

Small Birth Size

**Cardiovascular and metabolic profile of
young adults born SGA who have been treated
with growth hormone
and young adults born preterm**

Petra E. Breukhoven



ISBN: 9789461915214

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Lay-out: Legatron Electronic Publishing, Rotterdam

Printing: Ipskamp Drukkers BV, Enschede

The studies described in this thesis were supported by an investigator-initiated independent research grant provided by Novo Nordisk B.V., Pfizer Inc., Netherlands Organisation for Scientific Research (NWO), and by grants from Revolving Fund 2001; Vereniging Trustfonds, Erasmus University Rotterdam; Jan Dekkerstichting/Dr Ludgardine Bouwmanstichting; and Stichting De Drie Lichten, the Netherlands.

Publication of this thesis was financially supported by the SBOH, employer of GP trainees, and the SGA platform.

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**Cardiovasculaire en metabole status van
SGA geboren jong volwassenen die zijn behandeld
met groeihormoon
en prematuur geboren jong volwassenen**

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. H.G. Schmidt
en volgens besluit van het college voor Promoties.

De openbare verdediging zal plaatsvinden op
vrijdag 21 december 2012 om 9.30 uur

door

Petra Esther Breukhoven

geboren te Rotterdam



Promotiecommissie

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

Overige leden: Prof.dr. S.L.S. Drop
Prof.dr. A.J. van der Lelij
Prof.dr. J. Dahlgren

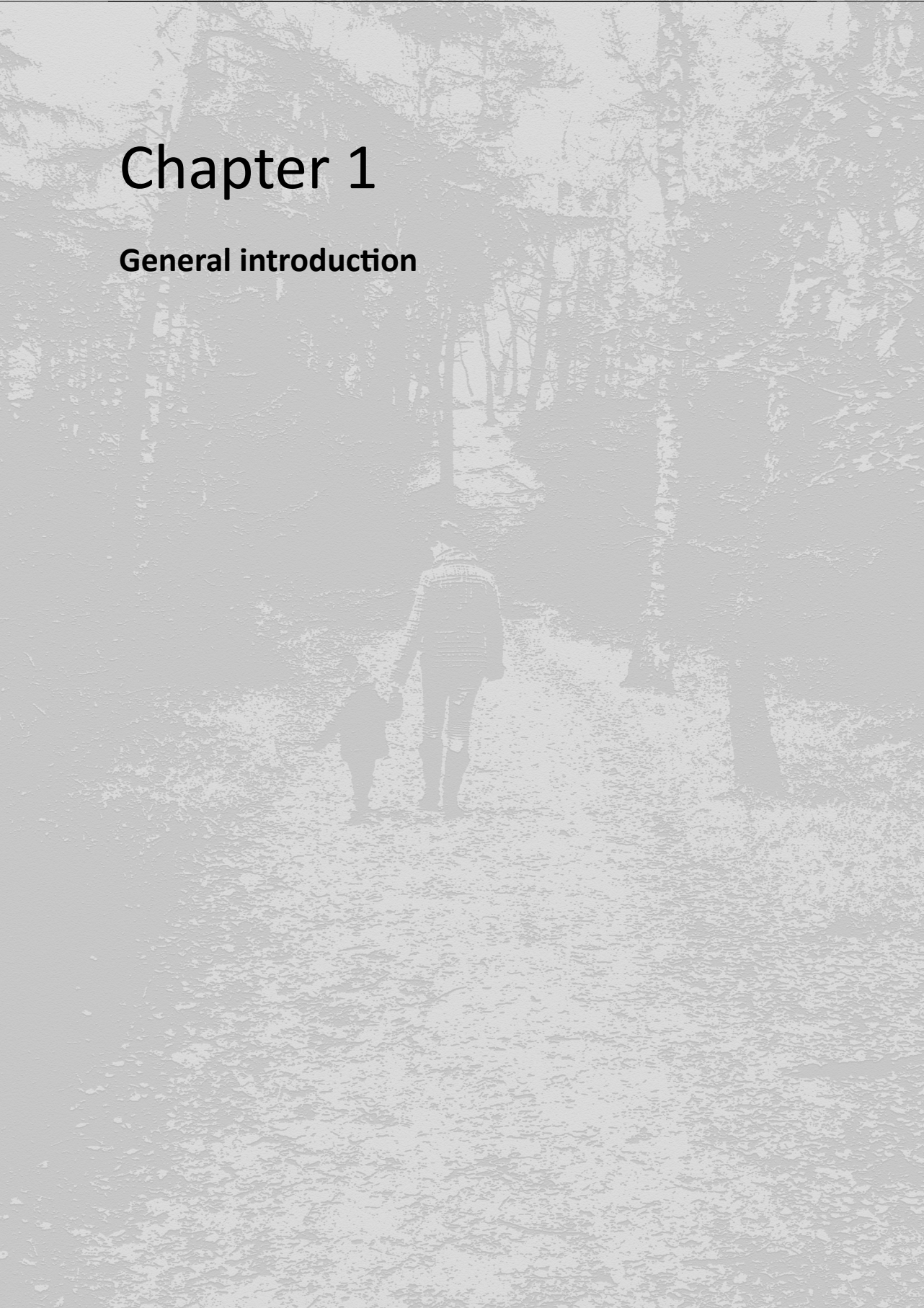
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Chapter 1

General introduction



This doctoral thesis describes cardiovascular and metabolic risk factors in children en young adults with a small birth size, either due to being born small for gestational age (SGA) or due to prematurity. For those born SGA with persistent short stature, the short and long-term effects of growth hormone (GH) treatment on these risk factors were studied. This chapter provides definitions and gives background information on SGA and prematurity. Finally, the aims of the study, the outline of this thesis, inclusion and exclusion criteria and study design of the IUGR-1, IUGR-2 and IUGR-3 study (Appendix A-C), and the PROGRAM/PREMS study (Appendix D) are presented.

PART 1 SMALL FOR GESTATIONAL AGE

1.1 Definition

In order to determine whether a child is born SGA the following is required: 1) an accurate knowledge of gestational age, 2) accurate measurements of weight and length at birth, and 3) an appropriate reference population in order to determine a standard deviation score (SDS) for birth weight and/or birth length (1). In 2001, the International SGA Advisory Board Panel reached consensus on the definition of SGA, by defining SGA as a birth length and/or birth weight below -2 SDS, adjusted for gestational age and gender (2).

The term SGA is used to define size at birth and does not refer to intra-uterine growth. Intra-uterine growth describes the growth velocity in fetal life, which is determined by at least two ultrasound measurements during pregnancy. The term intrauterine growth retardation (IUGR) is used when a fetus suffers from reduced fetal growth. A child born SGA has not necessarily suffered from IUGR, but may have been small from the beginning of fetal life. On the other hand, a child with IUGR late in gestation can have a normal size at birth. These different fetal growth patterns are shown in Figure 1. Because the intrauterine growth is often unknown, we nowadays prefer to use the term SGA for neonates with a birth size below -2 SDS.

1.2 Prevalence and aetiology

By definition, approximately 2.3% of all live-born neonates are born SGA. In 2007, 181.336 infants were live-born in the Netherlands (Central Bureau of Statistics, Voorburg, the Netherlands). According to the definition, 4171 of them were born SGA.

The etiology of SGA is multifactorial, including fetal, maternal, placental, and demographic factors (3-5). Identification of the underlying cause of SGA birth is important, since it may have consequences for prognosis and treatment. It is, however, important to realize that the cause of SGA birth remains unidentified in 40% of all cases. Table 1 shows some of the many factors associated with SGA birth.

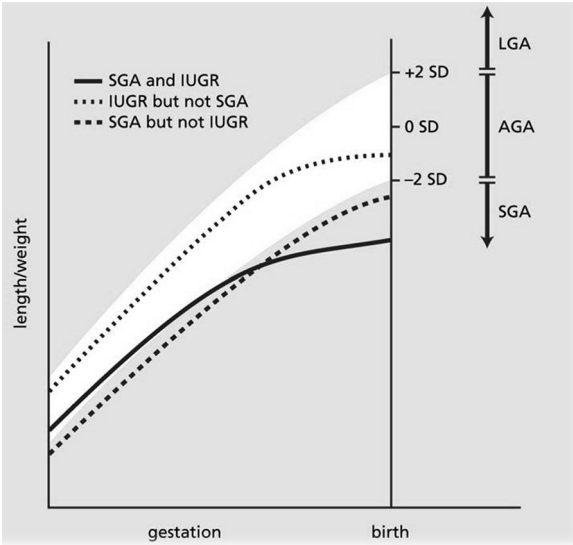


Figure 1. Fetal growth chart showing various intrauterine growth patterns.
LGA: large for gestational age, AGA: appropriate for gestational age, SGA: small for gestational age.

Table 1. Factors associated with SGA birth

Fetal factors	
Chromosomal disorders	Trisomy 21 (Down syndrome)
	Trisomy 18 (Edward syndrome)
	Monosomy X (Turner syndrome)
	Trisomy 13 (Patau syndrome)
Genetic disorders	Achondroplasia
	Bloom syndrome
Inborn errors of metabolism	
Congenital anomalies	Cardiac abnormalities
	Potter syndrome
Intrauterine infections	TORCHES (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes simplex, Syphilis)
	Malaria
	Trypanosomiasis
	Human immunodeficiency virus (HIV)
Maternal factors	
Medical conditions	Acute or chronic hypertension
	Pre-eclampsia
	Renal disease

Table 1. Factors associated with SGA birth *(continued)*

Maternal factors	
Environmental factors	Diabetes mellitus
	Collagen vascular disease (e.g. systemic lupus erythematosus)
	Antiphospholipid syndrome
	Cyanotic heart disease
	Chronic anemia
	Chronic pulmonary disease
	Abnormalities of the uterus
	Maternal nutrition
	Low pregnancy BMI
	Poor maternal weight gain
	Age at delivery (<16 or >35 years)
	Low socioeconomic status
	Cigarette smoking
	Alcohol abuse
	Therapeutic drugs (e.g. anticonvulsants, anticoagulants)
	Use of illicit drugs
Placental factors	
Structural abnormalities placenta	
Reduced blood flow	
Reduces area for exchange oxygen and nutrients	Placental hematomas, infarcts or local lesions
	Partial abruption
Demographic factors	
Maternal and paternal height	
Ethnicity	
Parity	Nulliparity
	Grand multiparity
Multiple gestation	
Previous delivery of SGA infants	
High altitude	

2 Clinical and endocrine aspects associated with SGA birth

2.1 Short stature

SGA birth is a common cause of short stature in childhood and adulthood, accounting for 22% of all cases (6). Most children show spontaneous catch-up to a normal height above -2 SDS. However, 10-15% of short children born SGA do not show sufficient catch-up growth and remain small (7, 8). Catch-up growth is most pronounced during the first six months of life and is usually completed in the first two years of life. However, catch-up growth may take longer in prematurely born SGA children since prematurely born children differ from children born term with regard to several parameters. For example, distance between height SDS at two years of age and target height SDS and distance between height SDS at the age of two years and birth length SDS (9). The absolute difference in duration of gestation had no effect on the timing of catch-up growth (7,9). By the age of 8 years, 91% of children born SGA have spontaneously reached a height above -2 SDS (9).

2.2 The GH-IGF-IGFBP axis

Fetal and postnatal growth and development are regulated by complex metabolic and endocrine processes, which are under the influence of genetic and environmental factors. The GH-IGF-IGFBP axis (Figure 2) plays a major role in this system.

The mechanisms underlying persistent short stature in children born SGA is still largely unknown, but disturbances in the GH-IGF-IGFBP axis may play a role. Many short SGA children show a reduced spontaneous GH secretion during a 24-hour GH-profile and/or low GH peaks during GH provocation tests (10-14). Serum IGF-I and IGFBP-3 levels are also lower in short SGA children than in healthy controls (15-17).

2.2.1 Growth hormone (GH)

GH is secreted by the pituitary gland under the control of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin, as well as ghrelin, which is predominantly secreted by the stomach (18). GHRH and ghrelin bind to their receptors in the pituitary and stimulate GH secretion. Somatostatin inhibits GH release. Most of the anabolic actions of GH are mediated by IGF-I, but GH has also many cellular effects that are independent of IGF-I (19).

2.2.2 Insulin-like growth factors (IGF)

The IGF system consists of IGF-I, IGF-II and insulin. They show structural similarity by sharing approximately 50% of their amino acids. The metabolic actions of insulin are mediated by binding to the insulin receptor. The growth-promoting effects of IGF-I and IGF-II are primarily mediated by binding to the IGF-I receptor.

Because of the strong homology between IGFs and insulin and between the insulin receptor and the IGF-I receptor, interactions between IGFs and the insulin receptor take place. The insulin-like effects of IGF-I are only 5% of that of insulin, but in case of excess of free IGF-I, it is capable of

lowering glucose levels 50 times more than insulin alone. This is, however, prevented by binding of IGF-I to specific IGF binding proteins (IGFBPs) in the circulation. Approximately 0.4% to 2% of IGF-I levels circulate as free IGF-I or at least very dissociable IGF-I. Acute as well as long-term biological effects of free IGF-I have been described, indicating that free IGF-I is the main biological active fraction (20).

IGF-I is a major regulator of growth and promotes proliferation, differentiation and cell survival. In addition, it promotes metabolic actions necessary for growth, such as protein synthesis, calcium accretion and fatty acid and glucose transport (21,22). Next to growth, IGFs are important in the development and function of the central nervous system, skeletal muscle and reproductive organs. The major source of circulating IGF-I is the liver, but it is also locally produced in various tissues (23).

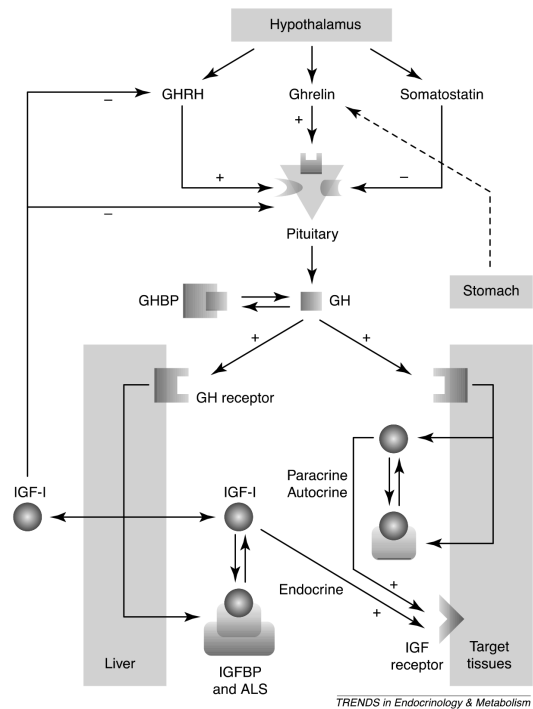


Figure 2. Physiology of the GH-IGF-IGFBP axis. Adapted from Holt (18).

2.2.3 IGF binding proteins

The majority of circulating IGF-I is bound to IGF binding proteins (IGFBPs), of which six classes have been identified. The majority of IGF-I (70-95%) is bound in a ternary complex with IGFBP-3 and an acid-labile subunit (ALS) (24). IGFBP-3 and ALS are both regulated by GH. Functions of IGFBPs are summarized in Table 2.

Table 2. Functions of the insulin-like growth factor binding proteins

All IGFBPs	Decrease bioavailability of free IGFs to bind to IGF receptors
IGFBP-1 and IGFBP-2	Prevent IGF-induced hypoglycaemia
IGFBP-1, IGFBP-2, IGFBP-3 and IGFBP-4	Regulate transport of IGFs between intravascular and extravascular spaces
IGFBP-3	Prolong the half-life of IGFs in the circulation
IGFBP-1, IGFBP-3 and IGFBP-5	Enhance actions of IGFs by forming a slow-releasing pool of IGFs
IGBBP-1 and IGFBP-3	Direct cellular effects via own IGFBP receptors

Adapted from Ferry et al (Horm Res 1999) and Collet-Solberg et al. (Endocrine 2000).

2.3 Cardiovascular disease and type 2 diabetes

2.3.1 Historical data and hypotheses

Epidemiological studies have shown that low birth weight is associated with the development of type 2 diabetes and associated disorders such as hypertension, dyslipidaemia and cardiovascular disease in adults (25-27). Insulin resistance plays an important role in the pathogenesis of these diseases (28,29), but the exact mechanism underlying these associations is still unknown. Several hypothesis have been proposed over time.

Fetal origins hypothesis: In 1989, Barker et al. were the first to postulate a hypothesis based on the inverse association found between birth weight and adult disease (30). They suggested that events leading to fetal malnutrition could result in permanent endocrine and metabolic changes in the fetus, called re-programming. At first, the fetus would benefit from these adaptations, as it would ensure to remain alive during fetal life, but on the long-term this re-programming might result in an increased risk of adult diseases.

Fetal insulin hypothesis: This hypothesis was formulated in 1999 by Hattersley et al. and states that the association between low birth weight and insulin resistance is principally genetically mediated (31). Insulin is an important intrauterine growth factor. Parental genes involved in insulin resistance, which are passed to the fetus, could result in both low-insulin-mediated growth and in insulin resistance leading to type 2 diabetes in later life.

Growth acceleration hypothesis: In 2004, Singhal and Lucas suggested that not low birth weight per se, but rapid postnatal growth is responsible for the increased risk for adult diseases in later life (32). Almost every child is genetically determined to grow to their own growth potential. Children born SGA, thus below their genetic growth potential, show later postnatal growth. According to this hypothesis, this catch-up growth will lead to the development of adult diseases.

Fat accumulation hypothesis: Based on detailed measurements of body composition using Dual Energy X-ray Absorptiometry (DXA) absorptiometry, our research group further specified

the growth acceleration hypothesis (33). It was shown that not catch-up growth per se, but rapid fat accumulation results in an increased risk for adult diseases. This indicated that small size at birth followed by growth in height and weight as such is not a problem when it is accompanied by a normal amount of fat mass. Leunissen et al. also demonstrated that rapid weight gain, especially during the first three months of life, is a risk factor for higher body fat percentage and associated risk for cardiovascular disease in adulthood (34).

2.3.2 Determinants of cardiovascular disease and type 2 diabetes

In order to determine the risks for cardiovascular disease and type 2 diabetes, various measurements and biomarkers were investigated in the studies described in this thesis.

Body composition

Body composition can be measured by DXA, which is explained in Appendix E. DXA gives more insight in the total amount of lean body mass and fat mass of the body. It is well known that obesity is an important risk factor for the development of cardiovascular disease and type 2 diabetes (35). In children born SGA with spontaneous catch-up in weight, early development of adiposity has been reported (36). However, short children born SGA have a different phenotype. They have a typical lean appearance, with a low body mass index SDS and a low sum of skinfolds SDS (37). Analysing DXA results showed that body fat percentage in short SGA children is significantly reduced compared with reference values (38).

Glucose homeostasis

Reduced insulin sensitivity also plays an important role in the pathogenesis of cardiovascular disease and usually precedes the first symptoms of disease by many years (28,29). 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 (39). An accurate way to measure insulin sensitivity is by means of a Frequently Sampled Intravenous Glucose Tolerance test (FSIGT), which is explained in Appendix E (40,41). In a group of prepubertal short SGA children (mean age 8 years), 8% had an impaired oral glucose tolerance test (42). Further studies indicated that short SGA children were more insulin resistant than children with short stature who were born appropriate for gestational age, and they had a compensatory higher insulin secretion (43-45).

Blood pressure and lipid metabolism

Increased blood pressure is an important determinant of cardiovascular disease (46). Next to increased diastolic and systolic blood pressure, an increased pulse pressure (the difference between systolic blood pressure and diastolic blood pressure), and blood pressure variability in time have also been associated with cardiovascular disease (47-49).

Raised serum levels of total cholesterol, low-density lipoprotein, and apolipoprotein B together with reduced levels of high-density lipoprotein and apolipoprotein A-I also increase the risk for CVD (50-52). Short SGA children had a higher systolic blood pressure than references (37,44). Also, SGA children reported to have more hypercholesterolemia (53) and more free fatty acid levels above the normal range (44) when compared with children born appropriate for gestational age.

Intima Media Thickness and Pulse Wave Velocity

Atherosclerosis is an important contributor to cardiovascular disease. The presence of atherosclerotic changes in the carotid arteries can be determined by investigating the intima media thickness (IMT, Appendix E) in the vessel wall of the carotid arteries by non-invasive ultrasound measurements (54). A greater thickness is associated with the development of atherosclerotic plaques and is positively correlated with cardiovascular events (55,56). Because development of atherosclerosis already starts in childhood, determining carotid IMT in early adulthood might give more insight in the risk of cardiovascular events in later life.

Arterial stiffness is another important determinant of cardiovascular disease, which can be quantified by assessing Pulse Wave Velocity (PWV) (57,58). A higher PWV indicates more arterial stiffness.

3 Growth Hormone (GH) treatment in children born SGA

3.1 Effects on linear growth

In 1991, the first Dutch multi-centre randomized double-blind dose-response GH trial was started to investigate the efficacy of GH treatment on growth in short SGA children (15,59). Adult height data demonstrated that 85% of these children reached a height above -2 SDS and 98% reached a height within the target height range (59). In 1996, the second Dutch GH trial was started with a randomized control group in which the children were not treated with GH. After three years, GH treatment with a dose of 1 mg/m²/day resulted in a normalization of height SDS, whereas children in the control group remained short (60). In addition to the Dutch GH trials, several other studies have demonstrated that GH treatment effectively induces catch-up growth in short SGA children (61-64).

GH-induced growth response is, however, highly variable (59). For that reason, several studies have been conducted to search for clinical predictors of the growth response to GH treatment. Patient characteristics found to be related to short-term response were: chronological age, bone age, height at start of GH treatment, IGFBP-3 levels and GH dose, explaining approximately 40% of the variability in growth response (65). It has also been suggested that pre-treatment insulin sensitivity significantly influence short-term growth response to GH treatment (66), suggesting that short SGA children who are more insulin resistant at baseline have a higher risk of glucose intolerance and a lower growth response, which could be considered a warning

against treatment (67). Since GH treatment is increasingly applied as a growth-promoting therapy in short SGA children, it is important to accurately investigate the relationship between insulin levels, insulin sensitivity and GH-induced growth response.

3.2 Effects on the GH-IGF-IGFBP axis

Previous reports showed that GH treatment of short SGA children leads to increases in serum IGF-I and IGFBP-3 levels, which are positively related to the GH dose (8,15,66). After one year of GH treatment with a dose of 1 mg/m²/day, a rise of 90% in IGF-I levels was reported, and after two years a rise of 123% (8). Another study reported a rise in IGF-I levels up to 1.2 SDS and a rise of IGFBP-3 levels up to 0.2 SDS during one years of GH treatment with a dose of 1 mg/m²/day (15). Treatment with 2 mg/m²/day resulted in mean IGF-I and IGFBP-3 levels of 1.9 SDS and 0.5 SDS, respectively (15). After three years of GH treatment, IGF-I and IGFBP-3 levels were similar in both GH dose groups (15). At discontinuation of GH treatment after attainment of adult height, mean IGF-I SDS was 1.0 in children treated with 1 mg/m²/day and 1.3 in children treated with 2 mg/m²/day, both significantly higher than the population mean (59). Mean IGFBP-3 levels were -0.8 SDS in children treated with 1 mg/m²/day, which is significantly lower than the population mean, and -0.06 SDS in children treated with 2 mg/m²/day (59). At 6.5 years after discontinuation of GH treatment, IGF-I (-0.4 SDS) and IGFBP-3 (-1.6 SDS) levels had decreased and were similar to levels in untreated SGA subjects, indicating that the GH-induced rise in IGF-I and IGFBP-3 was completely reversible after discontinuation of treatment (41).

During overnight GH profiles in short prepubertal SGA children, mean and maximum GH levels were 34.8 and 104 mU/l when treated with 1 mg/m²/day, and 64.4 and 161 mU/l when treated with 2 mg/m²/day (68). Because treatment with a GH dose of 1 mg/m²/day is as effective as treatment with 2 mg/m²/day with regard to reaching adult height (59) and the long-term risks of high GH levels in short SGA children are unknown, most short SGA children are nowadays treated with 1 mg/m²/day.

3.3 Effects on body composition

GH has well-documented anabolic effects on muscle mass and lipolytic effects on adipose tissue (69,70). GH deficiency has been associated with increased fat mass and truncal obesity (71), whereas GH excess, as in acromegaly, has been related to reduced fat mass and increased lean body mass (72).

Few studies investigated the effect of GH treatment on body composition in SGA children. Leger et al. measured body composition of the thighs during GH treatment by magnetic resonance imaging and reported an increase in muscle tissue and a decline in adipose tissue (73). Total body fat mass and muscle mass were not measured in this study and only the first three years of GH treatment were analysed. Willemsen et al. investigated the effect of GH treatment on body composition during six years of GH treatment. They found a significant decline in fat mass SDS and no change in lean body mass SDS (74).

Discontinuation of GH treatment was associated with significant changes in body composition six months after stop of treatment. Percentage body fat and fat mass SDS increased, whereas lean body mass SDS decreased (74). Fat distribution had not changed six months after discontinuation of GH treatment. All values remained within the normal range and therefore the clinical relevance of the observed changes is unclear (74). It remained to be elucidated how body composition changes many years after discontinuation of GH treatment.

