entered age, gender, adult height SDS, birth length SDS and birth weight SDS to the model, to investigate the influence of birth size on the outcomes (model A). The interaction term birth length SDS * adult height SDS was added to all MR models because the study group had been selected on birth length and adult height, in order to ensure that the effect of these variables was modeled correctly. Possible confounders like SES, smoking and alcohol use were also added to the models. Secondly, adult weight SDS was added for birth weight SDS, to relate adult size to the outcomes (model B). Thirdly, birth weight SDS was added again to the model. When birth size and adult size are shown in one model, it is possible to investigate if the change in weight...
Early Growth Related to Blood Pressure and cIMT

(c)entile crossing) between birth and adulthood is related to the outcomes (model C). For SBP and DBP, an additional model was made with fat mass (FM) and lean body mass (LBM) to specify adult weight SDS. For cIMT, corrections were made for SES, smoking, alcohol, SBP and artery diameter. Using a sample size of 243, we calculated that we could detect at least 2% extra variance in SBP, DBP and cIMT attributable to birth weight (with 80% power and a significance level of 0.05).

ANOVA was used to determine if there were differences between the subgroups with regard to the group characteristics. Bonferroni correction was used for pair wise group comparisons. An ANCOVA model was used to determine differences between the groups corrected for age, gender and for variables with a p-value < 0.1 in the MR analyses because they might act as confounders. Controls served as the reference group and SGA-S, SGA-CU and ISS were added as dummy variables. Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.

Results

The clinical characteristics of the total study population are shown in Table 1. The study population consisted of 243 subjects with a mean age (SD) of 20.9 (1.7) years.

Relationship between birth size and blood pressure in early adulthood

To determine the influence of birth size on blood pressure, we used multiple regression (MR) models (Table 2). The first model showed that male gender, alcohol use and heart rate were positively associated with SBP in early adulthood, while SES was negatively related. Birth length SDS and birth weight SDS were not related with SBP (Model A). In the second model, birth weight SDS was replaced by adult weight SDS to investigate the relationship between adult size and SBP (Model B). Adult weight SDS was a strong positive determinant of SBP in early adulthood. Birth weight SDS was added to Model B to determine whether weight gain from birth to early adulthood was related to SBP (Model C). Adult weight SDS remained significant and birth weight SDS was not, indicating that more weight gain during childhood is related with a higher SBP in early adulthood, independently of birth weight. Finally, adult weight was specified by fat mass and lean body mass (Model D). Fat mass was positively related to SBP and therefore indirectly showed that fat accumulation from birth to early adulthood was positively related to SBP. Furthermore, fat mass explained the gender difference in SBP. Alcohol use and heart rate remained other significant positive determinants of SBP, while SES was negatively related.

Table 1. Clinical characteristics of the total study group and the 4 subgroups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total group (n= 243)</th>
<th>SGA–S (n=35)</th>
<th>SGA–CU (n= 44)</th>
<th>ISS (n= 27)</th>
<th>controls (n= 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>37.9</td>
<td>31.4</td>
<td>38.6</td>
<td>37.0</td>
<td>40.6</td>
</tr>
<tr>
<td>Age</td>
<td>20.9 (1.7)</td>
<td>20.6 (1.7)</td>
<td>21.4 (1.4)</td>
<td>20.8 (1.8)</td>
<td>20.7 (1.8)</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.7 (3.1)</td>
<td>45.0 (2.2)</td>
<td>44.5 (2.0)</td>
<td>49.4 (1.2)</td>
<td>50.9 (1.8)</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>−1.47 (1.48)</td>
<td>−2.98 (0.87)</td>
<td>−2.88 (0.85)</td>
<td>−0.54 (0.48)</td>
<td>0.14 (0.73)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.82 (0.69)</td>
<td>2.48 (0.51)</td>
<td>2.17 (0.38)</td>
<td>2.33 (0.61)</td>
<td>3.36 (0.58)</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>−1.12 (1.43)</td>
<td>−1.96 (0.99)</td>
<td>−2.38 (0.75)</td>
<td>−0.16 (1.24)</td>
<td>0.08 (1.17)</td>
</tr>
<tr>
<td>Adult height (m)</td>
<td>1.69 (0.11)</td>
<td>1.57 (0.07)</td>
<td>1.75 (0.08)</td>
<td>1.59 (0.06)</td>
<td>1.78 (0.08)</td>
</tr>
<tr>
<td>Adult height SDS</td>
<td>−1.03 (1.41)</td>
<td>−2.62 (0.58)</td>
<td>−0.09 (0.75)</td>
<td>−2.39 (0.37)</td>
<td>0.38 (0.91)</td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>63.7 (12.4)</td>
<td>56.8 (12.1)</td>
<td>71.2 (13.2)</td>
<td>54.5 (10.5)</td>
<td>69.4 (11.1)</td>
</tr>
<tr>
<td>Adult weight SDS</td>
<td>−0.63 (1.43)</td>
<td>−1.52 (1.62)</td>
<td>0.21 (1.11)</td>
<td>−1.96 (1.69)</td>
<td>0.10 (0.93)</td>
</tr>
<tr>
<td>BMI</td>
<td>22.4 (3.5)</td>
<td>23.0 (4.9)</td>
<td>23.2 (3.6)</td>
<td>21.5 (4.1)</td>
<td>21.8 (2.8)</td>
</tr>
<tr>
<td>Fat mass (kg) *</td>
<td>15.2 (8.0)</td>
<td>15.9 (9.4)</td>
<td>18.3 (8.2)</td>
<td>14.0 (8.4)</td>
<td>15.0 (8.0)</td>
</tr>
<tr>
<td>Fat mass (SDS) *</td>
<td>0.12 (1.18)</td>
<td>0.40 (1.02)</td>
<td>−0.13 (1.05)</td>
<td>0.00 (1.00)</td>
<td>0.00 (1.00)</td>
</tr>
<tr>
<td>Trunk fat/total fat ratio *</td>
<td>0.48 (0.06)</td>
<td>0.47 (0.06)</td>
<td>0.49 (0.07)</td>
<td>0.46 (0.06)</td>
<td>0.47 (0.05)</td>
</tr>
<tr>
<td>Lean body mass (kg) *</td>
<td>45.4 (10.1)</td>
<td>38.1 (7.8)</td>
<td>49.3 (9.9)</td>
<td>37.6 (6.3)</td>
<td>51.2 (9.5)</td>
</tr>
<tr>
<td>Lean body mass (SDS) *</td>
<td>−1.38 (0.82)</td>
<td>−0.21 (1.04)</td>
<td>−1.43 (0.66)</td>
<td>−0.00 (1.00)</td>
<td>−0.00 (1.00)</td>
</tr>
<tr>
<td>Alcohol users (%)</td>
<td>75.7</td>
<td>77.1</td>
<td>80.0</td>
<td>81.5</td>
<td>78.1</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>25.5</td>
<td>22.9</td>
<td>35.0</td>
<td>37.0</td>
<td>20.3</td>
</tr>
<tr>
<td>cIMT (mm)*</td>
<td>0.52 (0.05)</td>
<td>0.50 (0.04)</td>
<td>0.53 (0.06)</td>
<td>0.50 (0.05)</td>
<td>0.52 (0.05)</td>
</tr>
<tr>
<td>Artery diameter (mm) *</td>
<td>6.66 (0.48)</td>
<td>6.37 (0.41)</td>
<td>6.76 (0.42)</td>
<td>6.44 (0.46)</td>
<td>6.79 (0.48)</td>
</tr>
<tr>
<td>cIMT/artery diameter ratio</td>
<td>0.078 (0.007)</td>
<td>0.078 (0.006)</td>
<td>0.079 (0.008)</td>
<td>0.077 (0.007)</td>
<td>0.076 (0.007)</td>
</tr>
<tr>
<td>Systolic BP (mmHg) *</td>
<td>110.0 (9.0)</td>
<td>108.0 (10.2)</td>
<td>116.2 (10.3)</td>
<td>107.3 (10.4)</td>
<td>110.0 (7.3)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg) *</td>
<td>66.1 (5.9)</td>
<td>65.9 (8.2)</td>
<td>66.4 (6.0)</td>
<td>65.4 (6.8)</td>
<td>66.1 (5.0)</td>
</tr>
<tr>
<td>Heart rate (beats/min) *</td>
<td>65.9 (0.9)</td>
<td>71.9 (7.2)</td>
<td>65.9 (1.1)</td>
<td>67.7 (6.6)</td>
<td>64.8 (4.4)</td>
</tr>
</tbody>
</table>

Values are given as means (sd). *Not corrected for age, gender and adult size.

1: p < 0.01 compared with the other subgroups, 2: p < 0.001 compared with SGA-S and SGA-CU, 3: p < 0.05 compared with SGA-S and ISS, 4: p < 0.05 compared with SGA-CU, 5: p < 0.05 compared with SGA-CU and controls.
### Table 2. Multiple regression of systolic and diastolic blood pressure in early adulthood.

<table>
<thead>
<tr>
<th>Variables</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model A</td>
<td>Model B</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>β</td>
<td>p-value</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.02</td>
<td>0.947</td>
</tr>
<tr>
<td>SES</td>
<td>-2.73</td>
<td>0.008</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.80</td>
<td>0.563</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>3.68</td>
<td>0.009</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adult height SDS</td>
<td>0.63</td>
<td>0.261</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>0.56</td>
<td>0.452</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>-0.73</td>
<td>0.273</td>
</tr>
<tr>
<td>Adult weight SDS</td>
<td>2.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.22</td>
<td>0.004</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>0.39</td>
<td>0.131</td>
</tr>
<tr>
<td>Overall</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.23</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Gender: 1 = men, 2 = women; SES: social economic score.

All models include the interaction term birth length SDS × adult height SDS and gender × lean body mass is included in model D.

No relationships were found between DBP in early adulthood and birth length SDS as well as birth weight SDS, while alcohol use and heart rate were significant positive determinants.

Comparison of subgroups

The clinical characteristics of the four subgroups are shown in Table 1. Differences between the subgroups with regard to anthropometric measurements and lean body mass were due to the selection criteria. There were no differences in SBP and DBP between the subgroups. Gestational length was significantly higher in SGA-CU subgroups compared to SGA-S subgroups. Gestational length was significantly smaller in the subgroups with short adult height, indicating that adult stature influences carotid artery diameters. This was confirmed by the fact that there was no difference in CM1/arterial wall thickness between the subgroups. Heart rate was significantly higher in SGA-CU and controls.

Relationship between birth size and CIMT in early adulthood

Table 3 shows the relationship between CIMT and birth size. Birth length SDS and birth weight SDS were no significant determinants of CIMT in early adulthood. Female gender and adult height SDS were significant positive determinants, whereas adult height SDS was negatively related with CIMT (Model A). Adult height SDS was replaced by adult weight SDS to investigate whether adult weight in early adulthood influenced CIMT, but this was not significantly related (Model B). Specifying adult weight in fat mass and lean body mass did not change the results (data not shown).

Notably, women had a greater CIMT than men in the final model, while it is generally known that men have greater CIMT and larger artery diameter than women. When we analyzed this gender difference without corrections, men had greater CIMT and larger artery diameter than women (0.53 mm vs. 0.51 mm, p = 0.006). However, the CIMT/artery diameter ratio was higher in women than in men (0.28 vs. 0.32, p = 0.006). This indirectly indicates that fat accumulation from birth to early adulthood is positively related to CIMT.
Table 3. Multiple regression for cIMT in early adulthood.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A</th>
<th></th>
<th>Model B</th>
<th></th>
<th>Model C</th>
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<tr>
<td></td>
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<td>p-value</td>
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<tr>
<td>SES</td>
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<td>0.523</td>
<td>-0.004</td>
<td>0.491</td>
<td>-0.005</td>
<td>0.533</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.013</td>
<td>0.075</td>
<td>0.013</td>
<td>0.087</td>
<td>0.013</td>
<td>0.094</td>
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<td>Alcohol use</td>
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<td>0.839</td>
<td>-0.002</td>
<td>0.803</td>
<td>-0.001</td>
<td>0.855</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
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<td>0.057</td>
<td>0.001</td>
<td>0.033</td>
<td>0.001</td>
<td>0.036</td>
</tr>
<tr>
<td>Artery diameter (mm)</td>
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<td>&lt;0.001</td>
<td>0.061</td>
<td>&lt;0.001</td>
<td>0.062</td>
<td>&lt;0.001</td>
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<tr>
<td>Adult height SDS</td>
<td>-0.006</td>
<td>0.044</td>
<td>-0.005</td>
<td>0.152</td>
<td>-0.005</td>
<td>0.143</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>0.000</td>
<td>0.969</td>
<td>-0.002</td>
<td>0.453</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>Birth weight SDS</td>
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<td>0.506</td>
<td>-0.003</td>
<td>0.500</td>
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<tr>
<td>Adult weight SDS</td>
<td>-0.003</td>
<td>0.363</td>
<td>-0.003</td>
<td>0.360</td>
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<tr>
<td>Overall</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R² adjusted</td>
<td>0.29</td>
<td></td>
<td>0.29</td>
<td></td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

Gender: 1 = men, 2 = women; SES: social economic score.
All models include the interaction term birth length SDS x adult height SDS.

After correction for age and gender, there were no significant differences between the subgroups with regard to SBP and DBP (Table 4). Additional corrections were made for variables with a p-value < 0.1 in the MR analyses because they might act as confounders. However, these corrections did not result in significant differences in SBP or DBP between the subgroups.

SGA-CU subjects had a higher cIMT than SGA-S and ISS subjects after correction for age and gender (Table 5). Additional correction for artery diameter resulted in a similar cIMT between subjects with short adult stature and subjects with normal adult stature, indicating that subjects with short stature have smaller carotid arteries and therefore a lower cIMT. SGA-CU subjects had a significantly higher cIMT than controls after correction for artery diameter (p= 0.017), which remained significant after corrections for variables with a p-value < 0.1 in the MR analyses (smoking and SBP, p= 0.047).

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Discussion

This study shows that birth weight and birth length SDS had no significant influence on blood pressure and cIMT in early adulthood. Fat mass was a positive determinant for both SBP and DBP in early adulthood, next to male gender, alcohol use and heart rate, while SES was negatively related. For cIMT, female gender, SBP and artery diameter were positive determinants. Subgroup
analyses showed that there was no difference in blood pressure between the subgroups, but cIMT was significantly higher in SGA-CU subjects than in controls.

The relationship between birth size and subsequent blood pressure has been previously investigated, with controversial results. According to the most recent review, this was due to different study designs, publication bias and different statistical approaches. In order to investigate the relationship between birth size and blood pressure, we did not only use the statistical model of Lucas et al., but also adjusted for several important confounding factors. Our results indicate that increased fat accumulation from birth to early adulthood is a significant risk factor for a higher blood pressure, independent of birth size. Although previous studies reported a positive association between weight gain in early life or childhood and later blood pressure, no other study was able to specify weight gain as accumulation of fat.

Infant nutrition is an important determinant of weight gain in early life. SGA infants who received nutrient-enriched formulas during early life showed more catch-up in weight, which was associated with increased blood pressure in childhood. Particularly the first five months of life might be a critical period for the effect of weight gain on blood pressure in later life, as weight gain during that period was associated with blood pressure at the age of 25 years (8). In view of these and our results, nutritional intervention studies should be undertaken to investigate which feeding regimen provide the optimal growth in early life with regard to blood pressure in later life. Awaiting these results, it seems best to avoid an exaggerated catch-up in weight for height during early life.

Birth size had no influence on cIMT in early adulthood. Adult weight or fat mass were also no determinants of cIMT, which was in contrast to our expectations. Previous reports showed that an increase in BMI during childhood associated with cIMT in adulthood. However, we could not confirm such a relation with either BMI or fat mass, and the small range of BMI in our study or the relatively young age might explain this difference. To our knowledge, no study has shown a direct relationship between cIMT and fat mass.

Men had greater cIMT than women when no corrections were made, however women had a higher cIMT/artery diameter ratio and female gender was positively related to cIMT in the MR analyses. This indicates that women have a relatively thicker carotid arterial wall. One explanation might be a different composition of the arterial wall in women which might preclude a different plaque phenotype in later life than men. Our data warrant further investigation.

The MR analyses and the subgroup analyses showed that artery diameter is a significant factor for cIMT. Subjects with short adult height had smaller cIMT and smaller artery diameter. This suggests that they have smaller carotid arteries, which was confirmed by a similar cIMT/artery diameter ratio as controls. These results might suggest that future studies investigating cIMT need to adjust for artery diameter, otherwise cIMT will be underestimated in short subjects.

Subgroup comparison showed no differences in blood pressure in early adulthood between the four clinically relevant subgroups. We expected that SGA-CU subjects would have a higher blood pressure than controls, because they had more fat mass than controls. Although the MR analyses showed a positive relationship between fat mass and blood pressure, blood pressure was not significantly different between the subgroups. This is probably due to the relatively small differences in fat mass between the subgroups. Both subgroups born SGA did not have a higher blood pressure, which is in line with the MR analyses showing that a lower birth weight does not increase the risk for higher blood pressure.

SGA-CU subjects had a greater cIMT than controls, even after correction for several confounders. In contrast, SGA subjects without catch-up growth had a similar cIMT as controls. Our results are in line with Oren et al., who previously reported that young adults with the lowest birth weight and the highest postnatal growth had a greater cIMT than young adults with normal birth size and postnatal growth. However, they were unable to make a distinction whether prenatal or postnatal growth influenced cIMT in later life. We show that not prenatal growth but postnatal growth influence cIMT in adulthood, as SGA-CU subjects and not SGA-S subjects have a greater cIMT in early adulthood.

Our study population consisted of a relatively high percentage of subjects born small for gestational age and of short adults. This created greater contrast in the study population, which contributed to a better statistical model in which relationships between various factors could be detected with more statistical power. In addition, this study population allowed comparison between clinically relevant subgroups. Thereby, no other study reflected the 24-hours blood pressure by 13 measurements, as most studies used two measurements.

