Growth Patterns and Adult Diseases

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

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The printing of this thesis and the investigator-initiated study described in Chapter 9 were financially supported by XXXXXXXXXXXX.

Cover: C. Renard (www.shutterstock.com)
Lay out: Legatron Electronic Publishing, Rotterdam
Printed by: Ipksmap Drukkers, Enschede (www.ppi.nl)

ISBN: XXXXXXXXXXXX

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

Groeipatronen en ziekten op de volwassen leeftijd

Groei tijdens de kindertijd en determinanten van het cardiovasculaire en metabole profiel in jong volwassenen

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam op gezag
van de rector magnificus Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 6 mei 2009 om 15.45 uur

door

Ralph Wilhelmus Jean Leunissen

geboren te Son en Breugel

ERASMUS UNIVERSITEIT ROTTERDAM

PROMOTIECOMMISSIE:

PROMOTOR:
Prof.dr. A.C.S. Hokken-Koelega

OVERIGE LEDEN:
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Prof.dr. A.J. van der Lelij and
Prof.dr. A.J. van der Heijden

Contents

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.

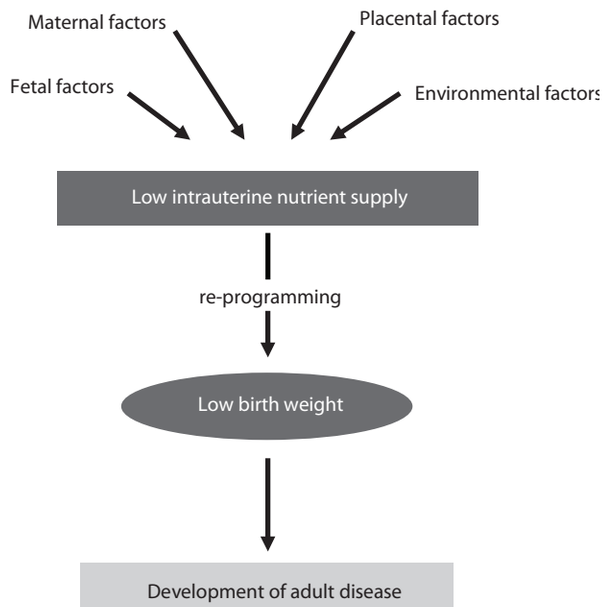


Figure 1. Representation of the fetal origin hypothesis. Adapted from Barker et al. (6,8,12).

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.

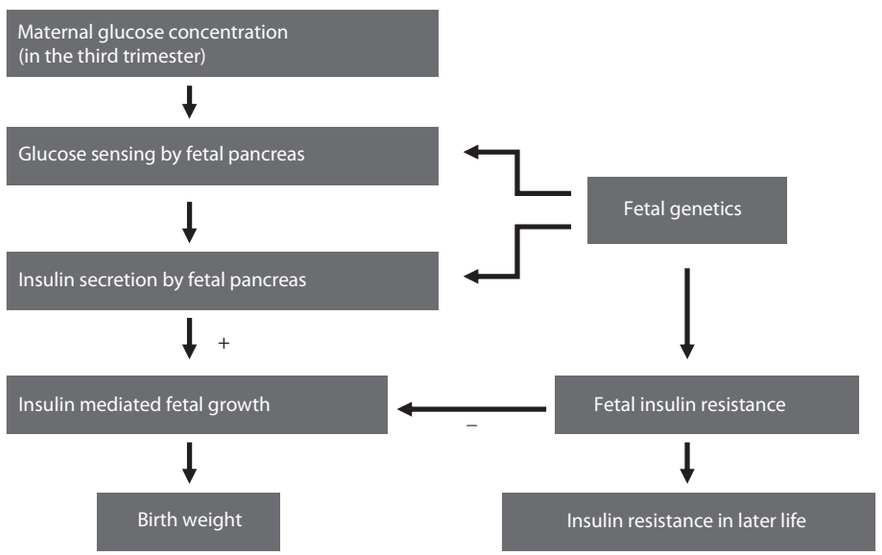


Figure 2. Simplified representation of the fetal insulin hypothesis. Adapted from Hattersley et al. (13).

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).

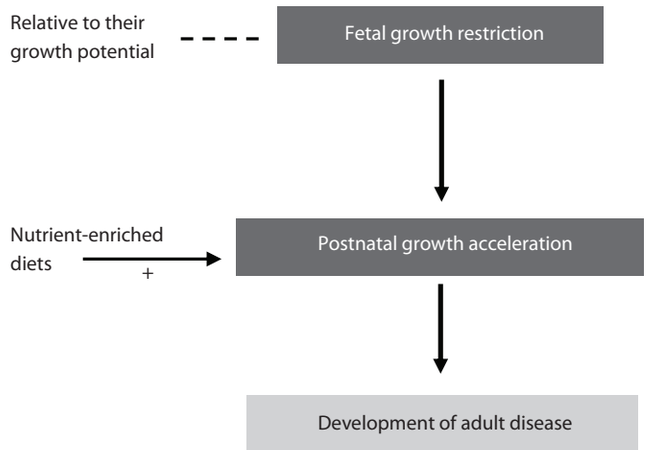


Figure 3. Representation of the growth acceleration hypothesis. Adapted from Singhal et al. (14).

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-I (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 adipsin 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). Cianflone 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 (gr/cm^2) (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.

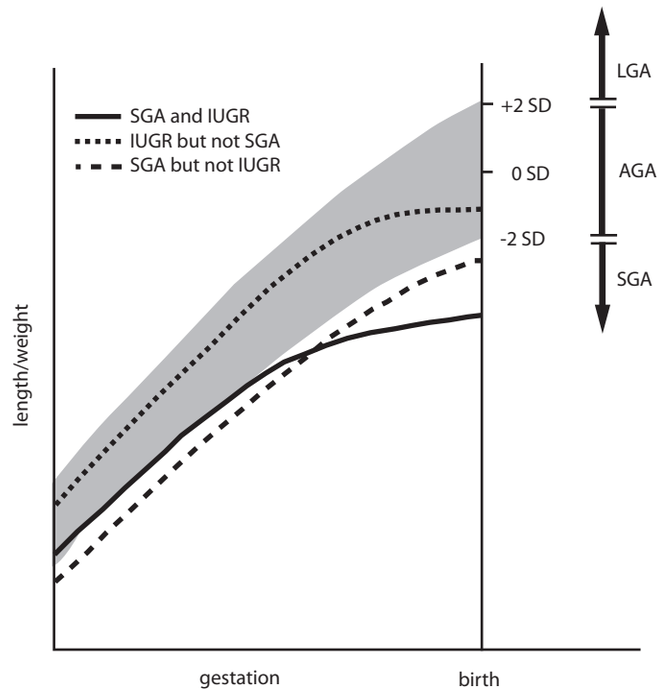


Figure 4. Fetal growth chart showing different growth patterns in IUGR and SGA newborns.

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 Ridder 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.

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

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 expectance 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.

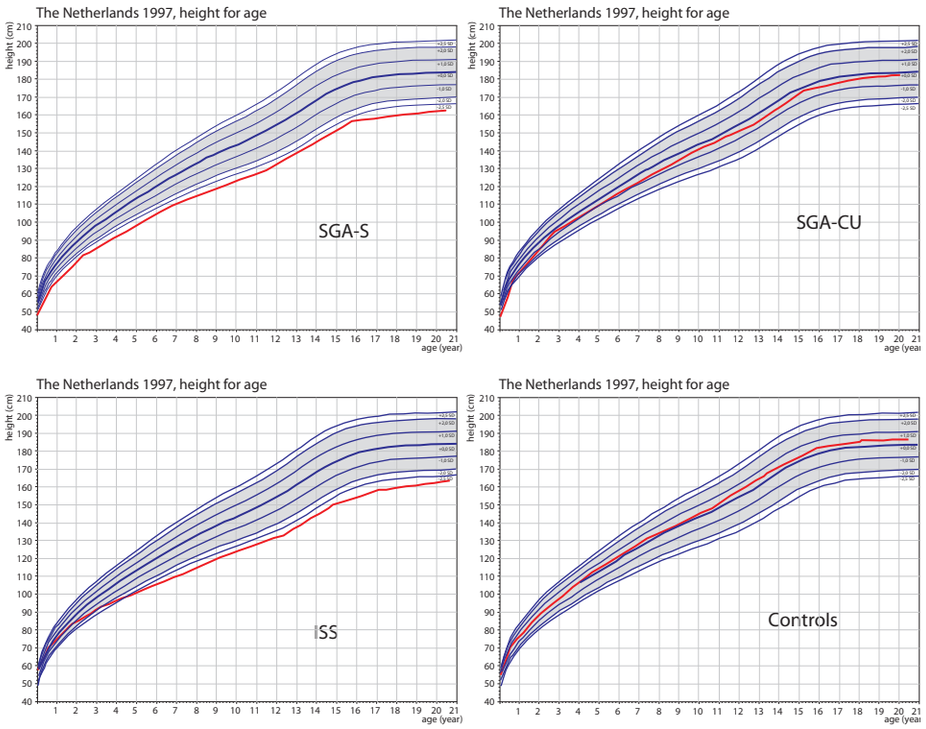


Figure 5. Growth patterns of the four subgroups. SGA-S: born SGA and short as adults, SGA-CU: born SGA with catch-up growth in childhood, ISS: born AGA and short as adults, Controls: born AGA with normal adult height.

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).

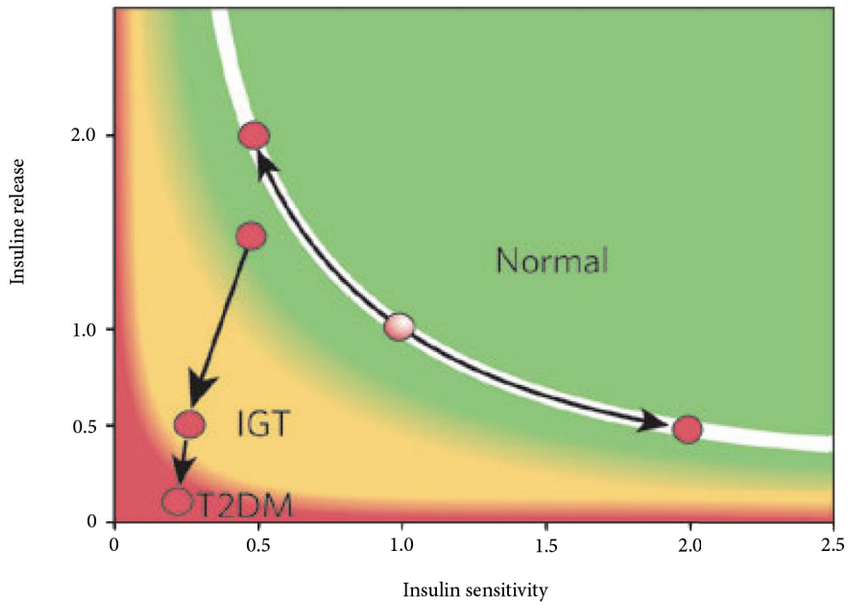


Figure 6. Hyperbolic association between insulin secretion and insulin sensitivity. Adapted from Kahn et al. (106). IGT: impaired glucose tolerance, T2DM: type 2 diabetes mellitus.

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).

References

1. **Yarbrough DE, Barrett-Connor E, Morton DJ** 2000 Birth weight as a predictor of adult bone mass in postmenopausal women: the Rancho Bernardo Study. Osteoporos Int 11:626-630
2. **Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M** 2002 Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation 105:1088-1092
3. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-67
4. **Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C** 1994 Thinness at birth and insulin resistance in adult life. Diabetologia 37:150-154
5. **Barker DJ, Bull AR, Osmond C, Simmonds SJ** 1990 Fetal and placental size and risk of hypertension in adult life. BMJ (Clinical research ed 301:259-262
6. **Barker DJ** 1990 The fetal and infant origins of adult disease. BMJ (Clinical research ed 301:1111
7. **Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D** 2000 Fetal and childhood growth and hypertension in adult life. Hypertension 36:790-794
8. **Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS** 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941
9. **Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH** 1993 Growth in utero and serum cholesterol concentrations in adult life. BMJ (Clinical research ed 307:1524-1527
10. **Martyn CN, Gale CR, Jespersen S, Sherriff SB** 1998 Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. Lancet 352:173-178
11. **Sayer AA, Cooper C** 2005 Fetal programming of body composition and musculoskeletal development. Early human development 81:735-744
12. **Barker DJ** 1995 Fetal origins of coronary heart disease. BMJ (Clinical research ed 311:171-174
13. **Hattersley AT, Tooke JE** 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789-1792
14. **Singhal A, Lucas A** 2004 Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 363:1642-1645
15. **Allender S, Scarborough P, O'Flaherty M, Capewell S** 2008 Patterns of coronary heart disease mortality over the 20th century in England and Wales: Possible plateaus in the rate of decline. BMC public health 8:148
16. **Stamler J** 1985 The marked decline in coronary heart disease mortality rates in the United States, 1968-1981; summary of findings and possible explanations. Cardiology 72:11-22
17. **Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM** 2004 Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. Jama 291:2847-2850
18. **Misra A, Khurana L** 2008 Obesity and the metabolic syndrome in developing countries. The Journal of clinical endocrinology and metabolism 93:S9-30
19. **Ogden CL, Carroll MD, Flegal KM** 2008 High body mass index for age among US children and adolescents, 2003-2006. Jama 299:2401-2405
20. **Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA** 1998 Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. The New England journal of medicine 338:1650-1656
21. **Tracy RE, Newman WP, 3rd, Wattigney WA, Srinivasan SR, Strong JP, Berenson GS** 1995 Histologic features of atherosclerosis and hypertension from autopsies of young individuals in a defined geographic population: the Bogalusa Heart Study. Atherosclerosis 116:163-179
22. **Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, Jarvisalo MJ, Uhari M, Jokinen E, Ronnemaa T, Akerblom HK, Viikari JS** 2003 Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Jama 290:2277-2283
23. **Cnop M, Vidal J, Hull RL, Utzschneider KM, Carr DB, Schraw T, Scherer PE, Boyko EJ, Fujimoto WY, Kahn SE** 2007 Progressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relatives of subjects with type 2 diabetes. Diabetes care 30:677-682
24. **Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP** 1994 Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia 37:624-631
25. **Boyko EJ** 2000 Proportion of type 2 diabetes cases resulting from impaired fetal growth. Diabetes care 23:1260-1264
26. **Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC** 2005 Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). Clinical endocrinology 62:44-50
27. **Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A** 2003 Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? The American journal of clinical nutrition 77:726-730
28. **Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M** 2005 Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. The American journal of clinical nutrition 82:980-987
29. **Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C** 2001 Intrauterine programming of adult body composition. The Journal of clinical endocrinology and metabolism 86:267-272
30. **Sayer AA, Syddall HE, Dennison EM, Gilbody HJ, Duggleby SL, Cooper C, Barker DJ, Phillips DI** 2004 Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. The American journal of clinical nutrition 80:199-203
31. **Sas T, Mulder P, Hokken-Koelega A** 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. The Journal of clinical endocrinology and metabolism 85:3786-3792
32. **Ibanez L, Ong K, Dunger DB, de Zegher F** 2006 Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. The Journal of clinical endocrinology and metabolism 91:2153-2158
33. **Vanhala M, Vanhala P, Kumpusalo E, Halonen P, Takala J** 1998 Relation between obesity from childhood to adulthood and the metabolic syndrome: population based study. BMJ (Clinical research ed 317:319
34. **Vanhala MJ, Vanhala PT, Keinanen-Kiukaanniemi SM, Kumpusalo EA, Takala JK** 1999 Relative weight gain and obesity as a child predict metabolic syndrome as an adult. Int J Obes Relat Metab Disord 23:656-659
35. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ (Clinical research ed 320:967-971
36. **Danesh J, Collins R, Peto R** 2000 Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 102:1082-1085
37. **Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang KY, Levine DM** 1993 Serum cholesterol in young men and subsequent cardiovascular disease. The New England journal of medicine 328:313-318
38. **Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E** 2001 High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 358:2026-2033
39. **Ariyo AA, Thach C, Tracy R** 2003 Lp(a) lipoprotein, vascular disease, and mortality in the elderly. The New England journal of medicine 349:2108-2115

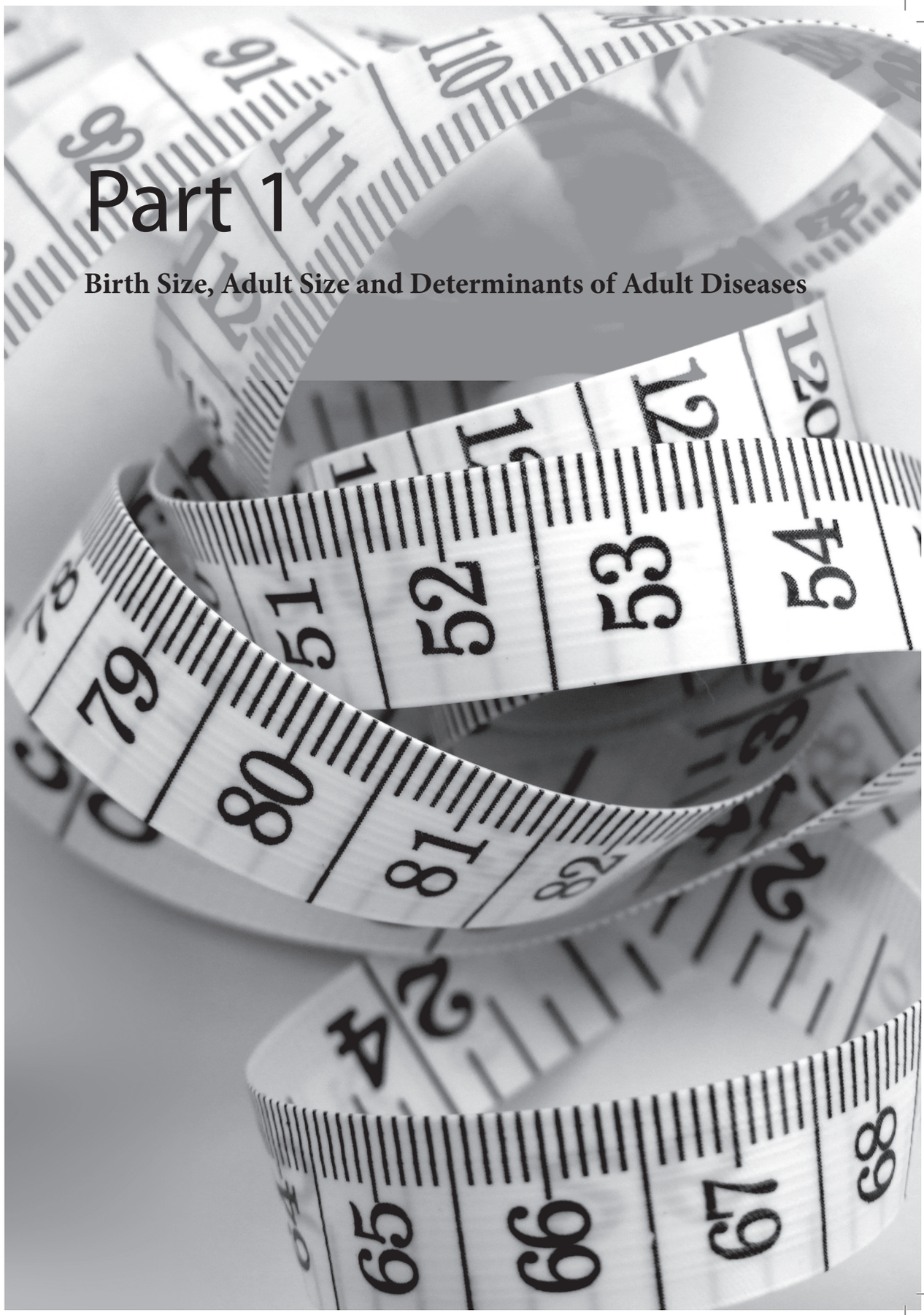
40. **Lauren L, Jarvelin MR, Elliott P, Sovio U, Spellman A, McCarthy M, Emmett P, Rogers I, Hartikainen AL, Pouta A, Hardy R, Wadsworth M, Helmsdal G, Olsen S, Bakoula C, Lekea V, Millwood I** 2003 Relationship between birthweight and blood lipid concentrations in later life: evidence from the existing literature. International journal of epidemiology 32:862-876
41. **Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R** 2004 Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. Jama 292:2755-2764
42. **Pulzer F, Haase U, Kratzsch J, Richter V, Rassoul F, Kiess W, Keller E** 1999 Lipoprotein(a) levels in formerly small-for-gestational-age children. Hormone research 52:241-246
43. **Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, Ong KK, Dunger DB, Mericq MV** 2003 Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. The Journal of clinical endocrinology and metabolism 88:3645-3650
44. **Lopez-Bermejo A, Casano-Sancho P, Fernandez-Real JM, Kihara S, Funahashi T, Rodriguez-Hierro F, Ricart W, Ibanez L** 2004 Both intrauterine growth restriction and postnatal growth influence childhood serum concentrations of adiponectin. Clinical endocrinology 61:339-346
45. **Mahley RW, Huang Y** 1999 Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. Current opinion in lipidology 10:207-217
46. **Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J** 2007 Association of apolipoprotein E genotypes with lipid levels and coronary risk. Jama 298:1300-1311
47. **Song Y, Stampfer MJ, Liu S** 2004 Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Annals of internal medicine 141:137-147
48. **Cianflone K, Xia Z, Chen LY** 2003 Critical review of acylation-stimulating protein physiology in humans and rodents. Biochimica et biophysica acta 1609:127-143
49. **Germinario R, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K** 1993 Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. Metabolism: clinical and experimental 42:574-580
50. **Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA** 1991 Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. Lipids 26:495-499
51. **Faraj M, Sniderman AD, Cianflone K** 2004 ASP enhances in situ lipoprotein lipase activity by increasing fatty acid trapping in adipocytes. Journal of lipid research 45:657-666
52. **Cianflone K, Zakarian R, Couillard C, Delplanque B, Despres JP, Sniderman A** 2004 Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance. Journal of lipid research 45:124-131
53. **Castro Cabezas M, Erkelens DW** 1998 The direct way from gut to vessel wall: atheroinitiation. European journal of clinical investigation 28:504-505
54. **Miura K, Nakagawa H, Tabata M, Morikawa Y, Nishijo M, Kagamimori S** 2001 Birth weight, childhood growth, and cardiovascular disease risk factors in Japanese aged 20 years. American journal of epidemiology 153:783-789
55. **Jarvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, Hartikainen AL, Laitinen J, Zitting P, Rantakallio P, Elliott P** 2004 Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. Hypertension 44:838-846
56. **Huxley RR, Shiell AW, Law CM** 2000 The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. Journal of hypertension 18:815-831
57. **Huxley R, Neil A, Collins R** 2002 Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360:659-665
58. **Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A** 2007 Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115:213-220

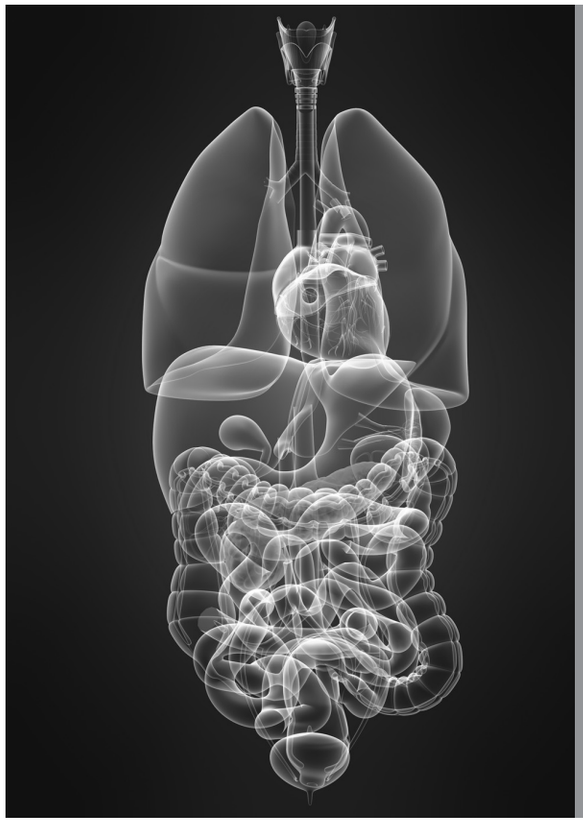
59. **de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, Kastelein JJ** 2004 Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation 109:III33-38
60. **O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr.** 1999 Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. The New England journal of medicine 340:14-22
61. **Burke GL, Evans GW, Riley WA, Sharrett AR, Howard G, Barnes RW, Rosamond W, Crow RS, Rautaharju PM, Heiss G** 1995 Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 26:386-391
62. **Carter DR, Bouxsein ML, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137-145
63. **Mazess RB, Barden H, Mautalen C, Vega E** 1994 Normalization of spine densitometry. J Bone Miner Res 9:541-548
64. **Kroger H, Vainio P, Nieminen J, Kotaniemi A** 1995 Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 17:157-159
65. **Looker AC, Orwoll ES, Johnston CC, Jr., Lindsay RL, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP** 1997 Prevalence of low femoral bone density in older U.S. adults from NHANES III. J Bone Miner Res 12:1761-1768
66. **Holt G, Khaw KT, Reid DM, Compston JE, Bhalla A, Woolf AD, Crabtree NJ, Dalzell N, Wardley-Smith B, Lunt M, Reeve J** 2002 Prevalence of osteoporotic bone mineral density at the hip in Britain differs substantially from the US over 50 years of age: implications for clinical densitometry. The British journal of radiology 75:736-742
67. **Dennison EM, Syddall HE, Sayer AA, Gilbody HJ, Cooper C** 2005 Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study. Pediatric research 57:582-586
68. **te Velde SJ, Twisk JW, van Mechelen W, Kemper HC** 2004 Birth weight and musculoskeletal health in 36-year-old men and women: results from the Amsterdam Growth and Health Longitudinal Study. Osteoporos Int 15:382-388
69. **Baker JL, Olsen LW, Sorensen TI** 2007 Childhood body-mass index and the risk of coronary heart disease in adulthood. The New England journal of medicine 357:2329-2337
70. **Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2006 Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). The American journal of clinical nutrition 83:324-330
71. **Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ** 1999 Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ (Clinical research ed 318:427-431
72. **Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG** 2005 Trajectories of growth among children who have coronary events as adults. The New England journal of medicine 353:1802-1809
73. **Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ** 1989 Weight in infancy and death from ischaemic heart disease. Lancet 2:577-580
74. **Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ** 2001 Early growth and coronary heart disease in later life: longitudinal study. BMJ (Clinical research ed 322:949-953
75. **Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2007 Association of weight gain in infancy and early childhood with metabolic risk in young adults. The Journal of clinical endocrinology and metabolism 92:98-103
76. **Chomtho S, Wells JC, Williams JE, Davies PS, Lucas A, Fewtrell MS** 2008 Infant growth and later body composition: evidence from the 4-component model. The American journal of clinical nutrition 87:1776-1784