3.4 Effects on insulin sensitivity and cardiovascular risk factors

GH has well-documented insulin-antagonistic effects and its use has been associated with a reduction in insulin sensitivity and an increase in insulin levels (75-77). Therefore, concerns were expressed regarding the long-term effects of GH treatment on risk factors for type 2 diabetes and associated comorbidities, especially in possibly predisposed subjects, such as children born SGA. Previous research had shown that short SGA children had a reduced insulin sensitivity before receiving GH and that GH treatment resulted in a further decline in insulin sensitivity and a compensatory increase in insulin secretion (43).

Previous studies reported conflicting results regarding the change in insulin sensitivity after GH was discontinued. De Zegher et al. observed a decrease in insulin sensitivity in eight short SGA children during high dose GH treatment, which was reversible after stop of treatment (66). Cutfield et al. measured insulin sensitivity in five short SGA children and reported that insulin sensitivity did not recover after discontinuation of GH treatment (43). Both studies investigated insulin sensitivity after stop of GH treatment, but still before adult height had been reached. Furthermore, it is questionable if the number of subjects was sufficient to draw firm conclusions. Willemssen et al. showed that GH-induced lower insulin sensitivity in SGA adolescents increased after stop of long-term GH treatment and became similar to that of AGA controls, six months after stop of treatment (74).

With respect to the other cardiovascular risk factors, GH treatment has been associated with a reduction in systolic blood pressure as well as a reduction in cholesterol levels which remained so until 6.5 years after discontinuation (41,78). In the same study, at an age of 22 years, insulin sensitivity and secretion, body mass index and waist circumference in previously GH-treated SGA subjects were comparable with those of untreated SGA subjects.

Reassuringly, the follow-up studies that have been performed in previously GH-treated SGA subjects by our research group do not indicate that GH treatment increased the risk for type 2 diabetes and cardiovascular disease. However, long-term surveillance of insulin sensitivity and other cardiovascular parameters in previously GH-treated SGA subjects remains important to exclude any negative effects of GH. This need for long-term follow-up was also emphasized during the SGA consensus meeting in 2007 (1).

3.5 Safety aspects of GH treatment

The National Cooperative Growth Study (NGCS) monitored the safety of GH treatment from 1984 until 1995 in children with various diseases. Reported adverse events included idiopathic intracranial hypertension, oedema and lymphedema, carpal tunnel syndrome, slipped capital femoral epiphysis, diabetes mellitus and glucose intolerance (79). However, the authors concluded that major adverse events in relation to GH treatment were rare and their frequency may be affected by pre-existing medical conditions.

In SGA children, several studies have shown that GH treatment was well tolerated and that side effects were uncommon (37,42,78). Nevertheless, all SGA children receiving GH treatment should be monitored regularly for changes in glucose metabolism, lipid profile, blood pressure and serum IGF-I levels to exclude any possible adverse effect of GH (1).

PART 2 PREMATURITY

4.1 Preterm birth

Preterm birth is defined as birth before 37 complete weeks of gestational age. The cause of preterm birth is in many situations elusive and unknown; many factors appear to be associated with preterm birth, making the reduction of preterm birth a challenging proposition. Table 3 shows some of the identifiable causes of preterm birth.

Nowadays, 5-13% of all newborns in developed countries are born preterm (80). Because of advances in neonatal care, survival of preterm infants has significantly improved and an increasing number of these children reach adulthood.

4.2 Preterm birth and risk for cardiovascular disease

Small size at birth may be due to preterm birth, poor fetal growth, or a combination of both. Most studies on the effect of small size at birth and risk for adult diseases, focused on subjects with a low birth weight and did not have information on gestational age, did not correct for it, or only included subjects born term. It is, therefore, difficult to determine whether the effect of small size at birth on cardiovascular risk factors in later life is due to a small size for gestational age (SGA) or to prematurity.

Preterm infants are frequently exposed to early postnatal growth restriction, glucocorticoid treatment, and stressful events. This may be important in the development of later organ dysfunction and adverse vascular outcome. To date, most long-term follow-up studies have focused on neurodevelopmental and respiratory complications of preterm infants, with little attention for the cardiovascular outcome.

Preterm birth has been associated with an increased risk of developing cardiovascular disease (81,82) and with increased cardiovascular mortality (83). We, therefore, investigated the relative contribution of prematurity and SGA on several parameters for cardiovascular diseases in young adulthood.

Table 3. Identifiable causes of preterm birth

Fetal	
Fetal distress	
Multiple gestation	
Erythroblastosis	
Nonimmune hydrops	
Placental	
Placental dysfunction	
Placenta previa	
Abruptio placentae	
Uterine	
Bicornuate uterus	
Incompetent cervix	Premature dilatation
Maternal	
Pre-eclampsia	
Chronic medical illness	Cyanotic heart disease
	Renal disease
Infection	Listeria monocytogenes
	Group B streptococcus
	Urinary tract infection
	Bacterial vaginosis
	Chorioamnionitis
Drug abuse	Cocaine
Other	
Premature rupture of membranes	
Polyhydramnios	
Iatrogenic	
Trauma	

Adapted from Nelson Textbook of Pediatrics, 18th edition.

4.3 Preterm birth and bone mineral density

Decreased mineralization of osteoid tissue during the early postnatal period is a known complication of very low birth weight and/or prematurely born infants (84-86). It comprises a variety of disturbances ranging from mild undermineralization to frank radiological rickets with fracture (87). Preterm infants are at an increased risk of low bone mineral density as bone mineralization, along with calcium and phosphorus accretion, mainly occurs during the third trimester of pregnancy (88).

Osteoporosis is an important and increasing cause of morbidity and mortality in developed countries. It is characterized by low bone mass, deterioration of bone tissue and disruption of bone architecture, compromised bone strength and an increase in the risk of fracture (89). Since early prevention of osteoporosis is likely to be more successful than treatment of an already established disorder, it is essential to identify potential risk factors, like preterm birth.

5 AIMS OF THE STUDY

PART 1 SMALL FOR GESTATIONAL AGE

Factors associated with GH-induced growth response

GH treatment induces catch-up growth and increases adult height in short SGA children. GH response is, however, highly variable. It has been suggested that pre-treatment fasting insulin sensitivity significantly influences short-term growth response to GH treatment. We, therefore, investigated the relationship between fasting insulin levels, insulin sensitivity and β -cell function, measured by a frequently sampled intravenous glucose tolerance test with Tolbutamide, and parameters of the GH-IGF-IGFBP axis, also including overnight GH profiles and free IGF-I, and the GH-induced growth response in short SGA children.

Health profile after discontinuation of GH treatment

Despite the fact that GH has been used for more than 25 years, there are only limited data available on the longitudinal effects after discontinuation of this treatment. We investigated longitudinal changes in several determinants for cardiovascular disease and type 2 diabetes until almost 7 years after discontinuation of GH treatment. Body composition and fat distribution were determined by DXA. Secondly, we evaluated insulin sensitivity and β -cell function, measured by a frequently sampled intravenous glucose tolerance test with Tolbutamide. Finally, lipid levels and blood pressure were measured.

PART 2 PREMATURITY

Since both SGA and preterm birth have been associated with an increased risk of developing adult diseases, we investigated the relative contribution of prematurity and SGA on several parameters for cardiovascular diseases and bone mineral density in young adulthood.

6 OUTLINE OF THIS THESIS

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

Chapter 2 reports on the effect of insulin sensitivity and the GH-IGF-IGFBP axis on the GH induced growth response in short children born SGA, before and during GH treatment.

Chapter 3 describes body composition and fat distribution in previously GH-treated young SGA adults at 6.8 years after discontinuation of GH treatment.

Chapter 4 reports on alterations in body composition, lipid levels and blood pressure until two years after discontinuation of GH treatment in subjects born SGA.

Chapter 5 reports on longitudinal changes in insulin sensitivity, insulin secretion and body composition in previously GH-treated young adults born SGA, until five years after discontinuation of GH treatment.

Chapter 6 describes the effect of preterm birth on body composition, especially fat mass, and lipid profile.

Chapter 7 describes the effect of preterm birth on several determinants of cardiovascular disease in early adulthood, including effects of birth size and growth patterns.

Chapter 8 describes the effect of preterm birth on bone mineral density in young adults.

Chapter 9 discusses our findings in relation to current literature and comments on the clinical implications and conclusions of our study results.

Chapter 10 summarizes our findings in English.

Chapter 11 summarizes our findings in Dutch.

APPENDIX A

IUGR-1 study

Inclusion criteria

1. Birth length and/or birth weight SDS below -1.88 (comparable to 3rd percentile) for gestational age (90);
2. An uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia;
3. Chronological age between 3.00 and 10.99 for boys and between 3.00 and 8.99 for girls at start of the study;
4. Height SDS for age below -1.88 according to Dutch reference values (91);
5. Height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (91);
6. Prepubertal, defined as Tanner stage 1 or testicular volume smaller than 4 ml (92);
7. Normal liver, kidney and thyroid functions.

Exclusion criteria

1. Any endocrine or metabolic disorder such as diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism, except for growth hormone deficiency;
2. Disorders of major organs;
3. Chromosomal abnormalities or signs of a syndrome, except Silver Russell Syndrome;
4. Chondrodysplasia;
5. Hydrocephalus;
6. Active malignancy or increased risk of leukaemia;
7. Serious suspicion of psychosocial dwarfism (emotional deprivation);
8. Previous anabolic sex steroid or GH treatment.

Design

The IUGR-1 study started in 1991. The study design was a multi-centre, randomized, double-blind, dose-response GH trial. After stratification for age and for spontaneous GH secretion, all children were randomly and blindly assigned to either 1 mg GH/m²/day (group A) or 2 mg GH/m²/day (group B). Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Bagsværd, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

APPENDIX B

IUGR-2 study

Inclusion criteria

1. Birth length and/or birth weight SDS below -1.88 (comparable to 3rd percentile) for gestational age (90);
2. An uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia;
3. Chronological age between 3.00 and 7.99 years at start of the study;
4. Height SDS for age below -1.88 according to Dutch reference values (91);
5. Height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (91);
6. Prepubertal, defined as Tanner stage 1 or testicular volume smaller than 4 ml (92);
7. Normal liver, kidney and thyroid functions.

Exclusion criteria

1. Any endocrine or metabolic disorder such as diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism, except for growth hormone deficiency;
2. Disorders of major organs;
3. Chromosomal abnormalities or signs of a syndrome, except Silver Russell Syndrome;
4. Chondrodysplasia;
5. Hydrocephalus;
6. Active malignancy or increased risk of leukaemia;
7. Serious suspicion of psychosocial dwarfism (emotional deprivation);
8. Previous anabolic sex steroid or GH treatment.

Design

The IUGR-2 study started in 1996. The study was an open-labelled, multi-centre study with a randomized control group, which included 174 short children born SGA. Before entering the study, the GH status was evaluated in all children using GH stimulation tests (arginine and/or clonidine). Children with GH deficiency, which was defined as a GH peak <10 mcg/l during two stimulation tests, were not randomized but started with treatment at a dose of 1 mg GH/m²/day (GHD-group). The non-growth hormone deficient children were stratified according to age (3.00-5.50 versus 5.50-7.99 years) and height of the parents (height of both parents above -1.88 SDS versus height of at least one parent below -1.88 SDS). After stratification, children were randomly assigned to either the GH-group (2/3 of the children) or the control group (1/3 of the children).

The GH-group started immediately with treatment at a dose of 1 mg GH/m²/day. The control group remained untreated for three years and subsequently received the same GH treatment as the GH-group. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Bagsværd, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

APPENDIX C

IUGR-3 study

Inclusion criteria

1. Birth length and/or birth weight SDS below -2.00 for gestational age (90);
2. An uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia;
3. Chronological age between 3.00 and 7.99 years at start of the study;
4. Height SDS for age below -2.50 according to Dutch reference values (91);
5. Height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (91);
6. Prepubertal, defined as Tanner stage 1 or testicular volume smaller than 4 ml (92);
7. Normal liver, kidney and thyroid functions.

Exclusion criteria

1. Any endocrine or metabolic disorder such as diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism, except for growth hormone deficiency;
2. Disorders of major organs;
3. Chromosomal abnormalities or signs of a syndrome, except Silver Russell Syndrome;
4. Chondrodysplasia;
5. Hydrocephalus;
6. Active malignancy or increased risk of leukaemia;
7. Serious suspicion of psychosocial dwarfism (emotional deprivation);
8. Previous anabolic sex steroid or GH treatment.

Design

The IUGR-3 study started in 2002. The study design was an open-labeled, randomized, multi-centre study. After stratification for gender, GH-status (maximum serum GH between 20-30 mU/l versus serum GH >30 mU/l during a GH stimulation test) and BMI (<-1 SDS versus >-1 SDS), children were randomized into 2 different groups. During 6 months, children in group A received 1 mg GH/m²/day and those in group B received 2 mg GH/m²/day. Subsequently, all children received the same dose of 1 mg GH/m²/day. Biosynthetic GH (r-hGH Norditropin® Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

APPENDIX D

The PROGRAM and PREMS study cohort

The PROgramming factors for Growth And Metabolism (PROGRAM) study consists of 323 healthy young adults born term, whereas the Prematurity and Small for Gestational Age (PREMS) study cohorts consists of 169 healthy young adults born preterm (gestational age <36 weeks). In these participants, several parameters for cardiovascular disease and type 2 diabetes were determined.

Inclusion criteria

1. Chronological age at inclusion: 18.00-23.99 years;
2. Neonatal period without signs of severe asphyxia (defined as an Apgar score <3 after 5 minutes), no serious diseases such as long-term artificial ventilation and oxygen supply, bronchopulmonary dysplasia or other chronic lung disease;
3. Well documented growth data;
4. Caucasian;
5. Born singleton;
6. Signed informed consent;
7. PROGRAM study: gestational age of 36 weeks or more;
8. PREMS study: gestational age of less than 36 weeks.

Exclusion criteria

1. Chromosomal disorders, known syndromes and serious dysmorphic symptoms suggestive for a yet unknown syndrome, except Silver-Russell Syndrome;
2. Any disease, endocrine or metabolic disorder that could interfere with growth during childhood (such as diabetes, growth hormone deficiency, malignancies, severe chronic disease);
3. Treatment that could have interfered with growth (such as radiotherapy or growth hormone treatment);
4. Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood.

Study design

To investigate the influence of different growth patterns during childhood on determinants of adult disease, we oversampled subjects with extreme variants of normal growth, such as subjects born small for gestational age (SGA) (with and without catch-up growth) and subjects with unknown growth retardation during childhood (idiopathic short stature (ISS)). This design created greater contrast in the study population, which contributed to more statistical power.

For subgroup analyses, the total study population was divided into four clinically relevant subgroups based on birth length and adult height. Two subgroups consisted of small for

gestational age (SGA) born adults, one without catch-up growth (SGA-S) and one with catch-up growth (SGA-CU). The last two subgroups consisted of young adults born appropriate for gestational age: one with short adult stature without known reason (idiopathic short stature) and one with normal adult height (controls).

APPENDIX E

Dual Energy X-ray Absorptiometry (DXA)

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 1/10th of a chest X-ray.

Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT)

Several values regarding glucose homeostasis can be measured by FSIGT: 1) insulin sensitivity (Si), which quantifies the capacity of insulin to promote glucose disposal; 2) glucose effectiveness (Sg), which reflects the capacity of glucose to mediate its own disposal; 3) acute insulin response to glucose (AIRg), which is an estimate of insulin secretory capacity; and 4) the disposition index (DI), the product of SI and AIRg 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 FSIGT with Tolbutamide (93-96).

When SI 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 3) (97). If insulin secretion does not change appropriately, impaired glucose tolerance and eventually type 2 diabetes will develop (39).

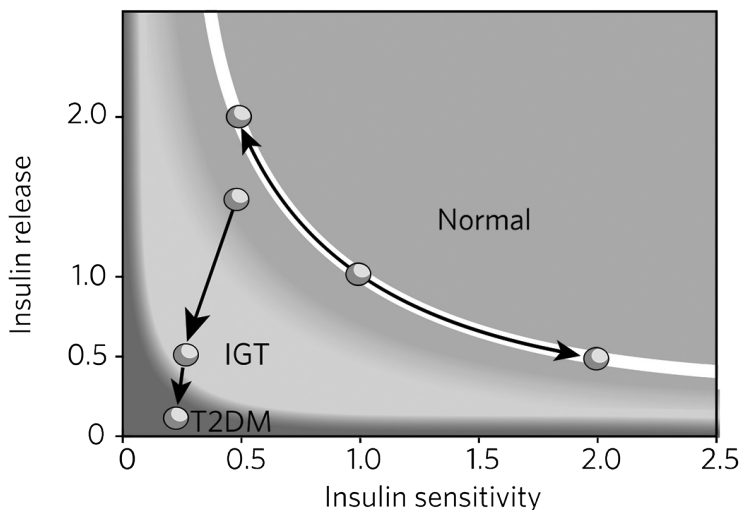


Figure 3. Hyperbolic association between insulin secretion and insulin sensitivity.

Adapted from Kahn et al. (74). IGT: impaired glucose tolerance, T2DM: type 2 diabetes mellitus

Carotid Intima Media Thickness

Intima media thickness (IMT) 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 atherosclerosis in later life (54,98). Carotid IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using a 7.5 MHz linear array transducer (ALT 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 (98).

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Part I

Small for gestational age



Chapter 2

Growth response during GH treatment is related with insulin levels, but not with insulin sensitivity in short children born small for gestational age

P.E. Breukhoven

J.S. Renes

A.C.S. Hokken-Koelega

Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia
Children's Hospital, Rotterdam, The Netherlands

Submitted

Chapter 3

Long-Term Impact of GH Treatment during Childhood on Body Composition and Fat Distribution in Young Adults Born SGA



P.E. Breukhoven

G.F. Kerkhof

M. van Dijk

A.C.S. Hokken-Koelega

Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia
Children's Hospital, Rotterdam, The Netherlands

J Clin Endocrinol Metab. 2011 Dec;96(12):3710-6

Abstract

Context: GH treatment of short children born small for gestational age (SGA) results in a decline in fat mass (FM) and an increase in lean body mass (LBM). It is, however, unknown whether these changes persist into adulthood.

Objective: Our objective was to assess the long-term impact of GH treatment during childhood on body composition and fat distribution.

Patients and Design: A total of 377 young adults participated in this cross-sectional study: 59 previously GH-treated young SGA adults compared to 52 untreated SGA adults with short stature (SGA-S), 161 SGA adults with spontaneous catch-up growth (SGA-CU), and 105 healthy normal-statured controls born appropriate for gestational age (AGA).

Outcome Measures: Body composition and fat distribution were determined by dual-energy x-ray absorptiometry.

Results: Mean (SD) duration of GH treatment was 7.7 (2.4) yr and period after discontinuation 6.8 (1.8) yr. FM, fat distribution, and LBM of GH-treated SGA adults were not significantly different from that of untreated SGA-S adults. GH-treated SGA adults also had a similar FM and fat distribution as SGA-CU adults but a lower LBM. All SGA subgroups had a lower LBM and tended to have a higher FM than healthy AGA controls.

Conclusion: Body composition and fat distribution of previously GH-treated SGA adults were similar to those of untreated SGA-S adults. GH-induced catch-up growth has no unfavorable effect on FM and fat distribution compared with spontaneous catch-up growth. However, our study shows that SGA adults in general may have a different body composition than healthy AGA controls.

Introduction

Studies on postnatal growth in infants born small for gestational age (SGA) or with intrauterine growth retardation have shown that, although these infants generally show spontaneous catch-up growth in height, approximately 10% of them remain short, with a height below -2 SD score (SDS) (1,2). SGA children who remain short tend to have a typical lean appearance with a low body mass index (BMI) (3). We previously showed that this leanness is characterized by a marked reduction of lean body mass (LBM) and to a lesser extent a lower total fat mass (FM) (4). GH treatment of these children resulted in a decline in FM and an increase in LBM, which is consistent with the lipolytic and anabolic properties of GH (4,5). De Kort et al. (6) showed that this decrease in FM is mainly located in the limbs of GH-treated SGA children, whereas the amount of trunk fat remains stable.

Obesity is a global and increasing problem with major public-health consequences (7). It is a risk factor for several adult diseases, including type 2 diabetes, the metabolic syndrome, and cardiovascular diseases. Previous studies showed that growth during infancy is an important determinant of body composition in young adulthood. Faster weight gain in infancy is associated with a greater risk of obesity later in life, whereas birth size is less important (8-10). Infants born SGA, however, are more likely to show catch-up growth in weight than infants born appropriate for gestational age (AGA) and could therefore be at greater risk of developing obesity later in life. Because GH treatment induces catch-up growth, concern has been expressed regarding the long-term effects of GH treatment on body composition (11).

The primary aim of this study was to investigate the impact of GH treatment during childhood on body composition and fat distribution in young adulthood in subjects born SGA, many years after discontinuation of GH. To answer this question, we compared previously GH-treated SGA adults with untreated SGA adults with short stature (SGA-S). We hypothesized that the body composition of GH-treated adults returns to levels of those who were never treated, especially because BMI SDS and waist circumference are comparable for GH-treated and untreated SGA adults with short stature (12). Our second aim was to evaluate whether GH-induced catch-up growth had a different effect on young adult body composition than spontaneous catch-up growth, and whether the various SGA groups had a different body composition and fat distribution than healthy AGA controls.

Subjects and Methods

Subjects

The total study group comprised 377 young adults, divided into 59 previously GH-treated adults born SGA who had previously been participating in a multicenter, double-blind, randomized, dose-response GH trial (13,14); 52 untreated SGA-S (adult height <-2 SDS); 161 SGA adults with

spontaneous catch-up growth (SGA-CU) (adult height >-2 SDS); and 105 healthy, normal-statured adult controls who were born AGA (defined as birth length and adult height >-2 SDS). The SGA-S, SGA-CU, and AGA adults were part of a cohort of young adults participating in a national study evaluating risk factors for diabetes mellitus type 2 and cardiovascular disease, the PROGRAM study (8).

The dose-response GH trial started in 1991 and evaluated the effect of two doses of GH, 1 and 2 mg/m²/day, on long-term growth and adult height. The inclusion criteria for the GH trial have been described (13). GH treatment was discontinued when height velocity had dropped below 0.5 cm over the preceding 6 months and when bone age was at least 15 yr for girls and at least 16.5 yr for boys. Patients were included in the present study when they had been treated with GH for more than 4 yr and had discontinued GH treatment for more than 4 yr. Thirty-one of the original 90 participants were not included for the following reasons: one subject had discontinued GH treatment less than 4 yr earlier; five children dropped out during the original GH trial due to lack of motivation (n=3), precocious puberty (n=1), or GH insensitivity (n=1); one subject was not included due to psychosocial problems; four subjects were lost to follow-up; two subjects emigrated; one subject had died due to a road accident; five persons did not respond to the invitation letter, and 12 subjects did not want to participate due to lack of interest. The clinical characteristics of the 59 GH-treated SGA subjects who were included were comparable with those of the 31 subjects who were not included, except for age at discontinuation of GH treatment (15.7 vs. 14.8 yr, respectively; $P=0.001$).

The studies were approved by the Medical Research Ethics Committees of the participating centers. Written informed consent was obtained from all participants or their parents.

Measurements

Standing height was measured in the upright position to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd., Crymmyth, UK). Sitting height (SH) was measured to the nearest 0.1 cm using a Harpenden SH table. SH to height ratio was then calculated and expressed as SDS adjusted for age and gender (15). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior, Katwijk, The Netherlands). BMI was calculated by dividing weight in kilograms by the square of height in meters and rounded to the nearest tenth. Waist circumference was measured at the level of the umbilicus using a nonextendable measuring tape. All anthropometric measurements were performed at least twice according to standardized methods, after which the mean was calculated. Height, BMI, and waist circumference were expressed as SDS adjusted for age and gender according to Dutch reference data for children (16-18).

In all participants, total FM, trunk FM, limb FM, and LBM were measured on one dual-energy x-ray absorptiometry (DXA) machine (Lunar Prodigy; GE Healthcare, Chalfont St. Giles, UK). Quality assurance was performed daily. For this type of DXA, the intraassay coefficient of variation has been reported to be 0.41-0.88% for fat tissue and 1.57-4.49% for LBM (19).

Statistics

Data are expressed as mean \pm SDS. Statistical analyses within the GH-treated SGA group were performed for the GH dosage groups separately (1 vs. 2 mg/m²/day) and for the groups together. Because the outcome variables were the same in both GH dosage groups, data are shown for the groups together.

Differences in clinical characteristics between the GH-treated SGA adults, and SGA-S, SGA-CU, and AGA adults were evaluated using independent-samples t tests. Differences in body composition and fat distribution between the GH-treated SGA adults, and the other three subgroups were determined using analyses of covariance, with correction for age, gender, gestational age, and adult height SDS. In the analyses, GH-treated SGA adults were defined as 1 and the other groups as 0.

The effect of the duration of GH treatment on total FM, trunk FM, limb FM, and LBM was analyzed using multiple regression analyses, corrected for possible confounders (age, gender, gestational age, height SDS, weight SDS, birth length SDS, and birth weight SDS). The interaction term birth length SDS * adult height SDS was added to the multiple regression model to ensure that the effect of these variables was modeled correctly (20). Oral contraceptive use and serum IGF-I levels at time of the present follow-up study (levels <-2 SDS used as proxy for GH deficiency) were added to the original models, but these did not change the results. Due to the lack of a significant contribution, these independent variables were deleted from the final models.