In conclusion, our study shows that catch-up growth in weight during childhood, specified as fat accumulation, influences blood pressure in later life. Birth size did not influence cIMT and blood pressure in early adulthood. Subgroup comparison showed that subjects born SGA with catch-up growth had a significantly greater cIMT in early adulthood than controls. Therefore, fat accumulation during childhood should be prevented to attain normal blood pressure in early adulthood and more research is warranted to investigate whether subjects born small for gestational age with catch-up growth have a higher risk for cardiovascular events in later life.
Acknowledgements

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Potential Financial Conflicts of Interest: none

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33. Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML 2003 Change in body mass index from adolescence to early adulthood and increased carotid intima-media thickness at 28 years of age: the Atherosclerosis Risk in Young Adults study. Int J Obes Relat Metab Disord 27:1383-1390
Abstract

Background/Objectives: Low bone mineral density (BMD) may lead to osteoporosis and is associated with increased fracture risk. Associations between BMD and various factors have been reported. Our objective was to investigate whether birth size, lean body mass (LBM) and fat mass (FM) are determinants of BMD_TB (total body) and BMD_LS (lumbar spine).

Methods: In the PROGRAM-study, a cohort of 312 young adults aged 18-24, BMD_TB and BMD_LS were determined by Dual Energy X-ray Absorptiometry (DXA). Subsequently, differences in BMD_TB and BMD_LS were analyzed in 4 subgroups, young adults either born Small for Gestational Age with short stature (SGA-S) or with catch-up growth (SGA-CU), or born Appropriate for Gestational Age (AGA) with Idiopathic Short Stature (ISS) or with normal stature (controls).

Results: Adult weight, LBM, FM as well as weight gain during childhood were the main positive determinants for BMD_TB in early adulthood, whereas birth size had no influence (adjusted R^2 = 0.50). Gender, adult weight, LBM, FM and weight gain were the significant determinants of BMD_LS. In the subgroups, after correction for age, gender and adult body size, the ISS group had a significantly lower BMD_TB than controls but there was no difference in BMD_LS between the subgroups.

Conclusions: Prenatal growth has no significant influence on BMD_TB and BMD_LS in early adulthood. Gender and postnatal growth, particularly weight gain, are the main positive determinants. To achieve a normal BMD in adulthood, health-care workers should aim for a normal weight gain in children.

Introduction

Low bone mineral density (BMD) may lead to osteoporosis and is associated with an increased fractures risk (1-5). The etiology of a low BMD is thought to be genetically and environmentally (6-12). Several studies evaluated associations between various factors and BMD (11-23). Most studies were, however, performed in elderly and did not correct for adult body size. Correction for adult weight and height is necessary, because Dual Energy X-ray Absorptiometry (DXA) will underestimate BMD in short subjects because DXA measures areal rather than volumetric bone density (24,25).

Only one study evaluated the association between birth length and BMD of the lumbar spine (BMD_LS) but not between birth length and BMD of the total body (BMD_TB) (17). No corrections for gestational age, LBM, FM or weight were made. Several studies evaluated the association between birth weight and BMD, but did not correct for adult weight (11,13-16). Other studies evaluated whether lean body mass (LBM) and fat mass (FM) were associated with BMD (18-23). Most studies corrected only for weight, not for height, although LBM and height are highly associated.

Growth might also be a significant factor in determining BMD (26,27). A lower growth velocity during childhood leads to an increased fracture risk in later life (28), whereas weight gain during childhood results in a higher BMD (29).

We therefore evaluated in a cohort of 312 young adults (PROGRAM-study) aged 18-24, the association between birth size and BMD_TB and BMD_LS measured by DXA, after correction for age, gender, adult weight, adult height and body composition. Subsequently, we evaluated the influence of LBM and FM on BMD_TB and BMD_LS after correction for adult size. In addition, we evaluated if there were differences with regard to BMD_TB and BMD_LS between four clinically recognizable subgroups: young adults, born short with either short adult height (SGA-S) or normal adult height (SGA-CU), or born AGA with either idiopathic short stature (ISS) or normal adult height (controls).
**Subjects and Methods**

**Subjects**
The PROgramming factors for GRowth And Metabolism (PROGRAM) study investigated a cohort of 323 healthy subjects with an age between 18 and 24 years. They were randomly selected from hospitals in the Netherlands, were they had been registered because of their being small at birth (SGA with a birth length <–2SD) (30) or showing short stature (after being born SGA or appropriate for gestational age (AGA) with an adult height <–2SD) (31). In addition, a group of healthy controls was invited to participate. Only those born at 36 weeks or more of gestation, being singleton and Caucasian, were invited to participate in order to exclude a potential influence of prematurity, parity and ethnicity, respectively. All subjects fulfilled the same inclusion criteria: an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia. Subjects were excluded if they had been suffering from any serious condition or had been receiving any treatment known to interfere with growth (e.g. growth hormone deficiency, severe chronic illness, emotional deprivation, growth hormone treatment, treatment with glucocorticosteroid, radiotherapy) or if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome. Birth data were taken from hospital records, community health services and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, The Netherlands, approved this study. Written informed consent was obtained from all the participants.

Of the 323 participants who entered the study, 11 were excluded from analysis because of incomplete data, resulting in 312 eligible subjects. Based on the SD-scores of birth length and adult height, the subjects were also assigned to one of four subgroups. Normal birth length and adult height was set at an SDS >–1 (± 0.1 SDS), in order to increase the statistical power to identify differences between subgroups (32). Of the 312 subjects, 217 were included in one of the following subgroups:

- Born small for gestational age (<–2 SDS) with a short adult height (<–2 SDS) (SGA-short, SGA-S),
- Born small for gestational age (<–2 SDS) with catch-up growth resulting in a normal adult height (≥–1 SDS) (SGA-CU),
- Born appropriate for gestational age (birth length ≥–1 SDS) with growth retardation resulting in a short adult height (<–2 SDS) (Idiopathic short stature (ISS)) and
- Born appropriate for gestational age (birth length ≥–1 SDS) and a normal adult height (≥–1 SDS) (controls).

**Methods**
All participants were invited to visit the Erasmus Medical Centre in Rotterdam. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer and weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice and the mean value was used for analysis. A questionnaire concerning subject’s substance use including alcohol and smoking was completed.

In all participants, bone mass of total body (TB), lumbar spine (LS), lean body mass (LBM) and fat mass (FM) was measured by one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was daily performed. The coefficient of variation was 0.64% for BMC, 1.04% for spine BMD and 0.64% for total body BMD (33). The coefficient of variation for lean tissue and fat tissue has been reported to be 1.57-4.49% and 0.41-0.88%, respectively (34). To account for differences in bone size, we calculated apparent BMD (BMAD) of the lumbar spine with the model BMAD<sub>LS</sub> = BMD<sub>LS</sub> x [4(π x width)], with the width as 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 the lumbar vertebrae (35).

**Statistical analysis**
In order to correct for gestational age, gender and age, SD-scores for birth length, birth weight, adult height and adult weight were calculated (30,31). Multiple linear regression (MR) analyses were performed to determine the associations between birth size and BMD<sub>TB</sub> and BMD<sub>LS</sub>, with corrections for age, gender, adult size and body composition. These corrections were made because these variables might act as possible confounders. Firstly, we entered age, gender, birth length SDS, adult height SDS and birth weight SDS to the model (model A). The interaction term birth length SDS * adult height SDS was added to all MR models because the study group had been selected on birth length and adult height, in order to ensure that the effect of these variables was modeled correctly. Secondly, weight was added, to correct for adult weight (model B). Thirdly, lean body mass (LBM) and fat mass (FM) were added as independent variables instead of adult weight (model C). Finally, we replaced FM and height by delta weight SDS during childhood (adult weight SDS – birth weight SDS) and delta height SDS during childhood (model D).
BMD in SGA-short, SGA-CU and ISS

Chapter 7

The BMC, BMC/height, BMD TB, BMDLS and the BMAD of the subgroups were expressed as SD-scores with controls as reference. ANOVA was used to determine if there were differences between the subgroups with regard to the group characteristics. Bonferroni correction was used for pairwise group comparisons. To determine differences between the groups corrected for age, gender, adult size and LBM, an ANCOVA model was used with controls as reference group and SGA-S, SGA-CU and ISS as dummy variables. Statistical package SPSS version 11.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.

Results

Clinical characteristics

The clinical characteristics of our study group are shown in Table 1. Mean (SD) age was 20.9 (1.67) years and more women than men were included (191 vs. 121). The characteristics of men and women are given separately.

Due to the selection criteria of the subgroups, birth length, birth weight, height and weight were different between the groups. The SGA-CU group had a lower birth weight than the SGA-S group, but this difference was not significant when birth weight was expressed as SDS. Adult height SDS of the SGA-CU group was significantly lower than controls. The gain in weight and height SDS of the SGA-CU group and the loss in weight and height SDS in the ISS group during childhood were significantly different compared to the other groups. Alcohol use and smoking were not significantly different between the subgroups.

Diff erences in bone mineral density between the subgroups

The differences in bone mineral density between the subgroups are shown in Table 2. Mean (SD) age was 20.9 (1.67) years and more women than men were included (191 vs. 121). The characteristics of men and women were given separately.

Due to the selection criteria of the subgroups, birth length, birth weight, height and weight were different between the groups. The SGA-CU group had a lower birth weight than the SGA-S group, but this difference was not significant when birth weight was expressed as SDS. Adult height SDS of the SGA-CU group was significantly lower than controls. The gain in weight and height SDS of the SGA-CU group and the loss in weight and height SDS in the ISS group during childhood were significantly different compared to the other groups. Alcohol use and smoking were not significantly different between the subgroups.

Table 1. Clinical characteristics of the total study group and the 4 subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Total group (n = 312)</th>
<th>Total men (n = 121)</th>
<th>Total women (n = 191)</th>
<th>SGA–S (n = 44)</th>
<th>SGA–CU (n = 56)</th>
<th>ISS (n = 38)</th>
<th>Controls (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>121/191</td>
<td>121</td>
<td>191</td>
<td>15/29</td>
<td>22/34</td>
<td>16/22</td>
<td>29/50</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>47.6 (3.08)</td>
<td>47.7 (3.0)</td>
<td>47.6 (3.0)</td>
<td>45.2 (1.98)*</td>
<td>44.6 (1.9)*</td>
<td>49.6 (1.45)</td>
<td>50.8 (1.7)</td>
</tr>
<tr>
<td>Birth length (SDS)</td>
<td>–1.5 (1.48)</td>
<td>–1.5 (1.42)</td>
<td>–1.5 (1.45)</td>
<td>–2.90 (0.79)*</td>
<td>–2.86 (0.80)*</td>
<td>–0.53 (0.47)*</td>
<td>0.08 (0.75)</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.78 (0.67)</td>
<td>2.75 (0.65)</td>
<td>2.80 (0.69)</td>
<td>2.50 (0.485)</td>
<td>2.19 (0.33)</td>
<td>3.25 (0.56)</td>
<td>3.30 (0.62)</td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>–1.2 (1.39)</td>
<td>–1.3 (1.39)</td>
<td>–1.2 (1.38)</td>
<td>–1.94 (0.94)*</td>
<td>–2.36 (0.70)*</td>
<td>–0.17 (1.14)*</td>
<td>–0.09 (1.27)</td>
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<tr>
<td>Age (years)</td>
<td>20.9 (1.68)</td>
<td>20.9 (1.65)</td>
<td>20.9 (1.70)</td>
<td>20.7 (1.76)</td>
<td>21.0 (1.5)</td>
<td>21.0 (1.7)</td>
<td>20.8 (1.71)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.2 (10.7)</td>
<td>175.1 (8.8)**</td>
<td>163.8 (9.4)</td>
<td>157.3 (6.7)**</td>
<td>174.7 (7.8)</td>
<td>159.3 (6.7)**</td>
<td>176.8 (7.7)</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>–1.1 (1.37)</td>
<td>–1.2 (1.23)</td>
<td>–1.0 (1.46)</td>
<td>–2.63 (0.55)**</td>
<td>–0.14 (0.77)</td>
<td>–2.48 (0.40)*</td>
<td>0.24 (0.90)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63.8 (12.5)</td>
<td>69.0 (12.8)**</td>
<td>60.4 (11.2)</td>
<td>56.7 (11.4)**</td>
<td>70.5 (14.4)</td>
<td>56.5 (12.2)*</td>
<td>68.8 (11.0)</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>–0.6 (1.42)</td>
<td>–0.7 (1.35)</td>
<td>–0.6 (1.46)</td>
<td>–1.55 (1.54)**</td>
<td>0.12 (1.17)</td>
<td>–1.77 (1.67)*</td>
<td>0.07 (0.98)</td>
</tr>
<tr>
<td>Delta weight1 (SDS)</td>
<td>0.57 (1.99)</td>
<td>0.60 (1.99)</td>
<td>0.55 (2.0)</td>
<td>0.39 (1.76)</td>
<td>2.48 (1.33)**</td>
<td>–1.60 (2.22)*</td>
<td>0.16 (1.43)</td>
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<tr>
<td>Delta height2 (SDS)</td>
<td>0.44 (1.72)</td>
<td>0.32 (1.66)</td>
<td>0.52 (1.75)</td>
<td>0.28 (0.93)</td>
<td>2.72 (0.99)**</td>
<td>–1.95 (0.57)*</td>
<td>0.16 (1.13)</td>
</tr>
</tbody>
</table>

Values are given as means (sd). M: Male, F: Female,

1: difference in weight from birth until early adulthood in SDS,
2: difference in height from birth until early adulthood in SDS,
* p < 0.001 compared with controls, #: p < 0.001 compared with ISS, §: p < 0.01 compared with SGA-CU.
Influence of birth size and body composition on bone mineral density

Bone mineral density of the total body (BMD$_{TB}$)

Table 3 shows the results of the multiple regression for BMD$_{TB}$. The first model showed no significant influence of birth length or birth weight on BMD$_{TB}$ whereas age, and adult height were significant determinants (Model A). Adding adult weight to the model showed that adult weight is also a significant determinant, next to adult height (Model B). Adult weight increased the explained variance from 28% to 44%. When LBM and FM replaced weight, the model showed that female gender, LBM and FM were the significant positive determinants of BMD$_{TB}$, whereas birth length and birth weight remained not significant (Model C, adjusted $R^2 = 0.49$). In Model D, the influence of weight gain and height gain during childhood on BMD$_{TB}$ is shown. This model showed that LBM and weight gain were significant determinants of BMD$_{TB}$ (adjusted $R^2 = 0.49$). Thus, the significant determinants of BMD$_{TB}$ were adult weight or LBM and FM, as well as weight gain during childhood. Birth length and birth weight were no significant determinants of BMD$_{TB}$.

Table 3. Multiple regression for BMD$_{TB}$ (g/cm$^2$).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
<th>Model D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>p-value</td>
<td>$\beta$</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.62</td>
<td>0.026</td>
<td>0.30</td>
<td>0.236</td>
</tr>
<tr>
<td>Gender</td>
<td>-1.66</td>
<td>&lt; 0.0005</td>
<td>-1.52</td>
<td>0.165</td>
</tr>
<tr>
<td>Height (cm)</td>
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<td>&lt; 0.0005</td>
<td>0.21</td>
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<tr>
<td>Birth length SDS</td>
<td>0.42</td>
<td>0.467</td>
<td>0.45</td>
<td>0.364</td>
</tr>
<tr>
<td>BL*AH (SDS)</td>
<td>0.44</td>
<td>0.058</td>
<td>0.33</td>
<td>0.107</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>-0.38</td>
<td>0.471</td>
<td>-0.24</td>
<td>0.607</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.38</td>
<td>&lt; 0.0005</td>
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<tr>
<td>LBM (kg)</td>
<td>0.87</td>
<td>&lt; 0.0005</td>
<td>0.67</td>
<td>&lt; 0.0005</td>
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<tr>
<td>Fat mass (kg)</td>
<td>0.22</td>
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<td>Delta weight (SDS)</td>
<td>1.82</td>
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<tr>
<td>Delta height (SDS)</td>
<td>-0.42</td>
<td>0.345</td>
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<tr>
<td>Overall</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.30</td>
<td>0.45</td>
<td>0.50</td>
<td>0.51</td>
</tr>
<tr>
<td>$R^2$ adjusted</td>
<td>0.28</td>
<td>0.44</td>
<td>0.49</td>
<td>0.49</td>
</tr>
</tbody>
</table>
Bone mineral density of the lumbar spine (BMD$_{LS}$)

We applied the same models on BMD$_{LS}$ (Table 4). The first model showed that age, adult height and birth weight SDS were significant determinants of BMD$_{LS}$, whereas birth length SDS was not (Model A). With adult weight in the model, gender, adult height, birth weight SDS and adult weight were significant determinants and the adjusted $R^2$ increased from 0.13 to 0.22 (Model B). When we replaced adult weight by LBM and FM, gender, birth weight SDS, LBM and FM were significant determinants (model C, adjusted $R^2=0.25$). Birth length SDS tended to be significant ($p=0.054$). To determine the influence of weight gain and height gain during childhood on BMD$_{LS}$, we replaced FM and adult height by delta weight SDS and delta height SDS. This model showed that female gender, LBM and the gain in weight during childhood were significant positive determinants of BMD$_{LS}$ (Model D, adjusted $R^2=0.25$). The significance of birth weight SDS and the trend of birth length SDS disappeared completely. Thus, the most significant determinants of BMD$_{LS}$ were gender, LBM and weight gain during childhood, whereas birth length and birth weight were no significant determinants for BMD$_{LS}$.

Table 4. Multiple regression for BMD$_{LS}$ (g/cm$^2$).