77. **Druet C, Ong KK** 2008 Early childhood predictors of adult body composition. Best practice & research 22:489-502
78. **Arenz S, Ruckerl R, Koletzko B, von Kries R** 2004 Breast-feeding and childhood obesity--a systematic review. Int J Obes Relat Metab Disord 28:1247-1256
79. **Armstrong J, Reilly JJ** 2002 Breastfeeding and lowering the risk of childhood obesity. Lancet 359:2003-2004
80. **Svenningsen NW, Lindroth M, Lindquist B** 1982 A comparative study of varying protein intake in low birthweight infant feeding. Acta Paediatr Scand Suppl 296:28-31
81. **Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F** 1995 Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Relat Metab Disord 19:573-578
82. **Gunther AL, Buyken AE, Kroke A** 2007 Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. The American journal of clinical nutrition 85:1626-1633
83. **Gunther AL, Remer T, Kroke A, Buyken AE** 2007 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? The American journal of clinical nutrition 86:1765-1772
84. **Hoppe C, Molgaard C, Thomsen BL, Juul A, Michaelsen KF** 2004 Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children. The American journal of clinical nutrition 79:494-501
85. **Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P** 2003 International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. Pediatrics 111:1253-1261
86. **Bernstein PS, Divon MY** 1997 Etiologies of fetal growth restriction. Clinical obstetrics and gynecology 40:723-729
87. **Keller C, Keller KR, Shew SB, Plon SE** 1999 Growth deficiency and malnutrition in Bloom syndrome. The Journal of pediatrics 134:472-479
88. **Pollack RN, Divon MY** 1992 Intrauterine growth retardation: definition, classification, and etiology. Clinical obstetrics and gynecology 35:99-107
89. **Wollmann HA** 1998 Intrauterine growth restriction: definition and etiology. Hormone research 49 Suppl 2:1-6
90. **Bryan SM, Hindmarsh PC** 2006 Normal and abnormal fetal growth. Hormone research 65 Suppl 3:19-27
91. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatric research 38:267-271
92. **Albertsson-Wikland K, Karlberg J** 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71
93. **Karlberg J, Albertsson-Wikland K** 1995 Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatric research 38:733-739
94. **de Ridder MA, Engels MA, Stijnen T, Hokken-Koelega AC** 2008 Small for gestational age children without early catch-up growth: spontaneous growth and prediction of height at 8 years. Hormone research 70:203-208
95. **Chaussain JL, Colle M, Ducret JP** 1994 Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. Acta Paediatr Suppl 399:72-73
96. **Hokken-Koelega A, van Pareren Y, Arends N, Boonstra V** 2004 Efficacy and safety of long-term continuous growth hormone treatment of children born small for gestational age. Hormone research 62 Suppl 3:149-154
97. **de Zegher F, Ong KK, Ibanez L, Dunger DB** 2006 Growth hormone therapy in short children born small for gestational age. Hormone research 65 Suppl 3:145-152
98. **Cutfield WS, Lindberg A, Rapaport R, Wajnrajch MP, Saenger P** 2006 Safety of growth hormone treatment in children born small for gestational age: the US trial and KIGS analysis. Hormone research 65 Suppl 3:153-159
99. **Jung H, Rosilio M, Blum WF, Drop SL** 2008 Growth hormone treatment for short stature in children born small for gestational age. Advances in therapy 25:951-978
100. **Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, Chernausek SD, Savage MO, Wit JM** 2008 Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. The Journal of clinical endocrinology and metabolism 93:4210-4217
101. **Bergman RN, Phillips LS, Cobelli C** 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 68:1456-1467
102. **Pacini G, Bergman RN** 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23:113-122
103. **Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN** 2003 MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol Ther 5:1003-1015
104. **Bergman RN** 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-1527
105. **Kahn SE** 2003 The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 46:3-19
106. **Kahn SE, Hull RL, Utzschneider KM** 2006 Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444:840-846

Part 1

Birth Size, Adult Size and Determinants of Adult Diseases





Chapter 2

Fat Mass Accumulation During Childhood Determines Insulin Sensitivity in Early Adulthood

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J Clin Endocrinol Metab. 2008 Feb;93(2):445-51

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, were 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 glucocorticosteriods, 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 R^2= 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 R^2= 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 R^2= 0.23), even after adding waist-to-hip ratio to the model (Model D, adjusted R^2= 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.

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

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

	Si							
	Model A		Model B		Model C		Model D	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Age (yrs)	–0.21	0.389	–0.15	0.516	–0.13	0.543	–0.11	0.620
Gender	–1.84	0.013	–2.75	0.001	–0.13	0.927	–0.79	0.614
Height SDS	0.34	0.381	0.87	0.038	0.47	0.314	0.49	0.305
Birth length SDS	0.01	0.987	0.001	0.998	0.12	0.801	0.12	0.791
BL*AH (SDS)	0.19	0.369	0.16	0.413	0.14	0.464	0.14	0.464
Birth weight SDS	0.89	0.040	0.66	0.116	0.55	0.169	0.51	0.209
Weight SDS			–0.74	0.018				
W/H ratio			–13.8	0.026			–7.70	0.210
Fat mass (kg)					–0.24	< 0.0005	–0.21	< 0.0005
LBM (kg)					0.02	0.758	0.02	0.799
Overall	0.026		< 0.0005		< 0.0005		< 0.0005	
R^2	0.10		0.22		0.28		0.29	
R^2 adjusted	0.06		0.17		0.23		0.24	

BL: Birth length SDS; AH: Adult height SDS

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*.

	Total group (n= 136)	SGA-S (n= 25)	SGA-CU (n= 23)	ISS (n= 23)	Controls (n= 26)
Si *10^{-4}/min (μU/ml)	6.9 (4.3)	6.3 (3.9)	4.9 (3.1)	6.7 (4.1)	8.0 (4.0)
Sg *10^{-2}/min (mg/d)	0.02 (0.01)	0.02 (0.01)	0.02 (0.01)	0.02 (0.00)	0.02 (0.00)
AIRg (mU/l)	540 (509)	615 (703)	782 (777)	401 (261)	456 (283)
DI (Si*AIRg)	2833 (1818)	2698 (1618)	2716 (1908)	2264 (1306)	3208 (2242)
Fasting glucose (mmol/l)	4.9 (0.51)	5.0 (0.45)	4.9 (0.50)	4.8 (0.5)	4.9 (0.4)
Fasting insulin (mU/l)	10.2 (4.7)	10.2 (5.1)	11.5 (4.1)	9.1 (3.9)	10.1 (5.0)

*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*.

	Model 1		Model 2	
Variables	**β**	**p-value**	**β**	**p-value**
Age (yrs)	–0.33	0.177	-0.23	0.316
Gender	–1.88	0.13	0.01	0.988
Height SDS	0.32	0.570	-0.19	0.723
Weight SDS	–0.94	0.001	0.89	0.077
FM (kg)			-0.33	< 0.0005
SGA-S[1]	–2.25	0.214	-1.34	0.421
SGA-CU[1]	–2.66	0.013	-2.28	0.021
ISS[1]	–2.13	0.237	-0.60	0.720
Overall		0.001		< 0.0005
R^2		0.23		0.36
R^2 adjusted		0.17		0.30

*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 5. Multiple regression for AIRg in various subgroups.

	Model 1		Model 2	
Variables	**β**	**p-value**	**β**	**p-value**
Age (yrs)	–60.0	0.077	–65.2	0.055
Gender	167.3	0.104	71.0	0.556
Height SDS	93.5	0.222	120.0	0.125
Weight SDS	169.7	< 0.0005	75.3	0.309
FM (kg)			17.5	0.138
SGA-S[1]	702.4	0.005	659.0	0.009
SGA-CU[1]	345.4	0.019	328.9	0.025
ISS[1]	526.8	0.034	451.3	0.073
Overall		< 0.0005		< 0.0005
R^2		0.32		0.33
R^2 adjusted		0.26		0.27

[1]: 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 $2.42*10^{-4}$/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.

Acknowledgements:

The authors wish to thank all participants. We acknowledge the support of many colleagues at the Erasmus University MC, Rotterdam, MCRZ Hospital Rotterdam, University MC Nijmegen, Catharina Hospital Eindhoven, Leiden University MC, Juliana Children's Hospital the Hague, University Medical Centre Groningen. We thank J.P. Sluimer and P. Kooy of the Department of Nuclear Medicine, Erasmus University MC and Dr. Hackeng for their support. We greatly acknowledge Mrs. J. Dunk and Mrs. I. van Slobbe, research nurses, for their technical assistance and support with data collection.

Grant support: The study was financially supported by Netherlands Organisation for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

References:

1. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-7
2. **Yajnik CS** 2004 Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr 134:205-10
3. **Barker DJ** 2007 The origins of the developmental origins theory. J Intern Med 261:412-7
4. **Boyko EJ** 2000 Proportion of type 2 diabetes cases resulting from impaired fetal growth. Diabetes Care 23:1260-4
5. **Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR** 1992 Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet 340:925-9
6. **Facchini FS, Hua N, Abbasi F, Reaven GM** 2001 Insulin resistance as a predictor of age-related diseases. J Clin Endocrinol Metab 86:3574-8
7. **Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP** 1994 Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia 37:624-31
8. **Ahren B, Pacini G** 2004 Importance of quantifying insulin secretion in relation to insulin sensitivity to accurately assess beta cell function in clinical studies. Eur J Endocrinol 150:97-104
9. **Bergman RN** 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-27
10. **Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC** 2005 Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). Clin Endocrinol (Oxf) 62:44-50
11. **Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD** 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82:402-6
12. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71
13. **Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S** 1993 Longitudinal follow-up of growth in children born small for gestational age. Acta Paediatr 82:438-43
14. **Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, Ong KK, Dunger DB, Mericq MV** 2003 Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab 88:3645-50
15. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10
16. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23
17. **Pacini G, Bergman RN** 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23:113-22
18. **Bergman RN, Phillips LS, Cobelli C** 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 68:1456-67
19. **Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN** 2003 MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol Ther 5:1003-15

20. **Jaquet D, Chevenne D, Czernichow P, Levy-Marchal C** 2000 No evidence for a major beta-cell dysfunction in young adults born with intra-uterine growth retardation. Pediatr Diabetes 1:181-5
21. **Mericq V, Ong KK, Bazaes R, Pena V, Avila A, Salazar T, Soto N, Iniguez G, Dunger DB** 2005 Longitudinal changes in insulin sensitivity and secretion from birth to age three years in small- and appropriate-for-gestational-age children. Diabetologia 48:2609-14
22. **Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C** 2000 Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 85:1401-6
23. **Ibanez L, Ong K, Dunger DB, de Zegher F** 2006 Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 91:2153-8
24. **Singhal A, Lucas A** 2004 Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 363:1642-5
25. **Van Gaal LF, Mertens IL, De Block CE** 2006 Mechanisms linking obesity with cardiovascular disease. Nature 444:875-80
26. **Cutfield WS, Jackson WE, Jefferies C, Robinson EM, Breier BH, Richards GE, Hofman PL** 2003 Reduced insulin sensitivity during growth hormone therapy for short children born small for gestational age. J Pediatr 142:113-6
27. **Cutfield WS, Bergman RN, Menon RK, Sperling MA** 1990 The modified minimal model: application to measurement of insulin sensitivity in children. J Clin Endocrinol Metab 70:1644-50
28. **Cianfarani S, Germani D, Branca F** 1999 Low birthweight and adult insulin resistance: the "catch-up growth" hypothesis. Arch Dis Child Fetal Neonatal Ed 81:F71-3
29. **Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, Kahn BB, Kahn CR, Shulman GI** 2000 Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. J Clin Invest 105:1791-7
30. **Cariou B, Postic C, Boudou P, Burcelin R, Kahn CR, Girard J, Burnol AF, Mauvais-Jarvis F** 2004 Cellular and molecular mechanisms of adipose tissue plasticity in muscle insulin receptor knockout mice. Endocrinology 145:1926-32
31. **Abbott WG, Foley JE** 1987 Comparison of body composition, adipocyte size, and glucose and insulin concentrations in Pima Indian and Caucasian children. Metabolism 36:576-9
32. **van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, Grobbee DE, de Jong FH, van Duyn CM, Pols HA, Lamberts SW** 2002 A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes 51:3128-34
33. **Barker DJ, Fall CH** 1993 Fetal and infant origins of cardiovascular disease. Arch Dis Child 68:797-9
34. **Weyer C, Bogardus C, Mott DM, Pratley RE** 1999 The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus. J Clin Invest 104:787-94
35. **Flanagan DE, Moore VM, Godsland IF, Cockington RA, Robinson JS, Phillips DI** 2000 Fetal growth and the physiological control of glucose tolerance in adults: a minimal model analysis. Am J Physiol Endocrinol Metab 278:E700-6

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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Clin Endocrinol (Oxf). 2008 Jun 23

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).

Subjects and Methods

Subjects

The PROgramming factors for GRowth And Metabolism study (PROGRAM) 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) (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 glucocorticosteriods, 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 a total number of 312 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 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.

	Total group (n= 312)	SGA-S (n=44)	SGA-CU (n=56)	ISS (n=38)	Controls (n=79)
Male (n) (%)	122 (39.1)	15 (34.1)	23 (41.1)	16 (42.1)	29 (36.7)
Birth length (cm)	47.6 (3.08)	45.2 (1.98)*	44.6 (1.9)*	49.6 (1.4)§	50.8 (1.7)
Birth length (SDS)	–1.5 (1.48)	–2.90 (0.79)*	–2.86 (0.80)*	–0.53 (0.47)$	0.08 (0.75)
Birth weight (kg)	2.78 (0.67)	2.50 (0.48)§	2.19 (0.33)§	3.25 (0.56)	3.30 (0.62)
Birth weight (SDS)	–1.2 (1.39)	–1.94 (0.94)*#	–2.36 (0.70)*	–0.17 (1.14)^	–0.09 (1.27)
Age (years)	20.9 (1.68)	20.7 (1.76)	21.0 (1.5)	21.0 (1.7)	20.8 (1.71)
Height (cm)	168.2 (10.7)	157.3 (6.7)*^	174.7 (7.8)	159.3 (6.7)*^	176.8 (7.7)
Height (SDS)	–1.1 (1.37)	–2.63 (0.55)*^	–0.14 (0.77)§	–2.48 (0.40)*^	0.24 (0.90)
Weight (kg)	63.8 (12.5)	56.7 (11.4)*^	70.5 (14.4)	56.5 (12.2)*^	68.8 (11.0)
Weight (SDS)	–0.6 (1.42)	–1.55 (1.54)*^	0.12 (1.17)	–1.77 (1.67)*^	0.07 (0.98)
Delta weight SDS	0.57 (1.99)	0.39 (1.76)	2.48 (1.33)$	–1.60 (2.22)$	0.16 (1.43)
BMI	22.5 (3.9)	22.9 (4.6)	23.1 (4.5)	22.2 (4.7)	21.9 (2.9)
Waist/Hip Ratio	0.88 (0.07)	0.87 (0.08)	0.90 (0.07)	0.87 (0.08)	0.89 (0.06)
FM (kg) **	15.7 (8.73)	15.7 (8.88)	18.7 (10.7)	14.7 (9.10)	15.6 (8.71)
FM (SDS) **		0.01 (1.02)	0.35 (1.23)	–0.10 (1.05)	0.00 (1.00)
Trunk fat / total fat ratio	0.48 (0.06)	0.47 (0.06)	0.49 (0.06)	0.47 (0.07)	0.48 (0.05)
LBM (kg) **	45.1 (9.85)	38.3 (7.28)*^	48.5 (9.86)	39.1 (7.17)*^	50.0 (9.78)
LBM (SDS) **		–1.20 (0.74)*^	–0.16 (1.01)	–1.11 (0.73)*^	0.00 (1.00)

Values are given as means (sd). **Not corrected for age, gender, adult height and adult weight.
$: p< 0.001 compared with the other subgroups, §: p< 0.02 compared with the other subgroups,
*: p< 0.001 compared with controls, #: p<0.001 compared with ISS, ^: p< 0.001 compared with SGA-CU.

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 no 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 R^2= 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 R^2= 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 R^2= 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 SDS were also significant determinants of FM.

Table 2. Multiple regression for log transformed FM.

	Log transformed FM							
	Model A		Model B		Model C		Model D	
Variables	β[1]	p-value	β[1]	p-value	β[1]	p-value	β[1]	p-value
Age (yrs)	4.36	0.011	3.40	0.001	3.50	0.001	3.53	0.001
Gender	52.1	< 0.0005	52.1	< 0.0005	52.9	< 0.0005	52.7	< 0.0005
Height SDS	5.00	0.083	–13.9	< 0.0005	–14.6	< 0.0005	–14.8	< 0.0005
Birth length SDS	1.11	0.753	–1.27	0.397	1.66	0.436	2.19	0.341
BL*AH (SDS)	0.68	0.631	0.49	0.598	0.32	0.709	0.81	0.446
Birth weight SDS	–5.65	0.082			–3.89	0.048	–4.28	0.046
Weight SDS			33.7	< 0.0005	33.4	< 0.0005	33.2	< 0.0005
BW*AW (SDS)							–0.23	0.810
Overall		< 0.0005		< 0.0005		< 0.0005		< 0.0005
R^2 adjusted		0.23		0.71		0.72		0.72

[1]: refers to the unstandardized regression coefficients (1/100).
BL: Birth length SDS, AH: Adult height SDS, BW: Birth weight, AW: Adult weight.

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

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 R^2= 0.79). Model B shows that adult weight was a strong significant determinant of LBM (Model B, adjusted R^2= 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 R^2= 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)

	LBM							
	Model A		Model B		Model C		Model D	
Variables	**β**	**p–value**	**β**	**p–value**	**β**	**p–value**	**β**	**p–value**
Age (yrs)	0.33	0.031	0.29	0.020	0.28	0.030	0.27	0.032
Gender	–15.6	< 0.0005	–15.5	< 0.0005	–15.5	< 0.0005	–15.6	< 0.0005
Height SDS	3.69	< 0.0005	2.35	< 0.0005	2.44	< 0.0005	2.45	< 0.0005
Birth length SDS	–0.39	0.223	0.19	0.287	–0.35	0.180	–0.29	0.303
BL*AH (SDS)	–0.04	0.723	0.04	0.737	0.02	0.833	0.05	0.691
Birth weight SDS	0.55	0.061			0.66	0.006	0.60	0.020
Weight SDS			2.09	< 0.0005	2.12	< 0.0005	1.99	< 0.0005
BW*AW (SDS)							–0.084	0.456
Overall		< 0.0005		< 0.0005		< 0.0005		< 0.0005
R^2 adjusted		0.79		0.86		0.86		0.86

BL: Birth length SDS, AH: Adult height SDS, BW: Birth weight, AW: Adult weight.

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.

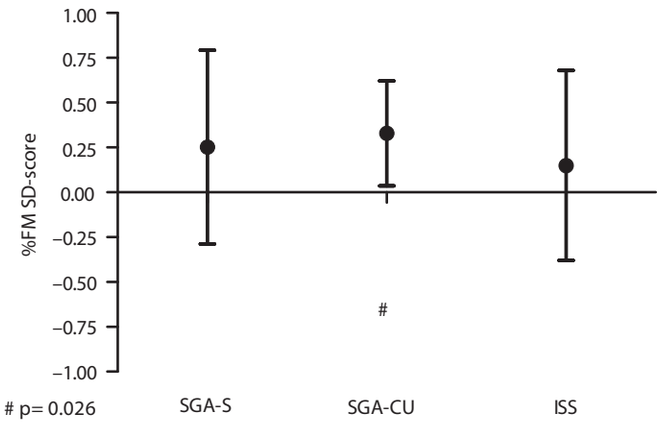

Figure 1. Difference in %FM SD-score between the 3 subgroups compared to controls (with 95% CI), after correction for age, gender and adult height. # p= 0.026

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.

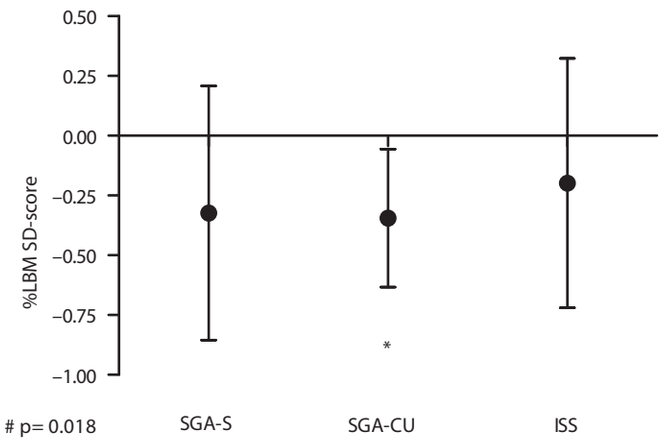

Figure 2. Difference in %LBM SD-score between the 3 subgroups compared to controls (with 95% CI), after correction for age, gender and adult height. * p= 0.018

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

We greatly thank Mrs. J. Dunk and Mrs. I. van Slobbe, research nurses, for their technical assistance and support with data collection. We acknowledge Mrs. J. Sluimer for checking the DXA results.