In addition, the means of the different parameters of body composition adjusted for age, gender, gestational age, and height SDS were calculated using univariate ANOVA, resulting in estimated marginal means (EMM). Based on the EMM, the differences in body composition between the three SGA subgroups and the healthy AGA controls were determined via pairwise comparisons.

Before the study, a power analysis with a significance level (α) of 0.05 and a chosen power of 80% estimated that there should be at least 22 subjects in each group to allow detection of a relevant difference of 10% in LBM. Results were regarded as statistically significant at $P<0.05$. Statistics were performed using the computer Statistical Package for Social Science (SPSS version 17.0; SPSS Inc., Chicago, IL).

Results

Clinical characteristics

The clinical characteristics of the four subgroups are shown in Table 1. GH-treated SGA adults were 22.5 (2.0) yr old. At the start of GH treatment, mean height SDS was -3.0, and mean age at start of GH treatment was 8.0 yr. The mean (SDS) duration of GH treatment was 7.7 (2.4) yr and the period after discontinuation of GH 6.8 (1.8) yr. GH-treated SGA adults had a significantly smaller size at birth (birth weight and/or birth length) than the SGA-S, SGA-CU, and AGA adults.

Table 1. Unadjusted clinical characteristics

	GH-treated SGA (n=59)	Untreated SGA-S (n=52)	P value^a	SGA-CU (n=161)	P value^b	AGA controls (n=105)	P value^c
Gender (male/female)	34/25	18/34		72/89		34/71	
Gestational age	36.6 (3.8)	37.9 (3.0)	0.051	36.6 (3.6)	0.892	39.4 (1.5)	<0.001
Birth weight SDS	-2.7 (1.0)	-2.3 (0.8)	0.013	-2.3 (0.7)	0.011	-0.1 (1.1)	<0.001
Birth length SDS	-3.5 (1.3)	-3.0 (0.9)	0.031	-2.7 (1.0)	<0.001	-0.3 (0.9)	<0.001
Age	22.5 (2.0)	20.9 (1.7)	<0.001	20.9 (1.7)	<0.001	20.9 (1.7)	<0.001
Height SDS	-1.6 (1.1)	-2.5 (0.6)	<0.001	-0.4 (0.9)	<0.001	0.1 (1.1)	<0.001
BMI SDS	0.3 (1.2)	0.3 (1.6)	0.799	0.3 (1.4)	0.857	0.1 (0.9)	0.191
Waist circumference SDS	-0.3 (1.3)	-0.2 (1.4)	0.923	0.4 (1.2)	<0.001	0.2 (1.0)	0.018
Ratio SH/height SDS	1.6 (1.3)	1.6 (1.2)	0.950	0.7 (2.8)	0.028	0.2 (1.7)	<0.001
IGF-I SDS	-0.4 (1.0)	-0.5 (0.9)	0.583	-0.4 (0.9)	0.828	-0.4 (1.0)	0.874

Data are expressed as mean (SDS).

^a Differences between GH-treated SGA and untreated SGA-S.

^b Differences between GH-treated SGA and SGA-CU.

^c Differences between GH-treated SGA and AGA controls.

At time of the present follow-up study, previously GH-treated SGA adults were 1.6 yr older than untreated SGA-S adults, SGA-CU adults, and AGA controls. GH-treated SGA adults were taller than the untreated SGA-S adults and shorter than the SGA-CU adults and AGA controls. GH-treated SGA adults had a similar SH to height ratio SDS as SGA-S and SGA-CU adults. This ratio was higher in SGA adults, either GH-treated or not, than in AGA controls. At the time of the present study, waist circumference SDS of GH-treated adults was similar to that of SGA-S adults but differed from those of SGA-CU and AGA adults. BMI SDS and serum IGF-I level SDS of the GH-treated SGA adults were comparable to those of the other three subgroups.

Body composition

Unadjusted body composition data are shown in Table 2. The differences between the GH-treated SGA adults and the other subgroups are given after correction for age, gender, gestational age, and adult height SDS.

GH-treated SGA vs. SGA-S

Total FM of GH-treated SGA adults was similar to that of untreated SGA-S adults. Fat distribution (trunk FM and limb FM) was also comparable for GH-treated SGA and untreated SGA-S adults. Unadjusted LBM seems to be higher in GH-treated SGA than in untreated SGA-S adults. However, after necessary adjustments for age, gender, gestational age and height SDS, the adjusted LBM of GH-treated SGA adults tended to be lower than untreated SGA adults (GH-treated vs. SGA-S

Table 2. Unadjusted body composition

	GH-treated SGA		Untreated SGA-S		β	P value ^a	SGA-CU	β	P value ^b	AGA controls	β	P value ^c
n	59	52					161			105		
Total FM (g)	15211 (8502)	15808 (8743)	-942	0.628			17210 (9923)	-1456	0.384	14296 (6671)	2752	0.098
Trunk FM (g)	8002 (4738)	7591 (4405)	-208	0.847			8522 (5168)	-419	0.649	6668 (3253)	1905	0.037
Limb FM (g)	6620 (3773)	7613 (4339)	-730	0.392			8043 (4784)	-996	0.184	6969 (3370)	836	0.262
LBM (g)	43715 (9788)	38004 (7529)	-2188	0.063			46909 (9494)	-1981	0.023	48200 (9431)	-3364	0.001

In the analyses, GH-treated SGA adults are defined as 1 and the other groups as 0. Data are expressed as mean (SDS). P values are adjusted for age, gender, gestational age, and adult height SDS.

^a Differences between GH-treated SGA and untreated SGA-S.

^b Differences between GH-treated SGA and SGA-CU.

^c Differences between GH-treated SGA and AGA controls.

$\beta=-2188$; $P=0.063$). After additional adjustment for birth weight SDS and birth length SDS, because GH-treated SGA adults were the smallest at birth, this difference disappeared ($P=0.130$).

GH-treated SGA vs. SGA-CU

GH-treated SGA adults had a similar total FM and fat distribution as SGA-CU. LBM was lower in GH-treated SGA adults than in SGA-CU adults.

SGA subgroups vs. healthy AGA controls

The EMM for all body composition parameters and differences between the SGA subgroups and the healthy AGA controls are shown in Figures 1-4. These EMM are adjusted for several variables such as adult height SDS and gender. Because the subgroups differ in, for example, gender and adult height SDS, it is important and more valuable to show these adjusted values. All SGA subjects had a higher adjusted total FM and adjusted limb FM than healthy AGA controls, but these differences were significant only for the SGA-CU adults compared with AGA controls ($P<0.001$). Adjusted trunk FM was also higher in all SGA subgroups but did not reach significance in the SGA-S adults ($P=0.100$), probably due to a lower number of subjects. In addition, all SGA subgroups had a lower adjusted LBM than healthy AGA controls.

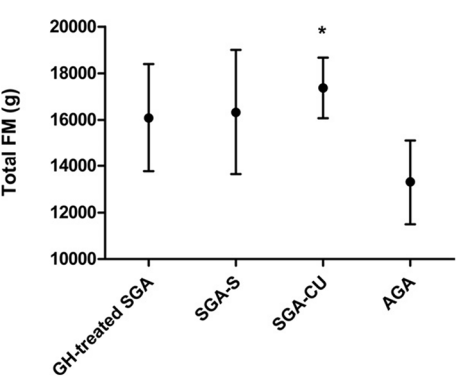


Figure 1. EMM of total FM of the three SGA subgroups compared with AGA controls (with 95% CI), adjusted for age, gender, gestational age and height SDS. *, $P<0.001$ compared with AGA controls.

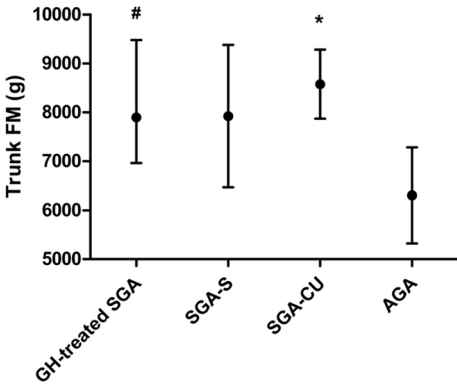


Figure 2. EMM of trunk FM of the three SGA subgroups compared with AGA controls (with 95% CI), adjusted for age, gender, gestational age, and height SDS. *, $P<0.001$ compared with AGA controls; #, $P<0.05$ compared with AGA controls.

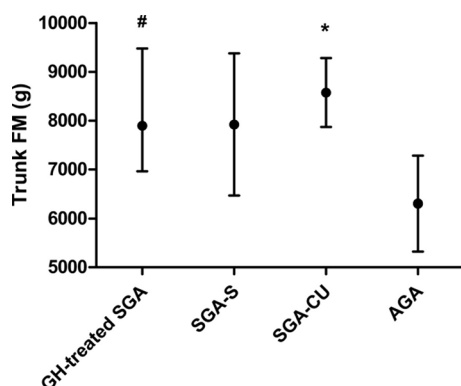


Figure 3. EMM of limb FM of the three SGA subgroups compared with AGA controls (with 95% CI), adjusted for age, gender, gestational age, and height SDS. *, $P < 0.001$ compared with AGA controls.

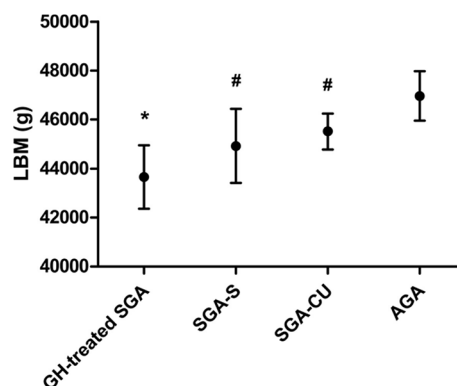


Figure 4. EMM of LBM of the three SGA subgroups compared with AGA controls (with 95% CI), adjusted for age, gender, gestational age, and height SDS. *, $P < 0.001$ compared with AGA controls; #, $P < 0.05$ compared with AGA controls.

Duration of GH treatment

Within the GH-treated SGA adults, multiple regression models were used to determine the influence of the duration of GH treatment on body composition. The duration of GH treatment did not have an effect on total FM ($\beta = 484$; $P = 0.199$), trunk FM ($\beta = 291$; $P = 0.199$), limb FM ($\beta = 183$; $P = 0.239$), and LBM ($\beta = -256$; $P = 0.523$), after adjustment for possible confounders (age, gender, gestational age, birth length SDS, birth weight SDS, adult height SDS, and adult weight SDS), many years after discontinuation of GH.

Discussion

This study compared the body composition and fat distribution of previously GH-treated SGA adults, at 6.8 yr after discontinuation of GH treatment, with those of untreated SGA-S, SGA-CU, and healthy AGA controls.

FM, fat distribution, and LBM of GH-treated SGA adults were not significantly different from those of untreated SGA-S. As indicated by Willemsen et al. (21), significant changes in body composition occur in previously GH-treated SGA adults after stopping GH treatment. They showed that at 6 months after GH discontinuation (mean age subjects 16 yr), fat percentage and FM had increased, whereas LBM had decreased. In a group of 10 SGA subjects who were treated

with GH for a period of 3 yr, Leger et al. (5) also showed a tendency to a decreased LBM and a significant increase in adipose tissue 3 months after GH discontinuation. These changes are opposite to those that occur during GH treatment in SGA children (22), i.e. an increase in LBM and a decrease in FM (4,5). In our cohort of young adults many years after discontinuation of GH treatment, FM and LBM levels were similar to those of untreated SGA-S. So despite an increase in FM and decrease in LBM after discontinuation of GH treatment, previously GH-treated SGA adults have a similar body composition as those who remained untreated. Furthermore, duration of GH treatment did not have any effect on body composition in young adulthood.

GH-treated SGA adults had a similar total FM and fat distribution as SGA-CU, which indicates that GH-induced catch-up growth has no unfavorable effect on FM compared with spontaneous catch-up growth. GH-treated SGA adults had, however, a lower LBM than SGA-CU adults, which is in line with the body composition of SGA children with short stature characterized by, among other things, a reduced LBM.

Furthermore, all SGA adults (GH-treated, SGA-S, and SGA-CU) tended to have a higher total FM and a higher trunk FM and limb FM than healthy AGA controls and had a lower LBM than the control subjects. These observed differences in body composition and fat distribution between SGA adults and controls probably indicate that SGA adults in general have a different body composition than healthy AGA controls. Using DXA trunk FM as a surrogate for abdominal FM and knowing that abdominal obesity is a risk factor for the development of metabolic abnormalities (23,24), our study suggests that all SGA-born adults might have a higher risk of developing diabetes mellitus type 2 and cardiovascular diseases than normal-statured healthy AGA controls.

The SGA-CU subjects had the highest amount of FM (total, trunk, and limb) of all three SGA subgroups and differed most significantly from healthy AGA controls. This is in line with previous research by Leunissen et al. (8) and Ibáñez et al. (25,26) who reported that SGA-CU gained more body adiposity and abdominal fat than AGA infants. Previous systematic reviews have also described a very consistent association between rapid weight gain during infancy and subsequent adiposity risk in childhood and later life (27-29).

To our knowledge, this is the first study investigating the long-term effects of GH on body composition many years after discontinuation of treatment in adults born SGA. Because body composition is greatly influenced by age, gender, and height (16), differences in body composition were evaluated after correction for these variables. Because gestational age was significantly different between the four subgroups and previous research suggests that gestational age might be of influence on later body composition (30), we also adjusted for this possible confounder in our analyses.

The GH-treated SGA subjects started GH treatment relatively late, around 8 yr of age. Our results demonstrate that GH treatment does not have an unfavorable effect on body composition in young adulthood, many years after stopping GH. However, the beneficial effects of GH on body composition during treatment, i.e. an increase in LBM and a decrease in FM, did not remain

either. It has been suggested that the adverse effects of poor intrauterine growth on the adipose tissue is most prominent in the first years of life. Ibáñez et al. (31) showed that girls born SGA, aged 2-8 yr, tend to follow an altered developmental trajectory that may lead to abnormal fat storage in puberty and adulthood. One could speculate that the lack of a positive effect of GH treatment on body composition, many years after its discontinuation, might be due to the fact that GH treatment was not started in the early years of life. Nowadays, some studies start GH treatment in (much) younger SGA children (32-34). It would be interesting to evaluate whether these children have a different adipose tissue development, particularly in the long term.

In conclusion, our study shows that at 6.8 yr after discontinuation of long-term GH treatment, body composition of previously GH-treated SGA adults is similar to that of untreated SGA-S. These data are reassuring, because they suggest that long-term GH treatment of SGA children with short stature does not have an unfavorable effect on body composition in young adulthood. Second, our study shows that SGA adults in general may have a different body composition from healthy AGA controls. It remains to be elucidated how body composition and fat distribution develop when these subjects become older.

Acknowledgments

We greatly acknowledge Mrs. J. Dunk, Mrs. J. van Nieuwkasteele, Mrs. M. Huibregtse-Schouten, Mrs. E. Lems, Mrs. I. van Slobbe, and Mrs. M. van Pieterse, research nurses, for their technical assistance and help with data collection; Prof. E. P. Krenning, Ph.D., head of the Nuclear Medicine Department, and Mrs. J. P. Sluimer for performing and analyzing the DXA measurements. Mrs. M. A. de Ridder is greatly acknowledged for her help with the calculations of the EMM. We also greatly acknowledge the physicians who participated in the original GH trial: E. C. A. M. Houdijk, M.D., Ph.D., VU University Medical Center, Amsterdam; M. Jansen, M.D., Ph.D., Wilhelmina Children's Hospital, Utrecht; H. M. Reeser, M.D., Ph.D., Juliana Children's Hospital, The Hague; and Y. K. van Pareren, M.D., Ph.D., Erasmus University Medical Center/Sophia Children's Hospital, Rotterdam, The Netherlands.

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Chapter 4

Changes in Body Composition, Lipid Levels and Blood Pressure after Discontinuation of GH in subjects born SGA

P.E. Breukhoven¹

M.A.J. de Ridder²

G.F. Kerkhof¹

A.C.S. Hokken-Koelega¹

¹Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia Children's Hospital, Rotterdam, The Netherlands

²Department of Biostatistics, ErasmusMC, Rotterdam, The Netherlands

Submitted

Chapter 5

Longitudinal changes in insulin sensitivity and β -cell function after discontinuation of growth hormone treatment: A 5-year follow-up study in subjects born SGA

P.E. Breukhoven

G.F. Kerkhof

A.C.S. Hokken-Koelega

Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia
Children's Hospital, Rotterdam, The Netherlands

Submitted

Part II

Prematurity



Chapter 6

Fat Mass and Lipid Profile in Young Adults Born Preterm

P.E. Breukhoven

G.F. Kerkhof

R.H. Willemsen

A.C.S. Hokken-Koelega

Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia
Children's Hospital Rotterdam, The Netherlands

J Clin Endocrinol Metab. 2012 Apr;97(4):1294-302

Abstracts

Context: Associations between small size at birth and abnormal cardiovascular parameters in later life have been reported. It is, however, unknown whether the effect of a small size at birth on cardiovascular risk factors in later life is due to a small size for gestational age or due to prematurity. Due to advances in neonatal care, survival of preterm infants has significantly improved, and nowadays an increasing number of these children reach adulthood. It is, therefore, of increasing importance to assess the long-term effect of prematurity on determinants for cardiovascular disease.

Objective: The aim of the study was to assess the long-term effects of gestational age and particularly preterm birth on lipid levels and fat mass in early adulthood.

Design and Patients: A cross-sectional study was conducted with 455 healthy subjects, aged 18 to 24 yr; 167 preterm subjects were compared with 288 full-term subjects.

Outcome Measure: Total fat mass, trunk fat mass, and limb fat mass were determined by dual-energy x-ray absorptiometry. Furthermore, fasting lipid levels (total cholesterol, low-density lipoprotein, triglyceride, apolipoprotein B, lipoprotein a, high-density lipoprotein, and apolipoprotein A-I) were measured.

Results: Preterm subjects had a significantly higher percentage of total fat mass, trunk fat mass, and limb fat mass than subjects born term. Furthermore, preterm subjects had significantly lower serum lipoprotein a levels and higher apolipoprotein A-I levels than term subjects. Multiple linear regression analyses to assess the association between gestational age and fat mass and lipid levels showed similar results.

Conclusion: In our cohort of 455 young adults, preterm birth was associated with more total fat mass, trunk fat, and limb fat mass but a relatively favorable lipid profile.

Introduction

Dyslipidemia is one of the major determinants for cardiovascular disease (CVD) and is characterized by raised levels of total cholesterol (TC), low-density lipoprotein (LDLc), triglyceride (Tg), apolipoprotein B (ApoB), and lipoprotein a [Lp(a)], together with reduced levels of high-density lipoprotein (HDLc) and apolipoprotein A-I (ApoA-I) (1-4). Several studies reported an association between a small size at birth and fat mass and/or components of the lipid profile in later life (5-12), which might indicate that subjects born with a low birth weight would have an increased risk for developing CVD. These reports have contributed to the hypothesis that impaired fetal growth may be associated with abnormal metabolic and cardiovascular parameters in later life.

Small size at birth may be due to preterm birth, poor fetal growth, or a combination of both. Most studies focused on subjects with a low birth weight and did not have information on gestational age, did not correct for it, or only included subjects born term (6-9). It is, therefore, difficult to determine whether the effect of a small size at birth on cardiovascular risk factors in later life is due to a small size for gestational age (SGA) or to prematurity. Previous research by Leunissen et al. (10) showed that low birth weight was no significant determinant of serum lipid levels in young adults born term. We, therefore, hypothesized that the previously described effect of a small birth size on serum lipid levels might be due to preterm birth rather than being born SGA.

Approximately 7% of all Dutch newborns are born preterm (gestational age <37 wk, Central Bureau of Statistics 2007, The Netherlands). Preterm infants are frequently exposed to early postnatal growth restriction, glucocorticoid treatment, and stressful events. This may be important in the development of later organ dysfunction and adverse vascular outcome and could increase the effect of prematurity on fat mass and lipid levels in later life. To date, most long-term follow-up studies have focused on neurodevelopmental and respiratory complications of preterm infants (13-16), with little attention to cardiovascular outcome. Due to advances in neonatal care, survival of preterm and very low birth weight infants has significantly improved, and an increasing number of these children reach adulthood. It is, therefore, of increasing importance to assess the long-term effect of prematurity on determinants for CVD.

In line with our hypothesis that preterm birth is associated with an adverse lipid profile in early adulthood, we also hypothesized that being born preterm is associated with more fat mass because most lipid levels are influenced by the amount of fat mass. Therefore, we investigated the long-term effects of gestational age and particularly preterm birth on these outcome parameters. We also assessed whether being born SGA, in addition to preterm birth, increases the risk for adverse cardiovascular parameters in this group.

Subjects and Methods

Subjects

The PREMS/PROGRAM study cohort consists of 492 healthy subjects, aged 18 to 24 yr. Preterm-born subjects (gestational age <36 wk, PREMS study) had been admitted to the neonatal intensive care unit of the Erasmus University Medical Centre shortly after birth. Term subjects (gestational age ≥36 wk, PROGRAM study) were randomly selected from hospitals in The Netherlands, where they had been registered because of being born SGA [defined as birth length below -2 SD score (SDS)]. In addition, healthy term subjects of different schools were randomly asked to participate (PROGRAM study).

The participation rate of the PREMS/PROGRAM study cohort was 79.5%. All participants fulfilled the same inclusion criteria: 1) age 18-24 yr; 2) Caucasian; 3) born singleton; 4) uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score <3 after 5 min), without sepsis or long-term complications of respiratory ventilation, such as bronchopulmonary dysplasia; and 5) maximum duration of respiratory ventilation and/or oxygen supply of 2 wk during the neonatal period. Subjects with a serious neonatal complication (e.g. necrotizing enterocolitis, respiratory problems requiring glucocorticoids, degree 3 or more intraventricular hemorrhage, spastic hemiplegia or quadriplegia), an endocrine or metabolic disorder, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive for a yet unknown syndrome were excluded. Subjects with a condition known to interfere with growth, including GH deficiency, severe chronic illness, emotional deprivation, GH treatment, glucocorticosteroids treatment, and radiotherapy, were also excluded.

Data regarding gestational age and birth size were taken from hospital records and records from community health services and general practitioners. Information regarding socioeconomic status (SES), cigarette smoking, alcohol consumption, and usage of oral contraceptives was obtained using questionnaires. SES was determined by using educational level of the participant, which was assessed by the highest grade of school completed or currently participating in, and categorized into: 1) high (higher general secondary education or higher); 2) median (junior general secondary education – secondary vocational education); and 3) low education (preparatory middle-level vocational education or lower).

Of the 492 participants who entered the study, 37 had incomplete data on body composition and lipid levels, resulting in a total number of 455 eligible subjects for analyses. There were no differences in anthropometric measurements between the included and excluded participants.

Based on SDS of birth length and adult height, the subjects were also assigned to one of three subgroups. To increase the statistical power for subgroup comparison, the cutoff values for small birth size and short adult height were set at -2 SDS, and the cutoff values for normal birth size and normal adult height were set at -1 SDS. This resulted in a total of 269 participants who were included in one of the three subgroups:

- Subjects born SGA (birth length below -2 SDS) with a short adult height (below -2 SDS) (SGA-S; n=49).
- Subjects born SGA (birth length below -2 SDS) with catch-up growth resulting in a normal adult height (above -1 SDS) (SGA-CU; n=85).
- Subjects born appropriate for gestational age (AGA; birth length above -1 SDS) with a normal adult height (above -1 SDS) (n=135).

All participants were invited to visit the Erasmus University Medical Centre in Rotterdam, The Netherlands. They had been fasting for at least 12 h and had abstained from smoking and alcohol for at least 16 h. The center's Medical Research Ethics Committee approved this study. Written informed consent was obtained from all the participants.

Anthropometry

Adult height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd., Crymch, UK). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, The Netherlands). All anthropometric measurements were performed twice, and the mean value was used for analysis. Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters and rounded to the nearest tenth.

Body composition

Body composition was measured by dual-energy x-ray ab-sorptiometry (DXA, type Lunar-Prodigy; GE Healthcare, Chalfont St.Giles, UK). All scans were made on the same machine, and quality assurance was performed daily. For this type of DXA, the intra assay coefficient of variation has been reported to be 0.41-0.88% for fat issue and 1.57-4.49% for lean body mass (LBM) (17).

Laboratory methods

After centrifugation, all samples were kept frozen until assayed (°80C). Fasting levels of TC, Tg, ApoB, Lp(a), HDLc, and ApoA-I were measured. LDLc was calculated using the Friedewald formula: $LDLc (mmol/liter) = TC - HDLc - 0.45 * Tg$ (18).

TC 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 homogeneous enzymatic colorimetric assay (Roche Diagnostics); ApoA-I, ApoB, and Lp(a) were determined by rate nephelometry on the Image Immunochemistry System, according to the manufacturer's instructions (Beckman Coulter, Mijdrecht, The Netherlands). The intraassay variations of measurements of TC, Tg, and HDLc were 2.9, 3.3, and 3.9%, respectively. Between-run coefficients of variation for ApoA-I, ApoB, and Lp(a) were 4.2, 2.8, and 6.9% at levels of 0.94, 0.53, and 0.35 g/liter, respectively.