<table>
<thead>
<tr>
<th>Variables</th>
<th>BMD$_{LS}$</th>
<th>Model A</th>
<th>p-value</th>
<th>Model B</th>
<th>p-value</th>
<th>Model C</th>
<th>p-value</th>
<th>Model D</th>
<th>p-value</th>
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<tr>
<td>Age (yrs)</td>
<td>0.77</td>
<td>0.044</td>
<td>0.41</td>
<td>0.331</td>
<td>0.42</td>
<td>0.310</td>
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<td>Gender</td>
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<td>0.439</td>
<td>0.63</td>
<td>0.001</td>
<td>12.5</td>
<td>&lt;0.0005</td>
<td>9.78</td>
<td>0.002</td>
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<td>Height (cm)</td>
<td>0.59</td>
<td>&lt;0.0005</td>
<td>0.29</td>
<td>0.013</td>
<td>0.02</td>
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<tr>
<td>Birth length SDS</td>
<td>1.35</td>
<td>0.119</td>
<td>1.39</td>
<td>0.104</td>
<td>1.63</td>
<td>0.054</td>
<td>1.49</td>
<td>0.176</td>
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<td>BL*AH (SDS)</td>
<td>0.51</td>
<td>0.138</td>
<td>0.39</td>
<td>0.255</td>
<td>0.39</td>
<td>0.241</td>
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<tr>
<td>Birth weight SDS</td>
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<td>0.026</td>
<td>−1.65</td>
<td>0.037</td>
<td>−2.11</td>
<td>0.007</td>
<td>0.19</td>
<td>0.866</td>
<td></td>
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<tr>
<td>Weight (kg)</td>
<td>0.42</td>
<td>&lt;0.0005</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LBM (kg)</td>
<td>0.95</td>
<td>&lt;0.0005</td>
<td>0.70</td>
<td>&lt;0.0005</td>
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<tr>
<td>Fat mass (kg)</td>
<td>0.23</td>
<td>0.007</td>
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<td></td>
<td></td>
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<tr>
<td>Delta weight (SDS)</td>
<td>2.25</td>
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<tr>
<td>Delta height (SDS)</td>
<td>−0.15</td>
<td>0.850</td>
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<tr>
<td>Overall</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.14</td>
<td>0.24</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
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<td></td>
</tr>
<tr>
<td>$R^2$ adjusted</td>
<td>0.13</td>
<td>0.22</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
</tbody>
</table>

Comparison of subgroups after correction

Our results clearly demonstrate that correction for adult height and weight is warranted before BMD$_{TB}$ and BMD$_{LS}$ between groups with a different body size can be compared. To determine the true difference between the four groups an analysis of covariance was performed with correction for body size. With an adjusted explained variance of 51%, it showed that the ISS group had a lower BMD$_{TB}$ than controls ($p=0.028$), after correction for age, gender, height, weight and LBM. The mean difference was more than 0.5 SDS between the ISS group and controls (Figure 1). There were no differences in BMD$_{LS}$ between the groups in young adulthood after correction for age, gender, adult size and LBM.

![Figure 1. Difference in BMD$_{TB}$ (SD-scores) between the subgroups, after correction for age, gender, adult height, adult weight and lean body mass. Controls are the reference group (0 SDS). SGA-S: SGA short, SGA-CU: SGA catch-up, ISS: idiopathic short stature. *: p<0.03 compared to controls.](image)

Discussion

Our study in 312 young adults showed that adult weight, LBM and FM, as well as weight gain during childhood had a significant influence on BMD$_{TB}$ in young adulthood, whereas age, gender, birth length, birth weight, adult height and height gain during childhood did not have BMD$_{LS}$ had gender, LBM and weight gain during childhood as most significant determinants,
without age, height, birth length and birth weight as significant factors. Analyses in four clinically relevant subgroups showed that after correction for age, gender, adult height, adult weight and LBM, ISS subjects had a significantly lower BMD_{TB} than controls. No significant differences between the subgroups were found with regard to BMD_{LS}.

After correction for age, gender, adult height, adult weight, LBM and FM, there was no significant association between birth length or birth weight and BMD_{TB} in young adulthood. Cooper et al. showed that a lower birth length might lead to a higher fracture risk although this association did not reach statistical significance (28). There is no literature with regard to the relationship between birth length and BMD_{TB}. The lack of association between birth weight and adult BMD_{TB} is in line with previous reports (13-16).

After correction for age, gender, adult height, adult weight or LBM and FM, there was a significant association between birth weight and BMD_{LS} in young adulthood. Surprisingly, the regression coefficient of birth weight was negative. This indicated that of subjects with a similar adult weight, those with a lower birth weight had a higher BMD_{LS} in adulthood. This suggested that subjects with more weight gain during childhood would reach a higher BMD_{LS} because they started with a lower weight at birth. Several studies showed that subjects with a low birth weight had more weight gain during childhood (36-38). We therefore investigated if weight gain during childhood was a significant factor by replacing adult weight by delta weight SDS. Model D showed that among subjects with a similar weight gain during life, birth weight was not a significant determinant of BMD_{LS}. For this reason we conclude that birth weight is not a significant determinant of BMD_{LS}, which is in line with the literature (10,13,15,16). Instead, age, gender, LBM and weight gain during childhood were the most significant determinants of BMD_{LS}.

Birth length was not a relevant determinant of BMD_{LS} when we replaced height by delta height SDS during childhood. Jones et al. (17) showed an association between birth length and BMD_{LS} but no corrections were made for gestational age, LBM, FM or weight. Thus, postnatal growth defined as weight gain during childhood determines adult BMD_{LS} whereas birth length and birth weight, i.e. prenatal growth, has no influence on adult BMD.

Both LBM and FM were significant determinants of BMD_{TB} and BMD_{LS}. The larger contribution of LBM compared to FM in determining BMD_{TB} and BMD_{LS} is indicated by the larger regression coefficient of LBM. This has also been reported by Wang et al. (20). Men have more LBM, so after correction for LBM female gender had a positive influence on BMD_{TB} (Model C). With regard to BMD_{LS}, the influence of female gender was already positive after correction for adult weight and this increased further after correction of LBM. A possible explanation for the positive influence of female gender might be the fact that women start puberty earlier than men. They have been exposed to sex hormones like estrogen for a longer time, which has an positive influence on BMD. Thus, LBM and FM are significant determinants of BMD_{(TB+LS)}, and LBM is a significant factor in the gender difference with regard to BMD_{(TB+LS)}.

Short SGA subjects (SGA-S) had similar BMD_{TB} and BMD_{LS} as controls, after correction for age, gender, adult height, adult weight and LBM. Arends et al (39) investigated BMD_{TB} and BMD_{LS} in SGA-S children aged 3-8 before growth hormone was administered. Baseline BMD_{TB} SDS and BMD_{LS} SDS were lower in the SGA-S children than in the reference group. Their data was corrected for height, but not for weight, which might explain the different results between their and our study.

The SGA-CU subjects also had similar BMD_{TB} and BMD_{LS} as controls. Whereas SGA-CU subjects remained somewhat shorter than controls, their weight was the same as that of controls. Because weight is such a significant determinant of BMD, it might explain why we did not find a difference between the 2 groups. To our knowledge no study on BMD has been performed in SGA-CU subjects.

ISS subjects had a significantly lower BMD_{TB} than controls after correction for age, gender, adult height, adult weight and LBM. Only two other studies evaluated BMD in ISS subjects (40,41) and showed a lower BMD_{LS} in the ISS group but, unfortunately, no BMD_{TB} was given. The reason for a lower BMD might be a reduced bone turnover in ISS subjects (40). Other reasons might be the low weight gain during childhood in ISS subjects, or a yet unknown genetic variation in this population. Our results are in line with a previous study demonstrating that children with a reduced growth rate during childhood had an increased risk for fractures in later life (28).

Some reports stated that age, gender, height, weight, LBM and FM were relevant determinants of BMD in childhood and adolescence (16,18-23,26,42). Therefore, we selected them in our model. The limited influence of age and gender in the model compared to other studies (26,42) is explained by the use of SD-scores to correct for gestational age, age and gender. The explained variance of BMD_{TB} (50%) and BMD_{LS} (25%) might have been higher if variables like daily exercise and calcium intake would have been available. On the other hand, the reported influence of calcium intake and physical exercise is reported as small (36). Part of the variance might also be explained by genetic variations (6,7,12).

Our study population consisted of a relatively high percentage of subjects born small for gestational age and of short adults. This created greater contrast in the study population, which contributed to a better statistical model in which relationships between various factors could
be detected with more statistical power. In addition, this study population allowed comparison between clinically relevant subgroups.

Based on our data, we conclude that size at birth is not a significant determinant of BMD in later life whereas postnatal growth, especially weight gain, is. To achieve a normal BMD in adulthood, health-care workers should aim for a normal weight gain in children.

Acknowledgements:
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Potential Financial Conflicts of Interest: none

References


Part 2

Birth Size, Adult Size and Determinants of Adult Diseases
Chapter 8

Timing and Tempo of First Year Catch-up Growth Influence Cardiovascular and Metabolic Risk Profile in Early Adulthood

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Submitted
Abstract

Content: Growth during infancy appears to be an important determinant of cardiovascular disease (CVD) and type 2 diabetes in later life.

Objectives: To specify which period in the first year of life is related to determinants of CVD and type 2 diabetes in early adulthood. Additionally, the influence of tempo of early catch-up growth was investigated.

Design, setting and participants: Observational study with longitudinal data in a cohort (PROgramming factors for GRowth And Metabolism (PROGRAM) study) of 217 healthy young adults, aged 18-24 years, performed in the Erasmus Medical Centre, Rotterdam, the Netherlands, from August 2004 until September 2007.

Main outcome measures: An extensive set of determinants of CVD and type 2 diabetes in early adulthood, like body composition, insulin sensitivity, lipid levels and blood pressure.

Results: Increased weight gain relative to height gain in the first three months of life was inversely associated with insulin sensitivity and serum HDL level and positively associated with waist circumference, acute insulin response, cholesterol / HDL ratio and triglycerides in early adulthood. Fast catch-up growth in weight in the first three months resulted in a higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood than slower catch-up growth which took place during the entire first year.

Conclusion: Fast catch-up growth in the first three months of life is a risk factor for a higher % body fat in early adulthood. As this is associated with a worse cardiovascular and metabolic profile in early adulthood, efforts should be made to prevent rapid weight gain in early life.

Introduction

Low birth weight has been associated with cardiovascular diseases (CVD) and type 2 diabetes in later life (1), but other studies showed that different growth patterns in infancy and childhood might have more impact (2-5). Catch-up growth in the first year of life is associated with determinants of CVD and type 2 diabetes (6-8), while other studies showed that poor growth in early life and catch-up growth after age 2 years is related with an increased risk to develop cardiovascular events in later life (9,10). So far, no study investigated the relationship between a broad cardiovascular and type 2 diabetes risk profile and growth in early life. In addition, it is unclear whether there is a relationship between the tempo of early life growth and cardiovascular and metabolic risk in early adulthood.

We investigated which period in the first year of life is associated with determinants for CVD and type 2 diabetes in early adulthood. Therefore, we evaluated a study population of 217 young adults, which had a wide variation in growth in the first year, resulting in increased power to identify relationships between growth in early life and metabolic risk factors. The investigated determinants were % body fat, body mass index (BMI), fat distribution, insulin sensitivity, lipid levels and systolic blood pressure. Additionally, we investigated whether tempo of catch-up growth has an influence on metabolic and cardiovascular determinants.

Subjects and Methods

Subjects

The study cohort consists of 323 healthy subjects with an age between 18 and 24 years. They were randomly selected from several hospitals where they had been registered because of their being small at birth (birth length <–2SD) (11) or showing short stature (adult height <–2SD) (12). In addition, healthy subjects of different schools were randomly asked to participate. This increased the variation in growth patterns. A higher contrast in growth patterns increased the statistical power to find a relationship between early growth patterns and determinants of CVD and type 2 diabetes. The inclusion and exclusion criteria have been previously described in detail (13,14). Briefly, only Caucasians born singleton at 36 weeks or more of gestation, after an uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis or long-term complications of respiratory ventilation, such as broncho-pulmonary dysplasia were invited to participate. Subjects were excluded if they had been suffering from any serious condition, or disorder, or had been receiving any
treatment known to interfere with growth. Birth data and childhood growth data were obtained from records of hospitals, primary health care centers and general practitioners. The Medical Ethics Committee of Erasmus Medical Centre, Rotterdam the Netherlands, approved the study. Written informed consent was obtained from all participants.

Of the 323 study participants, data on first year growth were available for 217 subjects. There were no significant differences in clinical characteristics with regard to birth size, adult height and adult weight between the subjects with first year growth data and the ones without. Weight and height at the age of 3, 6, 9 and 12 months were measured at primary health care centers or hospitals.

Measurements
All participants were invited to visit Erasmus Medical Centre in Rotterdam. They had been fasting for 12-hours and had abstained from smoking and alcohol for 16 hours. All anthropometric measurements were performed twice and the mean value was used for analysis.

In all participants, lean body mass (LBM) and fat mass (FM) were measured on one DXA machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Insulin sensitivity index (SI), acute insulin response to glucose (AIRg) and the disposition index (DI), the product of insulin sensitivity and acute insulin response indicating the degree of glucose homeostasis, were determined by the Bergman’s minimal model (MINMOD 6.01 copyright RN Bergman) calculating paired glucose and insulin data obtained by frequent measurements during an intravenous glucose tolerance test (FSIGT) (15-17) with Tolbutamide (18). Blood pressure was measured in sitting position after 10 minutes rest in the none-dominant arm with an automatic device (Accutorr Plus, Datascopc Corp., Montvale NJ, USA), every 5 minutes for one hour and the average value of 13 measurements was taken.

Laboratory methods
Blood samples were drawn to determine serum lipid levels. The assays have been previously described in detail (13,14). Briefly, plasma glucose levels were determined on a VITROS analyzer 750 (Orthoclinical Diagnostics, Johnson & Johnson Company, Beere, Belgium) and plasma insulin levels were measured by IRMA (Medgenix, Biosource Europe). Total cholesterol and TG were measured using the CHOD-PAP and the GPO-PAP reagent kit (Roche Diagnostics, Mannheim, Germany). HDLc was measured using a homogenous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = total cholesterol – HDLc – 0.45 x triglycerides. ApoA-1 and apoB were determined by rate nephelometry on the Image Immunochemistry System, according to the manufacturer’s instructions (Beckman Coulter, Mijdrecht, the Netherlands).

Statistical analysis
SD-scores for birth length, birth weight, adult height and adult weight were calculated, in order to correct for gestational age, gender and age (11,12). First, we performed multiple linear regression (MR) analyses to investigate the influence of weight gain per three months in the first year of life on several determinants in the total group of young adults. Adjustments were made for gestational age, age, gender, social economic status (SES), height gain per three months and % body fat.

Additionally, the total study group was divided in 2 groups, one with catch-up growth in the first year and one without (Figure 1). Catch-up was defined as more than 0.67 SDS weight gain in the first year, because 0.67 SD scores represent the width of each percentile band on standard growth charts (second to ninth percentile, ninth to 25th percentile and so on) (2). Of the group with first year catch-up growth, two subgroups were formed based on fast (> 0.5 SDS) or slow (< 0.5 SDS) catch-up growth in weight in the first three months. A cut-off value of 0.5 SDS was chosen because it is clinically useful. Differences between these two subgroups were determined by regression analyses, with corrections for first year height gain to investigate the influence of weight gain on the variables independently of height gain. Our study has 87 eligible subjects and according to a power analysis performed prior to the study, with a level of significance (α) of 0.05 and a chosen power of 80%, at least 72 subjects should be included in the regression analyses to enable detection of a R-square change of 0.10. The variables tested were adjusted for an additional 5 independent variables. Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.
outcome variables independently of height gain. Weight gain in the first three months of life had an inverse association with insulin sensitivity and HDL levels in early adulthood. Positive associations were found between weight gain in the first three months and waist circumference, acute insulin response, cholesterol / HDL ratio as well as triglycerides levels in early adulthood. Weight gain from three to six months was only positively related to acute insulin response. The other three-month periods in the first year showed no significant associations. Height gain in the first year of life was not significantly related with the outcomes (data not shown).

Table 1. Clinical characteristics of the study population

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<tr>
<td>Birth weight (kg)</td>
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<td>0.67</td>
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<tr>
<td>Birth weight (SDS)</td>
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**Clinical characteristics at birth**

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**Gain in weight SDS**

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<td>3-6 months</td>
<td>216</td>
</tr>
<tr>
<td>6-9 months</td>
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**Clinical characteristics in early adulthood**

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<tr>
<td>Adult height (m)</td>
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<td>Adult height (SDS)</td>
<td>217</td>
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<td>1.38</td>
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Figure 1. Derivation of sample into subgroups.

Results

Clinical characteristics

The clinical characteristics of the study population are shown in Table 1. The average age in adulthood was 20.8 years.

Relationship between growth in the first year and determinants of CVD and type 2 diabetes in early adulthood

Associations between first year weight gain and several determinants in early adulthood are shown in Table 2. Adjustments were made for gestational age, gender, age, SES and height gain SDS. We adjusted for height gain to investigate the influence of weight gain on the
Running title

Table 1. Continued

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<th>Clinical characteristics in early adulthood</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult weight (kg)</td>
<td>217</td>
<td>63.9</td>
<td>12.6</td>
</tr>
<tr>
<td>Adult weight (SDS)</td>
<td>217</td>
<td>–0.63</td>
<td>1.43</td>
</tr>
<tr>
<td>BMI</td>
<td>217</td>
<td>22.5</td>
<td>3.4</td>
</tr>
<tr>
<td>% body fat</td>
<td>211</td>
<td>24.0</td>
<td>10.4</td>
</tr>
<tr>
<td>Trunk fat / total fat ratio</td>
<td>211</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>216</td>
<td>77.2</td>
<td>9.8</td>
</tr>
<tr>
<td>Waist / hip ratio</td>
<td>214</td>
<td>0.89</td>
<td>0.07</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>96</td>
<td>6.82</td>
<td>4.33</td>
</tr>
<tr>
<td>Acute insulin response</td>
<td>95</td>
<td>571</td>
<td>560</td>
</tr>
<tr>
<td>Disposition index</td>
<td>95</td>
<td>2934</td>
<td>1812</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>205</td>
<td>4.53</td>
<td>0.94</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>205</td>
<td>1.39</td>
<td>0.38</td>
</tr>
<tr>
<td>Cholesterol / HDL ratio</td>
<td>204</td>
<td>3.45</td>
<td>1.11</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>204</td>
<td>2.67</td>
<td>0.84</td>
</tr>
<tr>
<td>ApoA1 (g/l)</td>
<td>206</td>
<td>1.30</td>
<td>0.22</td>
</tr>
<tr>
<td>ApoB (g/l)</td>
<td>206</td>
<td>0.82</td>
<td>0.24</td>
</tr>
<tr>
<td>ApoB / ApoA1 ratio</td>
<td>206</td>
<td>0.64</td>
<td>0.22</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>206</td>
<td>1.03</td>
<td>0.49</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>159</td>
<td>110.1</td>
<td>9.0</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>159</td>
<td>66.1</td>
<td>6.1</td>
</tr>
</tbody>
</table>

To investigate whether the associations between weight gain in the first three months and determinants of CVD and type 2 diabetes were explained by fat mass in early adulthood, an additional adjustment was performed for % body fat. The associations between weight gain in the first three months and insulin sensitivity (p= 0.032), HDL (p= 0.007) as well as cholesterol / HDL ratio (p = 0.013) remained significant. The associations with waist circumference (p= 0.054), acute insulin response (p= 0.087) and triglyceride levels (p= 0.075) became trends. Acute insulin response in early adulthood remained significantly related with weight gain from 3 to 6 months (p= 0.008).