Grant support: The PROGRAM-study was financially supported by Netherlands Organisation for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

References

1. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-67
2. **Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS** 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941
3. **Barker DJ** 1995 Fetal origins of coronary heart disease. Bmj 311:171-174
4. **Parsons TJ, Power C, Logan S, Summerbell CD** 1999 Childhood predictors of adult obesity: a systematic review. Int J Obes Relat Metab Disord 23 Suppl 8:S1-107
5. **Ezzahir N, Alberti C, Deghmoun S, Zaccaria I, Czernichow P, Levy-Marchal C, Jaquet D** 2005 Time course of catch-up in adiposity influences adult anthropometry in individuals who were born small for gestational age. Pediatr Res 58:243-247
6. **Rogers I** 2003 The influence of birthweight and intrauterine environment on adiposity and fat distribution in later life. Int J Obes Relat Metab Disord 27:755-777
7. **Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD** 1996 Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 348:1478-1480
8. **Wannamethee SG, Shaper AG, Walker M** 2005 Overweight and obesity and weight change in middle aged men: impact on cardiovascular disease and diabetes. J Epidemiol Community Health 59:134-139
9. **Rogers IS, Ness AR, Steer CD, Wells JC, Emmett PM, Reilly JR, Tobias J, Smith GD** 2006 Associations of size at birth and dual-energy X-ray absorptiometry measures of lean and fat mass at 9 to 10 y of age. Am J Clin Nutr 84:739-747
10. **Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A** 2003 Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am J Clin Nutr 77:726-730
11. **Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M** 2005 Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. Am J Clin Nutr 82:980-987
12. **Labayen I, Moreno LA, Blay MG, Blay VA, Mesana MI, Gonzalez-Gross M, Bueno G, Sarria A, Bueno M** 2006 Early programming of body composition and fat distribution in adolescents. J Nutr 136:147-152
13. **Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C** 2001 Intrauterine programming of adult body composition. J Clin Endocrinol Metab 86:267-272
14. **Sayer AA, Syddall HE, Dennison EM, Gilbody HJ, Duggleby SL, Cooper C, Barker DJ, Phillips DI** 2004 Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. Am J Clin Nutr 80:199-203
15. **Te Velde SJ, Twisk JW, Van Mechelen W, Kemper HC** 2003 Birth weight, adult body composition, and subcutaneous fat distribution. Obes Res 11:202-208
16. **Yliharsila H, Kajantie E, Osmond C, Forsen T, Barker DJ, Eriksson JG** 2007 Birth size, adult body composition and muscle strength in later life. Int J Obes (Lond)
17. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. Bmj 320:967-971
18. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-910
19. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
20. **Guo Y, Franks PW, Brookshire T, Antonio Tataranni P** 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. Obes Res 12:1925-1929
21. **Lucas A, Fewtrell MS, Cole TJ** 1999 Fetal origins of adult disease-the hypothesis revisited. Bmj 319:245-249
22. **Li H, Stein AD, Barnhart HX, Ramakrishnan U, Martorell R** 2003 Associations between prenatal and postnatal growth and adult body size and composition. Am J Clin Nutr 77:1498-1505
23. **Garnett SP, Cowell CT, Baur LA, Fay RA, Lee J, Coakley J, Peat JK, Boulton TJ** 2001 Abdominal fat and birth size in healthy prepubertal children. Int J Obes Relat Metab Disord 25:1667-1673
24. **Ong KK, Emmett PM, Noble S, Ness A, Dunger DB** 2006 Dietary energy intake at the age of 4 months predicts postnatal weight gain and childhood body mass index. Pediatrics 117:e503-508
25. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-271
26. **Singhal A, Cole TJ, Fewtrell M, Lucas A** 2004 Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 363:1571-1578
27. **Keijzer-Veen MG, Finken MJ, Nauta J, Dekker FW, Hille ET, Frolich M, Wit JM, van der Heijden AJ** 2005 Is blood pressure increased 19 years after intrauterine growth restriction and preterm birth? A prospective follow-up study in The Netherlands. Pediatrics 116:725-731
28. **Klein S, Allison DB, Heymsfield SB, Kelley DE, Leibel RL, Nonas C, Kahn R** 2007 Waist circumference and cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. Am J Clin Nutr 85:1197-1202
29. **Haffner SM** 2007 Abdominal adiposity and cardiometabolic risk: do we have all the answers? Am J Med 120:S10-16; discussion S16-17
30. **Despres JP, Lemieux I** 2006 Abdominal obesity and metabolic syndrome. Nature 444:881-887
31. **Sas T, Mulder P, Hokken-Koelega A** 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-3792
32. **Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, De Zegher F** 2002 Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. J Clin Endocrinol Metab 87:5702-5705
33. **Ibanez L, Ong K, Dunger DB, de Zegher F** 2006 Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 91:2153-2158
34. **Hediger ML, Overpeck MD, Kuczmarski RJ, McGlynn A, Maurer KR, Davis WW** 1998 Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics 102:E60
35. **Hogler W, Briody J, Moore B, Lu PW, Cowell CT** 2005 Effect of growth hormone therapy and puberty on bone and body composition in children with idiopathic short stature and growth hormone deficiency. Bone 37:642-650

Chapter 4

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

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J Clin Endocrinol Metab. 2008 Nov;93(11):4307-14

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 ε3/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 glucocorticosteriods, 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 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, 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

(Model 1). The interaction term birth length SDS e adult height SDS, birth length SDS and adult height SDS were added to all following MR models because the study cohort had been selected on birth length and adult height. This ensured that the effect of these variables was modeled correctly. Secondly, we added adult weight SDS to the model (Model 2). Thirdly, weight SDS was replaced by fat mass (FM) and lean body mass (LBM) to specify weight (Model 3). The interaction term gender e LBM was added to the model to investigate if the effect of LBM on women was different than the effect of LBM on men. Finally, we added ApoE genotype to investigate if this had a significant influence on serum lipid levels (Model 4). ApoE genotype was added with ApoE ε3/ε3 as reference group and the other 5 genotypes as dummy variables. The model assumptions independence, linearity, normality and homoscedasticy were examined in a histogram of the residuals, and subsequently by plotting the residuals against each predictor and against predicted values.

Differences in prevalence of ApoE genotype between the subgroups were analyzed with the Fisher's exact test. 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, %body fat and ApoE genotype, 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

Total study population

The clinical characteristics and lipid levels of the total study population are shown in Table 1. The mean (sd) age at time of measurement was 20.9 (1.7) years. The data on body composition and lipid concentrations presented in Table 1 are not yet corrected for age, gender and adult size. Table 2 shows the prevalence of ApoE genotypes in the total study population.

By multiple linear regression, we evaluated the contribution of birth size to the serum lipid levels in young adulthood. Firstly, a multiple regression was performed with birth length SDS and birth weight SDS after correction for age, gender and adult height SDS (model 1). Secondly, adult weight SDS was added (model 2). Subsequently, we replaced weight SDS for fat mass (FM) and lean body mass (LBM) (model 3). Finally, ApoE genotype was added (model 4). The results are shown in Table 3.

Table 1. Clinical characteristics and lipid concentrations of the total study group, the 4 subgroups and the unclassified group.

Variables	Total group (n = 297)	SGA–S (n = 42)	SGA–CU (n = 54)	ISS (n = 36)	Controls (n = 76)	Unclassified (n = 89)
Gender (M/F)	116/181	14/28	21/33	15/21	28/48	38/51
Age	20.9 (1.7)	20.6 (1.7)	21.0 (1.5)	20.9 (1.7)	20.8 (1.7)	20.8 (1.7)
Birth length (cm)	47.6 (3.0)	45.2 (2.00) [a]	44.6 (1.87) [a]	49.5 (1.38) [b]	50.8 (1.75)	47.1 (2.27)
Birth length SDS	–1.50 (1.44)	–2.91 (0.80) [a]	–2.82 (0.77) [a]	–0.52 (0.46) [b]	0.11 (0.75)	–1.80 (1.02)
Birth weight (kg)	2.78 (0.7)	2.50 (0.49) [b]	2.19 (0.33) [b]	3.22 (0.56) [c]	3.29 (0.63) [c]	2.65 (0.55)
Birth weight SDS	–1.21 (1.38)	–1.94 (0.96) [a]	–2.35 (0.70) [a]	–0.20 (1.12) [c]	–0.09 (1.27) [c]	–1.54 (1.06)
Adult height (m)	1.68 (0.11)	1.57 (0.07) [d,e]	1.75 (0.08)	1.59 (0.07) [d,e]	1.77 (0.08)	1.66 (0.09)
Adult height SDS	–1.09 (1.36)	–2.61 (0.56) [d,e]	–0.16 (0.72) [b]	–2.48 (0.41) [d,e]	0.24 (0.86) [b]	–1.52 (0.97)
Adult weight (kg)	63.7 (12.6)	57.1 (11.5) [d,e]	70.2 (14.6)	56.4 (12.4) [d,e]	69.0 (11.0)	61.5 (9.2)
Adult weight SDS	–0.64 (1.41)	–1.47 (1.53) [d,e]	0.09 (1.17)	–1.78 (1.71) [d,e]	0.08 (0.97)	–0.86 (1.08)
Fat mass (kg) [1]	15.7 (8.87)	16.0 (8.93)	18.8 (10.86)	14.6 (9.34)	15.6 (8.78)	14.1 (6.89)
Fat mass (SDS) [1]		0.04 (1.02)	0.36 (1.24)	–0.12 (1.06)	0.00 (1.00)	–0.17 (0.78)
Lean body mass (kg) [1]	45.1 (9.89)	38.4 (7.37) [d,e]	48.1 (9.88)	39.2 (7.17) [d,e]	50.2 (9.89)	44.6 (8.77)
Lean body mass (SDS) [1]		–1.20 (0.75) [d,e]	–0.21 (1.00)	–1.12 (0.72) [d,e]	0.00 (1.00)	–0.57 (0.89)
Smokers (%)	27.3	26.3	30.4	45.2	23.6	23.7

Table 1. Continued

Variables	Total group (n = 297)	SGA–S (n = 42)	SGA–CU (n = 54)	ISS (n = 36)	Controls (n = 76)	Unclassified (n = 89)
Cholesterol (mmol/l) [1]	4.57 (0.95)	4.75 (0.93)	4.68 (1.06)	4.53 (0.97)	4.41 (0.85)	4.55 (0.95)
TG (mmol/l) [1]	1.03 (0.52)	1.02 (0.58)	1.11 (0.54)	1.06 (0.53)	0.99 (0.48)	1.02 (0.51)
LDLc (mmol/l) [1]	2.71 (0.83)	2.93 (0.81)	2.74 (0.96)	2.70 (0.86)	2.54 (0.71)	2.75 (0.83)
ApoB (g/l) [1]	0.83 (0.24)	0.89 (0.24)	0.84 (0.26)	0.84 (0.25)	0.77 (0.22)	0.83 (0.24)
HDLc (mmol/l) [1]	1.38 (0.37)	1.32 (0.28)	1.44 (0.41)	1.35 (0.36)	1.42 (0.37)	1.36 (0.39)
ApoA–1 (g/l) [1]	1.30 (0.23)	1.29 (0.20)	1.33 (0.24)	1.34 (0.25)	1.28 (0.23)	1.30 (0.24)
Lp(a) (g/l) [1,2]	0.21 (0.25)	0.25 (0.24)	0.16 (0.29)	0.25 (0.26)	0.18 (0.21)	0.22 (0.24)

Values are given as means (sd). M: Male, F: Female. TG: triglycerides, HDLc: high-density lipoprotein density cholesterol, LDLc: low density lipoprotein cholesterol, apoA-1: apolipoprotein A-1, apoB: apolipoprotein B, Lp(a): lipoprotein a.
[1]Not corrected for age, gender and adult size, [2]Lp(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 and ISS,
[b]: p< 0.02 compared with the other 3 subgroups,
[c]: p< 0.001 compared with SGA-S and SGA-CU,
[d]: p< 0.001 compared with SGA-CU,
[e]: p< 0.001 compared with controls.

Table 2. Prevalence of the 6 genotypes of ApoE (%) in the total study population, the 4 subgroups and the unclassified group.

ApoE genotype	Total group (n= 246)	SGA-S (n= 33)	SGA-CU (n= 44)	ISS (n= 26)	Controls (n= 70)	Unclassified (n= 73)
2/2	0.8 (2)	0 (0)	0 (0)	0 (0)	2.8 (2)	0 (0)
2/3	11.7 (29)	3.0 (1)	15.9 (7)	19.2 (5)	14.3 (10)	8.2 (6)
2/4	3.7 (9)	0 (0)	2.3 (1)	3.8 (1)	4.3 (3)	5.5 (4)
3/3	58.1 (143)	72.7 (24)	45.5 (20)	50 (13)	54.3 (38)	65.8 (48)
3/4	24.0 (59)	24.2 (8)	31.8 (14)	26.9 (7)	22.9 (16)	19.2 (14)
4/4	1.6 (4)	0 (0)	4.5 (2)	0 (0)	1.4 (1)	1.4 (1)

Data is given in percentages (n).

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 inversed, 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.

Variables	Model	Gender		Birth length SDS	Birth weight SDS	Adult height SDS		Adult weight SDS		Fat mass (kg)		LBM (kg)		ApoE genotype										Adj R^2
														ε2/ε2		ε2/ε3		ε2/ε4		ε3/ε4		ε4/ε4		
		β	P	P	P	β	P	β	P	β	P	β	P	β	P	β	P	β	P	β	P	β	P	
TC (mmol/l)	1	0.68	<0.001	n.s.	n.s.	−0.06	n.s.																	0.12
	2	0.68	**<0.001**	n.s.	n.s.	−0.13	**0.023**	0.11	**0.008**															0.14
	3	1.48	0.051	n.s.	n.s.	−0.10	n.s.			0.01	**0.049**	0.01	n.s											0.13
	4	1.15	n.s.	n.s.	n.s.	−0.07	n.s.			0.01	n.s.	0.02	n.s	−0.74	n.s.	−0.42	**0.019**	−0.44	n.s	0.35	**0.011**	0.65	n.s.	0.19
TG (log)	1	0.22	**<0.001**	n.s.	n.s.	−0.04	n.s.																	0.08
	2	0.22	**<0.001**	n.s.	n.s.	−0.08	**0.003**	0.08	**<0.001**															0.12
	3	0.49	n.s.	n.s.	n.s.	−0.08	**0.024**			0.01	**0.005**	0.01	n.s											0.11
	4	0.70	0.092	n.s.	n.s.	−0.06	0.093			0.01	**0.017**	0.02	n.s	0.23	n.s.	0.15	0.082	0.20	n.s	0.02	n.s.	0.03	n.s.	0.12
LDLc (log)	1	0.12	**<0.001**	n.s.	n.s.	−0.02	n.s.																	0.04
	2	0.12	**<0.001**	n.s.	n.s.	−0.04	**0.020**	0.04	**0.008**															0.06
	3	0.33	n.s.	n.s.	n.s.	−0.04	0.056			0.00	**0.044**	0.01	n.s											0.06
	4	0.11	n.s.	n.s.	n.s.	−0.03	n.s.			0.01	0.083	0.00	n.s	−0.41	**0.035**	−0.18	**0.001**	−0.16	0.078	0.11	**0.007**	0.22	n.s.	0.16
ApoB (log)	1	0.18	**<0.001**	n.s.	n.s.	−0.04	**0.014**																	0.12
	2	0.18	**<0.001**	n.s.	n.s.	−0.06	**0.001**	0.04	**0.002**															0.15
	3	0.42	0.056	n.s.	n.s.	−0.05	**0.007**			0.00	**0.018**	0.01	n.s											0.14
	4	0.27	n.s.	n.s.	n.s.	−0.04	**0.043**			0.01	0.073	0.01	n.s	−0.31	0.077	−0.15	**0.003**	−0.13	n.s	0.08	**0.026**	0.28	**0.023**	0.21
HDLc (mmol/l)	1	0.23	**<0.001**	n.s.	n.s.	0.03	n.s.																	0.10
	2	0.22	**<0.001**	n.s.	n.s.	0.05	**0.021**	−0.04	0.026															0.11
	3	0.43	n.s.	n.s.	0.069	0.10	**0.001**			−0.00	n.s.	0.00	n.s											0.14
	4	0.49	n.s.	n.s.	n.s.	0.11	**0.001**			−0.01	n.s.	−0.01	n.s	−0.09	n.s.	−0.05	n.s.	−0.07	n.s	−0.01	n.s.	0.04	n.s.	0.15
ApoA-1 (g/l)	1	0.20	**<0.001**	n.s.	n.s.	−0.02	n.s.																	0.17
	2	0.20	**<0.001**	n.s.	n.s.	0.01	n.s.	−0.01	n.s.															0.17
	3	0.39	**0.026**	n.s.	n.s.	0.02	n.s.			0.00	n.s.	0.00	n.s											0.19
	4	0.39	n.s.	n.s.	0.070	0.01	n.s.			0.00	n.s.	0.00	n.s	−0.07	n.s.	−0.04	n.s.	−0.09	n.s	−0.04	n.s.	0.11	n.s.	0.20

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.

	LDLc[2] (n= 206)				ApoB (log) (n= 208)				LDLc[2] (n= 171)				ApoB (log) (n= 173)			
	Model A								Model B							
Variables	β	p-value	[95% CI]		β	p-value	[95% CI]		β	p-value	[95% CI]		β	p-value	[95% CI]	
Age (yrs)	0.015	0.661			0.006	0.594			–0.034	0.354			–0.008	0.468		
Gender	0.327	0.005			0.164	< 0.0005			0.238	0.104			0.132	0.004		
%FM									0.009	0.180			0.004	0.074		
ApoE genotype									0.241	< 0.0005			0.112	< 0.0005		
SGA–S[1]	0.389	0.014	[0.076-0.702]		0.141	0.006	[0.019-0.225]		0.322	0.055	[–0.008-0.652]		0.111	0.034	[0.009-0.213]	
SGA–CU[1]	0.190	0.183	[–0.078-0.497]		0.091	0.051	[–0.003-0.171]		0.183	0.229	[–0.116-0.482]		0.070	0.141	[–0.024-0.163]	
ISS[1]	0.149	0.363	[–0.148-0.505]		0.091	0.091	[–0.020-0.194]		0.098	0.582	[–0.253-0.449]		0.046	0.407	[–0.063-0.156]	
Overall		0.014				< 0.0005				< 0.0005				< 0.0005		
R^2 adjusted		0.04				0.10				0.16				0.23		

[1]: The control group is the reference group, [2]: expressed as mmol/l.
CI: confidence interval, FM: fat mass, ApoE genotype tested as dummy's.

Comparison of subgroups

Of the 297 subjects, 208 could be included in one of the four subgroups. Their clinical characteristics and serum lipid levels are shown in Table 1. The significant differences in birth size and adult size between the subgroups are due to the selection criteria. SGA-S and ISS subjects had a significantly lower lean body mass than the other subgroups, when no corrections were made for age, gender and adult weight. Almost all uncorrected lipid levels were higher in the SGA and ISS groups compared to those of controls. However, levels were not significantly different and within the normal range.

Table 2 and Figure 1 show the prevalence of ApoE genotype in the subgroups. In SGA-S subjects, 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.

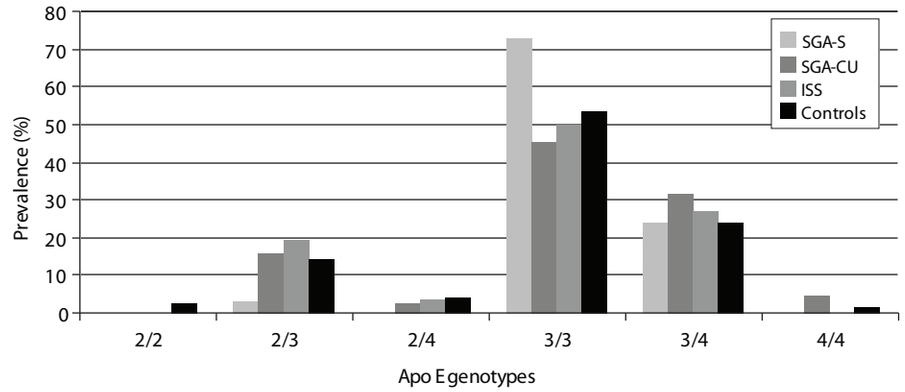


Figure 1. Prevalence of ApoE genotype per subgroup.

We also analyzed if there were differences in lipid 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) (Table 4). SGA-CU subjects almost had a significantly higher ApoB than controls (p= 0.051). There were no other significant differences in lipid levels between the four subgroups after correction for age and gender. Because of differences in fat mass and the prevalence of ApoE genotype between the subgroups, an additional correction for %body fat and ApoE genotype was performed. Only the difference in apoB levels remained significant between the SGA-S subjects and controls (p= 0.034).

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

We greatly thank Mrs. J. Dunk and Mrs. I. 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 Toorenenbergen for analyzing the lipid values.

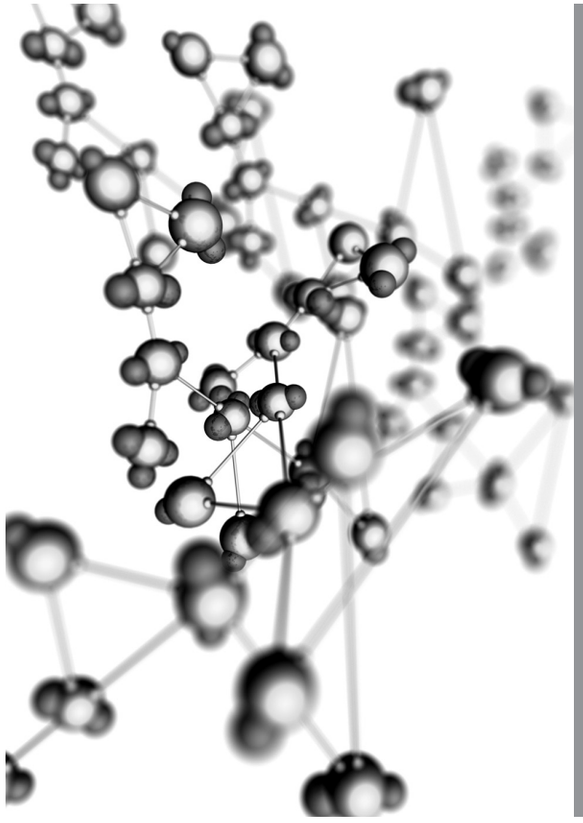
Grant support: The PROGRAM-study was financially supported by Netherlands Organisation for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

References

1. **Danesh J, Collins R, Peto R** 2000 Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 102:1082-5
2. **Klag MJ, Ford DE, Mead LA, He J, Whelton PK, Liang KY, Levine DM** 1993 Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 328:313-8
3. **Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E** 2001 High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 358:2026-33
4. **Ariyo AA, Thach C, Tracy R** 2003 Lp(a) lipoprotein, vascular disease, and mortality in the elderly. N Engl J Med 349:2108-15
5. **Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH** 1993 Growth in utero and serum cholesterol concentrations in adult life. Bmj 307:1524-7
6. **Davies AA, Smith GD, Ben-Shlomo Y, Litchfield P** 2004 Low birth weight is associated with higher adult total cholesterol concentration in men: findings from an occupational cohort of 25,843 employees. Circulation 110:1258-62
7. **Mogren I, Hogberg U, Stegmayr B, Lindahl B, Stenlund H** 2001 Fetal exposure, heredity and risk indicators for cardiovascular disease in a Swedish welfare cohort. Int J Epidemiol 30:853-62
8. **Martyn CN, Gale CR, Jespersen S, Sherriff SB** 1998 Impaired fetal growth and atherosclerosis of carotid and peripheral arteries. Lancet 352:173-8
9. **Lauren L, Jarvelin MR, Elliott P, Sovio U, Spellman A, McCarthy M, Emmett P, Rogers I, Hartikainen AL, Pouta A, Hardy R, Wadsworth M, Helmsdal G, Olsen S, Bakoula C, Lekea V, Millwood I** 2003 Relationship between birthweight and blood lipid concentrations in later life: evidence from the existing literature. Int J Epidemiol 32:862-76
10. **Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R** 2004 Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. Jama 292:2755-64
11. **Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC** 2005 Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). Clin Endocrinol (Oxf) 62:44-50
12. **Evagelidou EN, Giapros VI, Challa AS, Kiortsis DN, Tsatsoulis AA, Andronikou SK** 2007 Serum adiponectin levels, insulin resistance, and lipid profile in children born small for gestational age are affected by the severity of growth retardation at birth. Eur J Endocrinol 156:271-7
13. **Pulzer F, Haase U, Kratzsch J, Richter V, Rassoul F, Kiess W, Keller E** 1999 Lipoprotein(a) levels in formerly small-for-gestational-age children. Horm Res 52:241-6
14. **Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, Ong KK, Dunger DB, Mericq MV** 2003 Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab 88:3645-50
15. **Lopez-Bermejo A, Casano-Sancho P, Fernandez-Real JM, Kihara S, Funahashi T, Rodriguez-Hierro F, Ricart W, Ibanez L** 2004 Both intrauterine growth restriction and postnatal growth influence childhood serum concentrations of adiponectin. Clin Endocrinol (Oxf) 61:339-46
16. **Jaquet D, Deghmoun S, Chevenne D, Collin D, Czernichow P, Levy-Marchal C** 2005 Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. Diabetologia 48:849-55
17. **Mahley RW, Huang Y** 1999 Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. Curr Opin Lipidol 10:207-17
18. **Bennet AM, Di Angelantonio E, Ye Z, Wensley F, Dahlin A, Ahlbom A, Keavney B, Collins R, Wiman B, de Faire U, Danesh J** 2007 Association of apolipoprotein E genotypes with lipid levels and coronary risk. Jama 298:1300-11

19. **Song Y, Stampfer MJ, Liu S** 2004 Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. Ann Intern Med 141:137-47
20. **Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC** 2007 Fat Mass Accumulation During Childhood Determines Insulin Sensitivity in Early Adulthood. J Clin Endocrinol Metab
21. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10
22. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23
23. **Guo Y, Franks PW, Brookshire T, Antonio Tataranni P** 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. Obes Res 12:1925-9
24. **Slooter AJ, Houwing-Duistermaat JJ, van Harskamp F, Cruts M, Van Broeckhoven C, Breteler MM, Hofman A, Stijnen T, van Duijn CM** 1999 Apolipoprotein E genotype and progression of Alzheimer's disease: the Rotterdam Study. J Neurol 246:304-8
25. **Miura K, Nakagawa H, Tabata M, Morikawa Y, Nishijo M, Kagamimori S** 2001 Birth weight, childhood growth, and cardiovascular disease risk factors in Japanese aged 20 years. Am J Epidemiol 153:783-9
26. **Bavdekar A, Yajnik CS, Fall CH, Bapat S, Pandit AN, Deshpande V, Bhave S, Kellingray SD, Joglekar C** 1999 Insulin resistance syndrome in 8-year-old Indian children: small at birth, big at 8 years, or both? Diabetes 48:2422-9
27. **Suzuki T, Minami J, Ohrui M, Ishimitsu T, Matsuoka H** 2000 Relationship between birth weight and cardiovascular risk factors in Japanese young adults. Am J Hypertens 13:907-13
28. **Ziegler B, Johnsen SP, Thulstrup AM, Engberg M, Lauritzen T, Sorensen HT** 2000 Inverse association between birth weight, birth length and serum total cholesterol in adulthood. Scand Cardiovasc J 34:584-8
29. **Bergstrom E, Hernell O, Persson LA, Vessby B** 1995 Serum lipid values in adolescents are related to family history, infant feeding, and physical growth. Atherosclerosis 117:1-13
30. **Forrester TE, Wilks RJ, Bennett FI, Simeon D, Osmond C, Allen M, Chung AP, Scott P** 1996 Fetal growth and cardiovascular risk factors in Jamaican schoolchildren. Bmj 312:156-60
31. **Singhal A, Cole TJ, Fewtrell M, Lucas A** 2004 Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 363:1571-8
32. **Skidmore PM, Hardy RJ, Kuh DJ, Langenberg C, Wadsworth ME** 2004 Birth weight and lipids in a national birth cohort study. Arterioscler Thromb Vasc Biol 24:588-94
33. **Van Gaal LF, Mertens IL, De Block CE** 2006 Mechanisms linking obesity with cardiovascular disease. Nature 444:875-80
34. **Boomsma DI, Kempen HJ, Gevers Leuven JA, Havekes L, de Knijff P, Frants RR** 1996 Genetic analysis of sex and generation differences in plasma lipid, lipoprotein, and apolipoprotein levels in adolescent twins and their parents. Genet Epidemiol 13:49-60
35. **Sviridov D, Nestel PJ** 2007 Genetic factors affecting HDL levels, structure, metabolism and function. Curr Opin Lipidol 18:157-63
36. **Wang X, Cui Y, Tong X, Ye H, Li S** 2007 Glucose and lipid metabolism in small-for-gestational-age infants at 72 hours of age. J Clin Endocrinol Metab 92:681-4
37. **Quigley CA, Gill AM, Crowe BJ, Robling K, Chipman JJ, Rose SR, Ross JL, Cassorla FG, Wolka AM, Wit JM, Rekers-Mombarg LT, Cutler GB, Jr.** 2005 Safety of growth hormone treatment in pediatric patients with idiopathic short stature. J Clin Endocrinol Metab 90:5188-96
38. **Infante-Rivard C, Levy E, Rivard GE, Guiguet M, Feoli-Fonseca JC** 2003 Small babies receive the cardiovascular protective apolipoprotein epsilon 2 allele less frequently than expected. J Med Genet 40:626-9



Chapter 5

Growth Patterns During Childhood and the Relationship with Acylation Stimulating Protein

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Submitted

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 glucocorticosteriods, 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)) 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). 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, TG, LDLc, ApoB, insulin sensitivity and AIRg were log transformed. Multiple linear regression (MR) analyses were performed to determine the association between birth size, adult size and 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.