Statistics

To correct for gestational age, SDS for birth length and birth weight were calculated (19). To adjust for gender and age, SDS for adult height and adult weight were calculated (20). ANOVA was used to determine whether there were differences between subjects born either preterm or term with regard to clinical characteristics. Differences in smoking, alcohol use, SES, and oral contraceptive use were determined using a χ^2 test. Multiple linear regression analysis was used to determine differences between preterm and term subjects regarding fat mass (adjusted for age, gender, SES, birth length SDS, birth weight SDS, and adult height SDS) and regarding lipid levels (adjusted for age, gender, SES, alcohol, smoking, birth length SDS, adult height SDS, fat mass, and LBM). All lipid levels were log-transformed before analyses because of a skewed distribution. We also compared the SGA-S subjects born preterm and term, the SGA-CU subjects born preterm and term, and the AGA subjects born preterm and term.

The prevalences of raised TC levels ($TC > 6.5$ mmol/liter), raised LDLc levels ($LDLc > 4.12$ mmol/liter), raised Tg level ($Tg > 2.0$ mmol/liter), raised ApoB levels ($ApoB \geq 1.20$ g/liter), raised Lp(a) levels [$Lp(a) > 0.30$ g/liter], reduced HDLc levels ($HDLc < 0.9$ mmol/liter, males; or < 1.1 mmol/liter, females), and reduced ApoA-I levels ($ApoA-I \leq 1.20$ g/liter) were calculated for the preterm and term subjects. Differences in these prevalences were analyzed using a χ^2 test.

Multiple linear regression analysis was performed to determine the association between gestational age and fat mass (total fat mass, trunk fat mass, and limb fat mass). In model A, we entered gestational age, age, gender, birth length SDS, birth weight SDS, and adult height SDS. Next, we added SES, smoking, and alcohol use to model A (model B). Finally, adult weight SDS was added to the model, resulting in model C. By adding adult weight SDS into the model, we investigated the association between gestational age and fat mass, whereas adult weight SDS was assumed constant, thus indirectly demonstrating the association between gestational age and fat percentage. Oral contraceptive use was subsequently added and analyzed as a possible confounder only in the female subjects.

To study the effect of gestational age on TC, LDLc, Tg, ApoB, Lp(a), HDLc, and ApoA-I, we first entered gestational age, age, gender, birth length SDS, birth weight SDS, and adult height SDS (model A). Next, we added SES, smoking, alcohol use and adult weight SDS (model B). Finally, adult weight SDS was replaced by LBM and fat mass to investigate the relative effect of muscle and/or fat mass (model C). Oral contraceptive use was then added and analyzed as a possible confounder in the female subjects.

The interaction term birth length SDS*adult height SDS was added to all multiple linear regression models because the study group had been selected on birth length and adult height to ensure that the effect of these variables was modeled correctly.

To evaluate whether being born SGA had an effect on adult cardiovascular parameters in addition to being born preterm, preterm (yes or no) and SGA (yes or no) were added to a multiple linear regression model, with adjustments for possible confounders (age, gender, and SES in the analysis with fat mass as dependent variable; age, gender, SES, alcohol, smoking, fat mass, and

LBM in the analyses with lipid levels as dependent variables). Within the SGA subgroups, the effect of showing spontaneous catch-up growth to an adult height greater than -1 SDS (yes or no) was assessed by then adding catch-up growth as an independent parameter into this multiple linear regression analysis.

Statistical package SPSS version 16.0 (SPSS, Inc., Chicago, IL) was used for analyses. Results were regarded statistically significant if the P value was <0.05 .

Results

Clinical characteristics

The total study population consisted of 455 subjects. The clinical characteristics of the total study population, subdivided into adults born preterm vs. term, are shown in Table 1. Young adults born preterm had a higher birth weight SDS ($P<0.001$), adult height SDS ($P<0.001$), and adult weight SDS ($P=0.002$) than subjects born term. Mean BMI SDS was well within the normal range in both preterm and term subjects. The percentage of alcohol users and smokers was similar for preterm and term subjects, as was the SES. Females born preterm used oral contraceptives less frequently than those born term ($P=0.036$).

Preterm vs. term

Comparison of preterm and term subjects in the total study group showed that, after adjustment for possible confounders, preterm subjects had significantly more total fat mass ($P=0.017$), trunk fat mass ($P=0.021$), and limb fat mass ($P=0.017$) than subjects born term. However, preterm subjects had significantly lower Lp(a) levels ($P<0.001$) and higher ApoA-I levels ($P<0.01$) than term subjects. TC levels were significantly lower in preterm compared with term subjects ($P=0.030$) (Table 1).

Table 2 shows the prevalence of lipid levels above or below the normal range in the preterm and term subjects. There was a significant difference in prevalence of reduced ApoA-I levels between the preterm (12.6%) and term (33.3%) subjects. Abnormal TC, LDLc, Tg, ApoB, Lp(a), and HDLc levels occurred less frequently in preterm than in term subjects. However, these differences did not reach significance.

Table 1. Clinical characteristics of the total study population and subgroups (*continued*)

	Subgroups					
	Total study population			SGA-S		
	Preterm	Term		Preterm	Term	
LBM (kg)	47.3 [39.4-57.3] ^b	43.9 [37.5-50.8]		43.0 [35.6-50.4]	37.0 [33.8-45.0]	
Total FM (kg)	15.0 [8.9-22.0] ^c	14.1 [9.3-21.0]		16.6 [8.6-21.3]	15.5 [9.7-21.3]	
Trunk FM (kg)	7.0 [4.4-11.0] ^c	6.6 [4.2-9.8]		8.6 [4.6-11.4]	7.1 [5.0-9.8]	
Limb FM (kg)	8.0 [4.1-11.1] ^c	7.4 [4.8-10.6]		8.2 [4.1-10.0]	8.0 [4.9-10.7]	
TC (mmol/liter)	4.10 [3.70-4.80] ^c	4.40 [3.90-5.20]		5.00 [4.45-5.20]	4.64 [4.03-5.50]	
LDLc (mmol/liter)	2.38 [2.03-2.90]	2.61 [2.13-3.18]		2.96 [2.62-3.70]	3.07 [2.25-3.61]	
Tg (mmol/liter)	0.86 [0.63-1.19]	0.87 [0.68-1.23]		0.87 [0.59-1.14]	0.83 [0.69-1.13]	
ApoB (g/liter)	0.81 [0.68-0.92]	0.79 [0.66-0.96]		1.02 [0.89-1.12]	0.89 [0.70-1.05]	
Lp(a) (g/liter)	0.07 [0.02-0.18] ^a	0.10 [0.04-0.25]		0.03 [0.02-0.21]	0.12 [0.02-0.35]	
HDLc (mmol/liter)	1.34 [1.13-1.56]	1.34 [1.14-1.57]		1.38 [1.22-1.65]	1.29 [1.16-1.45]	
ApoA-I (g/liter)	1.40 [1.25-1.57] ^a	1.28 [1.17-1.42]		1.51 [1.31-1.86] ^a	1.23 [1.17-1.33]	
				1.38 [1.23-1.53]	1.29 [1.20-1.54]	

Data on clinical characteristics are expressed as mean (SDS). Data on body composition and lipid levels are expressed as median [interquartile range]. Lipid levels are log-transformed before analyses. Differences between preterm and term subjects regarding body composition are adjusted for age, gender, SES, birth length SDS, birth weight SDS, and adult height SDS. Differences between preterm and term subjects regarding lipid levels are adjusted for age, gender, SES, alcohol, smoking, birth length SDS, adult height SDS, fat mass, and LBM.

^a P<0.001 compared to term.

^b P<0.01 compared to term.

^c P<0.05 compared to term.

Gestational age

Using multiple linear regression analyses, we studied the effect of gestational age, as a continuous variable, on fat mass and lipid levels after adjustment for several confounders.

Table 2. Prevalence of abnormal lipid levels in early adulthood

Lipid levels	Preterm	Term	P value
High TC levels	2/165 (1.2%)	7/281 (2.4%)	0.363
High LDLc levels	2/165 (1.2%)	11/277 (3.8%)	0.106
High Tg levels	7/160 (4.2%)	14/274 (4.9%)	0.743
High ApoB levels	8/159 (4.8%)	22/266 (7.6%)	0.238
High Lp(a) levels	25/142 (15.0%)	60/228 (20.8%)	0.122
Low HDLc levels	18/149 (10.8%)	35/253 (12.2%)	0.660
Low ApoA-I levels	21/146 (12.6%)	96/192 (33.3%)	<0.001

Abnormal lipid levels are defined as TC >6.5 mmol/liter, LDLc >4.12 mmol/liter, Tg >2.0 mmol/liter, ApoB ≥1.20 g/liter, Lp (a) >0.30 g/liter, HDLc <0.9 (males) or 1.1 (females) mmol/liter, and ApoA-I ≤1.20 g/liter. Significant P values are indicated in boldface.

Relationship between gestational age and fat mass

Gestational age was inversely associated with total fat mass ($P=0.015$), trunk fat mass ($P=0.010$), and limb fat mass ($P=0.029$), after adjustment for age, gender, birth length SDS, birth weight SDS, and adult height SDS (Table 3). These associations remained significant after additional correction for SES, smoking, and alcohol use. Adjustment for oral contraceptive use in the female subjects did not change these results. Within the female subjects, oral contraceptive use did not significantly influence the variance in fat mass (total, trunk, and limb fat mass, data not shown).

By adding adult weight SDS to the analyses, we indirectly demonstrate the association between gestational age and fat percentage (see Subjects and Methods). Lower gestational age remained associated with a higher percentage of total fat mass ($P=0.003$), trunk fat mass ($P=0.003$), and limb fat mass ($P=0.006$).

Relationship between gestational age and lipid levels

Gestational age was not significantly associated with TC ($P=0.241$), LDLc ($P=0.211$), Tg ($P=0.227$), ApoB ($P=0.099$), and HDLc ($P=0.953$), also after adjustment for age, gender, birth length SDS, birth weight SDS, adult height SDS, SES, smoking, alcohol use, LBM, and fat mass (Tables 4-6). Adding oral contraceptive use to the model did not change these results. Oral contraceptive use was, however, a significant determinant of the variance in Tg ($P<0.001$), ApoA-I ($P=0.026$), and ApoB ($P=0.007$).

Table 3. Multiple regression for fat mass in early adulthood

	Total fat mass (kg)						Trunk fat mass (kg)						Limb fat mass (kg)					
	Model A			Model B			Model C			Model A			Model B			Model C		
	β	P value		β	P value		β	P value		β	P value		β	P value		β	P value	
Gestational age (wk)	-0.275	0.015		-0.271	0.031		-0.200	0.003		-0.156	0.010		-0.144	0.031		-0.107	0.003	
Age (yr)	0.706	0.004		0.844	0.002		0.626	<0.001		0.398	0.002		0.473	0.001		0.357	<0.001	
Gender	6.842	<0.001		6.902	<0.001		6.784	<0.001		2.433	<0.001		2.449	<0.001		2.386	<0.001	
Birth length SDS	0.917	0.047		0.956	0.051		0.301	0.245		0.501	0.043		0.515	0.049		0.168	0.234	
Birth weight SDS	-1.014	0.018		-1.058	0.022		-0.500	0.041		-0.578	0.012		-0.606	0.014		-0.310	0.020	
Adult height SDS	0.883	0.046		0.843	0.076		-2.511	<0.001		0.451	0.056		0.441	0.083		-1.342	<0.001	
SES				-1.511	0.029		-1.081	0.003					-0.906	0.015		-0.678	0.001	
Smoking				-0.342	0.747		0.263	0.640					-0.132	0.816		0.190	0.534	
Alcohol use				-0.608	0.599		-1.138	0.064					-0.274	0.658		-0.556	0.096	
Adult weight SDS							5.969	<0.001								3.172	<0.001	
Overall P value	<0.001			<0.001			<0.001			<0.001			<0.001			<0.001		
Adjusted R2	0.157			0.162			0.765			0.095			0.104			0.741		

Data are adjusted for the interaction term birth length SDS*adult height SDS. Significant P values are indicated in boldface.

Table 4. Multiple regression for lipid levels in early adulthood

	TC ^a						LDLc ^a						Tg ^a					
	Model A		Model B		Model C		Model A		Model B		Model C		Model A		Model B		Model C	
	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value
Gestational age (wk)	0.002	0.341	0.003	0.254	0.003	0.241	0.003	0.364	0.005	0.195	0.005	0.211	0.002	0.756	0.007	0.293	0.008	0.227
Age (yr)	0.015	0.006	0.015	0.009	0.013	0.029	0.018	0.028	0.020	0.029	0.016	0.077	0.015	0.251	0.008	0.533	0.001	0.954
Gender	0.124	<0.001	0.142	<0.001	0.153	<0.001	0.111	<0.001	0.131	<0.001	0.167	0.012	0.148	0.001	0.167	<0.001	0.164	0.103
Birth length SDS	-0.003	0.797	-0.005	0.602	-0.006	0.598	0.004	0.786	-0.002	0.916	-0.002	0.922	-0.031	0.205	-0.043	0.075	-0.045	0.067
Birth weight SDS	<0.001	0.960	-0.001	0.960	<0.001	0.987	-0.010	0.497	-0.006	0.691	-0.006	0.690	-0.013	0.568	-0.017	0.446	-0.015	0.523
Adult height SDS	-0.002	0.807	-0.013	0.237	-0.008	0.510	-0.013	0.387	-0.026	0.126	-0.023	0.223	-0.034	0.149	-0.087	0.001	-0.067	0.024
SES			-0.003	0.836	-0.001	0.930			-0.028	0.224	-0.026	0.256			0.037	0.288	0.045	0.201
Smoking			-0.001	0.949	-0.003	0.907			0.005	0.880	0.004	0.915			0.124	0.019	0.120	0.023
Alcohol use			0.040	0.108	0.044	0.078			0.031	0.414	0.036	0.340			0.102	0.077	0.115	0.046
Adult weight SDS			0.026	0.001					0.037	0.002					0.082	<0.001		
LBM (kg)					0.002	0.354					0.004	0.219					0.005	0.377
Fat mass (kg)					0.003	0.003					0.005	0.007					0.011	<0.001
Overall P value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001		0.002		<0.001		<0.001	
Adjusted R ²	0.104		0.147		0.144		0.055		0.090		0.090		0.035		0.112		0.114	

Data are adjusted for the interaction term birth length SDS*adult height SDS.

Significant P values are indicated in *boldface*.^a Levels are log-transformed for analyses.

Gestational age was positively associated with Lp(a) ($P=0.002$) and inversely associated with ApoA-I ($P<0.001$), also after adjustment for possible confounders (model C), indicating that subjects with a lower gestational age had lower levels of Lp(a) and higher levels of ApoA-I.

Preterm birth and SGA

Being born SGA, in addition to preterm birth, had no effect on total fat mass ($P=0.894$), trunk fat mass ($P=0.841$), and limb fat mass ($P=0.957$). Catch-up growth to a normal height after SGA birth was also not an additional contributor to the variance in total fat mass ($P=0.145$), trunk fat mass ($P=0.153$), and limb fat mass ($P=0.147$).

Furthermore, being born SGA did not additionally affect the variance in TC ($P=0.142$), LDLc ($P=0.086$), Tg ($P=0.370$), Lp(a) ($P=0.397$), HDLc ($P=0.201$), or ApoA-I ($P=0.536$). SGA birth did have a small positive effect on ApoB ($P=0.034$). Within the subjects who were born SGA, catch-up growth was not an additional contributor to the variance in Tg ($P=0.904$), Lp(a) ($P=0.752$), HDLc ($P=0.457$), and ApoA-I ($P=0.993$). Catch-up growth did contribute to the variance in TC ($P=0.033$), LDLc ($P=0.009$), and ApoB ($P=0.009$), in addition to being born preterm and SGA.

Discussion

Our study in 455 young adults shows that lower gestational age is associated with a higher percentage of total fat mass, trunk fat mass, and limb fat mass in early adulthood. In addition, our subgroup analysis shows that young adults born preterm have more fat mass. A shorter gestational age was, however, also associated with lower serum Lp(a) and higher ApoA-I levels. Thus, in accordance with our hypothesis, subjects born preterm are prone to develop a less favorable body composition later in life, but in contrast to our expectations they have a relatively favorable lipid profile. Being born SGA, in addition to being born preterm, had no effect on fat mass and most lipid levels, except for a small effect on ApoB levels.

Body fat mass and, in particular, trunk fat mass are major risk factors for cardiovascular disease (21). At the age of 18-24 yr, adults born preterm had significantly more fat mass (total fat mass, trunk fat mass, and limb fat mass) than adults born term, although BMI SDS was within the normal range and was similar in both groups. Some studies showed that children born preterm were shorter and lighter during childhood than children born term (22,23). We could not confirm such results in our population of young adults. Our results are in line with previous research in a cohort of healthy young adults. At the age of 18-27 yr, subjects born preterm had significantly more total fat mass and trunk fat mass than subjects born term, measured by whole body magnetic resonance imaging (12). Doyle et al. (24) also described a relative increase in BMI in subjects born preterm before the subjects reached the age of 20 yr. It might well be that, under the influence of hormonal changes in puberty and adolescence, preterm subjects undergo a relatively large increase in fat mass. Further longitudinal follow-up studies in childhood and adolescence are important to confirm these findings.

Table 5. Multiple regression for lipid levels in early adulthood

	ApoB ^a						Lp(a) ^a					
	Model A		Model B		Model C		Model A		Model B		Model C	
	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value
Gestational age (wk)	-0.007	0.035	-0.006	0.090	-0.006	0.099	0.059	0.001	0.063	0.002	0.065	0.002
Age (yr)	0.013	0.085	0.012	0.132	0.008	0.314	0.009	0.819	0.031	0.470	0.030	0.498
Gender	0.149	<0.001	0.173	<0.001	0.207	<0.001	0.308	0.021	0.273	0.066	0.152	0.637
Birth length SDS	-0.004	0.760	-0.007	0.596	-0.007	0.609	0.007	0.923	0.044	0.570	0.042	0.593
Birth weight SDS	-0.010	0.447	-0.010	0.471	-0.010	0.472	0.000	0.999	-0.039	0.594	-0.034	0.643
Adult height SDS	-0.025	0.067	-0.037	0.012	-0.033	0.054	-0.014	0.847	-0.027	0.744	0.001	0.990
SES			-0.018	0.376	-0.016	0.434			0.082	0.455	0.091	0.415
Smoking			0.049	0.109	0.047	0.123			-0.273	0.106	-0.274	0.105
Alcohol use			0.040	0.226	0.046	0.166			-0.040	0.826	-0.035	0.850
Adult weight SDS			0.042	<0.001					0.021	0.711		
LBM (kg)					0.004	0.156					-0.006	0.731
Fat mass (kg)					0.005	0.001					0.004	0.610
Overall P value		<0.001		<0.001		<0.001		0.009		0.022		0.033
Adjusted R ²		0.090		0.152		0.149		0.027		0.030		0.028

Data are adjusted for the interaction term birth length SDS*adult height SDS.

Significant P values are indicated in boldface.

^a Levels are log-transformed for analyses.

Table 6. Multiple regression for lipid levels in early adulthood

	HDLc ^a						ApoA-1 ^a					
	Model A		Model B		Model C		Model A		Model B		Model C	
	β	P value	β	P value	β	P value	β	P value	β	P value	β	P value
Gestational age (wk)	0.001	0.735	-0.001	0.829	0.000	0.953	-0.014	<0.001	-0.015	<0.001	-0.014	<0.001
Age (yr)	0.007	0.312	0.009	0.256	0.011	0.163	0.008	0.065	0.010	0.044	0.010	0.035
Gender	0.125	<0.001	0.140	<0.001	0.059	0.300	0.138	<0.001	0.152	<0.001	0.105	0.003
Birth length SDS	-0.002	0.899	0.004	0.799	0.002	0.877	0.002	0.849	0.006	0.485	0.005	0.548
Birth weight SDS	0.020	0.101	0.014	0.286	0.016	0.219	0.005	0.556	-0.001	0.946	0.001	0.909
Adult height SDS	0.030	0.019	0.043	0.003	0.052	0.002	-0.006	0.487	0.001	0.954	0.008	0.447
SES			0.013	0.510	0.016	0.425			0.003	0.835	0.005	0.695
Smoking			-0.079	0.008	-0.087	0.009			-0.027	0.143	-0.027	0.143
Alcohol use			0.047	0.147	0.046	0.162			0.047	0.021	0.047	0.020
Adult weight SDS			-0.021	0.038					-0.005	0.421		
LBM (kg)					-0.006	0.049					-0.003	0.105
Fat mass (kg)					-0.002	0.219					0.000	0.896
Overall P value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Adjusted R ²	0.069		0.104		0.107		0.223		0.256		0.258	

Data are adjusted for the interaction term birth length SDS*adult height SDS.
Significant P values are indicated in boldface.
^a Levels are log-transformed for analyses.

Another study found no differences in total fat mass between preterm and term subjects in 87 young adults (5). This might be due to the fact that fat mass was estimated using bioelectrical impedance analysis, which is known to underestimate total body fat percentage and fat tissue compared with DXA (25). Another reason for the difference might be that our cohort comprised a larger group of preterm subjects, which enables us to find differences with more statistical power.

The multiple linear regression analysis showed that the amount of fat mass was a significant contributor to the variance in most lipid levels. Because we showed that subjects born preterm had more fat mass, we expected a more adverse lipid profile. Gestational age was, however, not associated with TC, LDLc, Tg, ApoB, and HDLc. In our subgroup analyses, adults born preterm did have lower TC than those born term, but this difference is not likely to be clinically significant because preterm and term subjects had the same prevalence of abnormal TC levels. Our study was performed in young adults, and clinical manifestations of CVD do not usually occur before middle age. Assuming that the amount of fat mass of preterm subjects remains higher throughout adulthood, they might develop a more adverse lipid profile on the longer term.

A shorter gestational age was associated with lower Lp(a) levels and higher ApoA-I levels, and subjects born preterm had a significantly lower prevalence of adverse ApoA-I levels (12.6%), compared with those born term (33.3%). This might indicate that young adults born preterm have a more favorable lipid profile than those born term, although their body composition seems to be disadvantageous. Noteworthy, Lp(a) and ApoA-I levels were the only lipids not influenced by adult weight SDS. This indicates that Lp(a) and ApoA-I levels are not directly influenced by fat or fat accumulation but are determined by other factors, like genetic factors (10,26,27).

A relatively high percentage of our study population consisted of subjects born SGA (36.7%), compared with the normal population in which the prevalence of SGA is only 2.3%. This enabled us to study the additional effect of a small size at birth with more statistical power. In practice, preterm infants are more prone to impaired fetal growth than infants born term (28). We found no additional effect of being born SGA on the variance of fat mass and only a marginal effect of being born SGA on ApoB levels, whereas SGA had no effect on all other lipid levels. This is in accordance with our previous report by Leunissen et al. (10), who showed in subjects born term that fat mass of young adults born SGA was similar to fat mass of controls born AGA and that fat accumulation during childhood significantly determined serum lipid levels, whereas birth size did not. Irving et al. (11) also showed that among premature babies, those with intrauterine growth restriction were not measurably more disadvantaged regarding cardiovascular risk factors, e.g. lipid levels, than those born with birth weights AGA. This confirms our hypothesis that the previously described effect of small birth size on fat mass and lipid levels is due to preterm birth rather than being born SGA.

Family history is a well-known independent risk factor for future abnormal lipid profiles, as well as for CVD (29). It would have been of additional value to include this determinant of CVD in

our analyses. Unfortunately, we did not have sufficient information to assess family history in our cohort of young adults. However, none of the subjects who fully completed the questionnaires mentioned a family history of hyperlipidemia, CVD, hypertension, or type 2 diabetes mellitus.

In conclusion, in our cohort of 455 young adults, preterm birth was associated with more total fat mass, trunk fat, and limb fat mass, but also with a relatively favorable lipid profile. Further research is needed to evaluate whether or not these differences predispose subjects born preterm to CVD later in life. Such data are of major importance as an increasing number of children born preterm reach adulthood.

Acknowledgments

We thank all participants. We greatly acknowledge Mrs. J. Dunk, Mrs. M. Huibregtse-Schouten, and Mrs. I. van Slobbe, research nurses, for their technical assistance and support with data collection.

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Chapter 7

Does Preterm Birth Influence Cardiovascular Risk in Early Adulthood?

G.F. Kerkhof
P.E. Breukhoven
R.W.J. Leunissen
R.H. Willemsen
A.C.S. Hokken-Koelega

Department of Paediatrics, Subdivision of Endocrinology, ErasmusMC/Sophia
Children's Hospital, Rotterdam, The Netherland

J Pediatr. 2012 Sep;161(3):390-6

Abstract

Introduction: Both preterm birth and small birth size for gestational age (SGA) have been associated with an increased risk for developing cardiovascular disease (CVD), but controversies still exist. Our aim was to investigate the effect of preterm birth on risk factors for CVD, independent of birth size.

Patients and Methods: Observational study using data of 406 healthy participants aged 18 to 24 years, from the PROGRAM/PREMS study. Associations between gestational age and systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure, blood pressure variability, heart rate, Pulse Wave Velocity (PWV), and carotid Intima Media Thickness (cIMT) were studied. To study the differential effects of preterm and SGA birth, these parameters were also analyzed in subgroups born either preterm or term: young adults born SGA with short or normal adult stature, and young adults born appropriate for gestational age with normal adult stature.