Table 2. Regression coefficients for gain in weight SDS in the first year of life and determinants of CVD and type 2 diabetes in early adulthood

<table>
<thead>
<tr>
<th>Outcome in young adults</th>
<th>Gain in weight SDS from 0-3 months</th>
<th>Gain in weight SDS from 3-6 months</th>
<th>Gain in weight SDS from 6-9 months</th>
<th>Gain in weight SDS from 9-12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>% body fat</td>
<td>β 0.639 P-value 0.275</td>
<td>β –0.759 P-value 0.495</td>
<td>β 1.342 P-value 0.388</td>
<td>β –3.149 P-value 0.082</td>
</tr>
<tr>
<td>BMI*</td>
<td>β 0.014 P-value 0.174</td>
<td>β –0.021 P-value 0.312</td>
<td>β 0.033 P-value 0.241</td>
<td>β –0.019 P-value 0.540</td>
</tr>
<tr>
<td>Trunk fat / total fat ratio</td>
<td>β 0.006 P-value 0.085</td>
<td>β –0.005 P-value 0.463</td>
<td>β 0.007 P-value 0.481</td>
<td>β 0.004 P-value 0.756</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>β 1.437 P-value 0.040</td>
<td>β –0.098 P-value 0.942</td>
<td>β 1.995 P-value 0.293</td>
<td>β 0.082 P-value 0.968</td>
</tr>
<tr>
<td>Waist / hip ratio</td>
<td>β 0.002 P-value 0.718</td>
<td>β 0.009 P-value 0.345</td>
<td>β 0.000 P-value 0.993</td>
<td>β 0.017 P-value 0.261</td>
</tr>
<tr>
<td>Insulin sensitivity*#</td>
<td>β –0.223 P-value 0.008</td>
<td>β –0.155 P-value 0.323</td>
<td>β 0.157 P-value 0.490</td>
<td>β –0.021 P-value 0.936</td>
</tr>
<tr>
<td>Acute insulin response*#</td>
<td>β 0.210 P-value 0.028</td>
<td>β 0.403 P-value 0.019</td>
<td>β 0.307 P-value 0.219</td>
<td>β –0.091 P-value 0.749</td>
</tr>
<tr>
<td>Disposition index*#</td>
<td>β –0.011 P-value 0.916</td>
<td>β 0.261 P-value 0.180</td>
<td>β 0.463 P-value 0.090</td>
<td>β –0.140 P-value 0.641</td>
</tr>
<tr>
<td>Total cholesterol*</td>
<td>β –0.002 P-value 0.892</td>
<td>β 0.022 P-value 0.402</td>
<td>β 0.014 P-value 0.717</td>
<td>β 0.017 P-value 0.677</td>
</tr>
<tr>
<td>LDL*</td>
<td>β 0.013 P-value 0.538</td>
<td>β 0.008 P-value 0.836</td>
<td>β 0.017 P-value 0.784</td>
<td>β 0.017 P-value 0.790</td>
</tr>
<tr>
<td>HDL*</td>
<td>β –0.053 P-value 0.005</td>
<td>β 0.038 P-value 0.292</td>
<td>β –0.025 P-value 0.626</td>
<td>β 0.081 P-value 0.142</td>
</tr>
<tr>
<td>Cholesterol / HDL ratio*</td>
<td>β 0.052 P-value 0.014</td>
<td>β –0.020 P-value 0.625</td>
<td>β 0.040 P-value 0.497</td>
<td>β –0.061 P-value 0.335</td>
</tr>
<tr>
<td>ApoB*</td>
<td>β 0.026 P-value 0.178</td>
<td>β 0.020 P-value 0.574</td>
<td>β 0.012 P-value 0.826</td>
<td>β 0.022 P-value 0.697</td>
</tr>
<tr>
<td>ApoA1</td>
<td>β –0.019 P-value 0.194</td>
<td>β 0.029 P-value 0.300</td>
<td>β –0.004 P-value 0.924</td>
<td>β 0.005 P-value 0.900</td>
</tr>
<tr>
<td>ApoB / ApoA1 ratio*</td>
<td>β 0.039 P-value 0.085</td>
<td>β 0.008 P-value 0.859</td>
<td>β 0.014 P-value 0.827</td>
<td>β 0.013 P-value 0.845</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>β 0.066 P-value 0.040</td>
<td>β 0.024 P-value 0.700</td>
<td>β 0.136 P-value 0.121</td>
<td>β –0.009 P-value 0.460</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>β –1.08 P-value 0.122</td>
<td>β 0.461 P-value 0.733</td>
<td>β 1.825 P-value 0.316</td>
<td>β –2.704 P-value 0.176</td>
</tr>
</tbody>
</table>

All correlations were corrected for gestational age, age, gender, SES and height gain in the same period.

*Log transformed variables, # measured in 94 subjects.

Influence of tempo of catch-up growth in the first year on determinants of CVD and type 2 diabetes in early adulthood

To assess if tempo of catch-up growth influences determinants in early adulthood, subgroups were formed based on fast or slow catch-up growth in the first three months of life. Of all subjects with a clinically relevant weight gain of at least 0.67 SDS in the first year, some had a weight gain of more than 0.5 SDS in the first three months (fast catch-up (n= 65)), while others had a weight gain in the first three months of less than 0.5 SDS (slow catch-up (n= 22)). The clinical characteristics of these two subgroups are shown in Table 3. Birth size was not significantly different between the subgroups. First year growth patterns are shown in Figure 2. Both subgroups attained a similar adult height, which was significantly shorter than average (p< 0.05). However, the fast catch-up group attained a higher adult weight than the slow catch-
up group (p=0.075). When weight for height was expressed as weight SDS - height SDS, the fast catch-up group had more weight than height gain in the first three months, while the slow catch-up group had more height than weight gain (p=0.1).

Table 3. Clinical characteristics of subjects with fast versus slow first year catch-up growth in weight*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fast catch-up growth (n=65)</th>
<th>Slow catch-up growth (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (%)</td>
<td>35</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>38.5 (1.51)</td>
<td>39.0 (1.97)</td>
<td>0.164</td>
</tr>
<tr>
<td>Birth length SDS</td>
<td>-2.22 (1.28)</td>
<td>-2.13 (1.62)</td>
<td>0.777</td>
</tr>
<tr>
<td>Birth weight SDS</td>
<td>-2.23 (0.74)</td>
<td>-2.05 (1.04)</td>
<td>0.376</td>
</tr>
<tr>
<td>Height SDS at 3 months</td>
<td>-1.13 (1.06)</td>
<td>-1.66 (1.23)</td>
<td>0.058</td>
</tr>
<tr>
<td>Weight SDS at 3 months</td>
<td>-0.76 (0.93)</td>
<td>-2.12 (1.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight SDS - height SDS at 3 months</td>
<td>0.37 (0.82)</td>
<td>-0.46 (0.54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delta length SDS from birth to 3 months</td>
<td>1.07 (1.02)</td>
<td>0.47 (1.01)</td>
<td>0.020</td>
</tr>
<tr>
<td>Delta weight SDS from birth to 3 months</td>
<td>1.47 (0.62)</td>
<td>-0.07 (0.47)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Delta length SDS from 3 months to 1 year</td>
<td>0.58 (0.62)</td>
<td>0.57 (0.66)</td>
<td>0.962</td>
</tr>
<tr>
<td>Delta weight SDS from 3 months to 1 year</td>
<td>0.43 (0.67)</td>
<td>1.37 (0.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height SDS at 1 year</td>
<td>-0.55 (0.97)</td>
<td>-1.08 (1.09)</td>
<td>0.033</td>
</tr>
<tr>
<td>Weight SDS at 1 year</td>
<td>-0.33 (0.79)</td>
<td>-0.74 (1.02)</td>
<td>0.054</td>
</tr>
<tr>
<td>Weight SDS – height SDS at 1 year</td>
<td>0.22 (0.77)</td>
<td>0.34 (0.77)</td>
<td>0.514</td>
</tr>
<tr>
<td>Delta length SDS from birth to 1 year</td>
<td>1.68 (1.17)</td>
<td>1.05 (1.11)</td>
<td>0.030</td>
</tr>
<tr>
<td>Delta weight SDS from birth to 1 year</td>
<td>1.90 (0.68)</td>
<td>1.31 (0.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adult height SDS</td>
<td>-0.74 (1.02)</td>
<td>-0.79 (1.14)</td>
<td>0.833</td>
</tr>
<tr>
<td>Adult weight SDS</td>
<td>-0.09 (1.16)</td>
<td>-0.59 (1.05)</td>
<td>0.075</td>
</tr>
<tr>
<td>Weight SDS - height SDS</td>
<td>0.65 (1.25)</td>
<td>0.19 (0.91)</td>
<td>0.125</td>
</tr>
<tr>
<td>Adult BMI</td>
<td>23.2 (3.46)</td>
<td>21.7 (2.27)</td>
<td>0.095</td>
</tr>
<tr>
<td>% body fat</td>
<td>27.4 (9.2)</td>
<td>19.4 (11.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>80.6 (10.6)</td>
<td>75.0 (8.3)</td>
<td>0.028</td>
</tr>
<tr>
<td>Trunk fat / total fat ratio</td>
<td>0.49 (0.05)</td>
<td>0.48 (0.07)</td>
<td>0.040</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>5.38 (3.75)</td>
<td>8.13 (4.76)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Data is given in mean (sd).
*All subjects had a first year catch-up growth in weight of more than 0.67 SDS. They were divided in two subgroups based on fast (> 0.5 SDS) or slow (< 0.5 SDS) catch-up growth in weight in the first three months.

1: after correction for gestational age, gender, age, SES and height gain in the first year.

Figure 2. Height SDS, weight SDS and weight SDS relative to height SDS in the first year of life and in early adulthood between subjects with slow and fast catch-up growth in the first three months of life. Values are means (± SEM). *p < 0.05 between the two subgroups.
Interestingly, the fast catch-up group had a significantly higher % body fat ($\beta= 6.00$, $p= 0.006$), a greater waist circumference ($\beta= 6.19$, $p= 0.028$), a higher trunk fat / total fat ratio ($\beta= 0.029$, $p= 0.040$) and a reduced insulin sensitivity ($\beta= -3.54$, $p= 0.039$) in early adulthood than the slow catch-up group, after adjustment for gestational age, gender, age, SES and height gain in the first year. The difference in waist circumference, fat distribution and insulin sensitivity between the 2 subgroups disappeared after adjustment for adult % body fat, thus % body fat explained the differences. Additional adjustment for birth weight SDS and birth length SDS did not change these results.

Discussion

Our study shows that increased weight gain relative to height gain in the first three months of life is associated with reduced insulin sensitivity and serum HDL, and an increased waist circumference, acute insulin response, cholesterol / HDL ratio and serum triglyceride in early adulthood. These are all important determinants of CVD and type 2 diabetes in later life. Even after adjustment for adult % body fat, the associations with insulin sensitivity, HDL levels and cholesterol / HDL ratio remained significant. A subgroup analysis showed that of all subjects with first year catch-up in weight (> 0.67 SDS), those with fast catch-up growth in weight in the first three months of life had a higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood than those with slower catch-up growth. Percentage body fat explained the differences in central adiposity and insulin sensitivity in early adulthood between the two subgroups.

Low birth weight has previously been associated with an increased risk of CVD and type 2 diabetes in later life (19-21). Although this was initially thought to be due to an unfavorable fetal environment (1), other studies reported that postnatal catch-up growth influenced the risk as well (6,8,22-24). Our study shows that increased weight gain in the first three months of life had a significant influence on several determinants for CVD and type 2 diabetes in early adulthood. Of all subjects born small for gestational age (SGA), ninety percent experience catch-up growth in the first two years of life (25). We previously showed that young adults with catch-up growth between birth and adulthood had a higher risk for CVD or type 2 diabetes, while young adults born SGA without catch-up growth did not have an increased risk (13,14,26). This indicates that having a low birth weight for gestational age is not directly related with an unfavorable cardiovascular and metabolic profile, but increased weight gain during early childhood is.

Until now, it is unknown whether tempo of catch-up growth is of influence on cardiovascular or metabolic determinants. Of all subjects who showed postnatal catch-up growth in the first year, the ones with fast catch-up growth had a higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood, while both subgroups reached a similar adult height. The fast catch-up group was fatter in early adulthood and this explains the difference in central adiposity and insulin sensitivity. Therefore, it is important to investigate which factors determine weight gain in early life, because this might lead to intervention strategies to prevent cardiovascular events in later life. At this moment it is unclear whether (epi)genetic factors are involved. Early nutrition might be a major factor. Generally, nutrient-enriched diets lead to rapid weight gain in early life, and subsequently have adverse effects on cardiovascular risk factors in later life (6,27,28). Also, formula-fed infants grow at a faster rate than breast-fed infants and have a higher risk of being overweight later in life (29,30). Unfortunately, our study did not have nutritional data to investigate the relationship between early nutrition, growth in infancy and cardiovascular determinants later in life. Nevertheless, our findings suggest that the use of nutrient-enriched formulas which induce rapid weight gain in early life might increase the risk for CVD and type 2 diabetes in later life (27). Nutritional intervention, like stimulating breastfeeding during the first three months, might decrease the prevalence of CVD and type 2 diabetes.

In our study, we investigated the influence of early weight gain, but other periods in life might be important as well. As shown in reports of the Helsinki Birth Cohort Study, subjects with a slow gain in weight from birth to two years followed by catch-up weight during childhood also had an increased risk for development of CVD and type 2 diabetes (10,31,32). Similar results have been found in a cohort study in India (33). Interestingly, these subjects experienced growth retardation in weight in early life before they showed catch-up growth during childhood. This might indicate that catch-up growth after a period of growth retardation, either during intrauterine or early life, increases the risk for the development of CVD and type 2 diabetes in later life. Our study suggests that the tempo of catch-up growth might be more relevant than the timing. This merits further study of factors influencing tempo of catch-up growth.

In conclusion, we found that increased weight gain relative to height gain in the first three months of life is associated with unfavorable cardiovascular and metabolic profile in early adulthood. Furthermore, fast catch-up growth in early life is more detrimental than slow catch-up growth. For clinical practice, the first three months of life might provide a sensitive time-window to apply intervention strategies to prevent fast catch-up growth and later cardiovascular events. Additionally, more studies are required to investigate which factors determine catch-up growth in early infancy.
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Author contributions: Prof. Hokken-Koelega is the principal investigator and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Leunissen, Stijnen, Hokken-Koelega

Acquisition of data: Leunissen, Kerkhof

Analysis and interpretation of the data: Leunissen, Kerkhof, Stijnen, Hokken-Koelega

Drafting of the manuscript: Leunissen

Critical revision of the manuscript for important intellectual content: Leunissen, Kerkhof, Stijnen, Hokken-Koelega

Statistical analysis: Leunissen, Stijnen, Hokken-Koelega

Obtained funding: Hokken-Koelega

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Potential Financial Conflicts of Interest: none

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Chapter 9

Association Between Infant Dairy Milk Protein Intake and Adiposity at Age 12 Months


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Submitted.
Abstract

Background: Rapid weight gain and larger body size during infancy predict subsequent increased risk for childhood obesity. High protein intakes during infancy have been proposed to increase adiposity in early life, however previous studies have been inconsistent.

Objective: To investigate the associations between protein intake, and specifically dairy milk protein intakes (from dairy protein-based infant formula and cow’s milk), on body size and adiposity at age 12 months.

Design: Nutrient intakes at age 12 months, assessed by three-day diet records in 396 infants participating in the Cambridge Baby Growth Study, were related to length, weight, BMI and adiposity assessed by skinfold thickness at 12 months. Analyses were adjusted for age, sex, and maternal BMI, and also other nutritional factors (breast feeding at 3 months and total energy intake at 12 months).

Results: Total energy intake at 12 months was positively related to length, weight and BMI at 12 months, but not to adiposity. After adjustment for total energy intake, total protein intake was not related to any anthropometric outcome. However, specifically dairy milk protein intake was positively related to BMI (p=0.04) and adiposity (0.16 ± 0.06 SDS per 10g; P= 0.005) at 12 months, and the association with adiposity persisted after adjustment for other nutritional factors (0.13 ± 0.06; P= 0.02).

Conclusion: Higher dairy milk protein intake, rather than total protein intake, at age 12 months appeared to be associated with greater adiposity.

Introduction

Rapid weight gain in the first year of life has been related to increased adiposity in childhood and adverse cardiovascular risk profiles in young adults (1-5). Therefore, early interventions to avoid excessive infancy weight gain could potentially prevent obesity and its co-morbidities.

Various nutritional factors in early life have been shown to influence later obesity risk. Breast feeding is associated with both slower infant weight gain and lower risk of obesity (6,7). Among infants who are formula-fed, higher total energy intakes at age 4 months were associated with higher rates of infant weight gain and higher childhood BMI in a large UK study (8). Follow-up of randomised trials of different infant milk formulas in preterm infants also demonstrate the long-term body composition and metabolic outcomes related to early feeding (9-11). With regard to specific infant nutrients, protein intake at age 2 years was positively related to adiposity at 8 years in a study of 112 French children (12), leading to the proposal that higher protein intakes during infancy may increase adiposity and subsequent obesity risks. However, other studies did not confirm this association (13,14). Recently, the DONALD study reported that the apparent effect of infant total protein intake on increased adiposity at age 7 years could be driven specifically by infant dairy protein intake (15).