Variables	Total group (n= 285)	SGA–S (n= 31)	SGA–CU (n= 58)	ISS (n= 30)	Controls (n= 79)
Males (%)	38	36	38	37	38
Age	20.9 (1.7)	20.6 (1.8)	21.1 (1.5)	20.8 (1.7)	20.8 (1.7)
Birth length (cm)	47.7 (3.1)	45.0 (2.2)	44.5 (2.0)	49.7 (1.1) [1]	50.7 (1.7) [1]
Birth length SDS	–1.45 (1.4)	–2.96 (0.85)	–2.85 (0.83)	–0.51 (0.5) [1]	0.07 (0.7) [1]
Birth weight (kg)	2.78 (0.7)	2.44 (0.50)	2.17 (0.35)	3.28 (0.6) [2]	3.29 (0.6) [2]
Birth weight SDS	–1.19 (1.4)	–2.04 (1.0)	–2.36 (0.72)	–0.15 (1.2) [2]	–0.11 (1.2) [2]
Adult height (m)	1.69 (10.4)	1.58 (6.8)	1.74 (7.7) [3]	1.59 (6.4)	1.77 (7.8) [3]
Adult height SDS	–0.95 (1.3)	–2.60 (0.6)	–0.18 (0.7) [1]	–2.44 (0.4)	0.23 (0.9) [1]
Adult weight (kg)	64.2 (12.3)	57.0 (12.6)	69.8 (13.2) [3]	55.0 (11.3)	68.7 (11.0) [3]
Adult weight SDS	–0.56 (1.4)	–1.52 (1.5)	0.09 (1.1) [3]	–1.90 (1.7)	0.05 (1.0) [3]
BMI	22.4 (3.5)	23.0 (4.9)	22.9 (3.6)	21.8 (4.9)	21.9 (2.9)
ASP (nM)*	15.7 (11–21)	12.9 (9–21)	15.6 (11–21)	13.8 (10–18)	15.8 (11–21)
TC (mmol/l)	4.52 (0.93)	4.67 (0.95)	4.67 (1.05)	4.44 (0.98)	4.41 (0.85)
Triglycerides (mmol/l)	1.03 (0.52)	0.99 (0.56)	1.11 (0.53)	1.03 (0.53)	0.99 (0.48)
FFA (mmol/l)	0.61 (0.25)	0.60 (0.25)	0.67 (0.22)	0.69 (0.28)	0.53 (0.24) [4]
LDL (mmol/l)	2.66 (0.81)	2.83 (0.83)	2.73 (0.95)	2.59 (0.83)	2.53 (0.72)
ApoB (g/l)	0.82 (0.23)	0.87 (0.25)	0.84 (0.25)	0.83 (0.26)	0.76 (0.22)
HDL (mmol/l)	1.39 (0.37)	1.34 (0.30)	1.43 (0.41)	1.39 (0.38)	1.42 (0.36)
ApoA1 (g/l)	1.31 (0.23)	1.29 (0.21)	1.32 (0.24)	1.36 (0.26)	1.28 (0.23)
Insulin sensitivity**	6.95 (4.40)	6.19 (3.97)	4.88 (3.11)	7.10 (4.49)	7.98 (4.05) [5]
Acute insulin response**	556 (536)	686 (790)	782 (777)	415 (279)	456 (283)

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

	ASP*					
	Model A		**Model B**		**Model C**	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Age (yrs)	–0.01	0.597	–0.01	0.518	–0.02	0.379
Gender	0.26	< 0.0005	0.26	< 0.0005	–0.83	0.102
Birth length SDS	–0.04	0.291	–0.04	0.274	–0.04	0.279
Birth weight SDS	0.02	0.591	0.03	0.431	0.02	0.644
Height SDS	0.02	0.668	–0.05	0.197	–0.09	0.056
Weight SDS			0.11	< 0.0005		
Fat mass (kg)					0.01	0.060
LBM (kg)					–0.02	0.233
Overall		0.012		< 0.0005		< 0.0005
R^2 adjusted		0.04		0.08		0.09

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.

Variables	**ASP***	**FFA**	**TG***	**TC**	**LDLc***	**ApoB***	**HDLc**	**ApoA1**	**Si***
FFA	–0.06								
TG*	**0.16** [1]	0.11							
TC	**0.13** [1]	0.02	**0.34** [2]						
LDLc*	0.10	–0.01	**0.18** [2]	**0.86** [2]					
ApoB*	**0.15** [1]	0.02	**0.41** [2]	**0.87** [2]	**0.90** [2]				
HDLc	0.02	–0.04	**–0.13** [1]	**0.27** [2]	–0.11	–0.10			
ApoA1	0.11	–0.13	0.10	**0.27** [2]	–0.07	0.03	**0.78** [2]		
Si*	**–0.28** [1]	**–0.31** [2]	**–0.25** [1]	**–0.25** [1]	**–0.24** [1]	**–0.26** [1]	0.14	0.06	
AIRg*	**0.19** [1]	–0.16	**0.20** [1]	0.19	0.17	0.17	–0.03	–0.03	**–0.41** [2]

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 lower 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.

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

	SGA–S		SGA–CU		ISS	
Variables	**Model A**	**Model B**	**Model A**	**Model B**	**Model A**	**Model B**
ASP*	–0.12 (0.35)	–0.16 (0.22)	0.01 (0.90)	–0.03 (0.82)	–0.17 (0.18)	–0.19 (0.14)
FFA	0.07 (0.16)	0.07 (0.19)	**0.15 (< 0.001)**	**0.15 (0.001)**	0.17 (0.001)	0.17 (0.001)
TG*	–0.03 (0.73)	–0.07 (0.50)	0.12 (0.15)	0.09 (0.30)	0.01 (0.89)	0.00 (0.99)
TC	0.26 (0.18)	0.23 (0.24)	0.28 (0.09)	0.25 (0.13)	0.07 (0.71)	0.06 (0.75)
LDLc*	0.11 (0.09)	0.10 (0.13)	0.07 (0.22)	0.06 (0.31)	0.03 (0.63)	0.03 (0.68)
ApoB*	**0.13 (0.03)**	0.11 (0.06)	0.09 (0.07)	0.08 (0.12)	0.09 (0.16)	0.08 (0.18)

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 gender and % body fat.

Discussion

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 showed that the association was mainly based on fat mass, indicating that subjects with more fat have a higher risk to develop ASP resistance and therefore delayed TG clearance in early adulthood. This is supported by our previous report showing that fat accumulation during childhood was related to higher TG levels in early adulthood (21). ASP was positively related to TG levels, indicating that fat accumulation during childhood could result in ASP resistance. Women had higher ASP levels but this was due to a higher % body fat in women because after adjustment for % body fat, the difference disappeared.

Higher levels of ASP are seen in obese subjects, consistent with production from fat tissue. However, metabolic dysfunction also leads to higher ASP levels, even in lean subjects, such as lean subjects with type 2 diabetes mellitus or lean women with polycystic ovary syndrome (10,31). This may be a consequence of increased insulin secretion in subjects with reduced insulin sensitivity, as in vitro studies demonstrate that insulin increases the production of C3, the precursor of ASP (8). Our results support this hypothesis as insulin sensitivity was negatively and insulin secretion positively related to ASP levels in the young adults.

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.

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

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. I. 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 Toorenenbergen for analyzing the lipid values.

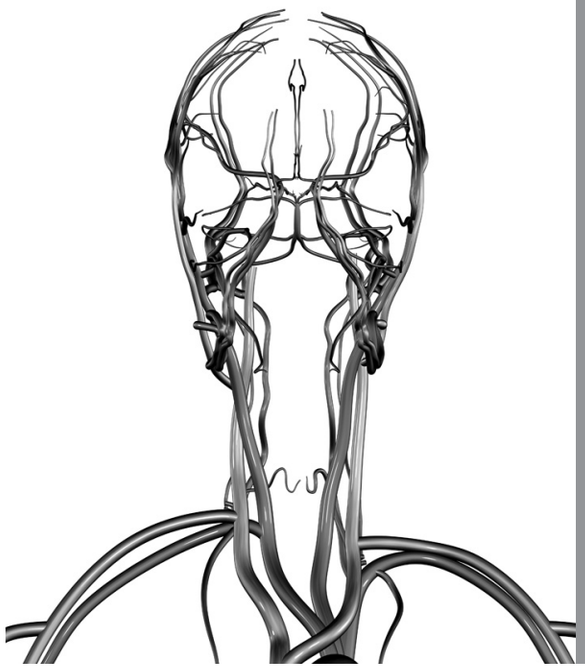
Grant support: The PROGRAM-study was financially supported by Netherlands Organization for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands. KC is supported by a Canada Research Chair and a grant from CIHR.

Potential Financial Conflicts of Interest: none

References

1. **Cianflone K, Xia Z, Chen LY** 2003 Critical review of acylation-stimulating protein physiology in humans and rodents. Biochim Biophys Acta 1609:127-143
2. **Germinario R, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K** 1993 Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. Metabolism 42:574-580
3. **Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA** 1991 Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. Lipids 26:495-499
4. **Faraj M, Sniderman AD, Cianflone K** 2004 ASP enhances in situ lipoprotein lipase activity by increasing fatty acid trapping in adipocytes. J Lipid Res 45:657-666
5. **Van Harmelen V, Reynisdottir S, Cianflone K, Degerman E, Hoffstedt J, Nilsell K, Sniderman A, Arner P** 1999 Mechanisms involved in the regulation of free fatty acid release from isolated human fat cells by acylation-stimulating protein and insulin. The Journal of biological chemistry 274:18243-18251
6. **Wamba PC, Mi J, Zhao XY, Zhang MX, Wen Y, Cheng H, Hou DQ, Cianflone K** 2008 Acylation stimulating protein but not complement C3 associates with metabolic syndrome components in Chinese children and adolescents. European journal of endocrinology / European Federation of Endocrine Societies 159:781-790
7. **Maslowska M, Vu H, Phelis S, Sniderman AD, Rhode BM, Blank D, Cianflone K** 1999 Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. European journal of clinical investigation 29:679-686
8. **Maslowska M, Scantlebury T, Germinario R, Cianflone K** 1997 Acute in vitro production of acylation stimulating protein in differentiated human adipocytes. J Lipid Res 38:1-11
9. **St-Pierre DH, Cianflone K, Smith J, Coderre L, Karelis AD, Imbeaut P, Lavoie JM, Rabasa-Lhoret R** 2008 Change in plasma acylation stimulating protein during euglycemic-hyperinsulinemic clamp in overweight and obese postmenopausal women: a MONET study. Clinical endocrinology
10. **Yang Y, Lu HL, Zhang J, Yu HY, Wang HW, Zhang MX, Cianflone K** 2006 Relationships among acylation stimulating protein, adiponectin and complement C3 in lean vs obese type 2 diabetes. Int J Obes (Lond) 30:439-446
11. **Cianflone KM, Maslowska MH, Sniderman AD** 1990 Impaired response of fibroblasts from patients with hyperapobetalipoproteinemia to acylation-stimulating protein. J Clin Invest 85:722-730
12. **Zhang XJ, Cianflone K, Genest J, Sniderman AD** 1998 Plasma acylation stimulating protein (ASP) as a predictor of impaired cellular biological response to ASP in patients with hyperapoB. Eur J Clin Invest 28:730-739
13. **Wen Y, Wang H, MacLaren R, Wu J, Lu H, Cianflone K** 2008 Palmitate and oleate induction of acylation stimulating protein resistance in 3T3-L1 adipocytes and preadipocytes. Journal of cellular biochemistry 104:391-401
14. **Bhattacharya S, Dey D, Roy SS** 2007 Molecular mechanism of insulin resistance. Journal of biosciences 32:405-413
15. **Cianflone K, Zakarian R, Couillard C, Delplanque B, Despres JP, Sniderman A** 2004 Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance. J Lipid Res 45:124-131
16. **Castro Cabezas M, Erkelens DW** 1998 The direct way from gut to vessel wall: atheroinitiation. Eur J Clin Invest 28:504-505
17. **Cianflone K, Lu H, Smith J, Yu W, Wang H** 2005 Adiponectin, acylation stimulating protein and complement C3 are altered in obesity in very young children. Clinical endocrinology 62:567-572
18. **Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC** 2008 Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. J Clin Endocrinol Metab 93:445-451

19. **Leunissen RW, Stijnen T, Hokken-Koelega AC** 2008 Influence of birth size on body composition in early adulthood. The PROGRAM-study. Clin Endocrinol (Oxf)
20. **Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2007 Association of weight gain in infancy and early childhood with metabolic risk in young adults. The Journal of clinical endocrinology and metabolism 92:98-103
21. **Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC** 2008 Fat Mass and ApoE Genotype Influence Serum Lipoprotein Levels in Early Adulthood, whereas Birth Size Does Not. J Clin Endocrinol Metab
22. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-271
23. **Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S** 1993 Longitudinal follow-up of growth in children born small for gestational age. Acta Paediatr 82:438-443
24. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-910
25. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
26. **Guo Y, Franks PW, Brookshire T, Antonio Tataranni P** 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. Obesity research 12:1925-1929
27. **Bergman RN, Phillips LS, Cobelli C** 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. The Journal of clinical investigation 68:1456-1467
28. **Pacini G, Bergman RN** 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Computer methods and programs in biomedicine 23:113-122
29. **Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN** 2003 MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes technology & therapeutics 5:1003-1015
30. **Bergman RN** 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-1527
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 .....



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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Submitted

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 glucocorticosteriods, 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, Datascope 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

(centile 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.

Variables	Total group (n= 243)	SGA–S (n=35)	SGA–CU (n= 44)	ISS (n= 27)	controls (n= 64)
Male (%)	37.9	31.4	38.6	37.0	40.6
Age	20.9 (1.7)	20.6 (1.7)	21.4 (1.4)	20.8 (1.8)	20.7 (1.8)
Birth length (cm)	47.7 (3.1)	45.0 (2.2)	44.5 (2.0)	49.4 (1.2) [1]	50.9 (1.8) [1]
Birth length SDS	–1.47 (1.48)	–2.98 (0.87)	–2.88 (0.85)	–0.54 (0.48) [1]	0.14 (0.73) [1]
Birth weight (kg)	2.82 (0.69)	2.48 (0.51)	2.17 (0.38)	3.23 (0.61) [2]	3.36 (0.58) [2]
Birth weight SDS	–1.12 (1.43)	–1.96 (0.99)	–2.38 (0.75)	–0.16 (1.24) [2]	0.08 (1.17) [2]
Adult height (m)	1.69 (0.11)	1.57 (0.07)	1.75 (0.08) [3]	1.59 (0.06)	1.78 (0.08) [3]
Adult height SDS	–1.03 (1.41)	–2.62 (0.58)	–0.09 (0.75) [1]	–2.39 (0.37)	0.38 (0.91) [1]
Adult weight (kg)	63.7 (12.4)	56.8 (12.1)	71.2 (13.2) [3]	54.5 (10.5)	69.4 (11.1) [3]
Adult weight SDS	–0.63 (1.43)	–1.52 (1.62)	0.21 (1.11) [3]	–1.96 (1.69)	0.10 (0.93) [3]
BMI	22.4 (3.5)	23.0 (4.9)	23.2 (3.6)	21.5 (4.1)	21.8 (2.8)
Fat mass (kg) *	15.2 (8.0)	15.9 (9.4)	18.3 (8.2)	14.0 (8.4)	15.0 (8.0)
Fat mass (SDS) *		0.12 (1.18)	0.40 (1.02)	–0.13 (1.05)	0.00 (1.00)
Trunk fat/total fat ratio *	0.48 (0.06)	0.47 (0.06)	0.49 (0.07)	0.46 (0.06)	0.47 (0.05)
Lean body mass (kg) *	45.4 (10.1)	38.1 (7.8)	49.3 (9.9)[3]	37.6 (6.3)	51.2 (9.5) [3]
Lean body mass (SDS) *		–1.38 (0.82)	–0.21 (1.04)[3]	–1.43 (0.66)	0.00 (1.00) [3]
Alcohol users (%)	75.7	77.1	80.0	81.5	78.1
Smokers (%)	25.5	22.9	35.0	37.0	20.3
cIMT (mm)*	0.52 (0.05)	0.50 (0.04)	0.53 (0.06)[4]	0.50 (0.05)	0.52 (0.05)
Artery diameter (mm) *	6.66 (0.48)	6.37 (0.41)	6.76 (0.42)[3]	6.44 (0.46)	6.79 (0.48)[3]
cIMT/artery diameter ratio	0.078 (0.007)	0.078 (0.006)	0.079 (0.008)	0.077 (0.007)	0.076 (0.007)
Systolic BP (mmHg) *	110.0 (9.0)	108.0 (10.2)	112.6 (10.3)	107.3 (10.4)	110.0 (7.3)
Diastolic BP (mmHg) *	66.1 (5.9)	65.9 (8.2)	66.4 (6.0)	65.4 (6.8)	66.1 (5.0)
Heart rate (beats/min) *	65 (9.0)	71 (9.7)[5]	65 (9.1)	67 (7.6)	64 (8.4)

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-S, [5]: p< 0.05 compared with SGA-CU and controls.

Table 2. Multiple regression of systolic and diastolic blood pressure in early adulthood.

Variables	SBP (mmHg)								DBP (mmHg)							
	Model A		Model B		Model C		Model D		Model A		Model B		Model C		Model D	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Age (yrs)	−0.02	0.947	−0.05	0.873	−0.01	0.969	−0.21	0.536	0.39	0.107	0.38	0.099	0.40	0.092	0.34	0.178
Gender	−7.48	**<0.001**	−7.41	**<0.001**	−7.33	**<0.001**	−3.50	0.667	−1.77	0.051	−1.72	**0.049**	−1.69	0.054	−2.58	0.661
SES	−2.73	**0.008**	−2.53	**0.008**	−2.41	**0.012**	−2.34	**0.020**	−1.09	0.122	−0.98	0.151	−0.94	0.173	−0.95	0.192
Smoking	−0.80	0.563	0.08	0.953	0.016	0.990	−0.18	0.892	−1.81	0.061	−1.38	0.140	−1.40	0.136	−1.65	0.088
Alcohol use	3.68	**0.009**	3.11	**0.018**	3.23	**0.014**	3.47	**0.010**	2.56	**0.009**	2.29	**0.015**	2.33	**0.014**	2.52	**0.010**
Heart rate (beats/min)	0.29	**<0.001**	0.27	**<0.001**	0.28	**<0.001**	0.29	**<0.001**	0.25	**<0.001**	0.24	**<0.001**	0.24	**<0.001**	0.23	**<0.001**
Adult height SDS	0.63	0.261	−0.66	0.257	−0.69	0.238	−1.04	0.148	−0.02	0.953	−0.67	0.109	−0.68	0.105	−0.64	0.223
Birth length SDS	0.56	0.452	0.13	0.780	0.69	0.321	0.72	0.311	0.38	0.466	0.26	0.463	0.44	0.377	0.42	0.407
Birth weight SDS	−0.73	0.273			−0.67	0.278	−0.82	0.192	−0.25	0.594			−0.22	0.625	−0.22	0.623
Adult weight SDS			2.37	**<0.001**	2.36	**<0.001**					1.18	**<0.001**	1.17	**<0.001**		
Fat mass (kg)							0.22	**0.004**							0.14	**0.011**
LBM (kg)							0.39	0.131							0.06	0.741
Overall		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
R^2 adjusted		0.23		0.33		0.33		0.32		0.15		0.21		0.20		0.18

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

All models include the interaction term birth length SDS x adult height SDS and gender x 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 (Model A). Adult weight was a strong positive determinant of DBP (Model B), and after adding birth weight SDS to the model, the attained weight in early adulthood remained a significant determinant of DBP independent of birth weight SDS. After specifying adult weight in fat mass and lean body mass, fat mass appeared to be a strong positive determinant of DPB in early adulthood, next to alcohol use and heart rate. This indirectly indicates that fat accumulation from birth to early adulthood is positively related to DBP.

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 artery diameter were significant positive determinants, whereas adult height SDS was negatively related with cIMT (Model A). Birth weight SDS was replaced by adult weight SDS to investigate whether weight in early adulthood influenced cIMT, but this was not significantly related (Model B). Weight gain from birth to early adulthood did not influence cIMT, while female gender, SBP and artery diameter remained positive determinants (Model C). Specification of adult weight SDS 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. 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 and 6.94 mm vs. 6.48 mm, p< 0.001, respectively). Nevertheless, the cIMT / artery diameter ratio was higher in women than in men (0.079 vs. 0.077, p= 0.006), indicating that women have a relatively thicker carotid arterial wall than men.

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. Carotid IMT was significantly higher in SGA-CU subjects compared to SGA-S subjects. Carotid artery diameter was significantly smaller in the subgroups with short adult height than those with normal adult height, indicating that adult stature influences carotid artery diameter. This is confirmed by the fact that there was no difference in cIMT/artery diameter ratio between the subgroups. Heart rate was significantly higher in SGA-S subjects than in SGA-CU and controls.

Table 3. Multiple regression for cIMT in early adulthood.

	cIMT (mm)					
	Model A		Model B		Model C	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
Age (yrs)	0.001	0.551	0.001	0.621	0.001	0.567
Gender	0.022	**0.006**	0.023	**<0.001**	0.023	**0.004**
SES	–0.004	0.523	–0.004	0.491	–0.005	0.533
Smoking	0.013	0.075	0.013	0.087	0.013	0.094
Alcohol use	–0.002	0.839	–0.002	0.803	–0.001	0.855
Systolic BP (mmHg)	0.001	0.057	0.001	**0.033**	0.001	**0.036**
Artery diameter (mm)	0.060	**< 0.001**	0.061	**< 0.001**	0.062	**< 0.001**
Adult height SDS	–0.006	**0.044**	–0.005	0.152	–0.005	0.143
Birth length SDS	0.000	0.969	–0.002	0.453	0.000	1.000
Birth weight SDS	–0.002	0.506			–0.003	0.500
Adult weight SDS			–0.003	0.363	–0.003	0.360
Overall		< 0.001		< 0.001		< 0.001
R^2 adjusted		0.29		0.29		0.29

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).

Table 4. Subgroup analyses of SBP and DBP.

	SBP (mmHg)				DBP (mmHg)			
	Model 1		Model 2		Model 1		Model 2	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
SGA-S	0.15	0.948	–0.06	0.977	0.73	0.644	0.06	0.969
SGA-CU	1.84	0.362	–0.26	0.888	0.51	0.723	–0.40	0.757
ISS	–2.79	0.217	–0.55	0.812	–0.66	0.678	0.44	0.793
Overall		0.001		<0.001		0.776		<0.001
R^2 adjusted		0.12		0.33		0.00		0.21

Reference group is controls.
Model 1: corrected for age and gender.
Model 2: corrected for age, gender, heart rate, SES, smoking, alcohol use and adult weight SDS.

Table 5. Subgroup analyses of cIMT.

	cIMT (mm)					
	Model 1		Model 2		Model 3	
Variables	**β**	**p-value**	**β**	**p-value**	**β**	**p-value**
SGA-S	–0.021	0.079	0.002	0.854	0.002	0.830
SGA-CU	0.020	0.058*	0.023	**0.017**	0.019	**0.047**
ISS	–0.020	0.099	0.001	0.957	0.000	0.972
Overall		0.002		<0.001		<0.001
R^2 adjusted		0.09		0.25		0.28

Reference group is controls.
Model 1: corrected for age, gender.
Model 2: corrected for age, gender, and artery diameter.
Model 3: corrected for age, gender, artery diameter, smoking and SBP.
*: p< 0.05 compared with SGA-S and ISS.