Results: Subjects born preterm (gestational age <36 weeks) had higher unadjusted SBP, pulse pressure, systolic and diastolic blood pressure variability, and heart rate, but a lower DBP than subjects born term. Gestational age was inversely associated with SBP, pulse pressure, blood pressure variability, and heart rate, and positively associated with DBP, also after adjustment for confounders. There was no effect of gestational age on PWV and cIMT, a marker of atherosclerosis. Of all the CVD risk factors measured, higher pulse pressure affected cIMT the most.

Conclusions: Young adults born preterm might have a higher risk for CVD than those born term.

Introduction

Small size at birth has been associated with an increased risk for developing cardiovascular disease (CVD) in later life (1). Both preterm birth and poor fetal growth can lead to small birth size. Thus, in unraveling the mechanism of this association, independent effects of gestational age as well as small birth size for gestational age (SGA) are important to determine.

Increased blood pressure and arterial stiffness (quantified by Pulse Wave Velocity (PWV) (2-3) are major determinants of CVD, and both preterm birth and SGA birth have been related to these CVD risk factors (1, 4-9).

A recent study showed increased carotid Intima Media Thickness (cIMT), which is a measure of atherosclerosis (10), in subjects born preterm, however, this was restricted to those with fetal growth restriction (11). Furthermore, low birth weight has been associated with increased cIMT in young adulthood (12). Although these results were not adjusted for gestational age, it was shown that exclusion of young adults born preterm strengthened the association, indicating that the effect of small birth size on cIMT was due to SGA rather than preterm birth. In contrast, others showed that birth weight SDS did not associate with cIMT in young adulthood (13).

We investigated differences between young adults born either preterm or term, using the following variables: systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (14), blood pressure variability (15-16), heart rate (17), PWV, and cIMT. We also investigated the influence of gestational age on these outcomes after adjustment for several confounders, including birth weight SDS and birth length SDS. Additionally, we studied the differential effects of preterm and SGA birth on CVD risk, by subdividing the total population in clinically relevant groups: born small for gestational age (either preterm or term) with short (SGA-S) or normal adult stature (SGA-CU), and born appropriate for gestational age (either preterm or term) with normal adult stature (AGA).

Methods

The PROGRAM (n=323) and PREMS (n=169) study cohorts consist of 492 healthy participants, aged 18-24 years. The PROGRAM- and PREMS study cohorts had similar inclusion and exclusion criteria, study center (Erasmus University Medical Centre in Rotterdam), and measurements, the only difference was that the PREMS study consists of participants born preterm (gestational age <36 weeks). Participants were recruited from several hospitals in the Netherlands, where they had been registered because of their small birth size (birth length < -2SDS) (18), short stature (adult height < -2SDS) (19), or being born preterm. By using advertisement, healthy subjects born AGA were asked to participate. The participation rate of the PROGRAM/PREMS study cohort was 79.5% (20). The study population has been previously described in detail (20-21). Birth data were

taken from medical records of hospitals, community health services and general practitioners. Information regarding socioeconomic status (SES), smoking and alcohol use was obtained using questionnaires. Education level of the participant was used as socioeconomic indicator to determine SES (22). The Medical Ethics Committee of Erasmus Medical Centre approved the study. Written informed consent was obtained from all participants.

Of the 492 participants who entered the study, 86 had incomplete data because the devices to measure blood pressure, cIMT and PWV were not available at all times, resulting in a total number of 406 eligible subjects for analyses.

Additionally, based on SD-scores of birth length and adult height, the subjects were assigned to one of three subgroups. In order to increase the statistical power for subgroup comparison, the cut-off values for small birth size and short adult height were set at -2 SDS, and the cut-off values for normal birth size and normal adult height were set at -1 SDS. This resulted in a total of 246 participants who were included in one of the three subgroups:

- SGA (birth length < -2 SDS) with a short adult height (< -2 SDS) (SGA-S): n=44,
- SGA (birth length < -2 SDS) with catch-up growth resulting in normal adult height (> -1 SDS) (SGA-CU): n=75,
- AGA (birth length > -1 SDS) with normal adult height (> -1 SDS) (AGA): n=127.

All participants fasted for 12 hours and abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer, weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice; the mean was used for analysis.

Blood pressure and heart rate were measured after 10 minutes at rest, in the supine position, using the nondominant arm with an automatic device (Accutorr Plus, Datascope Corp., Montvale NJ, USA (23)) every five minutes for one hour and the mean values of these 13 measurements were taken to reflect resting blood pressure and resting heart rate (HR). Measuring blood pressure using an automatic device has many advantages, however some factors influence the measurement accuracy such as the underlying algorithms used and size and material of the cuff (24). The device used in the present study has been validated by the Association for the Advancement of Medical Instruments (AAMI) and the British Hypertension Society (BHS), concluding that the device gives accurate measurements in greatest agreement with the mercury standard (25). The 13 blood pressure measurements were also used to calculate the coefficient of variation (15-16). Pulse pressure was calculated as the difference between mean systolic and diastolic blood pressure (14).

Carotid-femoral PWV was measured in supine position using SphygmoCor (AtCor Medical, Sydney Australia) (26). A pressure tonometer was used to simultaneously record carotid pulse wave and ECG. The femoral pulse wave and ECG were also recorded. Distance travelled by the pulse wave was determined by measuring the distances from sternal notch to the femoral location and from sternal notch to the carotid location of pulse wave recording (27).

Carotid IMT was measured in supine position by recording of ultrasonographic images of both left and right carotid artery, using one 7.5 MHz linear array transducer (ATL Ultramark IV, Advanced Tech. Laboratories, Bethel Washington, USA) (28). 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 cIMT was determined as the mean of the mean near and far wall measurements of both the left and right side common carotid artery (28).

Statistical analysis

SD-scores for birth length and birth weight were calculated to correct for gestational age and sex (18). SD-scores for adult height, and adult weight were calculated to correct for sex, and age (19). Variables were log-transformed (natural logarithm) if not normally distributed. ANOVA was used to determine if there were differences between participants born either preterm or term. Using the 13 blood pressure measurements, the coefficient of variation (CV) was calculated to determine the within-subject variation in SBP and DBP with time (blood pressure variability) (15,29).

Multiple linear regression (MR)-analysis was performed to determine the association of gestational age with SBP, DBP, pulse pressure, blood pressure variability, HR, PWV, and cIMT independent of birth size. In all MR-models, adjustments were made for birth length SDS, birth weight SDS, adult height SDS, age, sex, SES, smoking, alcohol use, and the interaction term birth length SDS*adult height SDS because the study group had been selected on birth length and adult height (Model A). To study the association with SBP, DBP, pulse pressure, and blood pressure variability, we additionally adjusted for weight SDS (model B), and heart rate (model C). To study PWV, we additionally adjusted for mean arterial pressure (MAP) (model B), weight SDS, the interaction term sex*weight SDS and age*weight SDS (model C), and heart rate (model D). To study cIMT, we additionally adjusted for artery diameter (model B), and weight SDS (model C). We tested which parameter (SBP, DBP, pulse pressure, blood pressure variability, HR, PWV) was the most important determinant of cIMT, by adding the parameters alternately to the final cIMT-model. All regression coefficients are presented as a percentage for better interpretation of the results. A positive value indicates that the dependent variable is increased by that % for every unit increase of the independent variable.

ANCOVA was used to determine differences in blood pressure among the subgroups corrected for age and sex (model 1), and additionally adjusted for alcohol use, smoking, SES, and weight SDS (model 2). In blood pressure analyses, heart rate was added to model 2. In HR analyses, systolic blood pressure was added to model 1. In PWV analyses, MAP and HR were added to model 1, and height SDS was added to model 2 (model 3). In cIMT analyses, artery diameter was added to model 1. AGA subjects born term served as reference group and SGA-S preterm, SGA-S term, SGA-CU preterm, SGA-CU term, and AGA preterm were added as dummy

variables. Statistical package SPSS version 15.0 (SPSS, Inc., Chicago, IL) was used for analyses. Results were regarded statistically significant if p was <0.05 .

Results

The clinical characteristics of the total study population are shown in Table 1. Young adults born preterm had higher unadjusted SBP ($p=0.007$), pulse pressure ($p<0.001$), systolic and diastolic blood pressure variability ($p=0.002$, and $p<0.001$ respectively), and heart rate ($p<0.001$) than subjects born term. Unadjusted DBP was lower in subjects born preterm ($p<0.001$).

Gestational age was inversely associated with SBP ($p=0.026$) and pulse pressure ($p=0.001$) after correction for age, sex, SES, smoking, alcohol use, adult height SDS, and birth size. These associations remained significant after additional correction for adult weight SDS (Table 2). In contrast with the association between gestational age and pulse pressure, which remained significant after additional correction for heart rate, the association of gestational age with SBP disappeared after correction for heart rate. Heart rate on itself was positively associated with SBP ($p<0.001$) (Table 2).

Gestational age was positively associated with DBP after correction for age, sex, SES, smoking, alcohol use, adult height SDS and birth size ($p=0.001$) (Table 2). This association remained significant after additional adjustment for weight SDS and heart rate ($p<0.001$), which were both positively associated with DBP ($p<0.001$).

Lower gestational age was associated with a higher coefficient of variation of both systolic ($\beta(\%)=-1.67$, $p=0.003$, adj. $R^2=0.058$) and diastolic blood pressure ($\beta(\%)=-2.85$, $p<0.001$, adj. $R^2=0.149$), after adjustment for age, sex, birth length SDS, birth weight SDS, adult height SDS, SES, smoking, alcohol use, heart rate, and weight SDS (data not shown).

In MR-analyses, gestational age was inversely associated with heart rate after adjustment for age, sex, birth size, adult height SDS, SES, smoking, and alcohol use ($\beta(\%)=-0.86$, $p<0.001$, adj. $R^2=0.176$). This association remained significant after additional adjustment for weight SDS and SBP ($\beta(\%)=-0.76$, $p<0.001$, adj. $R^2=0.213$) (data not shown).

After adjustments, gestational age was not significantly associated with PWV (Table 3). Adult height SDS showed a significant positive association with PWV ($p=0.029$) after adjustment for weight SDS. Smoking, higher mean arterial pressure, and higher heart rate, were also related to a higher PWV.

Lower gestational age showed a trend towards lower cIMT after adjustment for age, sex, SES, smoking, alcohol use, adult height SDS, and birth size ($p=0.074$) (Table 3). However, this disappeared after adjustment for artery diameter, which was positively associated with cIMT.

Table 1. Unadjusted clinical characteristics of the total study population and subgroups

	Total study population		Subgroups					
	Preterm (n=163)	Term (n= 243)	SGA-S		SGA-CU		AGA	
			Preterm (n=9)	Term (n=34)	Preterm (n=31)	Term (n=44)	Preterm (n=63)	Term (n=64)
Male/female #	83/80§	92/151	5/4	10/24	15/16	17/27	37/26	26/38
Age (yrs)	20.8(1.7)	20.9(1.7)	21.6(1.8)	20.6(1.7)	20.4(1.9)§	21.4(1.4)	21.0(1.6)	20.7(1.8)
Gestational age (wks)	32.0(2.2)†	39.2(1.7)	32.3(1.5)†	39.3(1.6)	32.3(2.1)†	38.3(1.6)	32.3(2.4)†	39.4(1.6)
Birth length SDS	-1.22(1.9)	-1.46(1.5)	-3.58(1.0)	-2.99(0.9)	-3.16(0.8)	-2.85(0.8)	0.38(0.9)	0.14(0.7)
Birth weight SDS	-0.42(1.8)†	-1.12(1.4)	-2.49(0.9)	-2.02(0.9)	-2.11(1.1)	-2.36(0.7)	0.79(1.1)§	0.08(1.2)
Adult height SDS	-0.42(1.0)†	-1.03(1.4)	-2.31(0.3)	-2.61(0.6)	-0.10(0.6)	-0.11(0.8)	0.13(0.6)	0.38(0.9)
Adult weight SDS	-0.28(1.2)‡	-0.63(1.4)	-1.08(1.2)	-1.50(1.6)	-0.32(1.2)	0.21(1.1)	0.27(0.8)	0.10(0.9)
SBP(mmHg)*	112.3(8.0)§	110.0(9.0)	109.2(6.6)	107.8(10.2)	113.4(7.2)	112.4(10.2)	113.1(8.2)‡	110.1(7.2)
DBP(mmHg)	63.3(5.3)†	66.1(5.9)	58.2(3.3)§	66.2(8.0)	64.5(5.2)	66.6(6.1)	63.6(5.4)§	66.1(5.0)
PP(mmHg)*	48.9(6.2)†	43.8(5.8)	51.0(6.1)†	41.7(5.2)	48.9(6.0)‡	45.8(6.7)	49.5(6.1)†	43.9(5.5)
CV SBP(%)*	5.17(1.8)§	4.77(2.7)	5.90(1.9)	5.49(4.4)	5.07(1.7)	4.42(1.8)	5.00(1.5)	4.62(2.1)
CV DBP(%)*	9.77(3.2)†	7.98(3.7)	9.00(2.1)	8.36(3.1)	9.64(2.8)§	7.87(3.1)	9.21(3.0)§	7.59(4.3)
HR (beats/minute)	70(9.1)†	65(9.0)	67(9.1)	71(9.6)	72(11.0)§	65(9.1)	69(8.6)§	64(8.4)
PWV (m/sec)*	7.60(1.0)	7.59(0.9)	8.00(0.8)	7.16(1.0)	7.65(1.1)	7.62(1.1)	7.67(0.9)	7.76(1.2)
cIMT (mm)*	0.52(0.1)	0.52(0.05)	0.52(0.1)	0.50(0.05)	0.52(0.1)	0.53(0.1)	0.53(0.1)	0.52(0.05)
Supplement								
GA Median (IQR)	32(29-34)	40(38-40)	32(32-34)	40(38-41)	33(31-34)	38(37-40)	34(32-36)	40(40-41)
BMI	22.2(3.4)	22.4(3.5)	23.5(3.6)	23.1(5.0)	21.5(3.7)	23.2(3.6)	22.7(2.7)	21.8(2.8)
Alcohol users (%)#	84.5‡	75.7	77.8	76.5	80.7	80.0	88.9	78.1
Smokers (%)#	27.0	25.5	22.2	20.6	19.4	35.0	27.0	20.3
SES(%) 1	13.0	9.4	14.3	13.3	14.3	15.6	7.5	3.2
2	30.5‡	20.8	28.6	33.3	39.3	21.9	26.4§	6.5
3	56.5	69.8	57.1	53.3	46.4	62.5	66.0	90.3
MAP(mmHg)*	83.4(7.3)	83.3(7.7)	81.7(5.2)	81.9(8.2)	84.0(7.7)	86.3(9.4)	84.4(7.4)	83.0(5.8)
AD (mm)	6.66(0.4)	6.66(0.5)	6.49(0.4)	6.38(0.4)	6.65(0.4)	6.76(0.4)	6.77(0.4)	6.79(0.5)
cIMT/AD	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)	0.08(0.01)

Values are given as means (SD). GA: gestational age, SDS= standard deviation score, SBP= systolic blood pressure, DBP= diastolic blood pressure, PP= pulse pressure, CV= Coefficient of Variation, HR= heart rate, PWV= Pulse Wave Velocity, cIMT= carotid Intima Media Thickness.

*log transformed for ANOVA, # Chi-square test used to determine differences between subjects born preterm and term.

†: p<.001 compared to term (same subgroup), ‡: p<.05 compared to term (same subgroup), §: p<.01 compared to term (same subgroup)

Table 2. Multiple regression for Systolic blood pressure, Diastolic blood pressure and Pulse pressure in early adulthood

	Systolic blood pressure						Diastolic blood pressure						Pulse pressure					
	Model A		Model B		Model C		Model A		Model B		Model C		Model A		Model B		Model C	
	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P
Gestational age	-0.246	0.026	-0.230	0.026	-0.127	0.223	0.451	0.001	0.462	0.001	0.631	<0.001	-1.207	<0.001	-1.184	<0.001	-1.167	<0.001
Birth length SDS	-0.257	0.556	-0.496	0.227	-0.413	0.302	-0.703	0.193	-0.866	0.104	-0.733	0.153	0.293	0.664	-0.058	0.928	-0.045	0.944
Birth weight SDS	0.060	0.883	0.155	0.690	0.106	0.781	0.213	0.677	0.361	0.475	0.280	0.563	-0.466	0.465	-0.152	0.802	-0.159	0.792
Adult height SDS	0.121	0.774	-1.021	0.018	-0.956	0.023	-0.599	0.249	-1.379	0.014	-1.272	0.018	1.254	0.056	-0.431	0.520	-0.421	0.531
SES 1	5.180	<0.001	4.557	0.001	3.408	0.013	3.678	0.043	3.256	0.068	1.415	0.414	7.354	0.001	6.425	0.003	6.238	0.005
SES 2	1.629	0.112	1.159	0.228	0.815	0.385	2.019	0.113	1.695	0.176	1.133	0.348	1.299	0.412	0.634	0.681	0.559	0.709
Smoking	-1.057	0.286	-0.531	0.569	-0.096	0.917	-1.949	0.111	-1.592	0.187	-0.890	0.448	0.447	0.772	1.228	0.403	1.298	0.380
Alcohol use	2.118	0.045	1.596	0.107	1.449	0.133	2.319	0.077	1.960	0.128	1.720	0.164	1.850	0.256	1.089	0.479	1.065	0.489
Adult weight SDS			2.108	<0.001	2.110	<0.001			1.442	0.001	1.447	<0.001			3.099	<0.001	3.099	<0.001
HR					0.176	<0.001					0.288	<0.001					0.028	0.688
Overall P-value	<0.001		<0.001		<0.001		0.004		<0.001		<0.001		<0.001		<0.001		<0.001	
R ² adjusted	0.172		0.272		0.307		0.046		0.079		0.149		0.386		0.452		0.451	

Regression coefficients are shown as a percentage, a positive value indicates that the dependent variable is increased with that % for every unit increase of the independent variable.

Bolded p-values are p-values below 0.05.

Adjusted for age, sex and the interaction term birth length SDS * adult height SDS.

SES 3 (highest socioeconomic class) is used as reference for SES analyses.

SDS= standard deviation score, SES= socioeconomic status, HR= heart rate.

Because gestational age had an effect on several markers that have been previously associated with atherosclerosis, we tested which marker was the most important determinant of cIMT, by adding the markers alternately to model C (data not shown). The effects of SBP ($\beta(\%)=0.16$, $p=0.002$, $\text{adj.}R^2=0.198$), DBP ($\beta(\%)=0.02$, $p=0.818$, $\text{adj.}R^2=0.172$), pulse pressure ($\beta(\%)=0.48$, $p<0.001$, $\text{adj.}R^2=0.228$), SBP variability ($\beta(\%)=0.015$, $p=0.475$, $\text{adj.}R^2=0.174$), DBP variability ($\beta(\%)=0.28$, $p=0.072$, $\text{adj.}R^2=0.181$), HR ($\beta(\%)=-0.06$, $p=0.352$, $\text{adj.}R^2=0.176$), and PWV ($\beta=-0.62$, $p=0.259$, $\text{adj.}R^2=0.172$) on cIMT were determined. The model with the highest adjusted R^2 , thus explaining the largest proportion of variation in cIMT, was the model including pulse pressure.

Unadjusted differences between the subgroups are shown in Table 1. Comparisons of preterm and term SGA-subgroups, after adjustment for age, sex, alcohol use, smoking, SES, heart rate and weight SDS, showed that SGA-S subjects born preterm had a significantly lower diastolic blood pressure ($p=0.002$), and a higher pulse pressure ($p=0.016$) than those born term. Also, SGA-CU subjects born preterm had a lower diastolic blood pressure ($p=0.046$), and a higher pulse pressure ($p=0.028$) and systolic and diastolic blood pressure variability ($p=0.035$ and $p=0.004$ respectively) than those born term. There were no significant differences in systolic blood pressure between preterm and term SGA-subgroups.

After adjustment for age, sex, alcohol use, smoking, SES, systolic blood pressure, and weight SDS, SGA-CU subjects born preterm had a higher heart rate than those born term ($p=0.009$). There was, however, no significant difference in heart rate between SGA-S subjects born preterm or term. After adjustment for confounders, PWV and cIMT did also not differ significantly between subjects born preterm or term, in any of the subgroups.

Table 4 shows comparisons of systolic blood pressure, diastolic blood pressure, pulse pressure, blood pressure variability, heart rate, PWV, and cIMT of the subgroups after adjustment for possible confounders, with AGA subjects born term as reference group. In the final model, all preterm subgroups had a significantly lower diastolic blood pressure, but higher pulse pressure and diastolic blood pressure variability than the reference group. After correction, there were no differences in systolic blood pressure variability and cIMT. SGA-CU and AGA subjects born preterm had a higher heart rate than the reference group (AGA, born term). SGA-S and SGA-CU subjects born term had a lower PWV than the reference group, but this significant difference disappeared after correction for adult height SDS.

Table 3. Multiple regression for PWV and cIMT in early adulthood

	PWV						cIMT					
	Model A		Model B		Model C		Model D		Model A		Model B	
	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P	β (%)	P
Gestational age	0.147	0.460	0.141	0.442	0.145	0.405	0.261	0.145	0.263	0.074	0.095	0.481
Birth length SDS	-0.062	0.935	-0.061	0.932	0.400	0.553	0.493	0.461	0.113	0.847	-0.084	0.875
Birth weight SDS	-0.101	0.890	0.193	0.776	-0.104	0.872	-0.173	0.787	-0.330	0.550	-0.234	0.643
Adult height SDS	0.005	0.995	-0.041	0.952	1.556	0.032	1.574	0.029	0.502	0.372	-0.337	0.519
SES1	2.064	0.259	0.740	0.755	2.540	0.268	1.365	0.555	0.531	0.775	0.432	0.799
SES2	-1.814	0.317	-3.084	0.066	-2.453	0.126	-2.725	0.087	1.488	0.271	1.538	0.213
Smoking	2.343	0.185	4.006	0.017	3.770	0.017	4.136	0.009	0.645	0.623	1.214	0.313
Alcohol use	-0.116	0.950	-0.552	0.749	0.600	0.716	0.443	0.787	0.757	0.586	1.001	0.431
Artery diameter											10.66	<0.001
Adult weight SDS					5.496	0.376	6.247	0.312				-0.202
MAP			0.638	<0.001	0.740	<0.001	0.692	<0.001				0.636
HR							0.192	0.015				
Overall P-value	<0.001		<0.001		<0.001		<0.001		0.099		<0.001	
R ² adjusted	0.108		0.236		0.317		0.329		0.019		0.183	

Regression coefficients are shown as a percentage, a positive value indicates that the dependent variable is increased with that % for every unit increase of the independent variable.

Adjusted for age, sex and the interaction term birth length SDS*adult height SDS, the model with PWV as dependent variable is additionally adjusted for the interaction terms age*adult weight SDS and gender*adult weight SDS

SES 3 (highest socioeconomic class) is used as reference for SES analyses.

PWV= pulse wave velocity, cIMT= carotid intima media thickness, SES= socioeconomic status, SBP= systolic blood pressure, HR= heart rate, MAP= mean arterial pressure

Table 4. Subgroup analyses of blood pressure, pulse pressure, blood pressure variability, heart rate, PWV, and cIMT compared to AGA term controls

	SGA-S Preterm			SGA-S Term			SGA-CU preterm			SGA-CU term			AGA preterm			R ² adjusted
	β (%)	P		β (%)	P		β (%)	P		β (%)	P		β (%)	P		
Systolic BP	Model 1	-2.67	0.355	-0.45	0.802		2.47	0.149		1.20	0.464		1.02	0.486		0.129
	Model 2 ^{1,2}	-3.00	0.256	0.09	0.959		-0.12	0.937		-0.34	0.819		-1.33	0.300		0.314
Diastolic BP	Model 1	-12.9	<0.001	0.27	0.901		-1.45	0.469		0.63	0.745		-4.57	0.005		0.107
	Model 2 ^{1,2}	-14.3	<0.001	-0.75	0.721		-5.57	0.003		-1.02	0.554		-7.66	<0.001		0.318
Pulse Pressure	Model 1	12.9	0.008	-1.95	0.483		8.34	0.002		2.87	0.371		9.36	<0.001		0.358
	Model 2 ^{1,2}	14.4	0.003	0.71	0.817		8.08	0.005		0.87	0.729		8.17	<0.001		0.396
CV SBP	Model 1	34.2	0.038	24.4	0.013		8.73	0.303		-9.94	0.182		8.91	0.204		0.057
	Model 2 ^{1,2}	32.5	0.055	20.4	0.060		6.60	0.470		-11.1	0.145		7.55	0.300		0.051
CV DBP	Model 1	36.4	0.042	23.0	0.028		30.4	0.003		2.95	0.730		29.8	<0.001		0.109
	Model 2 ^{1,2}	38.5	0.037	22.5	0.053		29.1	0.007		0.89	0.917		28.2	0.001		0.127
Heart rate	Model 1 ³	9.13	0.083	10.1	0.002		13.5	<0.001		0.67	0.811		8.72	0.001		0.234
	Model 2 ^{1,3}	4.52	0.377	5.11	0.138		9.58	0.002		-0.79	0.774		7.70	0.002		0.283
PWV	Model 1 ⁴	0.07	0.990	-6.96	0.019		-4.73	0.101		-5.59	0.033		-4.47	0.060		0.275
	Model 2 ^{1,4}	-2.24	0.681	-11.2	<0.001		-5.10	0.070		-4.90	0.055		-3.65	0.114		0.342
cIMT	Model 3 ^{1,4,5}	2.48	0.691	-6.41	0.145		-4.48	0.114		-4.18	0.106		-3.22	0.163		0.348
	Model 1 ⁶	2.25	0.374	1.35	0.525		1.75	0.409		2.28	0.261		1.82	0.298		0.132
	Model 2 ^{1,6}	2.57	0.503	0.03	0.989		1.43	0.522		2.31	0.265		2.02	0.260		0.131

Regression coefficients are shown as a percentage, a positive % indicates that the dependent variable is increased with that %, compared to AGA term controls.