We therefore investigated the associations between infant energy, protein and fat intakes on body size and adiposity at age 12 months. In particular we tested the hypothesis that dairy milk protein intake (from dairy protein-based infant formula milk and cow’s milk) might be specifically associated with infant adiposity.

Subjects and Methods

Study design

The current study is part of a large ongoing birth cohort study. Inclusion criteria were mothers attending a single antenatal centre in Cambridge UK. Exclusion criteria were mothers aged < 16 years, or unable to give informed consent. Mothers were approached and recruited at their first antenatal clinic appointment during early pregnancy by trained paediatric research nurses. Offspring were followed up during infancy. Weight, length, and skinfold thicknesses were measured at 0, 3, and 12 months by the research nurses.

At the time of the current analysis, the cohort included 1526 infants born between August 2001 and July 2008. The current dataset was based on a sub-cohort of 431 infants born between
July 2004 and June 2007, with information on infant dietary intakes at age 12 months, which were introduced to the study protocol from 2004 onwards. Complete data were available in 396 infants, including mother’s BMI and infant anthropometry at 3 and 12 months. Details of their birth weights and birth lengths and body size at 12 months are shown in Table 1. This sample was representative of the whole cohort with regard to birth weight, parity, mother’s BMI, mother’s age and type of milk feeding at age 3 months (data not shown).

The study was approved by the local Cambridge research ethics committee and all mothers gave informed written consent.

**Anthropometry**
Infant weight, length and skinfolds were measured at birth and at each research clinic visit by trained paediatric research nurses. Weight was measured to the nearest 1 g using electronic scales. Supine length was measured to the nearest 0.1 cm using a Kiddimeter (Holtain Ltd, Crosswell, Pembs., UK.). Skinfold thickness was measured in triplicate at four sites (triceps, subscapular, flank and quadriceps) using a Holtain Tanner/Whitehouse Skinfold Caliper (Holtain Ltd).

**Dietary assessment**
Mode of infant feeding (breast milk only or using formula milk) at age 3 months was assessed by questionnaire completed by the parents at that clinic visit.

At age 12 months, mothers were asked to record all food and drink consumed, in household measures, over a 3 day period. These estimated (unweighed) dietary intakes were recorded by mothers and carers in diet diary booklets, which were sent by mail prior to the 12 month clinic visit, and collected at that visit. At the visit, the mothers were interviewed by one Research Assistant (FK) to clarify responses and portion sizes recorded in the diaries. These diet diaries had been adapted from the ALSPAC study infant food diary for age 18 months with further examples and guidance on foods now available for infants (16).

Food diaries were coded by trained Dietary Assessment Assistants using our in-house program DINO (Diet in Nutrients out). Household measures (teaspoon, cup etc.), if specified, or age appropriate portion sizes were assigned to each food. Home-made foods with recipes supplied by parents were entered as individual ingredients. The output generated a food code and weight for each food item recorded. Checking of the coded data was carried out by nutritionally qualified editors. Programs within DINO were used to analyse the coded data to output food and nutrient intakes. The in-house food composition database is based on McCance and Widdowson “The Composition of Foods” 6th Edition (17). Foods that were not on the database were matched to existing foods with a similar composition. A new food code was created if the food was commonly consumed or contributed significantly to a respondent’s intake, in which case that food item was added to the database, with nutrients as listed on the packaging and other micronutrients calculated from listed ingredients. Thus a large number of currently available commercial infant and baby products have been added to the database.

**Calculations**
BMI was calculated as weight / height$^2$ (kg/m$^2$). Age and sex-appropriate standard deviation scores (SDS) for weight, height and BMI at each visit were calculated using internal means and standard deviations, including adjustment for precise age at each visit. Measurements of skinfolds at each anatomical site were used to derive separate internal SDS, adjusted for age and gender, and an overall skinfold SDS was calculated as the mean SDS of the 4 sites as an indicator of adiposity.

**Statistics**
Differences in clinical characteristics between boys and girls were calculated with an independent t-test and chi-square test. The relationships between nutritional data and anthropometric data were analysed by linear regression models. In Model 1, adjustments were made for age, sex, maternal BMI and infant length, weight, BMI or adiposity, respectively at age 3 months. In Model 2 additional adjustments were made for other nutritional factors: type of milk feeding at 3 months (breast-fed, mixed-fed or bottle-fed) and total energy intake at 12 months (except when this was the exposure variable). Statistical package SPSS version 16.0 (SPSS, Inc., Chicago, IL) was used for analysis. Results were regarded statistically significant if p was < 0.05.

**Results**
The characteristics of the study population are shown in Table 1. The overall average total energy intake was 0.44 MJ/kg/d, which is comparable to current UK recommendations (18). Almost all infants received some dairy protein-based milk (dairy protein-based infant formula or cow’s milk) at 12 months and this comprised around one third of the daily energy intake.
Early Nutrition Related to Adiposity at Age 1 Year

Total energy, protein and fat intakes
Total energy intake was positively associated with length (P<0.001), weight (P<0.001) and BMI (p= 0.01), but not adiposity at 12 months (Model 1: adjusted for age, sex, maternal BMI and infant length, weight, BMI or adiposity respectively at age 3 months). The associations with length (P= 0.001), weight (P< 0.001) and BMI (p= 0.01) persisted after further adjustment for type of milk feeding at 3 months.

Table 1. Characteristics of the study population by gender. Means (SD) or percentages.

<table>
<thead>
<tr>
<th></th>
<th>Boys (n= 208)</th>
<th>Girls (n= 188)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (kg)</td>
<td>3.61 (0.51)</td>
<td>3.38 (0.52)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.9 (1.6)</td>
<td>39.8 (1.5)</td>
<td>0.6</td>
</tr>
<tr>
<td>Milk feeding at 3 months (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– breast</td>
<td>38.7</td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>– bottle &amp; breast</td>
<td>28.9</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td>– bottle</td>
<td>32.4</td>
<td>19.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Size at 12 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (cm)</td>
<td>76.9 (2.4)</td>
<td>74.8 (2.8)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.3 (1.0)</td>
<td>9.5 (1.1)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.3 (1.2)</td>
<td>16.9 (1.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean skinfolds SDS</td>
<td>–0.09 (0.73)</td>
<td>0.04 (0.79)</td>
<td>0.1</td>
</tr>
<tr>
<td>% receiving breast milk at 12 months</td>
<td>21.2</td>
<td>22.3</td>
<td>0.8</td>
</tr>
<tr>
<td>% receiving dairy milk at 12 months</td>
<td>93.8</td>
<td>91.0</td>
<td>0.3</td>
</tr>
<tr>
<td>% receiving formula milk</td>
<td>70.1</td>
<td>71.2</td>
<td>0.8</td>
</tr>
<tr>
<td>% receiving cow's milk</td>
<td>63.5</td>
<td>58.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Dietary intakes at 12 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total energy (MJ/d)</td>
<td>4.48 (0.81)</td>
<td>4.19 (0.80)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Total protein (g/d)</td>
<td>38.9 (9.5)</td>
<td>35.7 (9.9)</td>
<td>0.002</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>44.0 (10.7)</td>
<td>41.8 (10.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Dairy milk protein (MJ/d)</td>
<td>11.3</td>
<td>9.7 (6.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>– Formula milk protein (MJ/d)</td>
<td>5.6</td>
<td>5.8 (5.0)</td>
<td>0.7</td>
</tr>
<tr>
<td>– Cow's milk protein (MJ/d)</td>
<td>5.7</td>
<td>3.9 (5.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>– Formula milk energy (g/d)</td>
<td>0.89</td>
<td>0.92 (0.76)</td>
<td>0.7</td>
</tr>
<tr>
<td>– Cow's milk energy (g/d)</td>
<td>0.45</td>
<td>0.31 (0.48)</td>
<td>0.02</td>
</tr>
<tr>
<td>– Cow's milk fat (g/d)</td>
<td>6.3 (8.7)</td>
<td>4.4 (6.9)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 2. Daily total energy, total protein and total fat intakes related to length, weight, BMI and adiposity at 12 months. Partial regression coefficients (β± SE).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>SE</td>
</tr>
<tr>
<td>Total energy (per MJ)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (SDS)</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.22</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Adiposity (SDS)**</td>
<td>–0.08</td>
<td>0.05</td>
</tr>
<tr>
<td>Total protein (per 10g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (SDS)</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.17</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Adiposity (SDS)**</td>
<td>–0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Total fat (per 10g)</td>
<td></td>
<td></td>
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<tr>
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<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.10</td>
<td>0.04</td>
</tr>
<tr>
<td>Adiposity (SDS)**</td>
<td>–0.03</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Model 1: adjusted for sex, age, maternal BMI and also infant length, weight, BMI or adiposity, respectively at age 3 months.
Model 2: further adjusted for nutritional factors (type of milk feeding at 3 months and total energy intake at 12 months, except where this was the exposure variable*).
**Adiposity was also adjusted for length at 12 months

Dairy milk protein intake
Specifically, dairy milk protein intake was positively associated with length (P= 0.003), weight (P< 0.001), BMI (P= 0.04) and also adiposity (P= 0.005); the association with adiposity persisted
after further adjustment for other nutritional factors (P=0.02). In fully adjusted models, both protein from dairy protein-based infant formula milk (0.21 ± 0.08 SDS per 10g; P=0.02) and protein from cow’s milk (0.11 ± 0.06; P=0.08) showed positive trends with adiposity.

Table 3. Daily dairy milk protein intake (from dairy protein-based infant formula and cow’s milk) related to length, weight, BMI and adiposity at 12 months. Partial regression coefficients (β ± SE).

<table>
<thead>
<tr>
<th>Total protein (per 10g)</th>
<th>(\beta)</th>
<th>SE</th>
<th>p-value</th>
<th>(\beta)</th>
<th>SE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy milk protein (per 10g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length (SDS)</td>
<td>0.17</td>
<td>0.06</td>
<td>0.003</td>
<td>0.07</td>
<td>0.06</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.20</td>
<td>0.06</td>
<td>&lt;0.001</td>
<td>0.10</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>0.13</td>
<td>0.06</td>
<td>0.04</td>
<td>0.08</td>
<td>0.07</td>
<td>0.2</td>
</tr>
<tr>
<td>Adiposity (SDS)*</td>
<td>0.16</td>
<td>0.06</td>
<td>0.005</td>
<td>0.13</td>
<td>0.06</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Model 1: adjusted for sex, age, maternal BMI and also infant length, weight, BMI or adiposity, respectively at age 3 months.
Model 2: further adjusted for nutritional factors (type of milk feeding at 3 months and total energy intake at 12 months).
*Adiposity was also adjusted for length at 12 months

Discussion

In this UK birth cohort study, higher infant intakes of dairy milk protein (from dairy protein-based infant formula and cow’s milk) were related to increased adiposity at 12 months. In contrast, length, weight and BMI in the first year of life appeared to be associated with total energy intake rather than to any specific nutrient or nutrient source.

A link between early total protein intake and childhood adiposity, assessed by skinfolds, was first suggested by Rolland-Cachera et al. (12). Protein intakes in infants tend to be far greater than is required for growth and metabolism (13,19). The mean protein intake in our study, 3.8 g/kg/day, was far greater than the Reference Nutrient Intake at this age (1.4 g/kg/d) (20). Such observations have lead to the hypothesis that higher protein intakes in early life might causally increase fat mass by stimulating the secretion of IGF-I and/or insulin, which in turn promote adipocyte maturation and lipid uptake storage (21-23). Repeated follow-up reports from theDONALD study have also described consistent positive associations between early protein intakes and adiposity in childhood in German children (15,19,24). In that study, total protein, animal protein and dairy protein intakes at ages 1 year and 5-6 years were positively associated with BMI and % body fat, measured by skinfolds at age 7 years (15,24) and with higher BMI at the start of adiposity rebound (19). However, Hoppe et al. did not find an association between total protein intake at 9 months and fat mass at age 10 years, measured by DXA, in 105 Danish children (13), and in the UK ALSPAC study, total protein intake at 18 months was not associated with early adiposity rebound in 772 children (14). Furthermore, Skinner et al. showed that total fat intake (% energy), but not total protein intake (% energy), at 2 years was positively associated with BMI at 8 years (25). The DONALD study also reported a positive relationship between fat intake at 2 years and % body fat at 5 years, although this has been questioned by other studies (12,26-28).

Our findings suggest that different sources of infant nutrients might contribute to the heterogeneity in results between studies. We found no associations between total energy, total protein or total fat intakes and adiposity at age 1 year, but rather specifically dairy milk protein intake appeared to correlate with infant adiposity, independent of various demographic and nutritional potential confounders. Too few infants were taking soya milk, or other milks, to be able to assess whether similar associations were seen with this source of protein. Our findings are consistent with a recent observation in 203 German children from the DONALD study that infant dairy protein intake was associated with adiposity at age 7 years, although this specific association reached only borderline statistical significance in that study (15).

Our study population was larger than most previous studies investigating the association between infant nutrient intakes and adiposity. Moreover we had very detailed dietary data, of 3 days duration, and carefully coded for all foods consumed with a large food composition database. We acknowledge that the sample was not designed to be representative of UK infants, although our population had similar birth weights to the British reference, and total energy intakes were almost identical to current UK recommendations. We were able to adjust for various potential confounding factors, including mother’s BMI, and type of milk feeding and body size and adiposity earlier in infancy at age 3 months, however, the reported associations are essentially cross-sectional. Furthermore, it is possible that the observations are residually confounded by other nutritional factors, including other components of infant milk formula and cow’s milk, or by parenting patterns associated with the use of these milks. The European Childhood Obesity Project (EARNEST) is trialling a novel infant milk formula with reduced protein content (29) and that study will hopefully eventually clarify the specific role of dairy milk protein on infant adiposity.
In addition to lower milk protein contents, reductions in overall intake of infant milk formula or cow’s milk may be an effective and alternative strategy to avoid excess infant gains in weight and adiposity. Higher intakes of formula milk at 4 months have been related to larger body weight and BMI at ages 1 to 5 year in 582 children in the UK ALSPAC study (8). The UK Department of Health recommends that 500–600 ml of either breast milk or infant formula should be given each day between the ages of 6 and 12 months (30). However, recently revised international guidance on energy intakes recommend substantially lower intakes in infants (0.33 MJ/kg/d at age 12 months) (31). Furthermore, current UK and European guidance recommends parents to avoid introducing unmodified cow’s milk to infant diets before age 12 months due to the risk of iron deficiency (32). However, data from a large UK study and an Icelandic study show that this recommendation is poorly adhered to, with 14–40% of infants receiving predominantly cow’s milk even at age 6-8 months (16,33). Our study indicates that increased adiposity in early life might be another reason to postpone and/or limit the use of cow’s milk in settings with a high prevalence of childhood obesity.

In conclusion, dairy milk protein intake was positively associated with adiposity at age 12 months. These findings highlight potential future strategies to reduce infant adiposity and childhood obesity.

Acknowledgements

We thank all the families in the Cambridge Baby Growth Study, the research study team and the staff at the Addenbrooke’s Wellcome Trust Clinical Research Facility and the dietary assessment team at MRC Human Nutrition Research, particularly Jenny Winster, who coded most of the diet diaries.

This project was supported by the European Union, the World Cancer Research Foundation International, the Medical Research Council and the NIHR Cambridge Biomedical Research Centre.

Potential Financial Conflicts of Interest: none

References

15. Gunther AL, Remer T, Kroke A, Bukeyen AE. Early protein intake and later obesity risk: which protein sources at which time points throughout infancy and childhood are important for body mass index and body fat percentage at 7 y of age? Am J Clin Nutr 2007;86:1765-72.


The present thesis describes in a large group of young adults, the influence of different growth patterns during childhood on determinants of cardiovascular disease (CVD), type 2 diabetes mellitus and osteoporosis. The first part reports the findings of six studies in which the influence of size at birth and postnatal growth on insulin sensitivity, beta cell function, body composition, serum lipid and acylation stimulating protein levels, blood pressure, carotid intima media thickness and bone mineral density were investigated. In addition, the differences in determinants of adult diseases are described between four clinically relevant subgroups with different growth patterns.

The second part describes two studies, one study in young adults and one study in infants. The study in young adults describes which period of first year growth is related to determinants of CVD and type 2 diabetes in early adulthood. In addition, the tempo of first year growth was investigated in relation to these determinants. The study in infants shows the relation between nutrients and body size and body composition at 12 months of age.

Statistical Approach
Several studies investigated the association between birth size and determinants of adult disease, but there is a great heterogeneity in the statistical approach. Adjustments for gestational age and adult size were not always made, and results were interpreted in different ways. To determine the association between birth size and determinants of adult disease, we used the multiple regression models as suggested by Lucas et al (1). First, birth weight was added to the model, to investigate the influence of birth size on the outcomes (model A). Secondly, birth weight was replaced by adult weight, to relate adult size to the outcomes. Thirdly, birth weight was added to the model. When birth size and adult size are shown in one model, this indirectly shows the change in weight (centile crossing) between birth and adulthood. Therefore, it is possible to investigate whether weight gain from birth to early adulthood is related to the outcomes. Finally, the interaction term birth weight * adult weight was added to the model to investigate whether birth weight modifies the effect of adult weight on outcome. This interaction term evaluated whether being growth retarded at birth and having weight gain during childhood is more serious in terms of outcome than having a normal birth size and an equivalent weight gain during childhood.

Part 1: Birth size, adult size and determinants of adult diseases

Insulin Sensitivity
Low birth weight has been associated with an increased risk for adult diseases, such as diabetes mellitus type II and CVD (2-5). This might indicate that malnutrition in fetal life could result in permanent endocrinologic and metabolic changes in the fetus with adverse effects in later life. However, rapid growth in childhood has also been related to increased insulin secretion and reduced insulin sensitivity (6).

We evaluated the contribution of birth size, adult size and body composition to insulin sensitivity and disposition index, measured by the frequently sampled intravenous tolerance (FSIGT) test, in 136 young adults. In addition, we evaluated if there were differences with regard to insulin sensitivity, insulin secretion and disposition index between 4 clinically recognizable subgroups of young adults.