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 (13-19), with controversial results. According to the most recent review, this was due to different study designs, publication bias and different statistical approaches (20). In order to investigate the relationship between birth size and blood pressure, we did not only use the statistical model of Lucas et al (31), 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 (8, 9), 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 (32). 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 (33). 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 (3,34). 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 (35). 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 (36). 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 (37). 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 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. 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

We greatly thank Mrs. J. Dunk and Mrs. I. 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 Mrs. I. Andringa for performing and analyzing the IMT measurements.

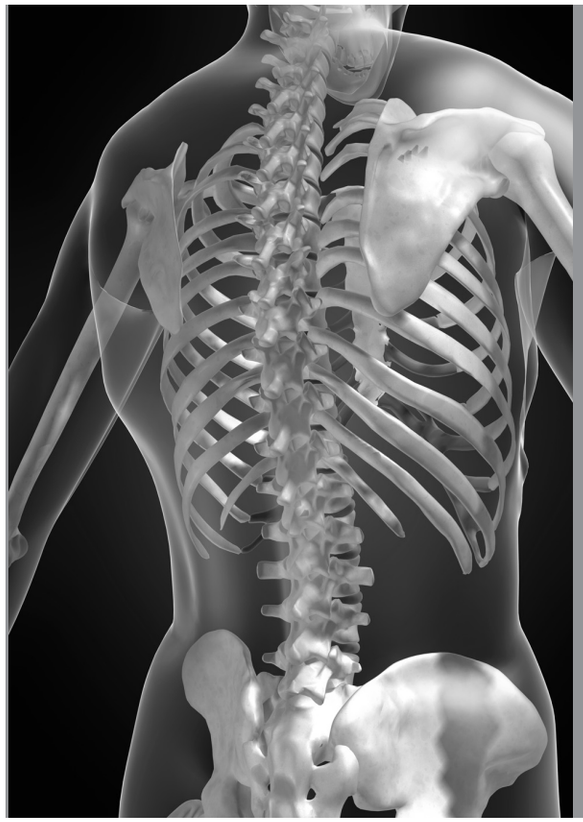
Grant support: The PROGRAM-study was financially supported by Netherlands Organisation for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

References

1. **Berenson GS, Srinivasan SR, Bao W, Newman WP, 3rd, Tracy RE, Wattigney WA** 1998 Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. The New England journal of medicine 338:1650-1656
2. **Tracy RE, Newman WP, 3rd, Wattigney WA, Srinivasan SR, Strong JP, Berenson GS** 1995 Histologic features of atherosclerosis and hypertension from autopsies of young individuals in a defined geographic population: the Bogalusa Heart Study. Atherosclerosis 116:163-179
3. **Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, Jarvisalo MJ, Uhari M, Jokinen E, Ronnemaa T, Akerblom HK, Viikari JS** 2003 Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Jama 290:2277-2283
4. **Barker DJ** 1990 The fetal and infant origins of adult disease. Bmj 301:1111
5. **Barker DJ** 2007 The origins of the developmental origins theory. J Intern Med 261:412-417
6. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. Bmj 320:967-971
7. **Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG** 2005 Trajectories of growth among children who have coronary events as adults. N Engl J Med 353:1802-1809
8. **Ben-Shlomo Y, McCarthy A, Hughes R, Tilling K, Davies D, Smith GD** 2008 Immediate postnatal growth is associated with blood pressure in early adulthood: the Barry Caerphilly Growth Study. Hypertension 52:638-644
9. **Uiterwaal CS, Anthony S, Launer LJ, Witteman JC, Trouwborst AM, Hofman A, Grobbee DE** 1997 Birth weight, growth, and blood pressure: an annual follow-up study of children aged 5 through 21 years. Hypertension 30:267-271
10. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-271
11. **Karlberg J, Albertsson-Wikland K** 1995 Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatr Res 38:733-739
12. **de Ridder MA, Engels MA, Stijnen T, Hokken-Koelega AC** 2008 Small for gestational age children without early catch-up growth: spontaneous growth and prediction of height at 8 years. Horm Res 70:203-208
13. **Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D** 2000 Fetal and childhood growth and hypertension in adult life. Hypertension 36:790-794
14. **Miura K, Nakagawa H, Tabata M, Morikawa Y, Nishijo M, Kagamimori S** 2001 Birth weight, childhood growth, and cardiovascular disease risk factors in Japanese aged 20 years. Am J Epidemiol 153:783-789
15. **Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M** 2002 Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation 105:1088-1092
16. **Jarvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, Hartikainen AL, Laitinen J, Zitting P, Rantakallio P, Elliott P** 2004 Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. Hypertension 44:838-846
17. **Menezes AM, Hallal PC, Horta BL, Araujo CL, Vieira Mde F, Neutzling M, Barros FC, Victora CG** 2007 Size at birth and blood pressure in early adolescence: a prospective birth cohort study. Am J Epidemiol 165:611-616
18. **Law CM, Shiell AW** 1996 Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. J Hypertens 14:935-941
19. **Huxley RR, Shiell AW, Law CM** 2000 The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. Journal of hypertension 18:815-831

20. **Huxley R, Neil A, Collins R** 2002 Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360:659-665
21. **Gamborg M, Byberg L, Rasmussen F, Andersen PK, Baker JL, Bengtsson C, Canoy D, Droyvold W, Eriksson JG, Forsen T, Gunnarsdottir I, Jarvelin MR, Koupil I, Lapidus L, Nilsen TI, Olsen SF, Schack-Nielsen L, Thorsdottir I, Tuomainen TP, Sorensen TI** 2007 Birth weight and systolic blood pressure in adolescence and adulthood: meta-regression analysis of sex- and age-specific results from 20 Nordic studies. Am J Epidemiol 166:634-645
22. **Davies AA, Smith GD, May MT, Ben-Shlomo Y** 2006 Association between birth weight and blood pressure is robust, amplifies with age, and may be underestimated. Hypertension 48:431-436
23. **Glynn RJ, L'Italien GJ, Sesso HD, Jackson EA, Buring JE** 2002 Development of predictive models for long-term cardiovascular risk associated with systolic and diastolic blood pressure. Hypertension 39:105-110
24. **de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, Kastelein JJ** 2004 Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation 109:III33-38
25. **O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr.** 1999 Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 340:14-22
26. **Stamler J** 1985 The marked decline in coronary heart disease mortality rates in the United States, 1968-1981; summary of findings and possible explanations. Cardiology 72:11-22
27. **Bots ML, Hofman A, De Jong PT, Grobbee DE** 1996 Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery. The Rotterdam Study. Ann Epidemiol 6:147-153
28. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-910
29. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
30. **Guo Y, Franks PW, Brookshire T, Antonio Tataranni P** 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. Obes Res 12:1925-1929
31. **Lucas A, Fewtrell MS, Cole TJ** 1999 Fetal origins of adult disease-the hypothesis revisited. Bmj 319:245-249
32. **Druet C, Ong KK** 2008 Early childhood predictors of adult body composition. Best practice & research 22:489-502
33. **Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A** 2007 Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115:213-220
34. **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
35. **Hellings WE, Moll FL, De Vries JP, Ackerstaff RG, Seldenrijk KA, Met R, Velema E, Derksen WJ, De Kleijn DP, Pasterkamp G** 2008 Atherosclerotic plaque composition and occurrence of restenosis after carotid endarterectomy. Jama 299:547-554
36. **Leunissen RW, Stijnen T, Hokken-Koelega AC** 2008 Influence of birth size on body composition in early adulthood. The PROGRAM-study. Clin Endocrinol (Oxf)
37. **Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML** 2004 Birth weight and carotid intima-media thickness: new perspectives from the atherosclerosis risk in young adults (ARYA) study. Ann Epidemiol 14:8-16



Chapter 7

Influence of Birth Size and Body Composition on Bone Mineral Density in Early Adulthood. The PROGRAM-study

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Clin Endocrinol (Oxf). 2008 Sep;69(3):386-92

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 glucocorticosteriods, 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 $BMAD_{LS} = BMD_{LS} \times [4(\pi \times width)]$, 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_{TB} and BMD_{LS}, 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).

The BMC, BMC/height, BMD_{TB}, BMD_{LS} 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 pair wise 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 dummies. 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.

Differences in bone mineral density between the subgroups

BMD_{TB} and BMD_{LS} of our study population are shown in Table 2. The differences between the subgroups are given after correction for age and gender. BMC and BMD_{TB} were significantly lower in the SGA-S and ISS groups than in controls and in the SGA-CU group. After correction for height, the difference in BMC between the 2 shorter groups and the 2 taller groups became less, but remained significant (BMC/height). BMD_{LS} was significantly lower in the SGA-S and ISS groups than in controls and in the SGA-CU group. However, when BMD_{LS} was corrected for bone size (BMAD) in each subgroup, the significant differences between the subgroups disappeared.

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

	Total group (n= 312)	Total men	Total women	SGA–S (n= 44)	SGA–CU (n= 56)	ISS (n= 38)	Controls (n= 79)
Gender (M/F)	121/191	121	191	15/29	22/34	16/22	29/50
Birth length (cm)	47.6 (3.08)	47.7 (3.0)	47.6 (3.0)	45.2 (1.98)*	44.6 (1.9)*	49.6 (1.4)§	50.8 (1.7)
Birth length (SDS)	–1.5 (1.48)	–1.5 (1.42)	–1.5 (1.45)	–2.90 (0.79)*	–2.86 (0.80)*	–0.53 (0.47)§	0.08 (0.75)
Birth weight (kg)	2.78 (0.67)	2.75 (0.65)	2.80 (0.69)	2.50 (0.48)§	2.19 (0.33)§	3.25 (0.56)	3.30 (0.62)
Birth weight (SDS)	–1.2 (1.39)	–1.3 (1.39)	–1.2 (1.38)	–1.94 (0.94)*#	–2.36 (0.70)*	–0.17 (1.14)^	–0.09 (1.27)
Age (years)	20.9 (1.68)	20.9 (1.65)	20.9 (1.70)	20.7 (1.76)	21.0 (1.5)	21.0 (1.7)	20.8 (1.71)
Height (cm)	168.2 (10.7)	175.1 (8.8)**	163.8 (9.4)	157.3 (6.7)*^	174.7 (7.8)	159.3 (6.7)*^	176.8 (7.7)
Height (SDS)	–1.1 (1.37)	–1.2 (1.23)	–1.0 (1.46)	–2.63 (0.55)*^	–0.14 (0.77)§	–2.48 (0.40)*^	0.24 (0.90)
Weight (kg)	63.8 (12.5)	69.0 (12.8)**	60.4 (11.2)	56.7 (11.4)*^	70.5 (14.4)	56.5 (12.2)*^	68.8 (11.0)
Weight (SDS)	–0.6 (1.42)	–0.7 (1.35)	–0.6 (1.46)	–1.55 (1.54)*^	0.12 (1.17)	–1.77 (1.67)*^	0.07 (0.98)
Delta weight[1] (SDS)	0.57 (1.99)	0.60 (1.99)	0.55 (2.0)	0.39 (1.76)	2.48 (1.33)§	–1.60 (2.22)§	0.16 (1.43)
Delta height[2] (SDS)	0.44 (1.72)	0.32 (1.66)	0.52 (1.75)	0.28 (0.93)	2.72 (0.99)§	–1.95 (0.57)§	0.16 (1.10)

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 between men and women, §: p< 0.02 compared with the other subgroups,

*: p< 0.001 compared with controls, #: p< 0.001 compared with ISS, ^: p< 0.001 compared with SGA-CU.

Table 2. Mean (sd) BMC, BMC/height, BMD_{TB}, BMD_{LS} and BMAD of the total study group and the 4 subgroups.

	Total group (n= 312)	Total men	Total women	SGA–S (n= 44)	SGA–CU (n= 56)	ISS (n= 38)	Controls (n= 79)
BMC (g)	2612 (534)	2922 (548)*	2416 (421)	2182 (329)#	2798 (513)	2177 (346)#	2985 (491)
BMC (SDS)				–1.63 (0.67)#	–0.38 (1.04)	–1.64 (0.70)#	0.00 (1.0)
BMC/height (g/cm)	15.4 (2.43)	16.6 (2.54)*	14.7 (2.02)	13.8 (1.85)#	16.0 (2.43)	13.6 (1.95)#	16.8 (2.31)
BMC/height (SDS)				–1.29 (0.80)#	–0.38 (1.05)	–1.38 (0.84)#	0.00 (1.0)
BMD_{TB} (g/cm^2)	1.17 (0.10)	1.21 (0.11)*	1.14 (0.08)	1.12 (0.08)#	1.19 (0.10)	1.10 (0.08)#	1.21 (0.09)
BMD_{TB} (SDS)				–0.90 (0.9)#	–0.23 (1.07)	–1.18 (0.89)#	0.00 (1.0)
BMD_{LS} (g/cm^2)	1.20 (0.14)	1.20 (0.15)	1.19 (0.13)	1.14 (0.13)#	1.21 (0.15)	1.10 (0.12)#	1.24 (0.12)
BMD_{LS} (SDS)				–0.83 (1.10)#	–0.26 (1,22)	–1.20 (1.01)#	0.00 (1.0)
BMAD (g/cm^3)	0.38 (0.04)	0.36 (0.04)*	0.39 (0.04)	0.38 (0.05)	0.37 (0.04)	0.36 (0.04)	0.37 (0.04)
BMAD (SDS)				0.19 (1.21)	–0.06 (1.06)	–0.37 (0.92)	0.00 (1.0)

Values are given as means (sd). Controls have been taken as reference group to calculate the BMC, BMC/height, BMD_{TB}, BMD_{LS} and BMAD in sd-score.
*: p< 0.001 between men and women,
The differences between the subgroups are given after correction for age and gender.
# p< 0.02 compared with SGA-CU and controls.

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).

	BMD_{TB}							
	Model A		Model B		Model C		Model D	
Variables	β [1]	p-value	β [1]	p-value	β [1]	p-value	β [1]	p-value
Age (yrs)	0.62	0.026	0.30	0.236	0.30	0.205	0.45	0.055
Gender	–1.66	0.178	–1.52	0.165	0.40	0.004	2.73	0.136
Height (cm)	0.48	< 0.0005	0.21	0.002	–0.32	0.686		
Birth length SDS	0.42	0.467	0.45	0.364	0.67	0.166	0.29	0.648
BL*AH (SDS)	0.44	0.058	0.33	0.107	0.33	0.088	0.36	0.041
Birth weight SDS	–0.38	0.471	–0.24	0.607	–0.66	0.145	1.17	0.068
Weight (kg)			0.38	< 0.0005				
LBM (kg)					0.87	< 0.0005	0.67	< 0.0005
Fat mass (kg)					0.22	< 0.0005		
Delta weight (SDS)							1.82	< 0.0005
Delta height (SDS)							–0.42	0.345
Overall		< 0.0005		< 0.0005		< 0.0005		< 0.0005
R^2		0.30		0.45		0.50		0.51
R^2 adjusted		0.28		0.44		0.49		0.49

[1]: refers to the unstandardized regression coefficients (1/100). BL: Birth length SDS; AH: Adult height SDS

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).

	BMD_{LS}							
	Model A		Model B		Model C		Model D	
Variables	β [1]	p-value	β [1]	p-value	β [1]	p-value	β [1]	p-value
Age (yrs)	0.77	0.044	0.41	0.331	0.42	0.310	0.60	0.142
Gender	0.62	0.439	0.63	0.001	12.5	< 0.0005	9.78	0.002
Height (cm)	0.59	< 0.0005	0.29	0.013	0.02	0.861		
Birth length SDS	1.35	0.119	1.39	0.104	1.63	0.054	1.49	0.176
BL*AH (SDS)	0.51	0.138	0.39	0.255	0.39	0.241	0.40	0.195
Birth weight SDS	–1.80	0.026	–1.65	0.037	–2.11	0.007	0.19	0.866
Weight (kg)			0.42	< 0.0005				
LBM (kg)					0.95	< 0.0005	0.70	< 0.0005
Fat mass (kg)					0.23	0.007		
Delta weight (SDS)							2.25	0.001
Delta height (SDS)							–0.15	0.850
Overall		< 0.0005		< 0.0005		< 0.0005		< 0.0005
R^2		0.14		0.24		0.27		0.27
R^2 adjusted		0.13		0.22		0.25		0.25

[1]: refers to the unstandardized regression coefficients (1/100). BL: Birth length SDS; AH: Adult height SDS

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.

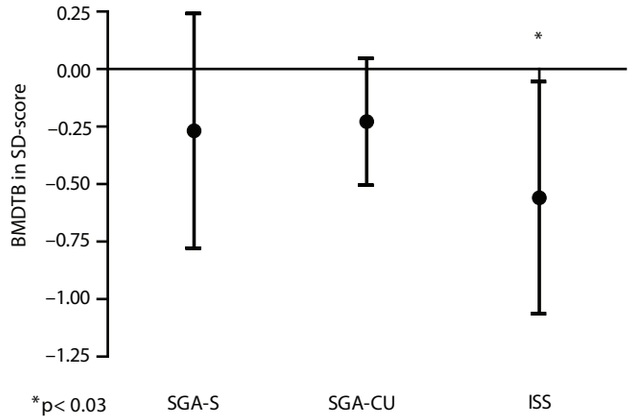


Figure 1. Difference in BMD_{TB} (SD-scores) between the subgroups, after correction for age, gender, adult height, adult weight and lean body mass. Controls are the reference group (0 SDS). SGA-S: SGA short, SGA-CU: SGA catch-up, ISS: idiopathic short stature. ***: p< 0.03 compared to controls.**

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 ($_{TB + LS}$), 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 estrogens 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 (26). 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:

The authors wish to thank all participants. We greatly thank Mrs. J. Dunk and Mrs. I. van Slobbe, research nurses, for their technical assistance and support with data collection. We acknowledge Mrs. J. Sluimer for checking the DXA results.

Grant support: The PROGRAM-study was financially supported by Netherlands Organisation for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

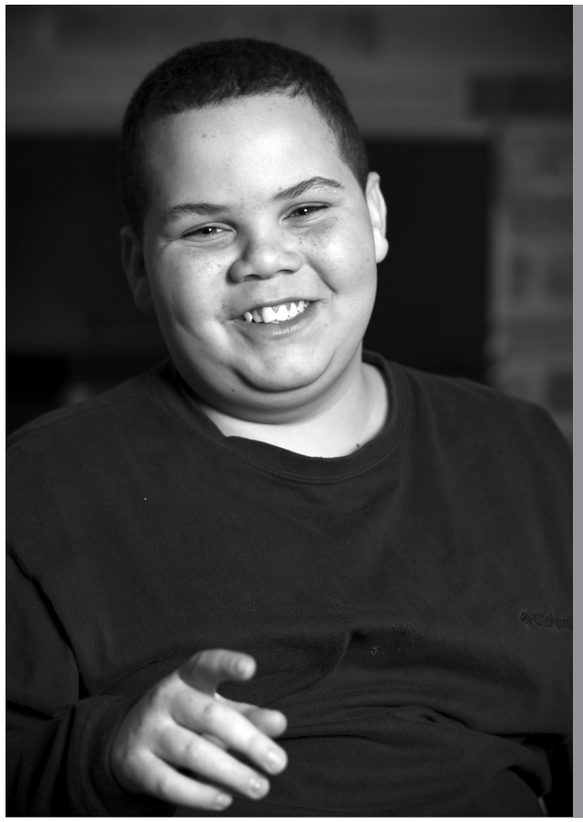
References

1. **Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM** 1993 Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet 341:72-75
2. **Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM** 2000 More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 15:2011-2018
3. **Ensrud KE, Lipschutz RC, Cauley JA, Seeley D, Nevitt MC, Scott J, Orwoll ES, Genant HK, Cummings SR** 1997 Body size and hip fracture risk in older women: a prospective study. Study of Osteoporotic Fractures Research Group. Am J Med 103:274-280
4. **Cummings SR, Marcus R, Palermo L, Ensrud KE, Genant HK** 1994 Does estimating volumetric bone density of the femoral neck improve the prediction of hip fracture? A prospective study. Study of Osteoporotic Fractures Research Group. J Bone Miner Res 9:1429-1432
5. **Melton LJ, 3rd, Atkinson EJ, O'Fallon WM, Wahner HW, Riggs BL** 1993 Long-term fracture prediction by bone mineral assessed at different skeletal sites. J Bone Miner Res 8:1227-1233
6. **Krall EA, Dawson-Hughes B** 1993 Heritable and life-style determinants of bone mineral density. J Bone Miner Res 8:1-9
7. **Ralston SH** 2002 Genetic control of susceptibility to osteoporosis. J Clin Endocrinol Metab 87:2460-2466
8. **Dennison EM, Syddall HE, Rodriguez S, Voropanov A, Day IN, Cooper C** 2004 Polymorphism in the growth hormone gene, weight in infancy, and adult bone mass. J Clin Endocrinol Metab 89:4898-4903
9. **Cooper C, Cawley M, Bhalla A, Egger P, Ring F, Morton L, Barker D** 1995 Childhood growth, physical activity, and peak bone mass in women. J Bone Miner Res 10:940-947
10. **Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D** 1997 Growth in infancy and bone mass in later life. Ann Rheum Dis 56:17-21
11. **Sayer AA, Cooper C** 2005 Fetal programming of body composition and musculoskeletal development. Early Hum Dev 81:735-744
12. **McGuigan FE, Murray L, Gallagher A, Davey-Smith G, Neville CE, Van't Hof R, Boreham C, Ralston SH** 2002 Genetic and environmental determinants of peak bone mass in young men and women. J Bone Miner Res 17:1273-1279
13. **Yarbrough DE, Barrett-Connor E, Morton DJ** 2000 Birth weight as a predictor of adult bone mass in postmenopausal women: the Rancho Bernardo Study. Osteoporos Int 11:626-630
14. **Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C** 2001 Intrauterine programming of adult body composition. J Clin Endocrinol Metab 86:267-272
15. **Dennison EM, Syddall HE, Sayer AA, Gilbody HJ, Cooper C** 2005 Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study. Pediatr Res 57:582-586
16. **te Velde SJ, Twisk JW, van Mechelen W, Kemper HC** 2004 Birth weight and musculoskeletal health in 36-year-old men and women: results from the Amsterdam Growth and Health Longitudinal Study. Osteoporos Int 15:382-388
17. **Jones G, Dwyer T** 2000 Birth weight, birth length, and bone density in prepubertal children: evidence for an association that may be mediated by genetic factors. Calcif Tissue Int 67:304-308
18. **Pietrobelli A, Faith MS, Wang J, Brambilla P, Chiumello G, Heymsfield SB** 2002 Association of lean tissue and fat mass with bone mineral content in children and adolescents. Obes Res 10:56-60
19. **Valdimarsson O, Kristinsson JO, Stefansson SO, Valdimarsson S, Sigurdsson G** 1999 Lean mass and physical activity as predictors of bone mineral density in 16-20-year old women. J Intern Med 245:489-496
20. **Wang MC, Bachrach LK, Van Loan M, Hudes M, Flegal KM, Crawford PB** 2005 The relative contributions of lean tissue mass and fat mass to bone density in young women. Bone 37:474-481

21. **Hogler W, Briody J, Woodhead HJ, Chan A, Cowell CT** 2003 Importance of lean mass in the interpretation of total body densitometry in children and adolescents. J Pediatr 143:81-88
22. **Crabtree NJ, Kibirige MS, Fordham JN, Banks LM, Muntoni F, Chinn D, Boivin CM, Shaw NJ** 2004 The relationship between lean body mass and bone mineral content in paediatric health and disease. Bone 35:965-972
23. **Lim S, Joung H, Shin CS, Lee HK, Kim KS, Shin EK, Kim HY, Lim MK, Cho SI** 2004 Body composition changes with age have gender-specific impacts on bone mineral density. Bone 35:792-798
24. **Carter DR, Bouxsein ML, Marcus R** 1992 New approaches for interpreting projected bone densitometry data. J Bone Miner Res 7:137-145
25. **Mazess RB, Barden H, Mautalen C, Vega E** 1994 Normalization of spine densitometry. J Bone Miner Res 9:541-548
26. **Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama SM** 1997 Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. J Clin Endocrinol Metab 82:57-62
27. **Blum M, Harris SS, Must A, Phillips SM, Rand WM, Dawson-Hughes B** 2001 Weight and body mass index at menarche are associated with premenopausal bone mass. Osteoporos Int 12:588-594
28. **Cooper C, Eriksson JG, Forsen T, Osmond C, Tuomilehto J, Barker DJ** 2001 Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporos Int 12:623-629
29. **Saito T, Nakamura K, Okuda Y, Nashimoto M, Yamamoto N, Yamamoto M** 2005 Weight gain in childhood and bone mass in female college students. J Bone Miner Metab 23:69-75
30. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-910
31. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
32. **Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC** 2007 Fat Mass Accumulation During Childhood Determines Insulin Sensitivity in Early Adulthood. J Clin Endocrinol Metab
33. **Johnson J, Dawson-Hughes B** 1991 Precision and stability of dual-energy X-ray absorptiometry measurements. Calcif Tissue Int 49:174-178
34. **Guo Y, Franks PW, Brookshire T, Antonio Tataranni P** 2004 The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. Obes Res 12:1925-1929
35. **Kroger H, Vainio P, Nieminen J, Kotaniemi A** 1995 Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 17:157-159
36. **Ezzahir N, Alberti C, Deghmoun S, Zaccaria I, Czernichow P, Levy-Marchal C, Jaquet D** 2005 Time course of catch-up in adiposity influences adult anthropometry in individuals who were born small for gestational age. Pediatr Res 58:243-247
37. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. Bmj 320:967-971
38. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-271
39. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. Clin Endocrinol (Oxf) 59:779-787
40. **Lanes R, Gunczler P, Esaa S, Weisinger JR** 2002 The effect of short- and long-term growth hormone treatment on bone mineral density and bone metabolism of prepubertal children with idiopathic short stature: a 3-year study. Clin Endocrinol (Oxf) 57:725-730
41. **Lanes R, Gunczler P, Weisinger JR** 1999 Decreased trabecular bone mineral density in children with idiopathic short stature: normalization of bone density and increased bone turnover after 1 year of growth hormone treatment. J Pediatr 135:177-181
42. **van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM** 2002 Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults. Arch Dis Child 87:341-347; discussion 341-347

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, Datascope 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, Beerse, 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.