BP= blood pressure, CV= coefficient of variation, Systolic blood pressure, DBP= diastolic blood pressure, PWV= pulse wave velocity, cIMT= carotid intima Media Thickness.

All models are adjusted for age and sex and additionally adjusted for: ¹ Alcohol use, smoking, SES and adult weight SDS, ² Heart rate, ³ Systolic blood pressure, ⁴ Mean arterial pressure, ⁵ Adult Height SDS, ⁶ Artery diameter

Discussion

Higher blood pressure in adults born preterm than in healthy controls has been reported (30). Also in the present study lower gestational age was associated with higher systolic blood pressure, but this disappeared after adjustment for heart rate. These findings suggest that the reported elevated systolic blood pressure in subjects born preterm is associated with an increased heart rate, indicating that both might share an underlying determinant. The mechanisms underlying these associations remain unknown (31), but might be explained by preterm birth being associated with an increased cardiac output, which might eventually lead to hypertension (32). In contrast, we showed a lower diastolic blood pressure in young adults born preterm, which remained significant after adjustment for several confounders. Lower diastolic blood pressure has been associated with less risk for CVD (33), although this was controversial in other studies (34).

To our knowledge, we are the first to report an increased pulse pressure in young adults born preterm. This new finding is in line with a study showing an inverse association between gestational age and pulse pressure in children (35). Elevated pulse pressure has been associated with increased risk for atherosclerosis, already in early adulthood (14,33). This was confirmed by our study showing that of all determinants of CVD examined, the effect of pulse pressure on cIMT was most pronounced, in contrast to the non-significant effect of DBP on cIMT. In addition, variability of systolic and diastolic blood pressure was higher in participants born preterm. Higher variability of blood pressure in time has also been associated with CVD (15-16).

Although one would expect a lower heart rate in combination with a higher pulse pressure, young adults born preterm had a higher heart rate than those born term. This finding is supported by previous studies (36-38). Johansson et al. hypothesized that an increased heart rate could be ascribed to altered sympathoadrenal function in subjects born small, either preterm or SGA (38). In the present study, higher heart rate was only found in subjects born preterm, regardless of birth weight. This implies that there is an effect of preterm birth on heart rate, rather than an effect of SGA birth. Determination of resting heart rate is of importance since it is associated with CVD (17). Unfortunately, the present study does not include tests to determine neural regulatory mechanisms. For future research it would be interesting to carry out spectral analyses in young adults born preterm, in order to determine whether the increased heart rate and blood pressure variability are due to sympathovagal imbalance (39-40).

We did not find an association of preterm birth with PWV. Adult height SDS was, however, positively associated with PWV. This association also explains the difference in PWV between SGA-S subjects born term and AGA subjects born term, as that difference disappeared after correction for height SDS. Only limited studies investigated the association between adult height SDS and PWV. One study showed a positive association between height and PWV in healthy children (41).

There was also no effect of preterm birth on cIMT. Previous studies reported controversial results regarding the association of cIMT with gestational age, preterm birth, and birth size (12-13). These studies, however, did not adjust for artery diameter, which is likely to be a confounder in the relationship of gestational age and birth size with cIMT. Also, it might well be that an effect of gestational age on cIMT will arise at an older age.

The great contrasts in birth size and adult stature in our study population enabled performing comparisons of clinically relevant subgroups. These comparisons showed that the effect of preterm birth on CVD risk can not be ascribed to SGA birth and/or catch up growth. We found significant differences in DBP, pulse pressure, and DBP variability, between the preterm subgroups and term AGA controls, irrespectively of SGA birth. The preterm groups had a significantly higher resting heart rate, except for the preterm SGA-S subgroup. There were no differences in CVD risk parameters between the SGA-groups born term and the healthy controls.

We acknowledge that the Datascope Accutorr Plus to determine blood pressure during one hour uses an algorithm to compute systolic and diastolic blood pressure. Although it has shown to be in greatest agreement with the mercury standard, this should be taken into account. Future studies are warranted to reproduce our results using directly measured systolic and diastolic blood pressure. We also acknowledge that our study population consists of subjects without serious postnatal complications and did not include extreme prematurely born subjects. Whether our results can be generalized to subjects with complications, such as broncho-pulmonary dysplasia, requires further research. Furthermore, it would be of additional value to include family history, as a risk factor of atherosclerosis, in our analyses. Unfortunately, we did not have sufficient information to assess family history in our cohort of young adults. However, none of the subjects who fully completed the questionnaires mentioned a family history of cardiovascular disease.

Our data show that young adults born preterm might have a higher risk to develop CVD due to a higher systolic blood pressure, resting heart rate, and a higher pulse pressure and blood pressure variability in time. Although we show that young adults born preterm have a lower diastolic blood pressure than adults born term, the lower diastolic blood pressure contributes to an increased pulse pressure in these subjects. Because the prevalence of preterm birth and survival is rapidly increasing, our results are of clinical relevance for an increasing number of subjects and are thus of major importance for public health.

Acknowledgements

We greatly thank Mrs. J. Dunk, research nurse, for her technical assistance and support with data collection.

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Chapter 8

Preterm birth does not affect bone mineral density in young adults

P.E. Breukhoven
R.W.J. Leunissen
S.W.K. de Kort
R.H. Willemsen
A.C.S. Hokken-Koelega

Subdivision of Endocrinology, Department of Pediatrics, ErasmusMC/Sophia
Children's Hospital, Rotterdam, The Netherlands

Eur J Endocrinol. 2011 Jan;164(1):133-8

Abstract

Objective: Previous studies showed conflicting data on the effect of prematurity on bone mineral density (BMD) in infants and children. Only a few studies investigated the long-term effects of prematurity on BMD in early adulthood. The objective of our study was to assess the long-term effects of preterm birth on BMD of the total body (BMD_{TB}), lumbar spine (BMD_{LS}) and bone mineral apparent density of the LS ($BMAD_{LS}$).

Design: Cross-sectional study.

Methods: It consists of two hundred and seventy-six healthy subjects without serious postnatal complications, aged 18-24 years. The contribution of gestational age to the variance in BMD in young adulthood and the differences in BMD between 151 subjects born preterm (median gestational age 32.2 weeks (interquartile range (IQR) 30.3-34.0)) and 125 subjects born at term (median gestational age 40.0 weeks (IQR 39.0-40.0)) were investigated. BMD was determined by dual-energy X-ray absorptiometry.

Results: There were no significant linear correlations between gestational age and BMD_{TB} ($r=0.063$, $P=0.30$), BMD_{LS} ($r=0.062$, $P=0.31$) and $BMAD_{LS}$ ($r=0.069$, $P=0.26$). Also after adjustment for possible confounders, gestational age was no significant contributor to the variance in BMD_{TB} ($P=0.27$), BMD_{LS} ($P=0.91$) and $BMAD_{LS}$ ($P=0.87$). No significant differences were found between preterm and term subjects with regard to BMD_{TB} , BMD_{LS} and $BMAD_{LS}$.

Conclusion: In our cohort of 276 young adults, aged 18-24 years, gestational age was not a significant determinant in the variance of BMD. Preterm birth without serious postnatal complications is not associated with a lower BMD in young adulthood.

Introduction

Decreased mineralization of osteoid tissue during the early postnatal period is a known complication of very low birth weight and/or prematurely born infants (1-3). It comprises a variety of disturbances ranging from mild undermineralization to frank radiological rickets with fractures (4). Preterm infants are at an increased risk of low bone mineral density (BMD) as bone mineralization, along with calcium and phosphorus accretion, mainly occurs during the third trimester of pregnancy (5).

Previous studies showed conflicting data on the effect of preterm birth on BMD in infants and children. Prematurity, irrespective of birth weight, was found to be associated with lower BMD in infancy and early childhood (1,6-9). In contrast, other studies in young children could not show differences in BMD due to prematurity (2,10).

Owing to advances in neonatal care, survival of preterm and very low birth weight infants has significantly improved and an increasing number of these children reach adulthood. In 2007, the preterm birth rate in the United States was 12.7%, which corresponds with ~550.000 preterm births per year (11). Moreover, this percentage is about 20% higher than the preterm birth rate in 1990 (11). It is, therefore, of increasing importance to assess the effect of prematurity on BMD in adulthood. Previous studies investigated the long-term effects of low birth weight and growth on BMD (12-20), but very few have studied the specific contribution of the duration of gestation on later BMD (14,21).

BMD in later life depends largely on the peak bone mass achieved in early adulthood and the subsequent bone loss (22). A high peak bone mass provides a larger reserve later in life (22,23). Osteoporosis is an important and increasing cause of morbidity and mortality in the developed countries. It is characterized by low bone mass, deterioration of bone tissue and disruption of bone architecture, compromised bone strength and an increase in the risk of fracture (24). According to the WHO diagnostic classification, osteoporosis is defined by BMD at the hip or spine that is ≤ 2.5 SDS below the young normal mean reference population (24). Since early prevention of osteoporosis is likely to be more successful than treatment of an already established disorder, it is essential to identify potential risk factors.

We hypothesized that prematurity is associated with lower BMD in early adulthood. Therefore, the aim of our study was to assess the long-term effects of gestational age and particularly preterm birth on BMD in a large group of young adults.

Subjects and methods

Subjects

This study investigated a cohort of 276 healthy subjects, aged 18-24 years. Subjects born preterm (gestational age <36 weeks, n=151) had been admitted to the neonatal intensive care unit of the Erasmus University Medical Centre shortly after birth. In total, 37.7% of all preterm subjects were born small for gestational age. Term controls of similar age (gestational age ≥ 36 weeks, n=125) were randomly asked to participate from different educational institutes.

All subjects fulfilled the same inclusion criteria: 1) age 18-24 years, 2) adult pubertal stage, 3) Caucasian, 4) born singleton, 5) a neonatal period without signs of severe asphyxia (defined as an Apgar score below 3 after 5 min) or long-term complications of respiratory ventilation, such as bronchopulmonary dysplasia, 6) maximum duration of respiratory ventilation and/or oxygen supply of 2 weeks in the neonatal period. Subjects with a serious neonatal complication (e.g. necrotizing enterocolitis, degree 3 or more intraventricular haemorrhage, spastic hemiplegia or quadriplegia), an endocrine or metabolic disorder, chromosomal defects, syndromes or dysmorphic symptoms suggestive for a yet unknown syndrome were excluded. Subjects with a condition known to interfere with growth, including GH deficiency, severe chronic illness, emotional deprivation, GH treatment, glucocorticosteroid treatment and radiotherapy were also excluded. Birth data were taken from hospital records, and records from community health services and general practitioners.

The Medical Research Ethics Committee of Erasmus University Medical Centre, Rotterdam, The Netherlands, approved this study. Written informed consent was obtained from all the participants.

Methods

Anthropometry

Adult height was measured in the upright position to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymmyth, UK). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S). All anthropometric measurements were performed twice, and the mean value was used for analysis.

Bone mineral density

Bone mass of the total body (TB), lumbar spine (LS), lean body mass (LBM) and fat mass (FM) were measured by dual-energy X-ray absorptiometry (DXA, type Lunar-Prodigy, GE Healthcare, Chalfont St Giles, UK). All scans were made on the same machine, and quality assurance was performed daily. The coefficient of variation (CV) was 0.5% for total body BMD and 1.0% for spine BMD (25-27). The CV for lean mass and FM has been reported to be 0.7 and 1.2% respectively (25,26).

To adjust for differences in bone size, we calculated bone mineral apparent density of the LS (BMAD_{LS}) (g/cm³) with the model $BMAD_{LS} = BMD_{LS} * (4/(\pi * width))$ (28). Width was 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.

Questionnaire

All subjects completed a structured questionnaire, which included questions on socioeconomic status of the participants and their parents, cigarette smoking, alcohol consumption and usage of oral contraceptives. Socioeconomic status was determined by using educational level of the participant, which was assessed by the highest grade of school completed or currently participating in, and categorized into 1) high (higher general secondary education or higher), 2) median (junior general secondary education – secondary vocational education) and 3) low education (preparatory middle-level vocational education or lower).

Statistical analysis

Clinical characteristics are expressed as median (interquartile range). Birth weight and length were adjusted for gestational age and gender (29), and baseline data in young adulthood were adjusted for age and gender (30). BMD data are expressed as mean (S.D.). SDSs for BMD_{TB}, BMD_{LS} and BMAD_{LS} were calculated using reference data of Boot et al. (31).

Independent samples t-tests were used to evaluate differences in clinical characteristics between preterm and term subjects. To test for linear relationships between gestational age and BMD_{TB}, BMD_{LS} and BMAD_{LS}, Pearson's correlation coefficient was used. The long-term effect of gestational age on BMD_{TB}, BMD_{LS} and BMAD_{LS} in young adulthood was analysed using multiple regression analyses, corrected for possible confounders (gender, age, birth weight SDS, birth length SDS, adult height SDS, LBM and FM). The interaction term birth length SDS * adult height SDS was added to the multiple regression model to ensure that the effect of these variables was modelled correctly.

To determine differences between preterm and term subjects with regard to BMD, multiple regression analysis was used with correction for age and gender and subsequently also with correction for gender, age, birth weight SDS, birth length SDS, adult height SDS, LBM and FM.

Results were regarded statistically significant at $P < 0.05$. Statistics were performed using the computer Statistical Package for Social Science (SPSS version 16.0; SPSS, Inc., Chicago, IL, USA).

Results

Clinical characteristics

Table 1 shows the baseline characteristics of the study group. The total group of 276 subjects had a median age of 20.9 years. Besides the obvious difference in gestational age, preterm subjects had a lower birth length SDS than term subjects.

Table 1. Clinical characteristics.

	Total group	Preterm	Term
n	276	151	125
Gender (M/F)	119/157	76/75	43/82
Gestational age (weeks)	34.9 (32.0 to 40.0)	32.2 (30.3 to 34.0)*	40.0 (39.0 to 40.0)
Birth length SDS	-0.6 (-1.7 to 0.3)	-1.1 (-2.8 to 0.3)*	-0.4 (-1.3 to 0.2)
Birth weight SDS	-0.3 (-1.6 to 0.6)	-0.3 (-2.1 to 0.8)	-0.3 (-1.2 to 0.4)
Age (years)	20.9 (19.6 to 22.3)	21.0 (19.7 to 22.3)	20.6 (19.4 to 22.4)
Adult height SDS	-0.3 (-1.2 to 0.3)	-0.3 (-1.1 to 0.2)	-0.4 (-1.5 to 0.5)
Adult weight SDS	-0.3 (-1.0 to 0.3)	-0.2 (-0.9 to 0.4)	-0.4 (-1.0 to 0.3)

* $P < 0.001$ between preterm and term. All values are given as median (interquartile range)

BMD and gestational age

There were no significant linear correlations between gestational age and BMD_{TB} ($r=0.063$, $P=0.30$), BMD_{LS} ($r=0.062$, $P=0.31$) and $BMAD_{LS}$ ($r=0.069$, $P=0.26$). Multiple regression analyses showed that gestational age was not a significant contributor to the variance in BMD_{TB} ($P=0.27$, $R^2=0.370$), BMD_{LS} ($P=0.91$, $R^2=0.165$) and $BMAD_{LS}$ ($P=0.87$, $R^2=0.262$), after adjustment for age, gender, birth weight SDS, birth length SDS, adult height SDS, LBM and FM as additional independent variables.

Furthermore, SDSs of BMD of the LS were not significantly correlated with gestational age (BMD_{LS} SDS: $r=0.069$, $P=0.25$ and $BMAD_{LS}$ SDS: $r=0.031$, $P=0.61$), whereas BMD_{TB} SDS was significantly correlated with gestational age ($r=0.137$, $P=0.023$). However, after adjustments for possible confounders, this correlation disappeared (BMD_{TB} SDS ($P=0.28$, $R^2=0.290$), BMD_{LS} SDS ($P=0.941$, $R^2=0.175$) and $BMAD_{LS}$ SDS ($P=0.815$, $R^2=0.064$)).

Further analysis showed that adult weight, specified as LBM and TB FM, was an important determinant of BMD in young adulthood, regardless of size at birth and gestational age. LBM was significantly associated with BMD_{TB} ($\beta=0.007$, $P<0.001$) and BMD_{LS} ($\beta=0.008$, $P<0.001$). FM was also associated with BMD_{TB} ($\beta=0.002$, $P<0.001$) and BMD_{LS} ($\beta=0.002$, $P=0.08$).

In addition, we evaluated the possible confounding effects of socioeconomic status, smoking, alcohol and the usage of oral contraceptives on these associations. The socioeconomic status was low in 8.8%, median in 22.6% and high in 68.8% of all subjects, and 22.8% of all subjects smoked.

Of all subjects reporting alcohol consumption (80.8%), 28.6% had an alcohol consumption ≥ 5 units/week. None of these factors had a significant influence on the BMD (TB and LS) outcomes.

BMD in preterm versus term subjects

No significant differences were found between preterm and term subjects with regard to BMD_{TB}, BMD_{LS} and BMAD_{LS} after correction for age and gender (Table 2). Even after additional adjustment for significant independent variables like birth length SDS, adult height SDS, birth weight SDS, LBM and FM, BMD outcomes did not significantly differ between preterm and term subjects.

Table 2. Bone mineral density in young adults born preterm vs term.

	Total group (n=276)	Preterm (n=151)	Term (n=125)	P value*	P value†
Total body					
BMD _{TB} (g/cm ²)	1.17 (0.09)	1.17 (0.08)	1.18 (0.10)	0.127	0.429
BMD _{TB} SDS	-0.50 (0.87)	-0.59 (0.83)	-0.40 (0.91)	0.184	0.534
Lumbar spine					
BMD _{LS} (g/cm ²)	1.20 (0.13)	1.20 (0.13)	1.21 (0.13)	0.462	0.878
BMD _{LS} SDS	-0.45 (0.80)	-0.49 (0.82)	-0.39 (0.78)	0.467	0.997
BMAD _{LS} (g/cm ³)	0.37 (0.04)	0.37 (0.05)	0.37 (0.04)	0.918	0.751
BMAD _{LS} SDS	-0.52 (0.93)	-0.55 (0.99)	-0.49 (0.84)	0.879	0.651

*Preterm vs term; P values are given after correction for age and gender. †Preterm vs term; P values are given after correction for age, gender, birth length SDS, adult height SDS, birth weight SDS, LBM and fat mass. All values are given as mean (SDS).

Discussion

Our study in 276 young adults showed that gestational age was not a significant contributor to the variance in BMD (BMD_{TB} and BMD_{LS}) and BMAD (BMAD_{LS}) in young adulthood. In addition, our subgroup analysis did not show differences between subjects born preterm or term. Thus, in contrast to our hypothesis, preterm birth was not associated with lower BMD in early adulthood.

Peak bone mass is an important determinant of BMD in later life, but the exact age at which peak bone mass is reached is not well established. Recent data show that peak bone mass is probably attained by the mid-twenties (32). We are one of the first to investigate BMD in subjects born preterm at a mean age of 21 years, thus around the age of reaching peak bone mass. Our results are, therefore, important for the evaluation of the long-term consequences of preterm birth.

Our subgroup analysis showed no differences in BMD between subjects born preterm or term. Dalziel et al. investigated a cohort of 174 adults (mean age 31 years) whose mothers had participated in a randomized trial of antenatal betamethasone treatment. Although the main aim of the study was to determine the effect of maternal betamethasone usage on later BMD, they also showed that prematurity had no effect on peak bone mass (21). Hamed et al. showed that, in a cohort of 230 women (aged 20-23 years), BMD was not influenced by prematurity (14), although in that study no adjustments for possible confounders (e.g. LBM, FM or weight) were made and the cohort consisted of female subjects only.

Weiler et al. (33) showed that preterm born adolescents had a lower bone mineral content (BMC) than those born at term. However, after correction for adult height and weight, these differences disappeared, indicating that the effect of gestational age on BMC was largely influenced by adult body size.

Previous studies in infancy and early childhood showed differences in BMD between preterm and term subjects (1,6,7,9), but we could not confirm such results in our young adults. One explanation might be that there was a catch-up in bone mineralization during infancy and childhood (34), but to prove this hypothesis, prospective longitudinal research in preterm and term subjects is mandatory.

A relative high percentage of our premature study population consisted of subjects born small for gestational age (SGA; 37.7%), compared to the normal population in which the prevalence of SGA is only 2.3%. In clinical practice, the majority of children born SGA are born preterm. Furthermore, our study comprises a relatively healthy population. Subjects with, for example, a complicated neonatal period were excluded from our analysis, and it might be that such subjects have a higher risk of lower BMD.

BMD was assessed for TB and LS. As there are differences in cell biology between these two sites (35,36), gestational age and/or prematurity could have different effects on BMD_{TB} and BMD_{LS} . Despite these differences, gestational age was no significant determinant of both BMD_{TB} and BMD_{LS} . Furthermore, it is important to realize that bone density is just one aspect of bone quality, as bones are complex three-dimensional structures (37).

Analysis of BMD showed that prematurity and also birth size had no long-term consequences on BMD. Instead, adult weight, LBM and TB FM were important determinants of BMD in young adulthood. So it seems important to ensure that appropriate growth and nutrition are maintained throughout childhood and adolescence. Although our data are reassuring, there is no guarantee that BMD will remain normal when subjects become older. Longer term follow-up is, therefore, still warranted.

DXA was used to assess BMD, as it is the most commonly used technique for BMD assessment because of low radiation exposure, great precision and accuracy, and short scanning time (6-10 min). Additionally, DXA performs whole body rather than slice measurements (CT) (31,38). A shortcoming of DXA is that it measures bone in two dimensions providing only an estimation of bone density. BMD is obtained by dividing BMC (g) by the projected bone image (area in cm^2).

BMD is, therefore, dependent on bone size, and this might lead to erroneous interpretations of BMD values. A widely used and validated model to correct for bone size is BMAD (volumetric BMD) (22,28,39). For that reason, we added BMAD_{LS} as one of the parameters reflecting bone mineralization.

Conclusions

In conclusion, in a cohort of 276 young adults, gestational age was not a significant determinant in the variance of BMD (TB and LS). BMD was not adversely affected in young adults born moderately preterm. Thus, in contrast to our hypothesis, preterm birth without postnatal complications is not associated with a lower BMD in young adulthood. These findings are of major importance as an increasing number of preterm born subjects reach adulthood.

Acknowledgements

We would like to thank all participants. We greatly acknowledge Mrs J Dunk, Mrs M Huibregtse-Schouten and Mrs I van Slobbe, research nurses, and M Eshuis, A Hellingsman, L Holl, C Nederstigt, P Oosterbeek, S Rambaran and N Verbeet, research nurses, for their technical assistance and support with data collection. We acknowledge the Erasmus University MC Rotterdam, Maastad Hospital Rotterdam, University MC Nijmegen, Catharina Hospital Eindhoven, Leiden University MC, Juliana Children's Hospital, the Hague, and University Medical Centre Groningen. We thank J P Sluimer and P Kooy of the Department of Nuclear Medicine, Erasmus University MC.