Birth weight was positively associated with insulin sensitivity, but the association disappeared when adult weight and waist/hip ratio were taken into account. Adult weight, particularly when specified as current fat mass, was the only significant determinant of insulin sensitivity, whereas birth size was not related. As adjustments were made for birth size, these results indirectly show that weight gain, specified as fat accumulation, from birth to early adulthood was related to reduced insulin sensitivity. None of the variables were related to disposition index at early adulthood.

SGA-CU subjects had lower insulin sensitivity and higher insulin secretion than controls, which attenuated after adjustment for current fat mass, but remained significantly different. There were no other differences in insulin sensitivity between the subgroups, but SGA-S subjects had higher insulin secretion than controls, even after adjustment for fat mass. The disposition index did not differ between the subgroups.

Only one other study has found a positive relationship between weight gain during childhood and insulin sensitivity in young adults, independent of birth weight (7). However, this study did not specify whether lean body or fat mass was responsible for this relationship. Our results show that fat mass was robustly and inversely related to insulin sensitivity. These results are in accordance with the growth acceleration hypothesis, indicating that fetal growth restriction relative to genetic growth potential could result in growth acceleration postnatally, which has potentially deleterious effects on the development of adult diseases in later life (8,9). Based on our data, we suggest to take this hypothesis a step further: i.e. that an increased accumulation of fat during childhood, independent of birth size, will result in reduced insulin sensitivity.
Generally, an increase in fat mass has been related to metabolic and endocrinologic changes, which might have deleterious effects in later life with regard to an increased risk for CVD and type 2 diabetes (10).

Reduced insulin sensitivity in SGA-CU subjects has been reported before, but only in children (11). Our results show that this persists into early adulthood. Fat mass did not completely explain this relationship, so other factors seem to be involved as well. Genetic factors, like a higher frequency of carriers of the 22/23EK allele of a glucocorticoid receptor gene polymorphism and environmental factors, such as physical activity, might also influence the relationship (12,13). The disposition index was similar between the subgroups, which indicates that all subgroups had normal functioning β-cells and were therefore able to maintain glucose tolerance. To be able to maintain glucose tolerance when subjects are less insulin sensitive, the β-cells have to produce more insulin. However, when β-cells start to fail, the disposition index will decline. This will lead to impaired glucose tolerance and eventually type 2 diabetes. Our data showed that in early adulthood, SGA-CU are able to maintain their glucose tolerance, as shown by their increased insulin secretion compared to controls.

In prepubertal SGA-S children, reduced insulin sensitivity has been reported before, but this could not be confirmed in young adults (14-16). This might have changed during puberty, when insulin sensitivity physiologically decreases in all subjects (17), but maybe less in SGA-S subjects. SGA-S subjects had increased insulin secretion, which is hard to explain. Possible explanation are an increased sensitivity of the beta cells for glucose or an increased clearance of insulin by the liver. The higher insulin secretion did not affect their glucose tolerance, because it was similar to the other subgroups.

Conclusions

Our data show that fat accumulation during childhood is the main predictor of insulin sensitivity in early adulthood. Prenatal growth, reflected by birth size, was not related to insulin sensitivity. In early adulthood, SGA-CU subjects had reduced insulin sensitivity compared to controls, while all other subgroups had similar insulin sensitivity as controls. Reduced insulin sensitivity was found in only one of the two SGA subgroups, which enhances our conclusion that birth size is not related to insulin sensitivity in later life.

Body Composition

We investigated the influence of birth length and birth weight on fat mass (FM) and lean body mass (LBM), measured by Dual Energy X-ray Absorptiometry (DXA), in 312 young adults. Additionally, we investigated if there were differences in FM and LBM between four subgroups of young adults. Birth length was not related to FM (kg) or LBM (kg), while lower birth weight tended to be weakly related to higher FM and weakly with lower LBM. However, adult weight as well as weight gain during childhood were strongly related to FM and LBM, next to age, gender and adult height. SGA-CU subjects had a significantly higher %FM and a significantly lower %LBM than controls.

Our study shows that birth weight has opposite effects on FM and LBM. One explanation might be that subjects who were born after fetal growth retardation use their energy for their vital organs and do not use their spare energy for accumulation of LBM, but rather for FM as a buffer for difficult periods later in life. With regard to LBM, our results are in line with other reports showing a positive association with birth weight (4,18-25). Recent studies did not find a conclusive answer with regard to the relation between birth weight and FM (4,20-25). This might be explained by the different ways of measuring body composition, different study populations and different statistical approaches. We used DXA to measure body composition and analyzed our data with and without adjustments for adult size. When adult height was taken into account, attained adult weight had the strongest association with FM, indicating that relatively more weight gain than height gain during childhood is related to FM. Birth weight only showed a weak negative association with FM, while adult weight was strongly positively related. This suggests that prenatal growth has little influence on later FM, in contrast to weight gain during childhood.

SGA-S subjects showed similar body composition as controls, which is in contrast to two reports showing less FM in SGA-S children (26,27). This difference might be due to a different statistical approach or SGA-S subjects might accumulate more fat than controls during puberty. The SGA-CU group had relatively more FM and less LBM than controls, indicating that not birth size, but especially postnatal weight gain explains the differences in body composition. Previously, higher %FM and less %LBM have been reported in children and older adults with low birth weight, but no data existed for young adults (11,28,29). The results of the ISS subgroup showed that growth retardation during childhood was not related to a different body composition in early adulthood. Högl found a lower amount of LBM in growth retarded children, but this might be due to the absence of corrections for current height (30). Our results showed less LBM in ISS subjects, which disappeared after adjustment for adult height.
Conclusions

Prenatal growth had a weak relationship with body composition in early adulthood, while weight gain was strongly associated with adult FM and LBM. SGA born subjects with catch-up growth had relatively more fat and less LBM than controls, while SGA born subjects with short stature had a similar body composition as controls. Therefore, body composition in early adulthood is mainly determined by postnatal growth, particularly gain in weight, instead of prenatal growth.

Lipid Profiles

The influence of birth size, adult size, body composition and ApoE genotype on fasting serum levels of total cholesterol (TC), triglycerides (TG), HDLc, LDLc, ApoA-1, ApoB and Lp(a) was investigated in 297 young adults. We also investigated if there were differences in serum lipid levels between the 4 subgroups. Birth length and birth weight were not related to any of the lipid levels in early adulthood. However, weight gain from birth to early adulthood, specified as fat accumulation, was positively related to serum levels of TC, TG, LDLc and ApoB. In addition, ApoE genotype was a strong predictor of TC, LDLc and ApoB, explaining on average 7% of the variance. In the subgroup analyses, no differences in ApoE genotype or lipid levels were found, except for SGA-S, who had higher serum levels of LDLc and ApoB than controls. However, the difference in LDLc disappeared after adjustment for %body fat and ApoE genotype. The difference in ApoB between SGA-S and controls attenuated after these adjustments, but remained significantly different from controls. There were no differences in ApoE genotype prevalence between the subgroups.

Our results are in line with two studies, which reported that birth weight was not related with serum lipid profiles in later life (33,34). The literature is less consistent with regard to the relationship between birth length and lipid profiles (33-35). An inverse relation was found in children born SGA, although previous studies did not differentiate between SGA with and without catch-up growth (6,14,36,38,40). Only Jaquet et al. investigated lipid levels in a mixed group of SGA young adults. They found slightly but significantly lower levels of HDLc and higher TG levels in 734 SGA subjects versus 886 controls (41).

Conclusions

Our study shows that birth size is not related to serum lipid levels in early adulthood, while weight gain during childhood, specified as fat accumulation, is. Genetic factors, like ApoE genotype, have a strong influence on lipid levels. Our subgroup analyses showed that not SGA-CU subjects, but SGA-S subjects had higher levels of LDLc and ApoB, but this disappeared or attenuated after adjustment for %body fat and ApoE genotype.

Acylation Stimulating Protein (ASP)

Acylation stimulating protein (ASP) is an adipose tissue-derived hormone, which stimulates glucose and free fatty acid (FFA) uptake into adipocytes (42-44). ASP levels might predict an early stage of dyslipidemia because fasting ASP is a predictor of triglyceride (TG) clearance (45,46). Postnatal catch-up growth might be associated with dyslipidemia in later life, and dyslipidemia might, in time, contribute to CVD. We therefore investigated the relationship between ASP and different growth patterns during childhood. In addition, differences in serum ASP levels were investigated between the 4 subgroups.

Weight gain during childhood, particularly fat accumulation, was positively related to serum ASP levels in early adulthood. Size at birth was not related to ASP levels. ASP was positively related to TG, TC, ApoB and insulin secretion and negatively to insulin sensitivity. Between the subgroups, no differences in ASP were found, but SGA-CU and ISS subjects had significantly higher levels of FFA.

ASP resistance is characterised by increased levels of both ASP and TG (42,45). This reflexes reduced free fatty acid (FFA) trapping into adipocytes because ASP is less active. This results in an increased FFA influx in the liver, which will lead to increased levels of LDLc and ApoB, as seen in dyslipidemia. ASP levels were positively related to TG levels, and both levels were related to fat accumulation during childhood. This might indicate that fat accumulation during childhood is related to ASP resistance. These results are in line with recent reports, which...
showed that higher levels of ASP and TG are related to obesity and %body fat in children, adolescents and adults (42,47,48).

ASP levels were negatively related to insulin sensitivity and positively to insulin secretion. This might suggest a link between ASP and insulin secretion as insulin stimulates C3 production, which is an ASP precursor (49). Higher levels of ASP were also found in lean subjects who were less insulin sensitive, like lean patients with diabetes mellitus type II or women with polycystic ovary syndrome (50,51), which strengthens the postulation there is a link between ASP and insulin metabolism. The effects of ASP on FFA metabolism are independent and additive to the effects of insulin (43), so a direct link was not yet found.

ASP levels were similar among the four subgroups, while we expected higher levels of ASP in SGA-CU subjects because they had a higher %body fat. This might be due to the relatively small variance in %body fat, as other studies only showed differences in ASP levels in obese subjects and not in controls (48,52). SGA-CU and ISS subjects had higher FFA levels than controls. Only in SGA-CU subjects this might be explained by an increased activity of hormone-sensitive-lipase, because they are less insulin sensitive. In ISS subjects, FFA trapping might be reduced as ASP was non-significantly lower.

Conclusions
Exaggerated weight gain during childhood should be prevented as this might lead to ASP resistance, characterised by higher levels of ASP and TG. In time, this might lead to postprandial hypertriglyceridemia, which has been associated with early dyslipidemia.

Blood Pressure and Carotid Intima Media Thickness (IMT)
Both blood pressure and carotid IMT are accurate predictors of cardiovascular events later in life (53-56). We, therefore, evaluated the influence of birth size, adult size and body composition on systolic (SBP), diastolic blood pressure (DBP) and carotid IMT in 243 young adults. We also investigated if there were differences in SBP, DBP and carotid IMT between the four clinically recognizable subgroups. Both blood pressure and carotid IMT were not related to birth length or birth weight. Fat accumulation during childhood was related to higher systolic and higher diastolic blood pressure in early adulthood, but not to carotid IMT. Comparison of the subgroups showed no difference in SBP or DBP, but carotid IMT was larger in SGA-CU than in controls.

The relationship between birth size and subsequent blood pressure is probably the most investigated association based on the ‘fetal origin’ hypothesis (57-61). Nevertheless, clarity is still lacking due to the impact of random error, selective emphasis of particular results and inappropriate adjustment for current weight and confounding factors (62). To correctly evaluate this association, we used the statistical approach as suggested by Lucas et al and adjusted for several important confounders (1). Our results showed that fat accumulation during childhood influenced SBP and DBP in early adulthood, while birth size was not related. This is in line with other studies, which previously showed that weight gain in early life or childhood was positively related to subsequent blood pressure (63,64), although no specification was made between lean body mass or fat mass.

Carotid IMT showed no association with birth size, but in contrast to our expectations, also no association was found with adult weight, weight gain or % body fat. Previous studies reported that an increase in BMI during childhood was associated with carotid IMT in adulthood, but they were unable to investigate whether there was a direct relation between carotid IMT and fat mass (65,66). We did not find a relation between carotid IMT and fat mass and that might be due to the small range of fat mass or relatively young age in our study population. We also found a correlation between artery diameter and carotid IMT, so we adjusted for artery diameter. No previous studies have taken artery diameter into account. This could explain the differences in study results.

Both subgroups born SGA did not have a higher blood pressure, which is in line with the multiple regression analyses showing that a lower birth weight does not increase the risk for higher blood pressure. No differences in SBP and DBP were seen between the four subgroups. This is probably due to the relatively small differences in fat mass between the subgroups, as the multiple regression analyses showed that fat mass was a predictor of blood pressure. Differences were seen in carotid IMT between the subgroups; SGA-CU had larger carotid IMT than controls. Oren et al previously reported that young adults with the lowest birth weight and the highest postnatal growth had a larger carotid IMT than young adults with normal birth size and postnatal growth (67). Our results indicate that prenatal growth does not influence carotid IMT in adulthood. If prenatal growth had an influence on carotid IMT, both SGA-S and SGA-CU subjects would have had a larger carotid IMT in early adulthood, instead of only SGA-CU subjects. This also indicates that other factors might influence carotid IMT in SGA-U subjects, like inflammatory factors or oxidative stress (68,69).

Conclusions
More fat accumulation during childhood leads to a higher blood pressure in early adulthood, while size at birth is not related to blood pressure. SGA-CU subjects had a larger carotid IMT
than controls, while SGA-S subjects had similar carotid IMT as controls. Therefore, postnatal weight gain is more important than prenatal growth in the development of a thicker intima media of the carotid arterial wall.

**Bone Mineral Density (BMD)**

We investigated in 312 young adults whether birth size, adult size and body composition, determined by DXA, were related to bone mineral density (BMD) in early adulthood. Current weight, specified as lean body mass and fat mass, and weight gain during childhood were positively related to BMD of the total body (TB) and the lumbar spine (LS). Birth length was not related to BMD (TB + LS) and birth weight was inversely related to BMD (LS), which disappeared after adjustment of weight gain. Comparison of the subgroups showed that after adjustments for adult size and body composition, ISS subjects had a lower BMD (TB) than controls. There were no differences in BMD (LS) and bone mineral apparent density (BMAD) between the subgroups.

Several reports showed that birth weight has no relationship with BMD (TB) (20,70-73), but no study investigated the relationship between birth length and BMD (TB), while it has been reported that shorter birth length might be related to a higher fracture risk (74). Birth weight was inversely related to BMD (LS) but this relationship disappeared after adjustment for weight gain during childhood. Therefore, weight gain has a greater influence on BMD (LS) than birth weight, which is in line with the literature (70,71,73,75).

Previously, Arends et al. reported lower BMD (TB) in 3-8 years old SGA-S children (76), but did not take current weight into account. Our results did not show any difference in BMD (TB + LS) between SGA-S and controls, but this difference might be due to the fact that we did correct for adult weight.

The SGA-CU subjects had also a similar BMD (TB) in 3-8 years old SGA-S children (76), but did not take current weight into account. Our results did not show any difference in BMD (TB + LS) between SGA-S and controls, but this difference might be due to the fact that we did correct for adult weight.

Conclusions

Postnatal growth, especially weight gain during childhood, determines BMD (TB + LS) in early adulthood, independent of birth size. Subjects with a reduced growth rate during childhood are at risk for lower BMD (TB), what might result in increased fracture risk in later life.

**Part 2: Growth in early life and determinants of adult diseases**

**Timing and Tempo of Catch-up Growth in Early Life**

It has been debated whether weight gain in early life, or poor growth in early life followed by catch-up growth between 2 and 10 years is related to increased risk for cardiovascular and metabolic diseases in later life (9,79-86). We investigated in 217 young adults whether a certain period in the first year of life was related to determinants of CVD and type 2 diabetes in early adulthood. Additionally, we evaluated whether the tempo of catch-up growth in weight was related to these determinants.

Our results showed that increased weight gain relative to height gain in the first three months of life associates with reduced insulin sensitivity and serum HDL, and an increased waist circumference, acute insulin response, cholesterol / HDL ratio and serum triglyceride in early adulthood, which are all important determinants of CVD and type 2 diabetes in later life. A subgroup analysis showed that of all subjects with first year catch-up in weight (> 0.67 SDS), those with fast catch-up growth in weight in the first three months of life had a significantly higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood than those with a more gradual catch-up growth. The differences in fat distribution and insulin sensitivity between the subgroups were explained by the % body fat.

Barker et al. postulated the fetal origin hypothesis, suggesting that an unfavourable fetal environment might lead to re-programming of the endocrine and metabolic pathways of the fetus, leading to the development of CVD and type 2 diabetes in later life (87,88). Recently, an increasing number of studies show a positive relationship between postnatal catch-up growth in early life and risk factors of CVD and type 2 diabetes in childhood and early adulthood (9,58,80-82,86,89). Previously, we showed that SGA born subjects with catch-up growth in weight had a higher risk for CVD or type 2 diabetes, while SGA born subjects without catch-up growth in weight did not have an increased risk. This indicates that having a low birth weight for gestational age is not directly related with an unfavourable cardiovascular and metabolic profile, but increased weight gain relative to height gain during early childhood is.
Our results show that particularly very early weight gain is positively related to several determinants of CVD in early adulthood, but other periods in life might be important as well. Previous studies showed that subjects with a slow gain in weight from birth to two years followed by catch-up in weight during childhood also had an increased risk for development of CVD and type 2 diabetes (79,90-92). Just like our subjects, who showed growth retardation in fetal life, these subjects also experienced catch-up in weight after a period of slow growth. As both groups showed catch-up growth in weight, this might indicate that catch-up in weight after a period of growth retardation, either during intra-uterine or early life, increases the risk for the development of CVD and type 2 diabetes in later life, independently of the period of catch-up growth.

As tempo of catch-up growth might be more important than the actual time period, factors influencing rapid weight gain in early life warrant investigation. So far, breast-fed infants appear to have slower weight gain than bottle-fed infants and infants with nutrient-enriched diets even have increased weight gain (9,36,93-95). The exact mechanism how nutrients influence growth and weight gain needs to be unravelled.