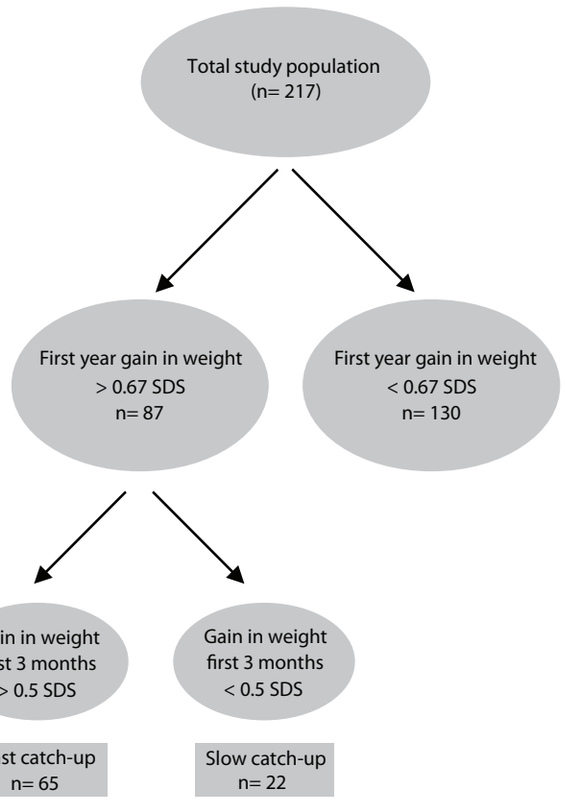

Figure 1. Derivation of sample into subgroups.

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 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

Clinical characteristics at birth			
Variable	**N**	**Mean**	**SD**
Males (%)	217	40.1	
Gestational age	217	39.3	1.7
Birth length (cm)	217	47.6	3.2
Birth length (SDS)	217	–1.54	1.45
Birth weight (kg)	217	2.78	0.67
Birth weight (SDS)	217	–1.21	1.34
First year growth characteristics			
Time period		**Gain in height SDS**	
	N	**Mean**	**SD**
Birth - 3 months	214	0.42	1.04
3-6 months	212	0.10	0.53
6-9 months	210	0.08	0.35
9-12 months	207	–0.03	0.30
		Gain in weight SDS	
	N	**Mean**	**SD**
Birth - 3 months	217	0.23	1.16
3-6 months	216	0.08	0.58
6-9 months	212	0.06	0.40
9-12 months	205	0.08	0.34
Clinical characteristics in early adulthood			
Variable	**N**	**Mean**	**SD**
Age (years)	217	20.8	1.67
Adult height (m)	217	1.68	0.11
Adult height (SDS)	217	–1.13	1.38

Table 1. Continued

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

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

Outcome in young adults	**Gain in weight SDS from 0-3 months**		**Gain in weight SDS from 3-6 months**		**Gain in weight SDS from 6-9 months**		**Gain in weight SDS from 9-12 months**	
	β	**P-value**	**β**	**P-value**	**β**	**P-value**	**β**	**P-value**
% body fat	0.639	0.275	–0.759	0.495	1.342	0.388	–3.149	0.082
BMI*	0.014	0.174	–0.021	0.312	0.033	0.241	–0.019	0.540
Trunk fat / total fat ratio	0.006	0.085	–0.005	0.463	0.007	0.481	0.004	0.756
Waist circumference	1.437	**0.040**	–0.098	0.942	1.995	0.293	0.082	0.968
Waist / hip ratio	0.002	0.718	0.009	0.345	0.000	0.993	0.017	0.261
Insulin sensitivity*#	–0.223	**0.008**	–0.155	0.323	0.157	0.490	–0.021	0.936
Acute insulin response*#	0.210	**0.028**	0.403	**0.019**	0.307	0.219	–0.091	0.749
Disposition index*#	–0.011	0.916	0.261	0.180	0.463	0.090	–0.140	0.641
Total cholesterol*	–0.002	0.892	0.022	0.402	0.014	0.717	0.017	0.677
LDL*	0.013	0.538	0.008	0.836	0.017	0.784	0.017	0.790
HDL*	–0.053	**0.005**	0.038	0.292	–0.025	0.626	0.081	0.142
Cholesterol / HDL ratio*	0.052	**0.014**	–0.020	0.625	0.040	0.497	–0.061	0.335
ApoB*	0.026	0.178	0.020	0.574	0.012	0.826	0.022	0.697
ApoA1	–0.019	0.194	0.029	0.300	–0.004	0.924	0.005	0.900
ApoB / ApoA1 ratio*	0.039	0.085	0.008	0.859	0.014	0.827	0.013	0.845
Triglycerides*	0.066	**0.040**	0.024	0.700	0.136	0.121	–0.069	0.460
Systolic BP	–1.08	0.122	0.461	0.733	1.825	0.316	–2.704	0.176

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*

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

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.

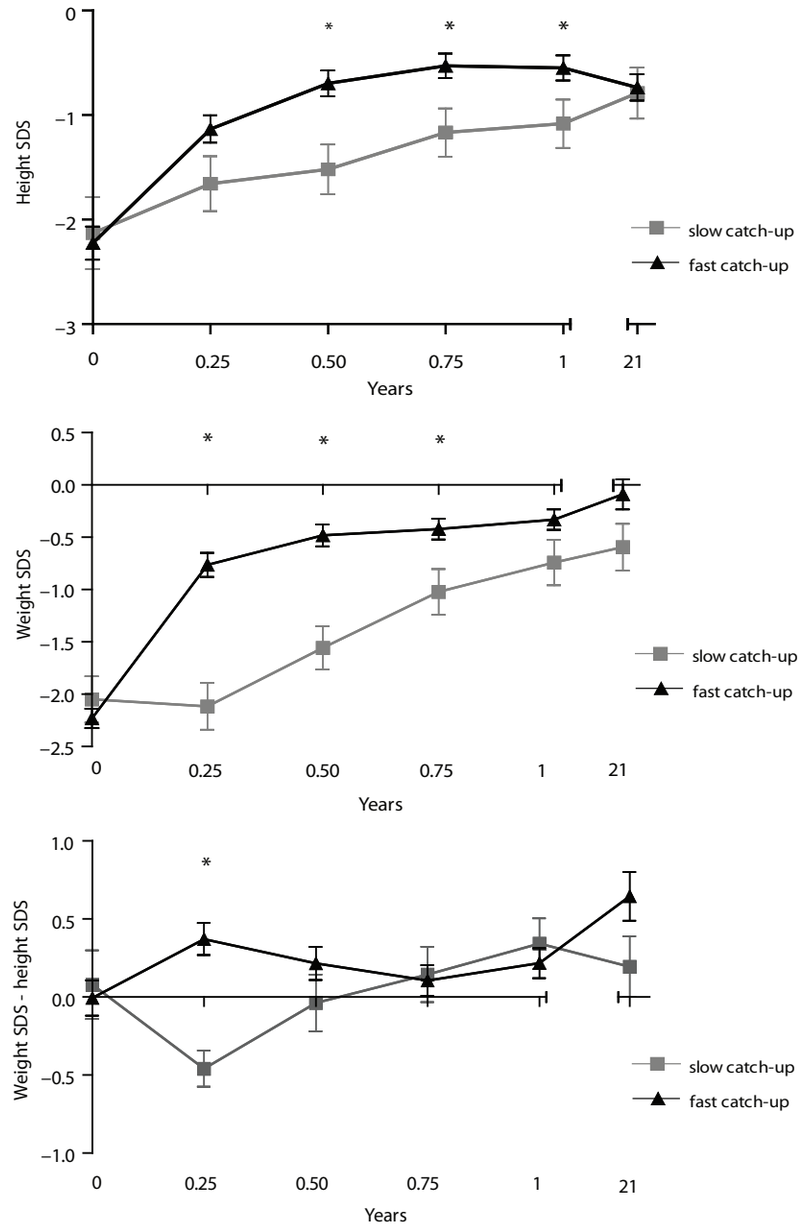

Figure 2. Height SDS, weight SDS and weight SDS relative to height SDS in the first year of life and in early adulthood between subjects with slow and fast catch-up growth in the first three months of life. Values are means (± SEM). *p< 0.05 between the two subgroups

Interestingly, the fast catch-up group had a significantly higher % body fat (**β**= 6.00, p= 0.006), a greater waist circumference (**β**= 6.19, p= 0.028), a higher trunk fat / total fat ratio (**β**= 0.029, p= 0.040) and a reduced insulin sensitivity (**β**= -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 in 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 intra-uterine 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 an 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.

Acknowledgements

We greatly thank Mrs. J. Dunk, Mrs. I. Andriga and Mrs. I. 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 Toorenenbergen for analyzing the lipid levels.

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.

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Acquisition of data: Leunissen, Kerkhof

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

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Obtained funding: Hokken-Koelega

Grant support: The PROGRAM-study was financially supported by Netherlands Organization for Scientific Research (NWO) (ACSHK received the ASPASIA-award (grant number 015 000 088)), and by grants from Revolving Fund 2001 and Vereniging Trustfonds, Erasmus University Rotterdam, and a grant by the Jan Dekkerstichting/ Dr Ludgardine Bouwmanstiching and the Stichting De Drie Lichten, The Netherlands.

Potential Financial Conflicts of Interest: none

References

1. **Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS** 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941
2. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. Bmj 320:967-971
3. **Baker JL, Olsen LW, Sorensen TI** 2007 Childhood body-mass index and the risk of coronary heart disease in adulthood. N Engl J Med 357:2329-2337
4. **Ibanez L, Ong K, Dunger DB, de Zegher F** 2006 Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab 91:2153-2158
5. **Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C** 2008 Consequences of being born small for gestational age on body composition: an 8-year follow-up study. J Clin Endocrinol Metab 93:3804-3809
6. **Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A** 2007 Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115:213-220
7. **Chomtho S, Wells JC, Williams JE, Davies PS, Lucas A, Fewtrell MS** 2008 Infant growth and later body composition: evidence from the 4-component model. Am J Clin Nutr 87:1776-1784
8. **Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2007 Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 92:98-103
9. **Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ** 1999 Catch-up growth in childhood and death from coronary heart disease: longitudinal study. Bmj 318:427-431
10. **Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG** 2005 Trajectories of growth among children who have coronary events as adults. N Engl J Med 353:1802-1809
11. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-910
12. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-323
13. **Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC** 2008 Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. J Clin Endocrinol Metab 93:445-451
14. **Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC** 2008 Fat Mass and ApoE Genotype Influence Serum Lipoprotein Levels in Early Adulthood, whereas Birth Size Does Not. J Clin Endocrinol Metab
15. **Bergman RN, Phillips LS, Cobelli C** 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 68:1456-1467
16. **Pacini G, Bergman RN** 1986 MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23:113-122
17. **Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN** 2003 MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. Diabetes Technol Ther 5:1003-1015
18. **Bergman RN** 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-1527

19. **Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ** 1989 Weight in infancy and death from ischaemic heart disease. Lancet 2:577-580
20. **Frankel S, Elwood P, Sweetnam P, Yarnell J, Smith GD** 1996 Birthweight, body-mass index in middle age, and incident coronary heart disease. Lancet 348:1478-1480
21. **Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ** 2001 Early growth and coronary heart disease in later life: longitudinal study. Bmj 322:949-953
22. **Belfort MB, Rifas-Shiman SL, Rich-Edwards J, Kleinman KP, Gillman MW** 2007 Size at birth, infant growth, and blood pressure at three years of age. J Pediatr 151:670-674
23. **Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2006 Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). Am J Clin Nutr 83:324-330
24. **Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M** 2002 Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation 105:1088-1092
25. **Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-271
26. **Leunissen RW, Stijnen T, Hokken-Koelega AC** 2008 Influence of birth size on body composition in early adulthood. The PROGRAM-study. Clin Endocrinol (Oxf)
27. **Singhal A, Cole TJ, Fewtrell M, Lucas A** 2004 Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 363:1571-1578
28. **Fewtrell MS, Morley R, Abbott RA, Singhal A, Stephenson T, MacFadyen UM, Clements H, Lucas A** 2001 Catch-up growth in small-for-gestational-age term infants: a randomized trial. The American journal of clinical nutrition 74:516-523
29. **Armstrong J, Reilly JJ** 2002 Breastfeeding and lowering the risk of childhood obesity. Lancet 359:2003-2004
30. **Gillman MW, Rifas-Shiman SL, Camargo CA, Jr., Berkey CS, Frazier AL, Rockett HR, Field AE, Colditz GA** 2001 Risk of overweight among adolescents who were breastfed as infants. Jama 285:2461-2467
31. **Kajantie E, Barker DJ, Osmond C, Forsen T, Eriksson JG** 2008 Growth before 2 years of age and serum lipids 60 years later: the Helsinki Birth Cohort study. Int J Epidemiol 37:280-289
32. **Eriksson JG, Osmond C, Kajantie E, Forsen TJ, Barker DJ** 2006 Patterns of growth among children who later develop type 2 diabetes or its risk factors. Diabetologia 49:2853-2858
33. **Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, Biswas SK, Ramji S, Prabhakaran D, Reddy KS** 2004 Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. N Engl J Med 350:865-875

Chapter 9

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

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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 diary 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.

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.

	Boys (n= 208)	Girls (n= 188)	p-value
Birth weight (kg)	3.61 (0.51)	3.38 (0.52)	<0.005
Gestational age (wk)	39.9 (1.6)	39.8 (1.5)	0.6
Milk feeding at 3 months (%)			
– breast	38.7	51.4	
– bottle & breast	28.9	29.5	
– bottle	32.4	19.1	0.007
Size at 12 months			
Length (cm)	76.9 (2.4)	74.8 (2.8)	<0.005
Weight (kg)	10.3 (1.0)	9.5 (1.1)	<0.005
BMI (kg/m^2)	17.3 (1.2)	16.9 (1.3)	0.001
Mean skinfolds SDS	–0.09 (0.73)	0.04 (0.79)	0.1
% receiving breast milk at 12 months	21.2	22.3	0.8
% receiving dairy milk at 12 months	93.8	91.0	0.3
– % receiving formula milk	70.1	71.2	0.8
– % receiving cow's milk	63.5	58.0	0.3
Dietary intakes at 12 months			
Total energy (MJ/d)	4.48 (0.81)	4.19 (0.80)	<0.005
Total protein (g/d)	38.9 (9.5)	35.7 (9.9)	0.002
Total fat (g/d)	44.0 (10.7)	41.8 (10.6)	0.04
Dairy milk protein (MJ/d)	11.3 (7.5)	9.7 (6.5)	0.03
– Formula milk protein (MJ/d)	5.6 (4.7)	5.8 (5.0)	0.7
– Cow's milk protein (MJ/d)	5.7 (7.8)	3.9 (5.9)	0.01
– Formula milk energy (g/d)	0.89 (0.75)	0.92 (0.76)	0.7
– Cow's milk energy (g/d)	0.45 (0.62)	0.31 (0.48)	0.02
– Cow's milk fat (g/d)	6.3 (8.7)	4.4 (6.9)	0.02

Total protein intake at 12 months appeared to be positively associated with length (P< 0.001), weight (P< 0.001) and BMI (P= 0.04), however these associations disappeared after adjustment for other nutritional factors. Total protein intake was not associated with adiposity.

Similarly, total fat intake appeared to be positively associated with length (P= 0.02), weight (P< 0.001) and BMI (P= 0.02) at 12 months, but these associations disappeared after adjusting for other nutritional factors. Total fat intake was not associated with adiposity.

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).

	Model 1			Model 2*		
	β	SE	p-value	β	SE	p-value
Total energy (per MJ)						
Length (SDS)	0.18	0.05	**<0.001**	0.17*	0.05	**0.001**
Weight (SDS)	0.22	0.05	**<0.001**	0.21*	0.05	**<0.001**
BMI (SDS)	0.14	0.05	**0.01**	0.14*	0.05	**0.01**
Adiposity (SDS)**	–0.08	0.05	0.1	–0.08*	0.05	0.1
Total protein (per 10g)						
Length (SDS)	0.16	0.04	**<0.001**	0.09	0.05	0.1
Weight (SDS)	0.17	0.04	**<0.001**	0.07	0.05	0.2
BMI (SDS)	0.09	0.04	0.04	0.01	0.06	0.8
Adiposity (SDS)**	–0.04	0.054	0.3	–0.01	0.05	0.8
Total fat (per 10g)						
Length (SDS)	0.09	0.04	0.02	–0.10	0.07	0.1
Weight (SDS)	0.14	0.04	<0.001	–0.03	0.07	0.7
BMI (SDS)	0.10	0.04	0.02	0.02	0.08	0.8
Adiposity (SDS)**	–0.03	0.04	0.4	0.06	0.07	0.4

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).

	Total protein (per 10g)					
	Model 1			Model 2		
	β	SE	p-value	β	SE	p-value
Dairy milk protein (per 10g)						
Length (SDS)	0.17	0.06	**0.003**	0.07	0.06	0.2
Weight (SDS)	0.20	0.06	**<0.001**	0.10	0.06	0.1
BMI (SDS)	0.13	0.06	**0.04**	0.08	0.07	0.2
Adiposity (SDS)*	0.16	0.06	**0.005**	0.13	0.06	**0.02**

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 the DONALD 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 diary 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

1. Ong KK, Loos RJ. Rapid infancy weight gain and subsequent obesity: systematic reviews and hopeful suggestions. Acta Paediatr 2006;95:904-8.
2. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ 2000;320:967-71.
3. Ekelund U, Ong KK, Linne Y, et al. Association of weight gain in infancy and early childhood with metabolic risk in young adults. J Clin Endocrinol Metab 2007;92:98-103.
4. Stettler N, Stallings VA, Troxel AB, et al. Weight gain in the first week of life and overweight in adulthood: a cohort study of European American subjects fed infant formula. Circulation 2005;111:1897-903.
5. Vanhala MJ, Vanhala PT, Keinanen-Kiukaanniemi SM, Kumpusalo EA, Takala JK. Relative weight gain and obesity as a child predict metabolic syndrome as an adult. Int J Obes Relat Metab Disord 1999;23:656-9.
6. Arenz S, Ruckerl R, Koletzko B, von Kries R. Breast-feeding and childhood obesity--a systematic review. Int J Obes Relat Metab Disord 2004;28:1247-56.
7. Armstrong J, Reilly JJ. Breastfeeding and lowering the risk of childhood obesity. Lancet 2002;359:2003-4.
8. Ong KK, Emmett PM, Noble S, Ness A, Dunger DB. Dietary energy intake at the age of 4 months predicts postnatal weight gain and childhood body mass index. Pediatrics 2006;117:e503-8.
9. Singhal A. Early nutrition and long-term cardiovascular health. Nutr Rev 2006;64:S44-9; discussion S72-91.
10. Singhal A, Fewtrell M, Cole TJ, Lucas A. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. Lancet 2003;361:1089-97.
11. Singhal A, Cole TJ, Fewtrell M, Lucas A. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 2004;363:1571-8.
12. Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F. Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Relat Metab Disord 1995;19:573-8.
13. Hoppe C, Molgaard C, Thomsen BL, Juul A, Michaelsen KF. Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children. Am J Clin Nutr 2004;79:494-501.
14. Dorosty AR, Emmett PM, Cowin S, Reilly JJ. Factors associated with early adiposity rebound. ALSPAC Study Team. Pediatrics 2000;105:1115-8.
15. Gunther AL, Remer T, Kroke A, Buyken 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.
16. Hopkins D, Emmett P, Steer C, Rogers I, Noble S, Emond A. Infant feeding in the second 6 months of life related to iron status: an observational study. Arch Dis Child 2007;92:850-4.
17. Agency FS. McCance and Widdowson's The Composition of Foods, Sixth summary edition: Cambridge: Royal Society of Chemistry, 2002.
18. Dietary reference values for food energy and nutrients for the United Kingdom. Report of the Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy. Rep Health Soc Subj (Lond) 1991;41:1-210.
19. Gunther AL, Buyken AE, Kroke A. The influence of habitual protein intake in early childhood on BMI and age at adiposity rebound: results from the DONALD Study. Int J Obes (Lond) 2006;30:1072-9.
20. Garlick PJ. Protein requirements of infants and children. Nestle Nutr Workshop Ser Pediatr Program 2006;58:39-47; discussion 47- 50.
21. Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF. Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. Am J Clin Nutr 2004;80:447-52.

22. Scavo LM, Karas M, Murray M, Leroith D. Insulin-like growth factor-I stimulates both cell growth and lipogenesis during differentiation of human mesenchymal stem cells into adipocytes. J Clin Endocrinol Metab 2004;89:3543-53.
23. Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS. Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. J Biol Chem 1988;263:9402-8.
24. Gunther AL, Buyken AE, Kroke A. Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. Am J Clin Nutr 2007;85:1626-33.
25. Skinner JD, Bounds W, Carruth BR, Morris M, Ziegler P. Predictors of children's body mass index: a longitudinal study of diet and growth in children aged 2-8 y. Int J Obes Relat Metab Disord 2004;28:476-82.
26. Magarey AM, Daniels LA, Boulton TJ, Cockington RA. Does fat intake predict adiposity in healthy children and adolescents aged 2--15 y? A longitudinal analysis. Eur J Clin Nutr 2001;55:471-81.
27. Karaolis-Danckert N, Gunther AL, Kroke A, Hornberg C, Buyken AE. How early dietary factors modify the effect of rapid weight gain in infancy on subsequent body-composition development in term children whose birth weight was appropriate for gestational age. Am J Clin Nutr 2007;86:1700-8.
28. Scaglioni S, Agostoni C, Notaris RD, et al. Early macronutrient intake and overweight at five years of age. Int J Obes Relat Metab Disord 2000;24:777-81.
29. Koletzko B, Broekaert I, Demmelmair H, et al. Protein intake in the first year of life: a risk factor for later obesity? The E.U. childhood obesity project. Adv Exp Med Biol 2005;569:69-79.
30. Weaning and the weaning diet. Report of the Working Group on the Weaning Diet of the Committee on Medical Aspects of Food Policy. Rep Health Soc Subj (Lond) 1994;45:1-113.
31. Human energy requirements: report of a joint FAO/ WHO/UNU Expert Consultation. Food Nutr Bull 2005;26:166.
32. Agostoni C, Decsi T, Fewtrell M, et al. Complementary feeding: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr Gastroenterol Nutr 2008;46:99-110.
33. Thorsdottir I, Gunnarsson BS, Atladottir H, Michaelsen KF, Palsson G. Iron status at 12 months of age -- effects of body size, growth and diet in a population with high birth weight. Eur J Clin Nutr 2003;57:505-13.

Chapter 10

General Discussion

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. Högler 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 (31,32). 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 and male adults, but not in adolescents. Some reports take adult size into account and others do not, which may account for these differences. Previously, a positive relationship between weight gain and lipid levels in later life has been shown (36,37). However, prior to our study, no one was able to substantiate whether this was due to a higher % body fat, as fat mass was not measured.

We expected an adverse lipid profile in SGA-CU subjects as they have a higher % body fat, but this was not found. An explanation for the similar lipid levels between SGA-CU subjects and controls might be that the difference in %body fat is not large enough to result in different lipid levels. SGA-S subjects had higher serum levels of LDLc and ApoB than controls. Only ApoB levels remained higher than controls after adjustment for % body fat and ApoE genotype. The reason for these higher levels remains unknown, but a reduced number of LDLc receptor in the liver might explain this difference. These results are in line with reported lipid levels in children born SGA, although previous studies did not differentiate between SGA with and without catch-up growth (6,14,38-40). 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$_{(TB+LS)}$ 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.

Our results showed that ISS subjects had lower BMD$_{(TB)}$ than controls. After correction for height and bone age, two studies showed lower BMD$_{(LS)}$ in ISS subjects, while no data were given about BMAD (77,78). The lower BMD$_{(TB)}$ might be due to a reduced bone turnover in ISS subjects (77). These results are in line with a report showing increased fracture risk in subjects with a reduced growth rate during childhood, which occurs in ISS subjects (74).

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-I 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

Chapter 10

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.