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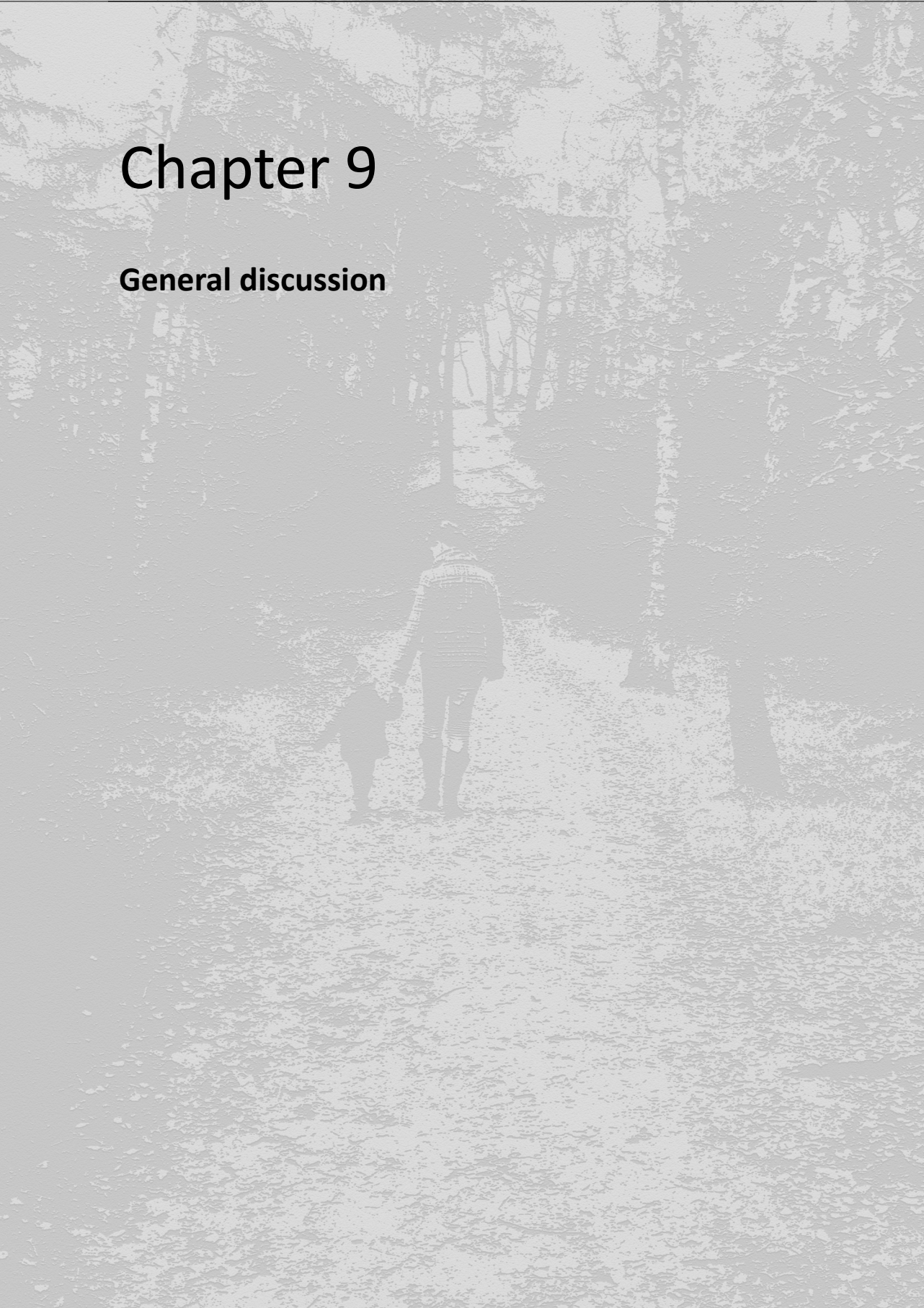
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Chapter 9

General discussion



This thesis describes several risk factors for adult diseases in two conditions associated with small size at birth: SGA and preterm birth. The first part consists of studies in SGA children with short stature, treated with growth hormone. The second part focuses on long-term consequences of preterm birth on cardiovascular risk factors and bone mineral density. In this chapter, results from the studies described in this thesis are discussed, also in view of current literature. Subsequently, clinical implications and conclusions are presented and directions for future research are given.

PART 1. SMALL FOR GESTATIONAL AGE

Factors associated with GH-induced growth response

GH-induced growth response is highly variable in short SGA children. It was suggested that higher pre-treatment insulin levels, assumedly reflecting lower insulin sensitivity, would lead to a lower growth response to GH treatment (1). In addition, it has been suggested that SGA children who are more insulin resistant before start of GH treatment, have a higher risk of glucose intolerance, which, in combination with a lower GH-induced growth response, could be considered a warning against treatment (2). In **Chapter 2** we demonstrated, in 76 short SGA children, that it is not the degree of insulin sensitivity or β -cell function, measured by a modified intravenous glucose tolerance test, but higher fasting serum insulin levels and a smaller increase in insulin levels that lead to a lower growth response during 6 months of GH treatment. No correlations were found between fasting insulin levels and measured insulin sensitivity, indicating that fasting insulin levels are no accurate proxy for insulin sensitivity in short children born SGA. Fasting insulin levels were, however, strongly related with overnight GH levels, which is also shown in *in vitro* studies (3), suggesting that not insulin, but pre-treatment overnight GH levels influence the GH-induced growth response.

It has been postulated that a higher GH dose would result in higher insulin levels due to a larger reduction in insulin sensitivity, and could be considered a reason not to treat short SGA children with GH if they have a low insulin sensitivity prior to start of GH treatment (2). We showed that GH treatment did result in a decrease in insulin sensitivity but, since insulin secretion simultaneously increased, the degree of glucose homeostasis remained unchanged. Furthermore, no differences in fasting insulin levels, insulin sensitivity and β -cell function were found between subjects receiving 1 mg or 2 mg GH/m²/day.

Conclusions, clinical implications, and directions for future research

Based on our findings, we conclude that higher pre-treatment insulin levels were associated with a lower growth response during GH treatment. Higher insulin levels were, however, not indicative for lower insulin sensitivity or less β -cell function and might, therefore, not be considered a contraindication for GH treatment in short SGA children. However, based on the lower growth response, expectations on the degree of catch-up growth in such patients should not be too high,

and a higher GH dose might be needed to achieve an adult height within the normal range. We showed that a higher GH dose was not associated with a more adverse metabolic outcome than a lower dose.

We also showed a strong relationship between pre-treatment overnight GH levels and fasting insulin levels in short SGA children. Further research is, however, needed to define this relationship in more detail.

Health profile after discontinuation of GH treatment

GH treatment in short children born SGA induces catch-up growth and increases adult height (4-6), but it is also accompanied by a reduction in several risk factors for cardiovascular disease which are consistent with the lipolytic and anabolic properties of GH (7,8). GH, however, also has well-documented insulin-antagonistic effects and its use has been associated with a reduction in insulin sensitivity and an increase in insulin levels (9-12). Over recent years, concerns have been expressed regarding the long-term effects of GH treatment on increasing the risk for type 2 diabetes and cardiovascular disease, but despite the fact that biosynthetic GH has been used for more than 25 years, there were no data available on the long-term effects after discontinuation of treatment in subjects born SGA.

In **Chapter 3** we describe a cross-sectional study in 377 young adults, investigating the long-term impact of GH treatment on body composition and fat distribution, more than 6 years after its discontinuation. We showed that body composition of previously GH-treated adults was similar to that of untreated SGA adults with short stature, and that fat mass and fat distribution of previously GH-treated SGA subjects were also similar to those of SGA adults who showed spontaneous catch-up growth. These data are reassuring, as they suggest that long-term GH treatment of short SGA children does not have an unfavourable effect on body composition in young adulthood.

The beneficial effects on body composition during GH treatment were, unfortunately, no longer present. It has been suggested that the adverse effects of poor intrauterine growth on adipose tissue is most prominent in the first years of life. A previous study showed that girls born SGA, aged 2-8 years, tended to follow an altered developmental trajectory that may lead to an abnormal fat storage in puberty and adulthood (13). It could, therefore, be that the loss of beneficial effects of GH treatment on body composition, many years after its discontinuation, might be due to the fact that GH treatment was not started in the early years of life, but around 8 years of age. Nowadays, some studies start GH treatment in (much) younger SGA children. It would be interesting to evaluate whether these children have a different adipose tissue development, particularly on the long term.

All SGA young adults, also those who did not receive GH treatment, had a lower lean body mass and tended to have a higher fat mass than healthy AGA controls, suggesting that SGA adults in general may have a different body composition than healthy controls.

Although the cross-sectional data described in Chapter 3 are reassuring, we cannot determine the exact influence of GH on the body composition in these young adults because we did not randomize for GH treatment in subjects born SGA. It might be that body composition in young adulthood was already 'programmed' in childhood, regardless of GH treatment. Since the efficacy of GH treatment has been well established, such a randomized controlled trial was considered unethical. Longitudinal data after discontinuation of GH treatment can provide insight in the effects of GH withdrawal on body composition and other cardiovascular risk factors.

We, therefore, longitudinally investigated changes in parameters of vascular and metabolic health, during the first 2 years after discontinuation of treatment in another cohort of 272 young adults (**Chapter 4**). Willemssen et al. previously described an increase in fat mass during the first 6 months after discontinuation of GH (14), but up until now no data were available on the years thereafter. We showed that fat mass continued to increase significantly until 2 years after stop of GH treatment. In addition, after an initial decrease, lean body mass returned to values comparable with stop of GH treatment. These changes in body composition are opposite to those occurring during treatment, i.e. a decrease in fat mass and an increase in lean body mass (7,8).

After an initial increase, blood pressure also returned to values comparable with stop of treatment. Since it has been shown in another cross-sectional cohort that blood pressure of SGA subjects, who had discontinued treatment for more than 6 years, was significantly lower than blood pressure of untreated SGA subjects with short stature (15), it might be that blood pressure continues to decrease beyond the period of 2 years.

We are the first to describe changes in lipid profile after discontinuation of GH treatment and showed that the lipid profile changed unfavourably. Nevertheless, previously GH-treated SGA subjects continued to have a more beneficial lipid profile compared to untreated SGA subjects with short stature.

Next to long-term effects of GH on risk factors for cardiovascular diseases, like body composition, lipid levels and blood pressure, we also studied the longitudinal changes in insulin sensitivity and β -cell function after discontinuation of GH treatment in 93 subjects born SGA until 5 years after stop of treatment (**Chapter 5**). In addition, we compared their data cross-sectionally at 5 years with those of 34 untreated SGA subjects and 44 healthy controls.

Our data show that after an initial increase after discontinuation of GH treatment, which is in line with previous reports (14,16), insulin sensitivity and β -cell function remained unchanged in the years thereafter. At 5 years after discontinuation, glucose homeostasis of previously GH-treated SGA subjects was similar to that of healthy AGA controls. These results are reassuring and confirm our previous cross-sectional data (15).

In addition, discontinuation of GH treatment was associated with a significant increase in fat percentage and total body fat mass, which, however, did not lead to a significant decrease in insulin sensitivity.

Conclusions, clinical implications, and directions for future research

Based on the findings presented in Chapters 3, 4 and 5 we conclude that body composition and glucose homeostasis of young adults previously treated with GH were largely similar to those of untreated SGA adults. Glucose homeostasis of previously GH-treated SGA adults was even similar to that of healthy AGA controls, indicating that GH does not seem to have unfavourable effects on various risk factors for cardiovascular disease and type 2 diabetes in young adulthood.

Discontinuation of GH treatment in SGA subjects, however, leads to significant changes in body composition and lipid levels, reflecting the loss of the anabolic and lipolytic properties of GH. The increase in fat mass highlights the importance to ensure more awareness to lifestyle and healthy diet in subjects who are treated with GH. Reassuringly, despite the significant increase in fat percentage and total body fat mass, the results of previously GH-treated SGA subjects were similar to those of untreated SGA-S subjects.

Recently, the French SaGhE study suggested that there might be an increased overall and cardiovascular mortality in adults treated with GH during childhood for isolated GH deficiency (17). It is, however, unknown whether these subjects were diagnosed with adult GH deficiency after discontinuation of GH treatment and whether or not GH treatment was continued in these subjects. It might be that they found an increased mortality rate due to lack of adult GH treatment in GH deficient patients, rather than due to GH treatment during childhood. The Belgian, Swedish and Dutch part of the SaGhE study reported no deaths due to cancer or cardiovascular disease (18). These studies highlight the importance of evaluating detailed long-term effects of GH treatment.

Based on our data we can conclude that GH does not negatively influence risk factors for cardiovascular disease and type 2 diabetes later in life. The 5-year duration of our follow-up studies is, however, too short to conclude on the lifetime risk of cardiovascular disease and type 2 diabetes. It is, therefore, important to continue to investigate the health profile of previously GH-treated subjects later in life, because the development of cardiovascular disease and type 2 diabetes might occur later in life.

PART 2. PREMATURITY

Preterm birth and risk for cardiovascular disease

Chapter 6 and 7 describe associations of preterm birth and postnatal growth with risk factors for cardiovascular disease. Both preterm birth and poor fetal growth can lead to small size at birth. Most studies focus on subjects with a low birth weight and did not have information on gestational age, did not correct for it, or only included subjects born term (19-22). It was, therefore, difficult to determine whether the effect of a small size at birth on cardiovascular risk factors in later life was due to a small size for gestational age (SGA) or due to prematurity.

In **Chapter 6** we demonstrate, in a cohort of 455 young adults, that those born preterm had a significantly higher percentage of total fat mass, trunk fat mass, and limb fat mass than subjects born term, but a relatively favourable lipid profile.

Our results are in line with previous results in a cohort of healthy young adults (23,24). It might well be that, under the influence of hormonal changes in puberty and adolescence, preterm subjects undergo a relatively large increase in fat mass. Further longitudinal follow-up studies in childhood and adolescence are important to confirm these findings.

Because we showed that young adults born preterm had more fat mass, we expected a more adverse lipid profile. In contrast, a shorter gestational age was associated with lower Lp(a) levels and higher ApoA-I levels, and subjects born preterm had a significantly lower prevalence of adverse ApoA-I levels, compared with those born term. This might indicate that young adults born preterm have a more favorable lipid profile than those born term, although their body composition is less advantageous. Noteworthy, Lp(a) and ApoA-I levels are the only lipids not influenced by adult weight SDS, indicating that these levels are not directly influenced by fat or fat accumulation but are determined by other variables, like genetic factors (25-27).

Our study was performed in young adults, while clinical manifestations of cardiovascular disease do usually not occur before middle age. Assuming that the amount of fat mass of preterm subjects remains higher throughout adulthood, they are expected to develop a more adverse lipid profile at a later age.

In **Chapter 7** we demonstrate that preterm birth is associated with higher systolic blood pressure, independent of birth weight SDS. In contrast, we report a lower diastolic blood pressure in young adults born preterm which, however, contributed to a higher pulse pressure (28). In addition, we found a higher blood pressure variability and heart rate in subjects born preterm, which might be due to an altered sympathoadrenal function (29). These results are in line with those of others (29-33). In order to determine whether the associations of gestational age with CVD risk factors can be ascribed to small for gestational age (SGA) birth, we additionally performed subgroup comparisons. These comparisons showed significant differences in blood pressure, pulse pressure, and blood pressure variability, between the preterm subjects and AGA controls, which was irrespective of SGA birth. Because the prevalence of preterm birth and the survival rate is rapidly increasing, our results are of clinical relevance for an increasing number of subjects and thus of major importance for public health (34).

Conclusions, clinical implications, and directions for future research

Based on the findings presented in Chapters 6 and 7, we conclude that preterm birth affects several determinants of cardiovascular disease, indicating that young adults born preterm might have increased risk for developing cardiovascular disease in later life, which is not due to being born SGA. This was confirmed by a recent study reporting increased cardiovascular mortality in subjects born preterm (35).

To date, most long-term follow-up studies have focused on neurodevelopmental and respiratory complications of preterm infants, with little attention for cardiovascular outcome. Since the survival of preterm and very low birth weight infants has significantly improved, it is of increasing importance to assess the long-term effects of prematurity on determinants of cardiovascular diseases.

Our study population consisted of subjects without serious postnatal complications and did not include extremely preterm born subjects. Thus, whether our results can be generalized to subjects with complications, such as those with bronchopulmonary dysplasia, requires further research.

The duration of follow-up in our study is too short to definitely conclude on the risk of cardiovascular disease. The development of cardiovascular disease and type 2 diabetes is age-dependent. Further research is, therefore, needed to evaluate whether or not these differences predispose subjects born preterm to cardiovascular diseases later in life.

Preterm birth and bone mineral density

In **Chapter 8** we studied the effect of prematurity on bone mineral density in early adulthood. Bone mineral density in later life depends largely on the peak bone mass achieved in early adulthood and the subsequent bone loss (36). A high peak bone mass provides a larger reserve later in life (36,37). Recent data show that peak bone mass is attained by the mid-twenties (38), around the age of our young adult cohort. Osteoporosis is an increasing cause of morbidity and mortality in the developed countries. It is characterized by low bone mass, deterioration of bone tissue and disruption of bone architecture, compromised bone strength and an increased fracture risk (39). Since early prevention of osteoporosis is likely to be more successful than treatment of an already established disorder, it is essential to identify potential risk factors.

We showed no differences in bone mineral density between young adults born preterm or term. These results are in line with those of others (40,41). Another study showed that preterm born adolescents had a lower bone mineral content than those born at term. However, after correction for adult height and weight, these differences disappeared, indicating that the effect of gestational age on bone mineral density was largely influenced by adult body size (42).

Conclusions, clinical implications, and directions for future research

Our data show that preterm birth is not associated with a lower bone mineral density in young adulthood, which is of major importance as an increasing number of preterm born subjects reach adulthood. Instead, adult weight, lean body mass and total body fat mass were important determinants of bone mineral density in young adulthood, indicating that it is important to ensure appropriate growth and nutrition throughout childhood and adolescence. Although our data are reassuring, there is no guarantee that bone mineral density will remain normal when subjects become older. Thus long-term follow-up is still warranted.

Our study comprises a relatively healthy population as extremely prematurely born subjects and those with a complicated neonatal period were excluded. Thus, whether our results can be generalized to subjects born very preterm or those with complications requiring e.g. dexamethasone treatment for bronchopulmonary dysplasia, requires further research.

GENERAL CONCLUSIONS

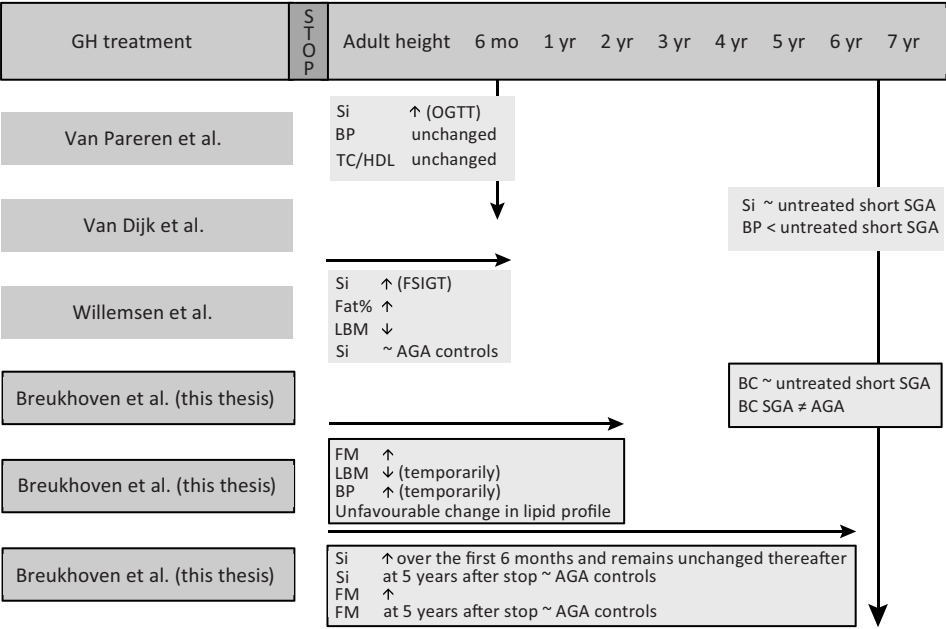
In the present thesis we describe the influence of several metabolic parameters on the variability in growth response to GH treatment and found that higher pre-treatment insulin levels were associated with a lower growth response during GH treatment. Higher pre-treatment insulin levels were, however, not indicative for lower insulin sensitivity or less β -cell function during GH treatment, and might, therefore, not be considered a contraindication for GH treatment in short SGA children.

We also describe longitudinal changes in several determinants for cardiovascular disease and type 2 diabetes until more than 6 years after discontinuation of GH in previously GH-treated SGA subjects. Figure 1 presents an overview of the results of this thesis and those previously reported by our research group. Based on these data, we conclude that GH has no unfavourable effects on body composition and glucose homeostasis in young adulthood. Discontinuation of GH treatment in subjects born SGA does, however, leads to significant changes in body composition and lipid levels, reflecting the loss of the anabolic and lipolytic properties of GH.

In addition, we report the long-term effect of prematurity on several parameters for vascular diseases and bone mineral density in young adulthood. Figure 2 summarizes the most important recent findings in young adults born preterm by our research group. Based on the data described in this thesis, we conclude that preterm birth affects several determinants of cardiovascular disease, indicating that young adults born preterm might have increased risk for developing cardiovascular disease in later life. Prematurity had no effects on bone mineral density in young adulthood.

It remains important to perform follow-up studies in previously GH-treated SGA subjects and in subjects born preterm, because development of cardiovascular disease and type 2 diabetes is age-dependent and might occur later in life, beyond young adulthood.

Figure 1. Follow-up studies in previously GH-treated subjects born SGA



SGA: small for gestational age, AGA: appropriate for gestational age, OGTT: oral glucose tolerance test, FSIGT: frequently sampled intravenous glucose tolerance test, Si: insulin sensitivity, BP: blood pressure, LBM: lean body mass, BC: body composition, TC: total cholesterol, HDL: high density lipoprotein cholesterol
→ longitudinal study, ↓ cross-sectional study

Figure 2. Effects of preterm birth in young adulthood

Willemsen et al.	Insulin sensitivity <ul style="list-style-type: none"> – Not associated with lower insulin sensitivity
Kerkhof et al.	Postnatal weight gain <ul style="list-style-type: none"> – Accelerated gain in weight relative to length during the period from birth to term age, as well as during the first three months of life, has adverse effects on body composition in early adulthood – Rapid weight gain after term age is an important determinant of body composition in later life
	Gonadal function <ul style="list-style-type: none"> – No effect on gonadal function in men – No effect on follicle pool size in women
Breukhoven et al.	Bone mineral density <ul style="list-style-type: none"> – Lower bone mineral density
	Parameters for cardiovascular disease <ul style="list-style-type: none"> – More fat mass – Relatively favourable lipid profile – Higher systolic blood pressure, pulse pressure, blood pressure variability and heart rate, and a lower diastolic blood pressure – No effect on pulse wave velocity and aortic intima media thickness – Not associated with combined biomarkers related to early stage atherosclerosis

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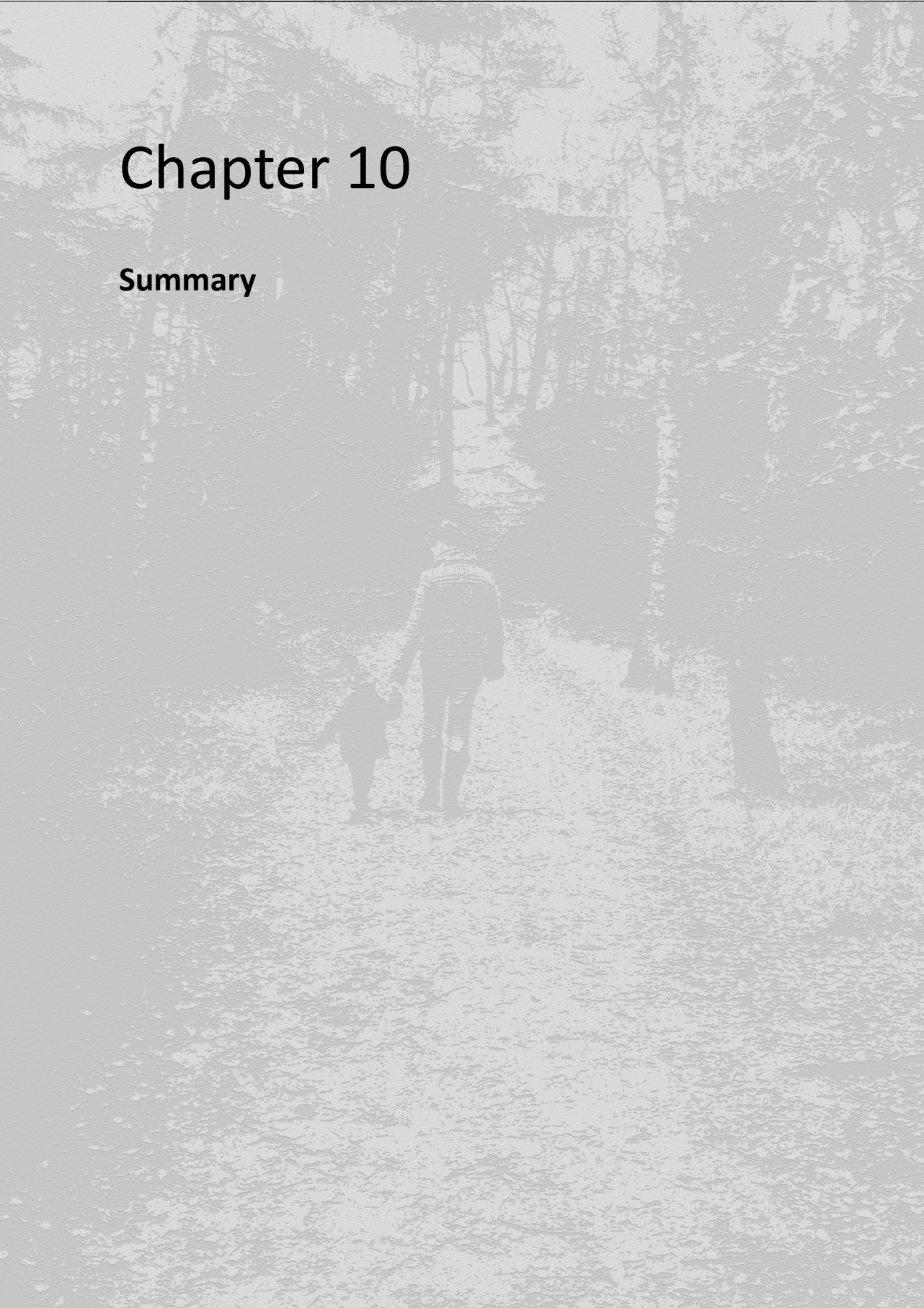
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Chapter 10

Summary



Chapter 1

This chapter provides a general introduction in the definitions, prevalence and possible causes of SGA birth. Furthermore, clinical and endocrinological aspects associated with SGA birth are described, such as short stature, the GH-IGF-IGFBP axis, and cardiovascular disease and type 2 diabetes. This chapter also gives a summary of previously described effects of GH on growth, the GH-IGF-IGFBP axis, body composition and risk factors for cardiovascular disease and type 2 diabetes. In addition it gives a general introduction in preterm birth and the associations between preterm birth and cardiovascular disease and bone mineral density. Finally, the aims of the studies performed and the outline of this thesis are presented.