Conclusions
Weight gain relative to height gain in the first three months of life is related to an unfavourable cardiovascular and metabolic profile in early adulthood. Thereby, fast catch-up growth in the first three months of life appears to be more detrimental than a more gradual catch-up growth over 1 year. This implicates that rapid weight gain during the first three months should be prevented.

Nutrient Intake and Body Size at 12 Months of Age
In 396 infants of the Cambridge Baby Growth Study, aged 12 months, we investigated the associations between infant energy, protein and fat intakes on body size and adiposity, measured by skinfolds, at age 12 months. In particular we tested the hypothesis that dairy milk protein intake (from dairy protein-based infant formula milk and cow’s milk) might be specifically associated with infant adiposity. Nutrient intakes were assessed by three-day diet records. Total energy intake at 12 months was positively related to length, weight, and BMI at 12 months, but not to adiposity. Total protein and total fat intakes were not related to any anthropometric outcome. However, dairy milk protein intake was positively related to adiposity at 12 months, and this association persisted after adjustment for other nutritional factors. Both protein from dairy protein-based infant formula milk and protein from cow’s milk showed similar trends with adiposity at 12 months.

Previously, Rolland-Cachera et al postulated a hypothesis that higher protein intakes in early life might causally increase fat mass by stimulating the secretion of IGF-1 and insulin, which in turn promote adipocyte maturation and lipid uptake storage (96-99). Several reports of the DONALD study have repeatedly shown a positive association between protein intake and adiposity in childhood in 203 German children (100-102). However, other studies did not find a relationship between total protein intakes in early life and fat mass at age 10 years or early adiposity rebound (103,104). Furthermore, Skinner et al. showed that total fat intake, but not total protein intake, at 2 years was positively associated with BMI at 8 years (105). However, this could not be confirmed by other studies (97,106-108).

Although we found that dairy milk protein intake was positively related to adiposity at 12 months, we must recognize that it is possible that the observations are residually confounded by other nutritional factors, including other components of infant milk formula and cow’s milk, or by parenting patterns associated with the use of these milks. Only one previous study reported an apparent positive association between infant dairy milk protein intake and adiposity, but they did not account for potential confounding by other related dairy nutrients (102).

Conclusions
Infant dairy milk protein intake, from both infant formula and cow’s milk sources, was positively associated with adiposity at age 12 months.

Higher intakes of formula milk at 4 months have also been related to larger body weight and BMI at ages 1 to 5y (81). Together with our data, this suggests that reduction of dairy milk intake might lead to less weight gain and less adiposity in early life.

General conclusions, implications and directions for future research
In the early 1990s, Barker et al. opened a new field of research by reporting a relationship between low birth weight and CVD and type 2 diabetes (2,5,87,88). Since, many studies have investigated this relationship and showed conflicting results. Singhal and Lucas formulated a new hypothesis in 2004, postulating that fetal growth restriction relative to genetic growth potential could result in growth acceleration during childhood, which is responsible for the increased risk for adult diseases in later life (8). In the PROGRAM-study, a cohort of 322 young adults,
we found that fat accumulation during childhood was related to reduced insulin sensitivity, an increase in serum levels of TC, TG, LDLc, ApoB and ASP, and systolic and diastolic blood pressure in early adulthood, independent of size at birth. We were therefore able to specify the hypothesis of Singhal and Lucas and postulated that increased accumulation of fat during childhood, independent of birth size, will result in an increased risk for development of CVD and type 2 diabetes (Figure 1). Weight gain during childhood was related to higher fat mass, lean body mass and bone mineral density(TB+LS) in early adulthood. Size at birth was not related to bone mineral density, but lower birth weight was moderately related to lower lean body mass and higher fat mass in early adulthood. However, the effect of weight gain during childhood was greater, indicating its importance. In conclusion, prenatal growth, reflected by size at birth seems to be of less influence compared to weight gain during childhood.

![Figure 1. Specified growth acceleration model of Singhal et al.](image)

Additionally, we investigated whether there was a sensitive time-window for weight gain in early life influencing determinants of CVD and type 2 diabetes, because this might be an option to intervene and start prevention programs. Our results show that more weight gain in the first three months of life related to reduced insulin sensitivity and serum HDL, and an increased waist circumference, acute insulin response, cholesterol / HDL ratio and serum triglyceride in early adulthood. Additionally, our results showed that of all subjects with first year catch-up in weight (> 0.67 SDS), those with fast catch-up in weight in the first three months of life had a significantly higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood than those with slower catch-up in weight. Therefore, rapid weight gain in early life should be prevented to reduce the risk for CVD and type 2 diabetes in later life. Future research should focus on which factors influence rapid infant weight gain. Secondly, intervention trials are required to investigate which intervention might prevent this rapid early weight gain.

It is clear that nutrition is a contributor to rapid weight gain, as breast-fed infants gain slower weight than formula-fed infants (93,94). Although it has been thought that this is due to the high protein content of formula milk, evidence is not conclusive. In our population-based cohort of 396 infants, higher intakes of dairy milk protein, cow’s milk protein and formula milk protein, were related to higher adiposity at 12 months. This indicates that reduced intakes of dairy milk might prevent rapid infant weight gain. Breast milk would be a good alternative for dairy milk. The observations might be residually confounded by other nutritional factors, including other components of infant milk formula and cow's milk, so it would be very interesting to perform intervention study to confirm whether intakes of dairy milk protein or intakes of other nutrients are responsible for this association.

Although we investigated the relation between nutrient intake and body composition in early infancy, this relationship needs to be investigated into childhood or even into adulthood. Food patterns may change throughout life and could therefore change the risk for adiposity in life. Another important factor influencing the risk for CVD and diabetes type 2 is the level of physical activity (109,110). Unfortunately, no data was collected with regard to physical activity, which might be considered as a limitation of the study. Another limitation is that there were no data on family history of cardiovascular disease and diabetes type 2 as well as maternal characteristics during pregnancy.

For clinical relevance, we assigned the study participants into four subgroups, based on growth during their fetal life and childhood. Although studies in SGA-S children showed that they might have an increased risk for CVD and type 2 diabetes (14), we could not confirm these results in young adults. We only found higher levels of ApoB compared to controls, while other lipid levels, insulin sensitivity, blood pressure and carotid IMT were similar to controls. Therefore, we conclude that subjects with a small size at birth who remain relatively short into adulthood do not have an increased risk for development of CVD or type 2 diabetes. On the other hand, we acknowledge that we investigated a relatively small group of SGA-S subjects and therefore other studies have to be performed to confirm our results. In addition, follow-up
studies need to investigate if the risk factors alter differently in SGA-S subjects compared to controls in later life, as we investigated these subjects at a relatively young age.

The results were different for subjects born SGA with catch-up growth during childhood. They had reduced insulin sensitivity, higher %body fat and greater carotid IMT than controls. This indicates that SGA-CU subjects might be at risk for cardiovascular events and type 2 diabetes in later life. Lipid levels and blood pressure were similar to controls, but these risk factors might start to differ from controls in the years to come. Recently, a study in a large French cohort of SGA adults showed a larger increase in BMI from 22 to 30 years in SGA born adults compared to AGA born adults (111). Therefore, follow-up studies have to be performed to investigate how these risk factors develop and whether these subjects have more cardiovascular events in later life. The differences in insulin sensitivity and carotid IMT with controls were independent of %body fat, suggesting that other factors or mechanisms are responsible for these differences. It would be of interest to investigate which factors, genetic or environmental, could explain these differences.

Subjects born appropriate for gestational age with idiopathic growth retardation during childhood had only a lower BMD than controls, but other risk factors for adult disease were similar to controls. These results might be reassuring for the risk of CVD, however, not for the risk of osteoporosis. Our ISS subjects have been investigated in their early twenties, when BMD is highest in life. As BMD deteriorates in later life, ISS subjects might need to be evaluated in later life to determine if osteoporosis begins sooner and is more severe than in controls.

Although we investigated a wide range of determinants of adult disease, it would be interesting to investigate additional determinants. With regard to cardiovascular disease, alterations in coagulation, fibrinolysis and inflammation might influence the development of CVD (10). Whether these determinants already differ between SGA-CU subjects and controls in early adulthood is yet unknown. Previously, low birth weight has been related to impaired lung and immune function and lower risk of cancer in later life (112). Therefore, relationships between growth patterns and adult disease needs further investigation as it might lead to specific interventions, which help to reduce the risk of adult disease.

Finally, we have made a new hypothetical model based on the existing literature and the results of the studies described in this thesis (Figure 2).

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**Figure 2.** Hypothetical model showing the different growth patterns and their linkage with adult disease.

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The model in Figure 2 shows that subjects who experienced fetal growth restriction are born relatively small for their growth potential. About 90% of them will show catch-up growth in the first two years of life and those who experience fast catch-up in weight in the first three months of life accumulate more fat mass and have a higher risk for development of reduced insulin sensitivity, unfavourable body composition and lipid profile and increased blood pressure in later life. These factors are all important determinants of cardiovascular disease and diabetes type 2. This model is not complete, but might be seen as a starting point for additional research. Future research should try to focus on which factors determine the growth acceleration and influence fat accumulation during childhood to complement this model.
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Chapter 1
This chapter provides an introduction in the different hypotheses with regard to the influence of birth size and childhood growth on adult diseases and their determinants. The relationships between birth size and several determinants of adult diseases are explained, like insulin sensitivity, body composition, serum lipid profiles, acylation stimulating protein (ASP), blood pressure, carotid intima media thickness (IMT) and bone mineral density (BMD). The relationship between growth in early life and cardiovascular and metabolic profile is discussed, together with the influence of nutrition on body size and body composition in infancy. In addition, the definitions, prevalence and etiologies of small for gestational age (SGA) and idiopathic short stature (ISS) are described together with the study design. Finally, the aims and outline of the thesis are presented.

Chapter 2
Low birth weight has been related to reduced insulin sensitivity, but it was unknown whether this was due to prenatal or postnatal factors. There was very limited information whether subjects born SGA who remain short (SGA-S) or subjects born SGA with catch-up growth (SGA-CU) were at risk for type 2 diabetes in later life. We therefore investigated in 136 young adults the relative contribution of birth size, adult size, body composition and waist-to-hip ratio on insulin sensitivity and disposition index, measured by Frequently Sampled Intravenous Glucose Tolerance test (FSIGT). In addition, we investigated if there were differences between four clinically relevant subgroups, subjects born SGA who remain short (SGA-S), and those with postnatal catch-up growth (SGA-CU), subjects with idiopathic short stature (ISS) and controls.

Neither birth length nor birth weight, but the accumulation of fat mass from birth to early adulthood appeared to be the main predictor of insulin sensitivity in early adulthood. After correction for age, gender, and adult body size, insulin sensitivity was significantly lower in subjects born SGA with catch-up growth than in controls. None of the variables had a significant influence on disposition index, and there was no significant difference in disposition index between the subgroups.

In conclusion, fat accumulation during childhood predicts insulin sensitivity in early adulthood. Prenatal growth, reflected by birth size, was not significantly related to insulin sensitivity. In early adulthood, SGA-CU subjects had reduced insulin sensitivity compared to controls, while all other subgroups had similar insulin sensitivity as controls. Reduced insulin sensitivity was found in only one of the two SGA subgroups, which supports our conclusion that fat mass accumulation during childhood is a major determinant of insulin sensitivity in later life.

Chapter 3
Several studies investigated the relationship between birth size and later body composition, but due to great differences in measuring body composition, different study populations and different statistical approaches, it remained hard to compare these studies. Short children born SGA have a typical lean appearance, while other reports showed that subjects born SGA with catch-up growth developed adiposity in early life. It was unknown if these differences in body composition persisted into adulthood. We investigated the relationship between birth size, adult size and body composition in 312 young adults. Body composition was measured by DXA.

Birth weight was inversely related to fat mass, but was not related to lean body mass. However, the influence of adult weight on fat mass and lean body mass was greater than birth weight, indicating that weight gain from birth to early adulthood was the main determinant of body composition in early adulthood. These results were confirmed by the results of the subgroup analyses, showing that SGA-CU subjects had relatively greater fat mass and less lean body mass than controls. SGA subjects who remained short as adults had a similar body composition as controls. Our results indicate that too much weight gain during childhood should be prevented as it may lead to an unfavorable body composition in later life. Intervention programs should focus on postnatal growth in weight as its effect on body composition is greater than that of prenatal growth.

Chapter 4
An unfavorable lipid profile increases the risk for cardiovascular disease and cardiovascular events in later life. No consistency was found with regard to the relationship between low birth weight and serum lipid profiles. If low birth weight would affect lipid levels in later life, this should be present in SGA subjects. No study found increased serum lipid levels in subjects born SGA, but no study differentiated between subjects born SGA with and without catch-up growth. This chapter describes the influence of birth size, adult size, body composition and ApoE genotype on fasting serum levels of total cholesterol (TC), triglycerides (TG), HDLc, LDLc, ApoA-1, ApoB and Lp(a) in 297 young adults. We also investigated if there were differences in serum lipid levels between the 4 subgroups.

Gender, ApoE genotype, adult height and adult weight, specified as fat mass, were significant determinants of serum lipid levels in early adulthood, while prenatal growth, reflected by birth
Summary

Weight gain during childhood, particularly fat accumulation, was positively related to serum ASP levels in early adulthood. Birth size was not related. ASP metabolism is positively related to insulin regulation and lipid metabolism. In early adulthood ASP metabolism was similar between the four clinically relevant subgroups.

Chapter 6
This chapter describes the relationship between birth size and blood pressure as well as carotid intima media thickness (IMT) in early adulthood. Blood pressure and carotid IMT are both highly correlated with cardiovascular events in later life. Development of higher blood pressure and greater carotid IMT in early life might increase the risk for cardiovascular events in later life. The relationship between birth size and blood pressure is one of the most investigated relationship within the 'fetal origin hypothesis', but controversy still remained. In SGA subjects, no study investigated carotid IMT. As SGA-CU subjects have reduced insulin sensitivity and an unfavourable body composition compared to controls, differences might be found in carotid IMT between SGA-CU subjects and controls. We investigated the relationship between growth patterns and blood pressure and carotid IMT in 243 young adults.

Birth size was not related to systolic blood pressure, diastolic blood pressure or carotid IMT. However, accumulation of fat mass from birth to early adulthood was related to systolic and diastolic blood pressure, but not to carotid IMT. Comparison of subgroups showed no differences in blood pressure between the four subgroups, but SGA-CU subjects had a greater carotid IMT than controls. This indicates that postnatal catch-up growth, and not prenatal growth, influences development of greater carotid IMT as SGA-CU subjects and not SGA-S subjects had higher carotid IMT. To diminish the risk for hypertension in later life, fat accumulation during childhood should be prevented.

Chapter 7
Osteoporosis is a condition characterized by reduced bone mineralization, resulting in an increased fracture risk. Although some studies showed a positive association between birth weight and bone mineral density (BMD), hardly any study had taken adult size into account and most of the subjects being studied were elderly. We therefore investigated in 312 young adults whether birth size, adult size and body composition were related to BMD in early adulthood.

Current weight, specified as lean body mass and fat mass, and weight gain during childhood were positively related to BMD of the total body (TB) and the lumbar spine (LS). Birth length was not related to BMD (TB) and birth weight was inversely related to BMD (LS), which disappeared after adjustment for childhood weight gain. This indicates that the influence of weight gain during childhood is greater than the influence of prenatal growth, reflected by birth weight. Comparison of the subgroups showed that after adjustments for adult size and body composition, ISS subjects had a lower BMD (TB) than controls. There were no differences in BMD (LS) and bone mineral apparent density (BMAD) between the subgroups.

In conclusion, weight gain during childhood determines BMD (TB+LS) in early adulthood, while birth weight hardly influences BMD (TB+LS). Birth length was not related to BMD (TB+LS) in early adulthood. In addition, subjects with a reduced growth rate during childhood are at risk for lower BMD (TB), what might result in increased fracture risk in later life.
Chapter 8

First year growth seems to be an important period in the development of cardiovascular disease and type 2 diabetes. Some even suggested that in this period a special ‘time window’ might be applicable. However, it remained unclear whether slow or fast growth in early life has an adverse effect on determinants of CVD. Some studies showed that slow growth in the first two years followed by rapid growth in childhood was related to an increased risk for cardiovascular events. Other studies showed that rapid weight gain in the first 3 to 6 months was related to increased fat mass in childhood and a disadvantageous metabolic profile in early adulthood. We investigated the relation between first year growth and an extensive set of cardiovascular and metabolic determinants in 217 young adults. In addition, we investigated whether tempo of catch-up growth was related to these determinants of CVD and type 2 diabetes.

Our data showed that increased weight gain relative to height gain in the first three months of life was associated with reduced insulin sensitivity and serum HDL, and an increased waist circumference, acute insulin response, cholesterol / HDL ratio and serum triglyceride in early adulthood. To investigate whether tempo of catch-up growth in the first year influenced the determinants, we made two subgroups out of all subjects with first year catch-up growth (> 0.67 SDS). Those with fast catch-up growth in the first three months of life (> 0.5 SDS) had a significantly higher % body fat, more central adiposity and reduced insulin sensitivity in early adulthood than those with a more gradual catch-up growth. The differences in fat distribution and insulin sensitivity between the subgroups were explained by the % body fat.

In conclusion, exaggerated weight gain relatively to height gain in the first three months should be prevented as it is related to several determinants of CVD and type 2 diabetes in early adulthood. In addition, slower weight gain in the first three months results in a more favorable metabolic profile than rapid weight gain in the first three months.

Chapter 9

Catch-up growth in early life seems to result in an increased fat mass in childhood, which is being tracked into adulthood. Factors influencing catch-up growth in early life need to be established and nutrition is one of these factors. Breast-fed infants have a slower weight gain than formula-fed infants and suggestions were made that this might be due to the higher protein content of formula milk. Others suggested that higher intakes of dairy milk, which is also high in protein content, were associated with increased weight gain and adiposity in childhood. We investigated in 396 infants of the Cambridge Baby Growth Study, the associations between infant energy, protein and fat intakes on body size and adiposity, measured by skin folds, at age 12 months. In particular we tested the hypothesis that dairy milk protein intake (from dairy protein-based infant formula milk and cow’s milk) might be specifically associated with infant adiposity.