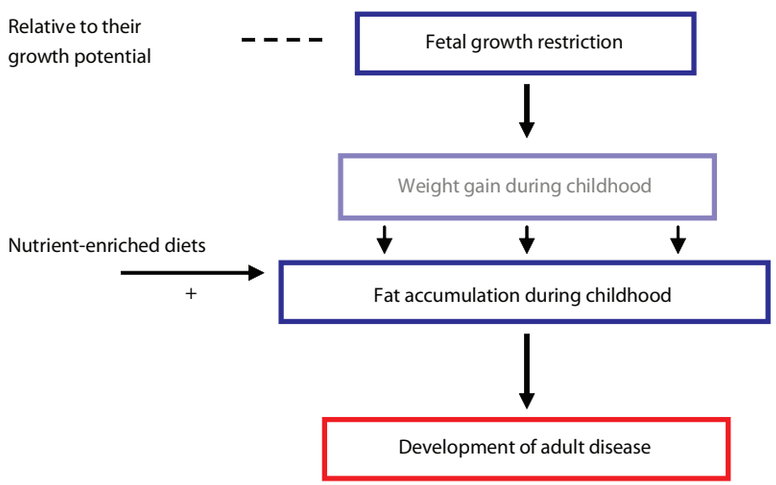


Figure 1. Specified growth acceleration model of Singhal et al.

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 trails 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).

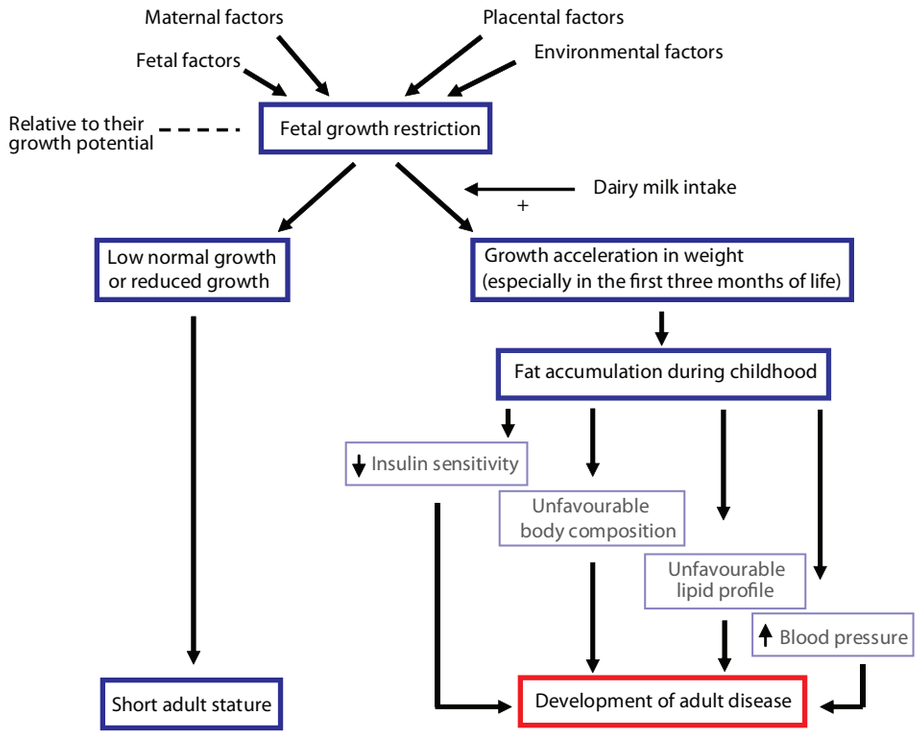


Figure 2. Hypothetical model showing the different growth patterns and their linkage with adult disease.

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.

References

1. **Lucas A, Fewtrell MS, Cole TJ** 1999 Fetal origins of adult disease-the hypothesis revisited. BMJ (Clinical research ed 319:245-249
2. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-67
3. **Yajnik CS** 2004 Early life origins of insulin resistance and type 2 diabetes in India and other Asian countries. J Nutr 134:205-210
4. **Yliharsila H, Kajantie E, Osmond C, Forsen T, Barker DJ, Eriksson JG** 2007 Birth size, adult body composition and muscle strength in later life. Int J Obes (Lond)
5. **Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS** 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-941
6. **Soto N, Bazaes RA, Pena V, Salazar T, Avila A, Iniguez G, Ong KK, Dunger DB, Mericq MV** 2003 Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. The Journal of clinical endocrinology and metabolism 88:3645-3650
7. **Jaquet D, Chevenne D, Czernichow P, Levy-Marchal C** 2000 No evidence for a major beta-cell dysfunction in young adults born with intra-uterine growth retardation. Pediatr Diabetes 1:181-185
8. **Singhal A, Lucas A** 2004 Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet 363:1642-1645
9. **Singhal A, Cole TJ, Fewtrell M, Kennedy K, Stephenson T, Elias-Jones A, Lucas A** 2007 Promotion of faster weight gain in infants born small for gestational age: is there an adverse effect on later blood pressure? Circulation 115:213-220
10. **Van Gaal LF, Mertens IL, De Block CE** 2006 Mechanisms linking obesity with cardiovascular disease. Nature 444:875-880
11. **Ibanez L, Ong K, Dunger DB, de Zegher F** 2006 Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. The Journal of clinical endocrinology and metabolism 91:2153-2158
12. **Donahue RP, Orchard TJ, Becker DJ, Kuller LH, Drash AL** 1988 Physical activity, insulin sensitivity, and the lipoprotein profile in young adults: the Beaver County Study. American journal of epidemiology 127:95-103
13. **van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, Grobbee DE, de Jong FH, van Duyn CM, Pols HA, Lamberts SW** 2002 A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes 51:3128-3134
14. **Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC** 2005 Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). Clinical endocrinology 62:44-50
15. **Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD** 1997 Insulin resistance in short children with intrauterine growth retardation. The Journal of clinical endocrinology and metabolism 82:402-406
16. **Cutfield WS, Jackson WE, Jefferies C, Robinson EM, Breier BH, Richards GE, Hofman PL** 2003 Reduced insulin sensitivity during growth hormone therapy for short children born small for gestational age. The Journal of pediatrics 142:113-116
17. **Cutfield WS, Bergman RN, Menon RK, Sperling MA** 1990 The modified minimal model: application to measurement of insulin sensitivity in children. The Journal of clinical endocrinology and metabolism 70:1644-1650
18. **Rogers IS, Ness AR, Steer CD, Wells JC, Emmett PM, Reilly JR, Tobias J, Smith GD** 2006 Associations of size at birth and dual-energy X-ray absorptiometry measures of lean and fat mass at 9 to 10 y of age. The American journal of clinical nutrition 84:739-747
19. **Singhal A, Wells J, Cole TJ, Fewtrell M, Lucas A** 2003 Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? The American journal of clinical nutrition 77:726-730
20. **Gale CR, Martyn CN, Kellingray S, Eastell R, Cooper C** 2001 Intrauterine programming of adult body composition. The Journal of clinical endocrinology and metabolism 86:267-272
21. **Garnett SP, Cowell CT, Baur LA, Fay RA, Lee J, Coakley J, Peat JK, Boulton TJ** 2001 Abdominal fat and birth size in healthy prepubertal children. Int J Obes Relat Metab Disord 25:1667-1673
22. **Labayen I, Moreno LA, Blay MG, Blay VA, Mesana MI, Gonzalez-Gross M, Bueno G, Sarria A, Bueno M** 2006 Early programming of body composition and fat distribution in adolescents. The Journal of nutrition 136:147-152
23. **Li H, Stein AD, Barnhart HX, Ramakrishnan U, Martorell R** 2003 Associations between prenatal and postnatal growth and adult body size and composition. The American journal of clinical nutrition 77:1498-1505
24. **Sayer AA, Syddall HE, Dennison EM, Gilbody HJ, Duggleby SL, Cooper C, Barker DJ, Phillips DI** 2004 Birth weight, weight at 1 y of age, and body composition in older men: findings from the Hertfordshire Cohort Study. The American journal of clinical nutrition 80:199-203
25. **Te Velde SJ, Twisk JW, Van Mechelen W, Kemper HC** 2003 Birth weight, adult body composition, and subcutaneous fat distribution. Obes Res 11:202-208
26. **Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, De Zegher F** 2002 Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. The Journal of clinical endocrinology and metabolism 87:5702-5705
27. **Sas T, Mulder P, Hokken-Koelega A** 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. The Journal of clinical endocrinology and metabolism 85:3786-3792
28. **Hediger ML, Overpeck MD, Kuczmarski RJ, McGlynn A, Maurer KR, Davis WW** 1998 Muscularity and fatness of infants and young children born small- or large-for-gestational-age. Pediatrics 102:E60
29. **Kensara OA, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M** 2005 Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. The American journal of clinical nutrition 82:980-987
30. **Hogler W, Briody J, Moore B, Lu PW, Cowell CT** 2005 Effect of growth hormone therapy and puberty on bone and body composition in children with idiopathic short stature and growth hormone deficiency. Bone 37:642-650
31. **Huxley R, Owen CG, Whincup PH, Cook DG, Colman S, Collins R** 2004 Birth weight and subsequent cholesterol levels: exploration of the "fetal origins" hypothesis. Jama 292:2755-2764
32. **Lauren L, Jarvelin MR, Elliott P, Sovio U, Spellman A, McCarthy M, Emmett P, Rogers I, Hartikainen AL, Pouta A, Hardy R, Wadsworth M, Helmsdal G, Olsen S, Bakoula C, Lekea V, Millwood I** 2003 Relationship between birthweight and blood lipid concentrations in later life: evidence from the existing literature. International journal of epidemiology 32:862-876
33. **Bergstrom E, Hernell O, Persson LA, Vessby B** 1995 Serum lipid values in adolescents are related to family history, infant feeding, and physical growth. Atherosclerosis 117:1-13
34. **Forrester TE, Wilks RJ, Bennett FI, Simeon D, Osmond C, Allen M, Chung AP, Scott P** 1996 Fetal growth and cardiovascular risk factors in Jamaican schoolchildren. BMJ (Clinical research ed 312:156-160

35. **Ziegler B, Johnsen SP, Thulstrup AM, Engberg M, Lauritzen T, Sorensen HT** 2000 Inverse association between birth weight, birth length and serum total cholesterol in adulthood. Scand Cardiovasc J 34:584-588
36. **Singhal A, Cole TJ, Fewtrell M, Lucas A** 2004 Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up of a prospective randomised study. Lancet 363:1571-1578
37. **Skidmore PM, Hardy RJ, Kuh DJ, Langenberg C, Wadsworth ME** 2004 Birth weight and lipids in a national birth cohort study. Arterioscler Thromb Vasc Biol 24:588-594
38. **Evagelidou EN, Giapros VI, Challa AS, Kiortsis DN, Tsatsoulis AA, Andronikou SK** 2007 Serum adiponectin levels, insulin resistance, and lipid profile in children born small for gestational age are affected by the severity of growth retardation at birth. European journal of endocrinology / European Federation of Endocrine Societies 156:271-277
39. **Lopez-Bermejo A, Casano-Sancho P, Fernandez-Real JM, Kihara S, Funahashi T, Rodriguez-Hierro F, Ricart W, Ibanez L** 2004 Both intrauterine growth restriction and postnatal growth influence childhood serum concentrations of adiponectin. Clinical endocrinology 61:339-346
40. **Pulzer F, Haase U, Kratzsch J, Richter V, Rassoul F, Kiess W, Keller E** 1999 Lipoprotein(a) levels in formerly small-for-gestational-age children. Hormone research 52:241-246
41. **Jaquet D, Deghmoun S, Chevenne D, Collin D, Czernichow P, Levy-Marchal C** 2005 Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. Diabetologia 48:849-855
42. **Cianflone K, Xia Z, Chen LY** 2003 Critical review of acylation-stimulating protein physiology in humans and rodents. Biochimica et biophysica acta 1609:127-143
43. **Germinario R, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K** 1993 Coordinate regulation of triacylglycerol synthesis and glucose transport by acylation-stimulating protein. Metabolism: clinical and experimental 42:574-580
44. **Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Walsh M, Rodriguez MA** 1991 Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. Lipids 26:495-499
45. **Cianflone K, Zakarian R, Couillard C, Delplanque B, Despres JP, Sniderman A** 2004 Fasting acylation-stimulating protein is predictive of postprandial triglyceride clearance. Journal of lipid research 45:124-131
46. **Castro Cabezas M, Erkelens DW** 1998 The direct way from gut to vessel wall: atheroinitiation. European journal of clinical investigation 28:504-505
47. **Maslowska M, Vu H, Phelis S, Sniderman AD, Rhode BM, Blank D, Cianflone K** 1999 Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. European journal of clinical investigation 29:679-686
48. **Wamba PC, Mi J, Zhao XY, Zhang MX, Wen Y, Cheng H, Hou DQ, Cianflone K** 2008 Acylation stimulating protein but not complement C3 associates with metabolic syndrome components in Chinese children and adolescents. European journal of endocrinology / European Federation of Endocrine Societies 159:781-790
49. **Maslowska M, Scantlebury T, Germinario R, Cianflone K** 1997 Acute in vitro production of acylation stimulating protein in differentiated human adipocytes. Journal of lipid research 38:1-11
50. **Yang Y, Lu HL, Zhang J, Yu HY, Wang HW, Zhang MX, Cianflone K** 2006 Relationships among acylation stimulating protein, adiponectin and complement C3 in lean vs obese type 2 diabetes. Int J Obes (Lond) 30:439-446
51. **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
52. **Cianflone K, Lu H, Smith J, Yu W, Wang H** 2005 Adiponectin, acylation stimulating protein and complement C3 are altered in obesity in very young children. Clinical endocrinology 62:567-572
53. **de Groot E, Hovingh GK, Wiegman A, Duriez P, Smit AJ, Fruchart JC, Kastelein JJ** 2004 Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. Circulation 109:III33-38
54. **Glynn RJ, L'Italien GJ, Sesso HD, Jackson EA, Buring JE** 2002 Development of predictive models for long-term cardiovascular risk associated with systolic and diastolic blood pressure. Hypertension 39:105-110
55. **O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK, Jr.** 1999 Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. The New England journal of medicine 340:14-22
56. **Stamler J** 1985 The marked decline in coronary heart disease mortality rates in the United States, 1968-1981; summary of findings and possible explanations. Cardiology 72:11-22
57. **Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D** 2000 Fetal and childhood growth and hypertension in adult life. Hypertension 36:790-794
58. **Law CM, Shiell AW, Newsome CA, Syddall HE, Shinebourne EA, Fayers PM, Martyn CN, de Swiet M** 2002 Fetal, infant, and childhood growth and adult blood pressure: a longitudinal study from birth to 22 years of age. Circulation 105:1088-1092
59. **Miura K, Nakagawa H, Tabata M, Morikawa Y, Nishijo M, Kagamimori S** 2001 Birth weight, childhood growth, and cardiovascular disease risk factors in Japanese aged 20 years. American journal of epidemiology 153:783-789
60. **Huxley RR, Shiell AW, Law CM** 2000 The role of size at birth and postnatal catch-up growth in determining systolic blood pressure: a systematic review of the literature. Journal of hypertension 18:815-831
61. **Law CM, Shiell AW** 1996 Is blood pressure inversely related to birth weight? The strength of evidence from a systematic review of the literature. Journal of hypertension 14:935-941
62. **Huxley R, Neil A, Collins R** 2002 Unravelling the fetal origins hypothesis: is there really an inverse association between birthweight and subsequent blood pressure? Lancet 360:659-665
63. **Ben-Shlomo Y, McCarthy A, Hughes R, Tilling K, Davies D, Smith GD** 2008 Immediate postnatal growth is associated with blood pressure in young adulthood: the Barry Caerphilly Growth Study. Hypertension 52:638-644
64. **Uiterwaal CS, Anthony S, Launer LJ, Witteman JC, Trouwborst AM, Hofman A, Grobbee DE** 1997 Birth weight, growth, and blood pressure: an annual follow-up study of children aged 5 through 21 years. Hypertension 30:267-271
65. **Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML** 2003 Change in body mass index from adolescence to young 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
66. **Raitakari OT, Juonala M, Kahonen M, Taittonen L, Laitinen T, Maki-Torkko N, Jarvisalo MJ, Uhari M, Jokinen E, Ronnemaa T, Akerblom HK, Viikari JS** 2003 Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. Jama 290:2277-2283
67. **Oren A, Vos LE, Uiterwaal CS, Gorissen WH, Grobbee DE, Bots ML** 2004 Birth weight and carotid intima-media thickness: new perspectives from the atherosclerosis risk in young adults (ARYA) study. Ann Epidemiol 14:8-16
68. **Martino F, Loffredo L, Carnevale R, Sanguigni V, Martino E, Catasca E, Zanoni C, Pignatelli P, Violi F** 2008 Oxidative stress is associated with arterial dysfunction and enhanced intima-media thickness in children with hypercholesterolemia: the potential role of nicotinamide-adenine dinucleotide phosphate oxidase. Pediatrics 122:e648-655
69. **Baldassarre D, De Jong A, Amato M, Werba JP, Castelnuovo S, Frigerio B, Veglia F, Tremoli E, Sirtori CR** 2008 Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. Ann Med 40:21-44
70. **Dennison EM, Syddall HE, Sayer AA, Gilbody HJ, Cooper C** 2005 Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire cohort study. Pediatric research 57:582-586

71. **te Velde SJ, Twisk JW, van Mechelen W, Kemper HC** 2004 Birth weight and musculoskeletal health in 36-year-old men and women: results from the Amsterdam Growth and Health Longitudinal Study. Osteoporos Int 15:382-388
72. **Yarbrough DE, Barrett-Connor E, Morton DJ** 2000 Birth weight as a predictor of adult bone mass in postmenopausal women: the Rancho Bernardo Study. Osteoporos Int 11:626-630
73. **Jensen RB, Vielwerth S, Frystyk J, Veldhuis J, Larsen T, Molgaard C, Greisen G, Juul A** 2008 Fetal growth velocity, size in early life and adolescence, and prediction of bone mass: association to the GH-IGF axis. J Bone Miner Res 23:439-446
74. **Cooper C, Eriksson JG, Forsen T, Osmond C, Tuomilehto J, Barker DJ** 2001 Maternal height, childhood growth and risk of hip fracture in later life: a longitudinal study. Osteoporos Int 12:623-629
75. **Cooper C, Fall C, Egger P, Hobbs R, Eastell R, Barker D** 1997 Growth in infancy and bone mass in later life. Ann Rheum Dis 56:17-21
76. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. Clinical endocrinology 59:779-787
77. **Lanes R, Gunczler P, Esaa S, Weisinger JR** 2002 The effect of short- and long-term growth hormone treatment on bone mineral density and bone metabolism of prepubertal children with idiopathic short stature: a 3-year study. Clinical endocrinology 57:725-730
78. **Lanes R, Gunczler P, Weisinger JR** 1999 Decreased trabecular bone mineral density in children with idiopathic short stature: normalization of bone density and increased bone turnover after 1 year of growth hormone treatment. The Journal of pediatrics 135:177-181
79. **Barker DJ, Osmond C, Forsen TJ, Kajantie E, Eriksson JG** 2005 Trajectories of growth among children who have coronary events as adults. The New England journal of medicine 353:1802-1809
80. **Chomtho S, Wells JC, Williams JE, Davies PS, Lucas A, Fewtrell MS** 2008 Infant growth and later body composition: evidence from the 4-component model. The American journal of clinical nutrition 87:1776-1784
81. **Ekelund U, Ong K, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2006 Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). The American journal of clinical nutrition 83:324-330
82. **Ekelund U, Ong KK, Linne Y, Neovius M, Brage S, Dunger DB, Wareham NJ, Rossner S** 2007 Association of weight gain in infancy and early childhood with metabolic risk in young adults. The Journal of clinical endocrinology and metabolism 92:98-103
83. **Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ** 2001 Early growth and coronary heart disease in later life: longitudinal study. BMJ (Clinical research ed 322:949-953
84. **Eriksson JG, Forsen T, Tuomilehto J, Winter PD, Osmond C, Barker DJ** 1999 Catch-up growth in childhood and death from coronary heart disease: longitudinal study. BMJ (Clinical research ed 318:427-431
85. **Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ** 1989 Weight in infancy and death from ischaemic heart disease. Lancet 2:577-580
86. **Belfort MB, Rifas-Shiman SL, Rich-Edwards J, Kleinman KP, Gillman MW** 2007 Size at birth, infant growth, and blood pressure at three years of age. The Journal of pediatrics 151:670-674
87. **Barker DJ** 1990 The fetal and infant origins of adult disease. BMJ (Clinical research ed 301:1111
88. **Barker DJ** 1995 Fetal origins of coronary heart disease. BMJ (Clinical research ed 311:171-174
89. **Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB** 2000 Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ (Clinical research ed 320:967-971
90. **Eriksson JG, Osmond C, Kajantie E, Forsen TJ, Barker DJ** 2006 Patterns of growth among children who later develop type 2 diabetes or its risk factors. Diabetologia 49:2853-2858
91. **Kajantie E, Barker DJ, Osmond C, Forsen T, Eriksson JG** 2008 Growth before 2 years of age and serum lipids 60 years later: the Helsinki Birth Cohort study. International journal of epidemiology 37:280-289
92. **Bhargava SK, Sachdev HS, Fall CH, Osmond C, Lakshmy R, Barker DJ, Biswas SK, Ramji S, Prabhakaran D, Reddy KS** 2004 Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. The New England journal of medicine 350:865-875
93. **Arenz S, Ruckerl R, Koletzko B, von Kries R** 2004 Breast-feeding and childhood obesity--a systematic review. Int J Obes Relat Metab Disord 28:1247-1256
94. **Armstrong J, Reilly JJ** 2002 Breastfeeding and lowering the risk of childhood obesity. Lancet 359:2003-2004
95. **Fewtrell MS, Morley R, Abbott RA, Singhal A, Stephenson T, MacFadyen UM, Clements H, Lucas A** 2001 Catch-up growth in small-for-gestational-age term infants: a randomized trial. The American journal of clinical nutrition 74:516-523
96. **Hoppe C, Udam TR, Lauritzen L, Molgaard C, Juul A, Michaelsen KF** 2004 Animal protein intake, serum insulin-like growth factor I, and growth in healthy 2.5-y-old Danish children. The American journal of clinical nutrition 80:447-452
97. **Rolland-Cachera MF, Deheeger M, Akrout M, Bellisle F** 1995 Influence of macronutrients on adiposity development: a follow up study of nutrition and growth from 10 months to 8 years of age. Int J Obes Relat Metab Disord 19:573-578
98. **Scavo LM, Karas M, Murray M, Leroith D** 2004 Insulin-like growth factor-I stimulates both cell growth and lipogenesis during differentiation of human mesenchymal stem cells into adipocytes. The Journal of clinical endocrinology and metabolism 89:3543-3553
99. **Smith PJ, Wise LS, Berkowitz R, Wan C, Rubin CS** 1988 Insulin-like growth factor-I is an essential regulator of the differentiation of 3T3-L1 adipocytes. The Journal of biological chemistry 263:9402-9408
100. **Gunther AL, Buyken AE, Kroke A** 2006 The influence of habitual protein intake in early childhood on BMI and age at adiposity rebound: results from the DONALD Study. Int J Obes (Lond) 30:1072-1079
101. **Gunther AL, Buyken AE, Kroke A** 2007 Protein intake during the period of complementary feeding and early childhood and the association with body mass index and percentage body fat at 7 y of age. The American journal of clinical nutrition 85:1626-1633
102. **Gunther AL, Remer T, Kroke A, Buyken AE** 2007 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? The American journal of clinical nutrition 86:1765-1772
103. **Dorosty AR, Emmett PM, Cowin S, Reilly JJ** 2000 Factors associated with early adiposity rebound. ALSPAC Study Team. Pediatrics 105:1115-1118
104. **Hoppe C, Molgaard C, Thomsen BL, Juul A, Michaelsen KF** 2004 Protein intake at 9 mo of age is associated with body size but not with body fat in 10-y-old Danish children. The American journal of clinical nutrition 79:494-501
105. **Skinner JD, Bounds W, Carruth BR, Morris M, Ziegler P** 2004 Predictors of children's body mass index: a longitudinal study of diet and growth in children aged 2-8 y. Int J Obes Relat Metab Disord 28:476-482
106. **Karaolis-Danckert N, Gunther AL, Kroke A, Hornberg C, Buyken AE** 2007 How early dietary factors modify the effect of rapid weight gain in infancy on subsequent body-composition development in term children whose birth weight was appropriate for gestational age. The American journal of clinical nutrition 86:1700-1708
107. **Magarey AM, Daniels LA, Boulton TJ, Cockington RA** 2001 Does fat intake predict adiposity in healthy children and adolescents aged 2--15 y? A longitudinal analysis. European journal of clinical nutrition 55:471-481

108. **Scaglioni S, Agostoni C, Notaris RD, Radaelli G, Radice N, Valenti M, Giovannini M, Riva E** 2000 Early macronutrient intake and overweight at five years of age. Int J Obes Relat Metab Disord 24:777-781
109. **Balkau B, Mhamdi L, Oppert JM, Nolan J, Golay A, Porcellati F, Laakso M, Ferrannini E** 2008 Physical activity and insulin sensitivity: the RISC study. Diabetes 57:2613-2618
110. **Church TS, Earnest CP, Skinner JS, Blair SN** 2007 Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial. Jama 297:2081-2091
111. **Meas T, Deghmoun S, Armoogum P, Alberti C, Levy-Marchal C** 2008 Consequences of being born small for gestational age on body composition: an 8-year follow-up study. The Journal of clinical endocrinology and metabolism 93:3804-3809
112. **Victora CG, Adair L, Fall C, Hallal PC, Martorell R, Richter L, Sachdev HS** 2008 Maternal and child undernutrition: consequences for adult health and human capital. Lancet 371:340-357

Chapter 11

Summary

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

length and birth weight, was not. Our subgroup analyses showed that short adults born SGA had higher levels of ApoB than controls. There were no differences in other serum lipid levels or ApoE genotype between the subgroups. These data imply that fat accumulation during childhood might result in unfavorable serum lipid levels in early adulthood and therefore enhance the development of cardiovascular disease in early life. Parents and their children need to be informed about the risk of fat accumulation during childhood.