Higher total energy intakes were related to higher length, weight and BMI at 12 months after adjustment for demographic and nutritional factors. There was no relation with adiposity. However, dairy milk protein intake was positively related to adiposity at 12 months, and this association persisted after adjustment for other nutritional factors. Both protein from dairy protein-based infant formula milk and protein from cow’s milk showed similar trends with adiposity at 12 months. Despite these associations, we must recognize that it is possible that the observations are residually confounded by other nutritional factors, including other components of infant milk formula and cow’s milk, or by parenting patterns associated with the use of these milks. These results indicate that reduction of dairy milk intake, from both infant formula and cow’s milk sources, might lead to less weight gain and less adiposity in early life.

Chapter 10

In the general discussion, our findings are discussed in relation to the current literature. Finally, our conclusions are described together with general considerations and directions for future research.
**Hoofdstuk 1**
In dit hoofdstuk worden verschillende hypotheses uitgelegd met betrekking tot de invloed van intra-uteriene groei en groei tijdens de kindertijd op ziektene die op oudere leeftijd optreden. Er wordt ingegaan op de relatie tussen grootte bij de geboorte en verschillende determinanten van deze ziektene, zoals insulinengevoeligheid, lichaamsafmetingen, serum lipidenprofielen, acylation stimulating protein (ASP), bloeddruk, de intima media dikte (IMT) van de arteria carotis en de bonthoekdichtheid (BMD). Vervolgens wordt de relatie tussen groei in het eerste jaar en het cardiovasculaire en metabole profiël op jong volwassen leeftijd besproken, samen met de invloed van voeding op de lichaamsgrootte en lichaamsafmetingen in het eerste jaar. De definities, prevalenties en etiologie van te klein zijn bij de geboorte (small for gestational age (SGA)) en idiopatische kleine gestalte (ISS) worden besproken, net als de studie opzet. Als laatste worden de doelstellingen van de studies vermeld, zoals die in dit proefschrift zijn beschreven.

**Hoofdstuk 2**
Verschillende studies lieten een relatie zien tussen een laag geboortegewicht en verminderde gevoeligheid voor insuline. Echter, het bleef onduidelijk of dit kwam door prenatale of postnatale factoren. Bij de start van de studie was het nog onduidelijk of SGA geboren mensen met een te kleine volwassen lengte (SGA-S) of juist SGA geboren mensen met inhaalgroei (SGA-CU) een verhoogde kans hadden op diabetes type 2 in het latere leven. Prenatale factoren. Bij de start van de studie was het nog onduidelijk of SGA geboren mensen met een verhoogde kans hadden op diabetes type 2 in het latere leven. Daarom werd in 136 jong volwassen onderzocht wat de bijdrage is van de lengte en het gewicht bij de geboorte, lengte en gewicht op de volwassen leeftijd, alsmede de lichaamsafmetingen en buik-heup ratio op insulinengevoeligheid en dispositie index (een maat voor de glucose homeostase), gemeten met behulp van Dual energy X-ray Absorptiometry (DXA).

De conclusie is dat meer vetaccumulatie van de geboorte tot aan jong volwassen leeftijd de belangrijkste determinant is van insulinengevoeligheid. Op jong volwassen leeftijd hadden SGA-CU mensen een verlaagde gevoeligheid voor insuline ten opzichte van controles en de andere subgroepen hadden een vergelijkbare insulinengevoeligheid dan de controles. Verminderde insulinengevoeligheid werd alleen aangetoond in een van de twee SGA subgroepen, hetgeen onze conclusie ondersteunt dat de grootte bij de geboorte niet geassocieerd is met de insulinengevoeligheid in het latere leven.

**Hoofdstuk 3**
De relatie tussen grootte bij de geboorte en de lichaamsafmetingen later in het leven is herhaaldelijk onderzocht. Maar door de grote verschillen in de manier van meten, verschillende studie populaties en verschillende statistische benaderingen blijft het moeilijk deze studies met elkaar te vergelijken en zodoende een conclusie te vormen over deze relatie. In SGA geboren kinderen die klein blijven wordt aangetoond dat ze een typisch mager voorkomen hebben, terwijl in SGA geboren kinderen met inhaalgroei werd aangetoond dat ze relatief vet zijn, zelfs al op jonge leeftijd. Voor de start van de studie was het nog onduidelijk of deze verschillen in lichaamsafmetingen blijvend zouden zijn tot in volwassenheid. Wij hebben de relatie tussen lengte en het gewicht bij de geboorte, lengte en gewicht op de volwassen leeftijd en de lichaamsafmetingen onderzocht in 312 jong volwassenen. De lichaamsafmeting werd gemeten met behulp van Dual energy X-ray Absorptiometry (DXA).

Geboortegewicht was negatief gerelateerd met vetmassa, maar was niet gerelateerd aan spiermassa. Echter, de associatie tussen het volwassen gewicht en de vetmassa met de spiermassa was groter dan die tussen het geboortegewicht en lichaamsafmetingen, wat laat zien dat de gewichtstoename van geboorte tot jong volwassen leeftijd de belangrijkste determinant is van de lichaamsafmetingen op jong volwassen leeftijd. De resultaten werden bevestigd door de resultaten in de subgroep analyse. Deze lieten zien dat SGA-CU mensen relatief meer vetmassa en minder spiermassa hebben dan controles. SGA-S jong volwassenen bleken een vergelijkbare lichaamsafmeting te hebben als controles. Deze resultaten implimeren dat teveel gewichtstoename tijdens de kindertijd voorkomen dient te worden, aangezien dit kan leiden tot een ongunstigere lichaamsaanpassing op jong volwassen leeftijd. Interventie programma's om een ongunstigere lichaamsaanpassing te voorkomen zouden zich voornamelijk op de postnatale gewichtstoename moeten focussen in plaats van op de prenatale groei, aangezien daar meer preventief effect te verwachten valt.
Hoofdstuk 4
Een ongunstig lipiden profiel verhoogt het risico van hart- en vaatziekten alsmede het risico van een hartaanval of een beroerte in het latere leven. Momenteel is er nog geen consensus bereikt over het feit of de grootte bij de geboorte invloed heeft op het lipiden profiel. Als een laag geboortegewicht een effect zou hebben op het lipiden profiel, dan zou men een ongunstig lipiden profiel verwachten bij mensen die SGA geboren zijn. Geen enkele studie heeft afwijkende lipiden spiegels gevonden bij SGA geboren mensen, maar deze studies hebben ook geen onderscheid gemaakt tussen SGA-S en SGA-CU. Dit hoofdstuk beschrijft de invloed van geboortegewicht en -grootte, volwassen lengte en gewicht, lichaamscomposiet en ApoE genotype op nuchtere serum waarden van totaal cholesterol (TC), triglyceriden (TG), HDLc, LDLc, ApoA-1, ApoB en Lp(a) in 297 jong volwassenen. Tevens werd onderzocht of er verschillen waren in serum lipiden spiegels tussen de vier klinisch relevante subgroepen.

Hoofdstuk 5
Acylation stimulating protein (ASP) is een door vetcellen geproduceerd hormoon dat de opname van glucose en vrije vetzuren (afkomenst van triglyceriden) in vetcellen bevordert. In fysiologische condities leidt een hogere serum ASP waarde tot een verlaging van de vrije vetzuur opname van glucose en vrije vetzuren (afkomenst van triglyceriden) in vetcellen bevordert. In het ontwikkelen van een hogere bloeddruk en een dikkere cIMT bij SGA-CU mensen men kan het risico van een cardiovasculair event in het latere leven verhogen. De relatie tussen grootte bij de geboorte en bloeddruk is een van de meest onderzochte relaties binnen de “fetal origin hypothesis”, maar er is nog steeds geen consensus bereikt. In SGA geboren mensen was nog geen studie verricht naar de cIMT. Aangezien SGA-CU mensen een verlaagde insulinegevoeligheid en een ongunstigere lichaamscomposiet hebben dan controles, zou men wellicht een dikkere cIMT bij SGA-CU mensen kunnen aantreffen ten opzichte van controles. We hebben de relatie tussen groeipatronen en bloeddruk en cIMT onderzocht in 243 jong volwassenen.

Grootte bij de geboorte en bloeddruk en cIMT onderzocht in 243 jong volwassenen. De conclusie is dat relatief teveel gewichtstoename tijdens de kindertijd kan leiden tot veranderingen in de ASP regulatie en vervolgens ook tot veranderingen in het vrije vetzuur metabolisme. ASP blijkt gerelateerd aan insuline secretie en –gevoeligheid alsmede aan het lipiden metabolisme. Er waren geen verschillen in ASP waarden tussen de vier verschillende subgroepen.
Hoofdstuk 7
Osteoporose is een aandoening die wordt gekarakteriseerd door een verminderde bot mineralisatie. Dit resultert in een verhoogd risico op botbreuken. Enkele studies hebben een positieve relatie aangetoond tussen grootte bij de geboorte en botdichtheid op latere leeftijd. Echter, veel van deze studies hielden geen rekening met de grootte van de volwassenen en de meeste studies werden verricht op ouderen. Daarom onderzochten wij bij 312 jong volwassenen of lengte en het gewicht bij de geboorte, lengte en gewicht op de volwassen leeftijd en de lichaamscompositie gerelateerd waren aan botdichtheid in jong volwassenen.

Huidig gewicht, zowel spiermassa als vetmassa, en gewichtstoename gedurende de kindertijd waren positief gecorreleerd met de botdichtheid van het hele lichaam (BMD(TB)) en van de lumbale wervelkolom (BMD(LS)). Geboortelengte was niet gerelateerd aan BMD(TB), maar geboortegewicht was negatief gerelateerd aan BMD(LS). Deze associatie verdween na correctie voor demografische factoren. Dit suggereert dat de invloed van gewichtstoename gedurende de kindertijd een grotere invloed heeft dan de invloed van prenatale groei, gerealiseerd door geboortelengte en -gewicht. Vergelijking van de subgroepen liet zien dat na correctie voor volwassen lengte, volwassen gewicht en de lichaamscompositie, ISS mensen een lagere BMD(LS) hadden dan controles. Er waren tussen de subgroepen geen verschillen in BMD(LS) gecorrigeerd voor lengte.

De conclusie is dat gewichtstoename tijdens de kindertijd de BMD(TB+LS) op jong volwassen leeftijd beïnvloedt, terwijl de invloed van geboortegewicht klein is. Enkele studies hadden bij ISS mensen een lagere BMD(LS) dan bij ISS-rijpers. Dit suggereert dat de invloed van gewichtstoename gedurende de kindertijd een grotere invloed heeft dan de invloed van prenatale groei, gerealiseerd door geboortelengte en -gewicht. De verschillen in BMD(LS) tussen ISS-rijpers en ISS-volwassenen waren negatief gerelateerd aan lengte en gewicht op de geboorte. Deze associatie verdween na correctie voor lengte.

Hoofdstuk 8
Groei in het eerste levensjaar blijkt een belangrijke periode te zijn in de ontwikkeling van hart- en vaatziekten en diabetes type 2. Echter, het was onduidelijk of langzame of juist snelle groei in het eerste levensjaar gerelateerd was aan deze determinanten. De conclusie is dat gewichtstoename gedurende de kindertijd een grotere invloed heeft dan de invloed van prenatale groei, gerealiseerd door geboortelengte en -gewicht. De verschillen in BMD(LS) tussen ISS-rijpers en ISS-volwassenen waren negatief gerelateerd aan lengte en gewicht op de geboorte. Deze associatie verdween na correctie voor lengte.

Teveel gewichtstoename ten opzichte van lengtetoonvalue in de eerste drie maanden moet worden voorkomen omdat dit gerelateerd is aan meer centrale vetdistributie en verminderde insulinegevoeligheid. Het blijkt dat een relatief kleine groeiverstoring, zoals het ontbreken van een lagere BMD(LS) bij ISS mensen aan de Большое русское слово, ten opzichte van het lagere BMD(LS) bij ISS-rijpers, is gerelateerd aan de inhaalgroei in de eerste 3 tot 6 maanden. De verschillen in BMD(LS) tussen ISS-rijpers en ISS-volwassenen waren negatief gerelateerd aan lengte en gewicht op de geboorte.}

Een verhoogde kans op botbreuken op latere leeftijd. Anderen vermeldden dat juist snelle inhaalgroei in de eerste 3 tot 6 maanden gerelateerd is aan een verminderde insulinegevoeligheid en een toename in buikomtrek, cholesterol/HDLc ratio en serum TG waarden op jong volwassen leeftijd. Om te onderzoeken of de snelheid van de inhaalgroei in het eerste levensjaar deze determinanten beïnvloedt, werden 2 subpopulaties gemaakt van alle mensen die inhaalgroei hadden vertoond in deze periode (> 0.67 SDS). De mensen met snelle inhaalgroei in de eerste 3 maanden (> 0.5 SDS) hadden een significant hoger vetpercentage, een meer centrale vetdistributie en verminderde insulinegevoeligheid op jong volwassen leeftijd ten opzichte van de mensen met een langzame inhaalgroei in de eerste 3 maanden. De verschillen in insulinegevoeligheid en vetdistributie konden verklard worden door het verschil in vetpercentage op jong volwassen leeftijd.

Hoofdstuk 9
Vroege inhaalgroei in gewicht lijkt te resulteren in een toegenomen vetmassa in de kindertijd, die persisteert in volwassenheid. De invloed van verschillende factoren die de vroege inhaalgroei beïnvloeden wordt daarom toenemend onderzocht. Een van die factoren is al bekend en dat is voeding. Kinderen die borstvoeding krijgen hebben een langzamere groeiverstoring dan kinderen die flesvoeding krijgen. Kinderen met flesvoeding krijgen een lagere BMD(LS) dan kinderen die borstvoeding krijgen. Kinderen met flesvoeding hebben een lagere BMD(LS) dan kinderen die borstvoeding krijgen. Kinderen met flesvoeding hebben een lagere BMD(LS) dan kinderen die borstvoeding krijgen.
met de hoeveelheid vet. Echter, een hogere inname van koemelk- eiwitten was wel gerelateerd aan meer lichaamsvet op 1 jaar en deze relatie bleef significant na correctie voor mogelijke confounders zoals totale energie inname. Zowel koemelk- eiwit van flesvoeding als van koemelk lieten dezelfde trend zien met betrekking tot lichaamsvet. Ondanks het feit dat deze associatie werd gevonden, kan deze associatie vertekend zijn door andere voedingsfactoren, inclusief andere voedingscomponenten uit flesvoeding en koemelk, of voedingspatronen van ouders die geassocieerd zijn met de inname van koemelk- eiwitten. De resultaten geven aan dat een verminderde inname van koemelk-eiwitten via flesvoeding en koemelk kan leiden tot een verminderde gewichtstoename en minder vetmassa in het vroege leven.

Hoofdstuk 10

In de algemene discussie worden de resultaten van de studies in relatie met de huidige literatuur besproken. Dit hoofdstuk wordt afgesloten met onze conclusies, algemene overwegingen en suggesties voor toekomstig onderzoek.

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Curriculum vitae of Ralph Leunissen

Ralph Leunissen was born in Son en Breugel on September 11th, 1979. In 1997 he graduated from high school (St. Thomascollege, Venlo) and started his medical training at the University of Maastricht. During his studies he rowed for two years at national level and was a coach of two lightweight men crew at the student rowing association M.S.R.V. Saurus, Maastricht. He performed a research project for four months at the Juliana Children’s Hospital, the Hague, investigating the effect of treatment of cystic fibrosis patients on growth (Drs. Kouwenberg). From 2002 to 2004 he did his internships. His final internship was at the Department of Pediatric Gastroenterology at the Royal Children’s Hospital in Brisbane, Australia (Prof. Cleghorn). After obtaining his medical degree in December 2004, he started to work as an resident at the Department of Pediatrics at the Amphia Hospital in Breda (Dr. Vaessen-Verberne). In May 2006, he began to work as a research fellow at the Department of Pediatrics, Division of Endocrinology, Erasmus MC Sophia Children’s Hospital, Rotterdam (Prof. Dr. Hokken-Koelega), which has resulted in this thesis. As a part of his research fellowship, he performed research from November 2008 until January 2009 at the Medical Research Council, Epidemiology Unit, Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, United Kingdom (K. Ong M.D. PhD.). In April 2009, he started his training in Pediatrics at the VUmc Amsterdam (Prof. Dr. Fetter). He is married to Marije Eijgendaal.
List of publications

1. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC 2009
   Timing and tempo of first year catch-up growth related to cardiovascular and metabolic risk profile in early adulthood. Submitted

   Association between infant dairy milk protein intake and adiposity at age 12 months. Submitted

3. Leunissen RW, Stijnen T, Hokken-Koelega AC 2009
   Blood pressure and carotid intima media thickness in early adulthood are related with catch-up in weight and not with birth size. Submitted

   Growth patterns during childhood and the relationship with acylation stimulating protein. Submitted

5. de Lind van Wijngaarden RF, Cianflone K, Leunissen RW, Gao Y, Hokken-Koelega AC 2009
   Cardiovascular and metabolic risk profile and ASP levels in children with Prader-Willi syndrome and effects of growth hormone treatment. Submitted

   Influence of Preterm Birth and Birth Size on Gonadal Function in Young Men. Submitted

   Type 2 diabetes gene TCF7L2 is not associated with insulin sensitivity and insulin secretion before and during growth hormone treatment in short subjects born small for gestational age. Submitted
   Bone mineral density in prematurely born young adults. Submitted

   Prematurity is not associated with reduced insulin sensitivity in adulthood. J Clin Endocrinol Metab, in press.

    Insulin-like Growth Factor-Binding Protein-1: Serum Levels, Promoter Polymorphism and Associations with Components of the Metabolic Syndrome in Short Subjects Born Small for Gestational Age. J Clin Endocrinol Metab, in press.


    Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. J Clin Endocrinol Metab 93:4307-4314


15. Leunissen RW, Stijnen T, Hokken-Koelega AC 2009
    Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. Clin Endocrinol (Oxf) 70:245-251