Chapter 5

Acylation stimulating protein (ASP) is hormone produced by adipocytes, which enhances the uptake of glucose and chylomicron-derived free fatty acid (FFA) in adipocytes. ASP influences the metabolism of FFA by increasing the uptake of FFA and reducing the release of FFA by adipocytes. Normally, higher serum ASP levels lead to lower serum FFA levels. However, 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. Serum ASP is considered as a predictor of delayed TG clearance and might therefore predict postprandial hypertriglyceridemia, which is an early stage of dyslipidemia. We investigated 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.

Weight gain during childhood, particularly fat accumulation, was positively related to serum ASP levels in early adulthood. Birth size was not related. Serum ASP levels were positively related to TG, TC, ApoB and insulin secretion and negatively to insulin sensitivity. The subgroup analyses showed no differences in serum ASP levels, but the SGA-CU subjects and the ISS subjects had higher levels of serum FFA than controls.

In conclusion, exaggerated weight gain during childhood might lead to changes in ASP regulation and therefore FFA metabolism. 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$_{(TB+LS)}$ 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 fist 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.

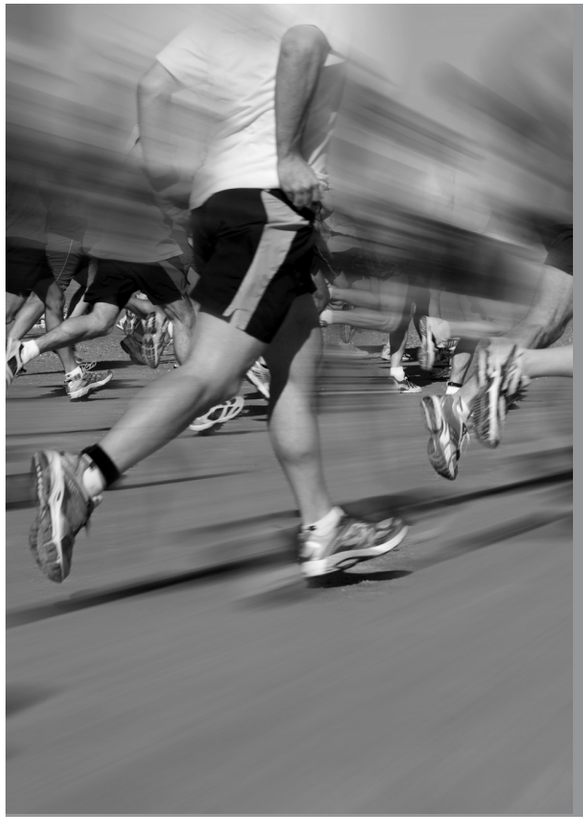
Chapter 9

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.



Chapter 12

Samenvatting

Hoofdstuk 1

In dit hoofdstuk worden verschillende hypotheses uitgelegd met betrekking tot de invloed van intra-uteriene groei en groei tijdens de kindertijd op ziekten die op oudere leeftijd optreden. Er wordt ingegaan op de relatie tussen grootte bij de geboorte en verschillende determinanten van deze ziekten, zoals insulinegevoeligheid, lichaamsamenstelling, serum lipiden profielen, acylation stimulating protein (ASP), bloeddruk, de intima media dikte (IMT) van de arteria carotis en de botdichtheid (BMD). Vervolgens wordt de relatie tussen groei in het eerste jaar en het cardiovasculaire en metabole profiel op jong volwassen leeftijd besproken, samen met de invloed van voeding op de lichaamsgrootte en lichaamsamenstelling 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. 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 lichaamsamenstelling en buik-heup ratio op insulinegevoeligheid en dispositie index (een maat voor de glucose homeostase), gemeten m.b.v. de Frequently Sampled Intravenous Glucose Tolerance test (FSIGT). Vervolgens werd onderzocht of er verschillen waren in vier klinisch relevante subgroepen, SGA-S, SGA-CU, ISS en controles.

De resultaten laten zien dat niet de grootte bij de geboorte, maar de hoeveelheid vetaccumulatie tijdens de kindertijd de belangrijkste determinant is van insulineongevoeligheid op de jong volwassen leeftijd. Na correctie voor leeftijd, geslacht en volwassen lichaamsgrootte, hadden SGA-CU mensen een verlaagde insulinegevoeligheid ten opzichte van controles. De dispositie index werd echter niet voorspeld door een van de variabelen en er was ook geen verschil in dispositie index tussen de vier subgroepen.

De conclusie is dat meer vetaccumulatie van de geboorte tot aan jong volwassenheid is geassocieerd met een lagere gevoeligheid voor insuline op jong volwassen leeftijd. Prenatale groei, gereflecteerd door lengte en gewicht bij de geboorte, heeft geen significante invloed op de insulinegevoeligheid. Op jong volwassen leeftijd hadden SGA-CU mensen een verlaagde gevoeligheid voor insuline ten opzichte van controles en de andere subgroepen hadden een vergelijkbare insulinegevoeligheid dan de controles. Verminderde insulinegevoeligheid 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 insulinegevoeligheid in het latere leven.

Hoofdstuk 3

De relatie tussen grootte bij de geboorte en de lichaamsamenstelling 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 word 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 lichaamsamenstelling 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 lichaamsamenstelling onderzocht in 312 jong volwassenen. De lichaamsamenstelling 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 en spiermassa was groter dan die tussen het geboortegewicht en lichaamsamenstelling, wat laat zien dat de gewichtstoename van geboorte tot jong volwassen leeftijd de belangrijkste determinant is van de lichaamsamenstelling 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 lichaamsamenstelling te hebben als controles. Deze resultaten impliceren dat teveel gewichtstoename tijdens de kindertijd voorkomen dient te worden, aangezien dit kan leiden tot een ongunstige lichaamsamenstelling op jong volwassen leeftijd. Interventie programma's om een ongunstige lichaamsamenstelling 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 geboortelengte en -gewicht, volwassen lengte en gewicht, lichaamsamenstelling 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.

Geslacht, ApoE genotype, volwassen lengte en gewicht, gespecificeerd als vetmassa, waren significante determinanten van serum lipiden spiegels op de jong volwassen leeftijd, terwijl prenatale groei, gereflecteerd door grootte bij de geboorte, niet significant was. De subgroep analyse wees uit dat SGA-S jong volwassenen hogere ApoB spiegels hadden dan controles. Er waren geen andere verschillen in serum lipiden spiegels of ApoE genotype tussen de subgroepen. Deze data laten zien dat vetaccumulatie tijdens de kindertijd kan resulteren in ongunstige serum lipiden spiegels op jong volwassen leeftijd en op deze manier de ontwikkeling van hart- en vaatziekten kan versterken. Ouders en hun kinderen moeten geïnformeerd worden over het risico van vetaccumulatie tijdens de kindertijd.

Hoofdstuk 5

Acylation stimulating protein (ASP) is een door vetcellen geproduceerd hormoon dat de opname van glucose en vrije vetzuren (afkomstig van triglyceriden) in vetcellen bevordert. In fysiologische condities leidt een hogere serum ASP waarde tot een verlaging van de vrije vetzuur spiegel. Echter, in geval van obesitas, insuline resistentie, dyslipidemie en cardiovasculaire ziekten worden zowel hoger serum waarden van ASP als triglyceriden gevonden. Dit heeft geresulteerd in de hypothese dat hoge ASP waarden in combinatie met normale of hogere TG waarden ASP resistentie reflecteert. Aangezien serum ASP wordt gezien als een voorspeller van een vertraagde TG klaring, kan de ASP spiegel postprandiale hypertriglyceridemie voorspellen. Postprandiale hypertriglyceridemie wordt gezien als een beginstadium van dyslipidemie. Wij hebben onderzocht welke factoren gerelateerd zijn nuchtere ASP waarden op de jong volwassen leeftijd en of verschillende groeipatronen tijdens de kindertijd zijn gerelateerd aan nuchtere ASP waarden in jong volwassenheid.

Relatief meer toename in gewicht dan in lengte tijdens de kindertijd, vooral vetaccumulatie, was positief gerelateerd aan de serum ASP spiegels in jong volwassenen. Grootte bij de geboorte had geen relatie met de serum ASP spiegels. Serum ASP was verder positief gecorreleerd met TG, TC, ApoB en de insuline secretie en negatief gecorreleerd met insulinegevoeligheid. De subgroep analyse liet geen verschil in ASP spiegels zien tussen de subgroepen, maar de SGA-CU mensen en de ISS mensen hadden wel een verhoogd vrije vetzuur waarde t.o.v. controles.

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 6

Dit hoofdstuk beschrijft de relatie tussen grootte bij de geboorte en de bloeddruk alsmede de intima media dikte van de carotis (cIMT) op jong volwassen leeftijd. Systolische bloeddruk, diastolische bloeddruk en cIMT zijn sterk gecorreleerd met cardiovasculaire events op oudere leeftijd. Het ontwikkelen van een hogere bloeddruk en een dikkere cIMT op jonge leeftijd 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 lichaamsamenstelling 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 was niet gerelateerd aan systolische bloeddruk, diastolische bloeddruk, of cIMT. Vetaccumulatie gedurende de kindertijd was gerelateerd aan een hogere systolische en diastolische bloeddruk, maar niet met cIMT. De subgroep analyse liet geen verschillen in bloeddruk zien, maar de cIMT van de SGA-CU mensen was groter dan die van de controles. Dit suggereert dat postnatale inhaalgroei, en niet prenatale groei, de ontwikkeling van een grotere cIMT versterkt, aangezien de SGA-CU mensen maar niet de SGA-S mensen een grotere cIMT hadden. Om het risico op een hogere bloeddruk op jong volwassen leeftijd te verminderen zal vetaccumulatie tijdens de kinderjaren moeten worden voorkomen.

Hoofdstuk 7

Osteoporose is een aandoening die wordt gekarakteriseerd door een verminderde bot mineralisatie. Dit resulteert 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 in 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 lichaamsamenstelling gerelateerd waren aan botdichtheid in jong volwassenheid.

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 $BMD_{(TB+LS)}$, maar geboortegewicht was negatief gerelateerd aan $BMD_{(LS)}$. Deze associatie verdween na correctie voor gewichtstoename. Dit suggereert dat de invloed van gewichtstoename gedurende de kindertijd een grotere invloed heeft dan de invloed van prenatale groei, gereflecteerd door geboortelengte en -gewicht. Vergelijking van de subgroepen liet zien dat na correctie voor volwassen lengte, volwassen gewicht en de lichaamsamenstelling, ISS mensen een lagere $BMD_{(TB)}$ hadden dan controles. Er waren tussen de subgroepen geen verschillen in $BMD_{(LS)}$ en $BMD_{(LS)}$ 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. Geboortelengte is niet gerelateerd aan $BMD_{(TB+LS)}$ op jong volwassen leeftijd. Vervolgens blijkt dat kinderen met een verminderde lengtegroei een verhoogde kans hebben op een lagere $BMD_{(TB)}$, wat kan resulteren in een verhoogde kans op botbreuken op latere leeftijd.

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 deze periode een negatief effect had op de determinanten van deze aandoeningen. Enkele studies lieten zien dat langzame groei in de eerste 2 jaar gevolgd door snelle groei in de kindertijd gerelateerd was aan een verhoogde kans op een cardiovasculair event op latere leeftijd. Anderen vermeldden dat juist snelle groei in de eerste 3 tot 6 maanden gerelateerd was aan een toename van de vetmassa tijdens de kindertijd en een ongunstig metabool profiel op de jong volwassen leeftijd. Wij onderzochten daarom de relatie tussen groei in het eerste levensjaar en een uitgebreide selectie van cardiovasculaire en metabole determinanten in 217 jong volwassenen. Vervolgens onderzochten we ook of de snelheid van inhaalgroei gerelateerd was aan deze determinanten van hart- en vaatziekten en diabetes type 2.

Onze data laten zien dat een relatieve gewichtstoename ten opzichte van lengtetoename in de eerste drie maanden van het leven was gerelateerd aan een verminderde insulinegevoeligheid en serum HDLc spiegel, en een toename in buikomtrek, insulinesecretie, 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 subgroepen 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 verklaard worden door het verschil in vetpercentage op jong volwassen leeftijd.

Teveel gewichtstoename ten opzichte van lengtetoename in de eerste drie maanden moet worden voorkomen omdat dit gerelateerd is aan meerdere determinanten van hart- en vaatziekten en diabetes type 2 in jong volwassenen. Gematigde inhaalgroei in gewicht in de eerste 3 maanden is gerelateerd aan een gunstiger metabool profiel dan snelle inhaalgroei in deze periode.

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 word daarom toenemend onderzocht. Een van die factoren is al bekend en dat is voeding. Kinderen die borstvoeding krijgen hebben een langzamere gewichtstoename dan kinderen met flesvoeding en er is geopperd dat dit verklaard zou kunnen worden door de verhoogde eiwit hoeveelheid in flesvoeding. Anderen suggereren dat een verhoogde inname van zuivelmelk, dat relatief veel eiwit bevat, is gerelateerd aan een toename in gewicht en adipositas in kinderen. In Cambridge, onderzochten wij 396 kinderen van de Baby Growth Study met de vraagstelling of energie, eiwit of vet inname op 1 jaar gerelateerd was aan lichaamsgrootte en vethoeveelheid op 1 jaar. Er werd specifiek gekeken of de inname van koemelk-eiwitten, uit flesvoeding en koemelk, gerelateerd was aan de vethoeveelheid op 1 jaar, gemeten met behulp van huidplooimetingen.

Hogere energie inname was positief gerelateerd aan lengte, gewicht en BMI op 1 jaar, na correctie voor demografische factoren en andere voedingsparameters. Er was geen relatie

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.

Acknowledgements

De afgelopen drie jaar heb ik met veel plezier mogen en kunnen werken aan deze interessante studie binnen de kindergeneeskunde met een link naar de volwassenen. In deze periode heb ik veel mooie momenten meegemaakt en vooral enorm veel geleerd. Dit is alleen mogelijk geweest omdat veel mensen hier een bijdrage aan hebben geleverd. Graag wil ik iedereen bedanken die deze studie tot een succes heeft gemaakt en ervoor heeft gezorgd dat dit proefschrift te realiseren is geweest!

Een aantal mensen wil ik in het bijzonder bedanken:

Allereerst alle deelnemers van de studie. Zonder jullie was de totale studie onmogelijk geweest. Jullie hebben een dag lang verschillende testen moeten ondergaan, sommige zelfs invasief, maar de sfeer bleef altijd positief. Door jullie inzet zijn we weer meer te weten gekomen over de relatie tussen groei en determinanten van cardiovasculaire ziekten en type 2 suikerziekte.

Mijn promotor, Prof. Dr. A.C.S. Hokken-Koelega. Beste Anita, allereerst enorm bedankt voor de kans die je me gegeven hebt om uit de Program-studie het succes te halen wat erin zat. Jouw begeleiding heeft ervoor gezorgd dat ik veel heb geleerd met betrekking tot het doen van onderzoek. Door je kritische instelling heb je het beste uit mij weten te halen. Vervolgens heb je door je enthousiasme en je onuitputtelijke energie mij gedurende de jaren blijvend geïnspireerd en mijn grenzen weten te verleggen. Op de valreep van mijn promotietraject heb je ook nog een fantastische plek weten te regelen in Cambridge bij een van de topresearch centra ter wereld met betrekking tot diabetes en andere metabole aandoeningen. Door mij de gelegenheid te geven om in een andere ‘keuken’ te kijken heb je van mij een veelzijdigere onderzoeker gemaakt. Ontzettend bedankt voor de onderzoekservaring die je me gegeven hebt.

Joke Dunk, research assistent van de Program-studie en paranimf. In het begin heb jij snel orde weten te brengen in een studie die de nodige orde miste. Samen hebben we in een rap tempo de moeilijkste inclusies binnengehaald zodat het een omvangrijke en degelijke studie is geworden. Door jouw positieve instelling en grondige aanpak was het altijd een feest om met je samen te werken, waarvoor veel dank. Daarnaast was er ook altijd tijd om even een ‘bakkie’ te doen en bij te kletsen. Al was de laatste periode niet meer zo intensief aangezien de data

verzameld en geordend was, het was altijd een prettig moment om even bij je langs te lopen en al je wandelverhalen te horen. Ik wens je nog een prettige tijd door op een andere studie en hopelijk krijg je nog veel buikspierpijn van het lachen met je (wandel)vriendinnen. Het zou fijn zijn als onze paden in de toekomst weer zouden kruisen.

Dr. Ken Ong, MRC Group Leader & Paediatric Endocrinologist at the Institute of Metabolic Science, Addenbrooke's Hospital in Cambridge. Dear Ken, thank you very much for giving me the chance to work with you and to see how your epidemiology unit handles with all the difficult problems in modern medical research. It has been a great experience and I've learned a lot about epidemiologic research in such a short period. Your hospitality and that of your colleagues at the unit made me feel welcome and made my time in Cambridge special. I'll definitely follow your work. Finally, thank you for taking place in the Reading Committee and considering the contents and quality of this thesis for its appropriateness for public defense.

Prof. Dr. A.J. van der Lelij en Prof. Dr. A.J. van der Heijden, beiden hartelijk dank voor de snelle beoordeling van mijn proefschrift en uw bereidheid om plaats te nemen in de kleine commissie.

Dr. A. Singhal and Dr. C. Levy-Marchal, thank you very much for your willingness to present at the symposium and for taking place in the Committee.

Prof. Dr. H.A.P. Pols, hartelijk dank voor het plaatsnemen in de grote commissie.

Prof. Dr. T. Stijnen, van u heb ik de belangrijkste onderdelen van de multipele regressies geleerd, waar ik erg dankbaar voor ben. U bent ook altijd bereid geweest om mijn artikelen te controleren op statistische inhoud. Veel dank voor deze snelle beoordelingen, de leerzame aanwijzingen en het plaatsnemen in de grote commissie.

Prof. Dr. K. Cianflone, thank you for collaborating with us with regard to the ASP manuscript. Your comments were very helpful.

Prof. Dr. S.L.S. Drop, Dr. S.M.P.F. de Muijnck Keizer-Schrama, Dr. A.M. Boot, Dr. E.L.T. van den Akker en Drs. J.C. van der Heijden; allen bedankt voor de aanvullingen en tips tijdens de leerzame endo-besprekingen.

Dr. W.H. Hackeng, bedankt voor de bepalingen van insuline en glucose van de FSIGT-testen. Dr. A.W. van Toorenenbergen en Dhr. G. Klein Heerenbrink wil ik graag bedanken voor het bepalen van de lipiden.

De research-assistenten die op de Program-studie gewerkt hebben; Ingrid, Merel, Janneke en Ineke. Dames, enorm bedankt voor alle data verzameling en verwerking. Dit heeft Joke en mij erg veel tijd gescheeld.

De student-assistenten van de studie; Patty, Lisette, Alwine, Merel, Christa, Hilde en Denise. Jullie hebben veel praktische zaken voor ons uit handen genomen waardoor de inclusies en dataverzameling veel sneller ging. Mede door jullie is het een mooie studie geworden, waarvoor veel dank.

De andere endo-research assistenten: Christel, Marianne, Esther, Marielle en Jolanda. Bedankt voor jullie hulp en ondersteuning waar nodig en de gezelligheid!

Prof. Dr. E.P. Krennig wil ik graag bedanken voor de mogelijkheid om DXA-scans uit te voeren op de afdeling Nucleaire Geneeskunde. Jopie Sluimer, bedankt voor het accorderen van de DXA-scans.

De Vereniging Trustfonds Erasmus Universiteit Rotterdam wil ik graag bedanken voor de financiële ondersteuning van mijn bezoeken aan de ESPE congressen in Helsinki en Istanbul.

Novo Nordisk Farma BV Nederland en Novo Nordisk Farma A/S Denemarken wil ik graag bedanken voor de financiële ondersteuning van dit proefschrift.

De (ex-)endo-onderzoekers: Ruben, Sandra, Daniëlle, Laura, Wietske, Dederieke, Daniëlle, Marije, Roderick, Elbrich, Petra en Annemieke. Jullie hebben mijn onderzoekstijd in het Sophia extra kleur gegeven door onze discussies over onderzoek, maar ook over alles en niks. We hebben aardig wat afgeborreld en gegeten, zowel in het Sophia, als in Rotterdam, bij mensen thuis en op congressen in Europa. De plaats maakte niet veel uit, we hebben het gezellig gehad. Hopelijk komen we elkaar in de toekomst nog vaak tegen zodat we deze mooie momenten kunnen blijven beleven!

Gerthe, mijn excellent-student en opvolgster. Jij hebt je in een korte periode snel thuis gemaakt in de onderzoekswereld. Je hebt me enorm geholpen met de verzameling van de groeidata bij de GGD's en andere praktische zaken. Je bent snel op je eigen 'onderzoeksbenen' gaan staan en ik heb er alle vertrouwen in dat jij een fantastisch mooi boekje gaat maken. Ik wens je alle succes toe met het onderzoek!

Mijn mede-Sophia-arts-onderzoekers van de pulmo, onco, neo, SEH en IC: het was gezellig tijdens de lunches en borrels en ik wens jullie veel succes toe met jullie eigen onderzoek en we komen elkaar zeker weer tegen!

Mijn vrienden in het Haagse, Amsterdam, het Gooi, Zurich en uiteraard Breda! Ondanks het feit dat de meeste van jullie niet echt begrepen waar ik mee bezig ben geweest, hebben jullie weldegelijk een belangrijke bijdrage geleverd aan dit boekje. Bij jullie heb ik juist kunnen ontspannen en het werk af en toe even kunnen vergeten. Of het nu samen sporten, borrelen, eten of op vakantie gaan was, ik heb er van genoten en we gaan er gewoon mee door!

Emile, paranimf, kamergenoot, maar vooral partner in crime! We hebben veel gelachen, elkaar van het werk afgehouden en mooie momenten meegemaakt, zoals het oprichten van de vrijdagmiddag borrel (VOBS). Uiteindelijk is het toch gelukt om een proefschrift te schrijven en ik weet zeker dat jij er binnenkort ook eentje hebt!

Mijn (schoon)ouders en lieve zus, bedankt voor jullie onvoorwaardelijke steun en stimulerende woorden. Ik heb jullie betrokkenheid enorm gewaardeerd. Jullie hebben mij de vrijheid gegeven om mij te blijven ontwikkelen en altijd gestimuleerd om het uiterste uit mijzelf te halen. Dankzij jullie ligt dit boekje nu voor je!

Lieve Marije, de uitdaging is afgerond! Na de nodige bergen en dalen is het boek af. Het was een mooie tocht om samen met jou te mogen afleggen. Dat onze volgende uitdagingen ook zo mooi afgesloten mogen worden! Op naar de volgende, met jou!

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*

2. **Leunissen RW, King F, Celia J. Prynne CJ, Reddy A, Hokken-Koelega AC, Hughes IA, Acerini CL, Dunger DB, Stephen AM, Ong KK** 2009
   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*

4. **Leunissen RW, Gao Y, Cianflone K, Stijnen T, Hokken-Koelega AC** 2009
   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*

6. **Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC** 2009
   Influence of Preterm Birth and Birth Size on Gonadal Function in Young Men. *Submitted*

7. **Kort SW, Leunissen RW, Mook-Kanamori DO, Jaddoe VW Hokken-Koelega AC** 2009
   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*

8. **Breukhoven PE, Leunissen RW, Kort SW, Willemsen RH, Hokken-Koelega AC** 2009
   Bone mineral density in prematurely born young adults. *Submitted*

9. **Willemsen RH, Leunissen RW, Stijnen T, Hokken-Koelega AC** 2009
   Prematurity is not associated with reduced insulin sensitivity in adulthood. J Clin Endocrinol Metab, *in press.*

10. **van der Kaay D, Deal C, de Kort S, Willemsen R, Leunissen R, Ester W, Paquette J, van Doorn J, Hokken-Koelega A** 2009
    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*.

11. **Kaay DC, Hendriks AE, Ester WA, Leunissen RW, Willemsen RH, Kort SW, Paquette JR, Hokken-Koelega AC, Deal CL** 2008
    Genetic and epigenetic variability in the gene for IGFBP-3 (IGFBP3): Correlation with serum IGFBP-3 levels and growth in short children born small for gestational age. Growth Horm IGF Res, *in press.*

12. **Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC** 2008
    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**

13. **Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC** 2008
    Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab 93:445-451*

14. **Leunissen RW, Stijnen T, Boot AM, Hokken-Koelega AC** 2008
    Influence of birth size and body composition on bone mineral density in early adulthood: the PROGRAM study. **Clin Endocrinol (Oxf) 69:386-392**

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*