Chapter 2

GH treatment induces catch-up growth and increases adult height in short children born SGA, but the growth response is variable. It has been suggested that higher pre-treatment insulin levels, assumedly reflecting lower insulin sensitivity, lead to a lower growth response to GH treatment. In the study described in chapter 2, we aimed to investigate whether the growth response during 6 months of GH treatment was related to baseline fasting insulin levels, insulin sensitivity and β -cell function and to assess whether insulin levels could serve as a proxy for insulin sensitivity in short children born SGA. In this prospective study, using data of 76 prepubertal short children born SGA, we showed that higher pre-treatment insulin levels were associated with a lower growth response during GH treatment. They were, however, not indicative for lower insulin sensitivity or less β -cell function during GH treatment. Based on our findings, we concluded that high insulin levels might not be considered a contraindication for GH treatment in short SGA children.

Chapter 3

GH treatment of short children born SGA results in a decline in fat mass and an increase in lean body mass. It was, however, unknown whether these changes persist into adulthood after cessation of GH. We, therefore, cross-sectionally investigated body composition and fat distribution, measured by dual-energy X-ray absorptiometry in 377 young adults. Fifty-nine previously GH-treated SGA subjects were compared to 52 untreated SGA subjects with short stature, 161 SGA adults with spontaneous catch-up growth, and 105 healthy normal-statured controls born appropriate for gestational age. Fat mass and fat distribution of previously GH-treated SGA subjects were not significantly different from those of untreated SGA subjects with short stature and those with spontaneous catch-up growth, at more than 6 years after discontinuation of GH treatment. All SGA subgroups had a lower lean body mass and tended to have a higher fat mass than healthy controls. We, therefore, concluded that GH-induced catch-up growth does not have unfavourable effects on fat mass and fat distribution compared with spontaneous catch-up growth. However, young adults born SGA in general have a different body composition than young adults born appropriate for gestational age.

Chapter 4

GH treatment induces a decrease in fat mass and blood pressure, and an increase in lean body mass, which is consistent with the lipolytic and anabolic properties of GH. Only limited data were available on the longitudinal changes after discontinuation of GH treatment. We, therefore, longitudinally investigated changes in body composition, lipid levels and resting blood pressure, until 2 years after discontinuation of GH treatment, in 101 subjects born SGA. Fat mass increased significantly over the first 2 years after stop of GH treatment and lean body mass temporarily decreased. Overall, the lipid profile showed an adverse change after discontinuation of GH treatment. In addition, after a temporarily increase in the first 6 months, blood pressure at 2 years after discontinuation of GH was comparable to levels at stop. We concluded that discontinuation of GH treatment in subjects born SGA leads to significant adverse changes in body composition and several metabolic parameters which continue for at least 2 years after discontinuation, reflecting the loss of GH properties.

Chapter 5

SGA birth has been associated with an increased risk of developing type 2 diabetes. It is known that GH treatment reduces insulin sensitivity, but no data were available on the long-term effects of GH treatment on insulin sensitivity and β -cell function after its cessation. Therefore, 93 previously GH-treated SGA adults were followed longitudinally until 5 years after discontinuation of GH treatment. Data at 5 years were compared with those of 34 untreated SGA adults with short stature, and 44 healthy controls. Insulin sensitivity was assessed by frequently sampled intravenous glucose tolerance tests and body composition by dual-energy X-ray absorptiometry.

We showed that after an initial increase after discontinuation of GH treatment, insulin sensitivity and β -cell function remain unchanged in the years thereafter. At 5 years after discontinuation of GH treatment, glucose homeostasis of previously GH-treated SGA subjects was similar to that of healthy AGA controls, which is reassuring. Discontinuation of GH treatment was, however, associated with a significant increase in fat percentage and total body fat mass.

Chapter 6

Associations between small size at birth and abnormal cardiovascular parameters in later life have been reported. It was, however, unknown whether the effects of a small size at birth on cardiovascular risk factors in later life was due to a small size for gestational age or due to prematurity. We, therefore, aimed to investigate the long-term effects of preterm birth on lipid levels and fat mass in early adulthood. We conducted a cross-sectional study in 455 healthy subjects, aged 18 to 24 years; 167 preterm subjects were compared with 288 full-term subjects. Young adults born preterm had a significantly higher percentage of total fat mass, trunk fat mass and limb fat mass than those born term. Furthermore, young adults born preterm had significantly lower serum lipoprotein a levels and higher apolipoprotein A-I levels than those

born term. Multiple linear regression analyses to assess the association between gestational age and fat mass and lipid levels showed similar results. Based on these results, we concluded that preterm birth is associated with more total fat mass, trunk fat, and limb fat mass in early adulthood, but also with a relatively favorable lipid profile.

Chapter 7

Both preterm birth and small birth size for gestational age have been associated with increased risk for cardiovascular disease, but controversies still existed. In the study described in Chapter 7, we aimed to investigate the effect of preterm birth on risk factors for cardiovascular disease, independent of birth size. In the study, using data of 406 young adults, we showed that preterm birth is associated with higher systolic blood pressure, pulse pressure, blood pressure variability, and heart rate, and with lower diastolic blood pressure in early adulthood, also after adjustment for confounders including size at birth. We, therefore, concluded that young adults born preterm might have a higher risk of cardiovascular disease than those born at term, independent of birth size. There was no effect of gestational age on pulse wave velocity and carotid intima-media thickness, which is a marker of early stage atherosclerosis, but our study population is still relatively young, and it might well be that this effect will arise at an older age.

Chapter 8

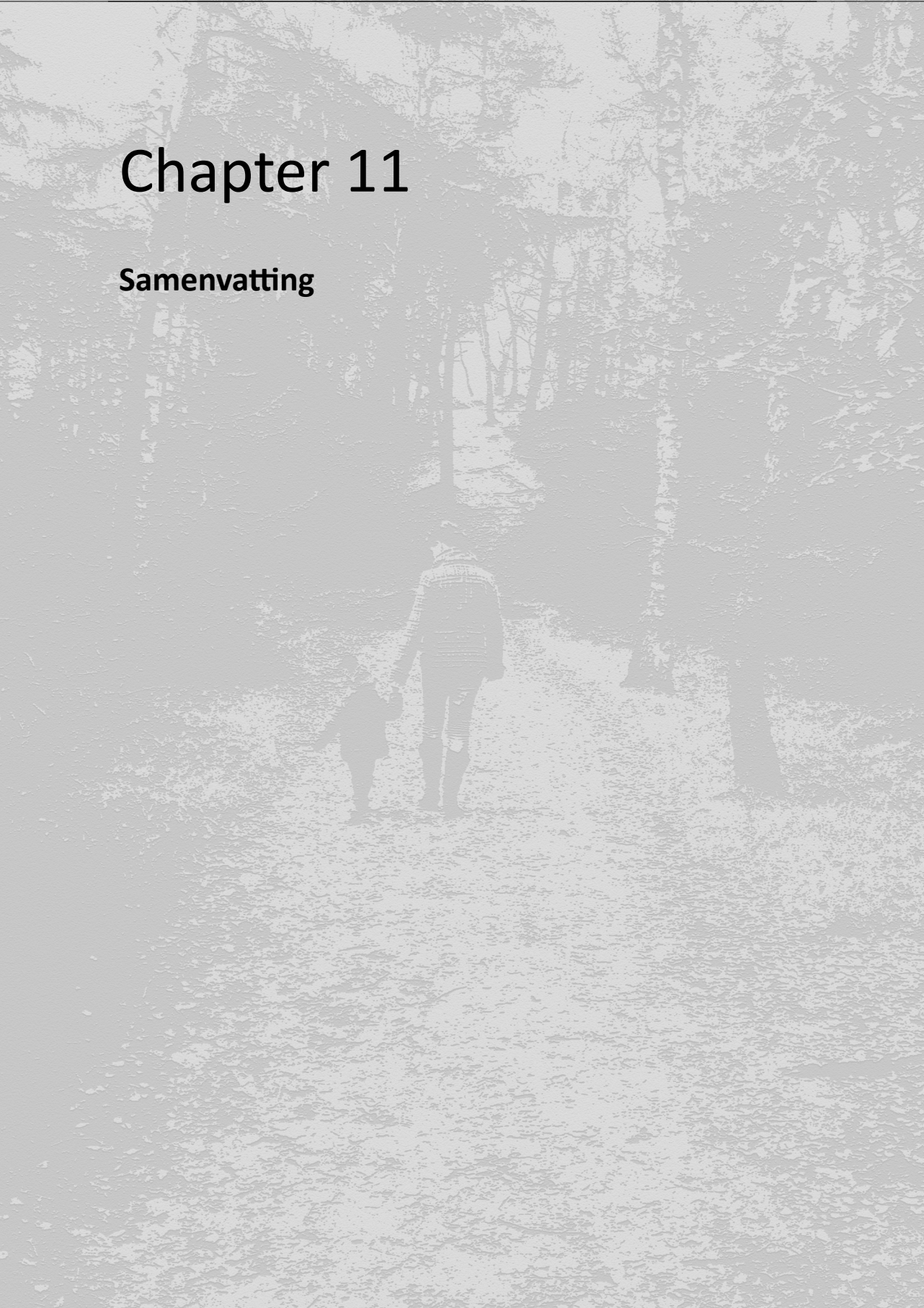
Previous studies showed conflicting data on the effect of prematurity on bone mineral density in infants and children and only limited data were available on the long-term effects of prematurity on bone mineral density in early adulthood. We, therefore, cross-sectionally investigated bone mineral density, measured by dual-energy X-ray absorptiometry, in 276 healthy subjects, aged 18-24 years (151 preterm subjects and 125 subjects born at term). Gestational age was not associated with bone mineral density of the total body and lumbar spine, also after adjustment for possible confounders. No significant differences were found between young adults born preterm or term. We concluded that preterm birth without serious postnatal complications is not associated with a lower bone mineral density in young adulthood.

Chapter 9

In this chapter, the main findings of the studies described in the present thesis are discussed, also in context of current literature. We emphasize on clinical implications and give suggestions for future research.

Chapter 11

Samenvatting



Hoofdstuk 1

Dit hoofdstuk geeft een algemene inleiding in de definities, de prevalentie en de mogelijke oorzaken van SGA geboorte. Bovendien worden de klinische en endocrinologische aspecten die samenhangen met SGA geboorte beschreven, zoals een kleine gestalte, de GH-IGF-IGFBP as, hart- en vaatziekten en type 2 diabetes. In dit hoofdstuk wordt ook een samenvatting gegeven van eerder beschreven effecten van GH op de groei, de GH-IGF-IGFBP as, de lichaamssamenstelling en risicofactoren voor hart- en vaatziekten en type 2 diabetes. Daarnaast geeft dit hoofdstuk een algemene inleiding in allerlei aspecten van prematuriteit en wordt er ingegaan op de associaties tussen prematuriteit en hart- en vaatziekten en botdichtheid. Aan het einde van dit hoofdstuk worden de doelstellingen van de studies en de inhoud van dit proefschrift besproken.

Hoofdstuk 2

GH behandeling induceert inhaalgroei en zorgt voor een betere volwassen lengte in te kleine SGA kinderen, maar de groeirespons is variabel. Er is gesuggereerd dat hogere insulinespiegels voor start van de behandeling, vermoedelijk als gevolg van een lagere insulinegevoeligheid, leiden tot een lagere groeirespons op GH behandeling. In de studie beschreven in hoofdstuk 2 hebben we onderzocht of de groeirespons gedurende de eerste 6 maanden van de GH behandeling was gerelateerd aan nuchtere insulinespiegels, insulinegevoeligheid en β -cel functie voor de start van de behandeling. Daarnaast hebben we onderzocht of insulinspiegels kunnen dienen als maat voor de insulinegevoeligheid in te kleine SGA kinderen. In deze prospectieve studie in 76 prepuberale SGA kinderen bleken hogere insulinespiegels geassocieerd met een lagere groeirespons tijdens GH behandeling. Hoge insulinespiegels waren echter geen maat voor een lagere insulinegevoeligheid of slechtere β -cel functie tijdens de GH behandeling. Wij concludeerden daarom dat hoge insulinespiegels voor de start van de behandeling niet mogen worden beschouwd als een contra-indicatie voor GH behandeling in te kleine SGA kinderen.

Hoofdstuk 3

GH behandeling in te kleine SGA kinderen resulteert in een daling van de vetmassa en een toename van de spiermassa. Het was echter niet bekend of deze veranderingen aanhouden tot de volwassen leeftijd. Wij hebben daarom de lichaamssamenstelling en vetverdeling, gemeten met behulp van een dual-energy X-ray absorptiometry, onderzocht in 377 jong volwassenen; 59 SGA jong volwassenen die in het verleden behandeld waren met GH werden vergeleken met 52 onbehandelde SGA jong volwassenen met een kleine gestalte, 161 SGA jong volwassenen met spontane inhaalgroei, en 105 gezonde controles. Vetmassa en vetverdeling van de GH-behandelde groep waren niet significant verschillend van die van de andere 2 SGA subgroepen, op meer dan 6 jaar na het stoppen van de GH behandeling. Alle SGA subgroepen hadden een lagere spiermassa en een trend tot een hogere vetmassa dan gezonde jong volwassenen. Wij concludeerde daarom dat GH-geïnduceerde inhaalgroei geen ongunstige effecten heeft op de vetmassa en vetverdeling in vergelijking met spontane inhaalgroei. Onze studie liet echter zien

dat alle SGA jong volwassenen mogelijk een andere lichaamssamenstelling hebben dan gezonde jong volwassenen.

Hoofdstuk 4

GH behandeling resulteert in een afname van de vetmassa, een verlaging van de bloeddruk en een toename van de spiermassa, in overeenstemming met de lipolytische en anabole eigenschappen van GH. Er waren echter maar weinig gegevens beschikbaar over de veranderingen hiervan na staken van de GH behandeling. Wij hebben daarom, in 101 jong volwassenen, gekeken naar de lichaamssamenstelling, het lipidenprofiel en de bloeddruk, gedurende 2 jaar na het stoppen van de GH behandeling. De vetmassa nam significant toe en de spiermassa nam tijdelijk af. Het lipidenprofiel toonde een nadelige verandering na het stoppen van de GH behandeling. Na een tijdelijke stijging, daalde de bloeddruk weer tot hetzelfde niveau als direct na het staken van de behandeling. Wij concludeerden dat staken van de GH behandeling in jonge SGA volwassenen leidt tot aanzienlijke veranderingen in de lichaamssamenstelling en een aantal metabole parameters, welke het gevolg zijn van het verlies van de eigenschappen van GH.

Hoofdstuk 5

SGA geboorte is in verband gebracht met een verhoogd risico op het ontwikkelen van type 2 diabetes. Het is bekend dat GH behandeling de gevoeligheid voor insuline vermindert, maar er waren geen gegevens beschikbaar over de lange-termijn effecten van GH behandeling op insulinegevoeligheid en β -cel functie. Daarom werden 93, voorheen met GH behandelde, SGA volwassenen longitudinaal gevolgd tot 5 jaar na het staken van de behandeling. Gegevens op 5 jaar na stop werden vergeleken met de gegevens van 34 onbehandelde SGA geboren volwassenen met een te kleine gestalte, en 44 gezonde controles. De insulinegevoeligheid werd gemeten met behulp van een zogenaamde 'frequently sampled intravenous glucose tolerance test' en de lichaamssamenstelling met behulp van een dual-energy X-ray absorptiometry. Wij toonden aan dat na een aanvankelijke stijging in de eerste maanden na het staken van de GH behandeling, de insulinegevoeligheid en β -cel functie onveranderd blijven in de jaren daarna. Vijf jaar na het staken van de GH behandeling was de glucosehuishouding van de eerder met GH behandelde SGA volwassenen vergelijkbaar met die van gezonde AGA controles. Dit is geruststellend. Staken van de GH behandeling was echter geassocieerd met een significante toename van het vetpercentage en de totale vetmassa.

Hoofdstuk 6

In het verleden zijn er associaties gemeld tussen klein zijn bij de geboorte en abnormale cardiovasculaire parameters op latere leeftijd. Het was echter niet bekend of de associaties tussen klein zijn bij de geboorte en cardiovasculaire risicofactoren het gevolg waren van SGA geboorte of van prematuriteit. In 455 gezonde jong volwassenen met een leeftijd tussen de 18 en 24 jaar, onderzochten wij de lange termijn effecten van prematuriteit op de lipidenspiegels

en de vetmassa. 167 prematuur geboren jong volwassenen werden vergeleken met 288 à term geboren jong volwassenen. Prematuur geboren jong volwassenen hadden significant hogere percentages totale vetmassa, romp vetmassa en vetmassa van de ledematen in vergelijking met jong volwassenen die à term waren geboren. Wel hadden prematuur geboren jong volwassenen significant lagere serum lipoproteïne A spiegels en hogere apolipoproteïne AI spiegels dan de à term geboren jong volwassenen. Multiële lineaire regressie-analyses om de associatie tussen zwangerschapsduur en de vetmassa en lipiden spiegels te beoordelen toonde vergelijkbare resultaten. Op basis van deze resultaten concludeerden wij dat prematuriteit is geassocieerd met meer totale vetmassa en meer romp vet en vet op de ledematen, maar met een relatief gunstig lipidenprofiel.

Hoofdstuk 7

Premature geboorte en te klein zijn bij de geboorte voor de zwangerschapsduur (SGA) veroorzaken beide een laag geboortegewicht. Verschillende studies hebben een relatie aangetoond tussen een laag geboortegewicht en een verhoogd risico op hart- en vaatziekten. Echter, tot op heden was de invloed van premature geboorte op deze relatie onbekend. In hoofdstuk 7 beschrijven we de studie waarin we de associatie hebben onderzocht tussen premature geboorte/zwangerschapsduur en verschillende risicofactoren voor hart- en vaatziekten op jong volwassen leeftijd, ongeacht het geboortegewicht. In deze studie hebben we laten zien dat premature geboorte geassocieerd is met een hogere systolische bloeddruk, polsdruk, bloeddrukvariabiliteit, hartslag en met een lagere diastolische bloeddruk. De resultaten bleven gelijk na correctie voor geboortegewicht en geboortelengte. Onze conclusie was dat prematuur geboren jong volwassenen mogelijk een hoger risico hebben op hart- en vaatziekten dan à term geboren jong volwassenen en dat dit onafhankelijk is van de grootte bij de geboorte. We vonden geen effect van zwangerschapsduur op de Pulse Wave Velocity (meting van de arteriele stijfheid) en de intima media dikte van de arteria carotis op jong volwassen leeftijd, maar onze studiepopulatie is nog relatief jong en het kan zijn dat deze associatie ontstaat op een latere leeftijd.

Hoofdstuk 8

Eerdere studies laten tegenstrijdige resultaten zien wat betreft het effect van prematuriteit op het botdichtheid bij zuigelingen en kinderen. Er zijn slechts beperkte gegevens beschikbaar over de lange-termijn effecten van prematuriteit op de botdichtheid op jong volwassen leeftijd. Wij hebben daarom de botdichtheid, gemeten met behulp van een dual-energy X-ray absorptiometrie, onderzocht in 276 gezonde proefpersonen in de leeftijd van 18 tot 24 jaar (151 prematuur geboren proefpersonen en 125 à term geboren personen). Zwangerschapsduur was niet geassocieerd met de botdichtheid van het totale lichaam en de lumbale wervelkolom, ook niet na correctie voor mogelijke confounders. Er werden geen significante verschillen gevonden tussen de prematuur en à term geboren proefpersonen. Wij concludeerden dat prematuriteit,

zonder ernstige postnatale complicaties, niet is geassocieerd met een lagere botdichtheid op jong volwassen leeftijd.

Hoofdstuk 9

In dit hoofdstuk worden de belangrijkste bevindingen van de studies in dit proefschrift besproken, ook in context van de huidige literatuur. We bespreken de klinische implicaties en geven suggesties voor toekomstig onderzoek.

Dankwoord

In 2008 begon ik aan mijn promotieonderzoek. Nu, terwijl ik dit dankwoord aan het schrijven ben, kom ik al snel tot de conclusie dat er veel veranderd is in de afgelopen jaren, dat er een hoop gebeurd is, en dat er veel verschillende mensen betrokken zijn geweest bij de totstandkoming van dit proefschrift. Ieder op een eigen manier. Mensen die mij geholpen hebben, gesteund en gestimuleerd. Mensen zonder wie het schrijven van van dit proefschrift niet mogelijk was geweest.

Allereerst wil ik graag alle ouders, kinderen en jong volwassenen bedanken die hebben deelgenomen aan de IUGR-, PROGRAM- en PREMS-studies. Mede dankzij jullie weten we nu meer over de lange termijn effecten van te klein zijn bij de geboorte.

Mijn promotor, Prof. Dr. A.C.S. Hokken-Koelega. Beste Anita, dankjewel voor je betrokkenheid, enthousiasme, motivatie en alle hulp de afgelopen jaren. Je passie voor het wetenschappelijk onderzoek is bewonderenswaardig en werkt aanstekelijk. Ik heb veel van je geleerd. Dank daarvoor!

Lieve Esther en Marianne, wat vond ik het super fijn om de afgelopen jaren met jullie te mogen samenwerken! Dank jullie wel voor jullie gezelligheid en alle hulp bij de polibezoeken, nacontroles en organisatie van de IUGR-2 studie. Esther, heel veel succes en plezier in je verdere carrière! Marianne, de endo mag blij zijn met zo'n researchverpleegkundige.

De andere endo-researchverpleegkundigen: Christel, Berber, Jose, Janneke en Jorien. Dank voor jullie hulp, inzet, betrokkenheid en ondersteuning. Ik vond het erg fijn om met jullie samen te werken!

Prof. dr. J. Dahlgren, thank you very much for taking place in the reading Committee.

Prof. dr. S.L.S. Drop en Prof. dr. A.J. van der Lelij, hartelijk dank voor het beoordelen van mijn manuscript.

Prof. dr. E. Steegers, Prof. dr. A.J. van der Heijden, Prof. dr. H. Lafeber, Prof. dr. A. Bos en dr. K. Joosten wil ik graag bedanken voor het plaatsnemen in de promotiecommissie.

Ook wil ik graag alle kinderartsen hartelijk bedanken met wie ik de afgelopen jaren heb mogen samenwerken.

Prof. dr. S.L.S. Drop, Dr. E.L.T. van de Akker, G.R.J. Zandwijken, J.C. van der Heijden, beste Sten, Erica, Gladys en Josine, dank jullie wel voor de gezelligheid en al jullie input tijdens de endobesprekingen.

Novo Nordisk B.V. Nederland wil ik graag bedanken voor de prettige samenwerking en de financiële ondersteuning van de studies en dit proefschrift.

Ineke Beukers, bedankt voor al je hulp en advies de afgelopen jaren!

Prof. dr. E.P. Krenning wil ik bedanken voor de mogelijkheid die wij hebben gekregen om DXA-scans te laten maken op de afdeling nucleaire geneeskunde. Jopie Sluimer wil ik bedanken voor het maken en analyseren van de DXA-scans.

Dr. M.A.J. de Ridder wil ik bedanken voor haar hulp en antwoorden op mijn statistische vragen.

Dr. W.H. Hackeng wil ik hartelijk bedanken voor het bepalen van de insulines en glucoses en zijn altijd aanwezige interesse in onze onderzoeken.

Alle (ex)onderzoekers uit het Sophia, hartelijk dank voor jullie gezelligheid, de koffietjes bij Doppio, de onderzoekersweekenden, kerstdiners etc. Denise, lieve paranimf, je bent een kanjer!! Dank voor al je steun, je gezelligheid, onze “brainstorm” sessies en het feit dat je een super collega bent geweest de afgelopen jaren. Binnenkort ben jij aan de beurt!

De (ex)endo-onderzoekers met wie ik de afgelopen jaren heb samengewerkt: Gerthe, Judith, Florentien, Annemieke, Elbrich, Yvonne, Nienke, Sinddie, Laura, Renske, Lidewij, Sandra, Ruben, Ralph, Roderick, Emile en Daniëlle. Dank voor jullie wetenschappelijke input, maar natuurlijk ook voor alle warmte en gezelligheid de afgelopen jaren. Manouk, heel veel plezier bij de IUGR-2 studie. Gerthe en Judith, dankzij jullie heb ik een onvergetelijke tijd gehad bij de endo. Dank voor jullie steun, enthousiasme en gezelligheid de afgelopen jaren. Ik zal die keer dat jullie onverwachts met butter-chicken voor mijn voordeur stonden nooit vergeten! En Gerthe, lieve vriendin en paranimf, ik ben er super trots op dat je naast me staat!

Mijn lieve vriendinnen, bedankt voor jullie interesse in mijn promotieonderzoek. Ann, alweer 27 jaar m’n vriendinnetje, bedankt dat je er altijd voor me bent! Co-dames, onze etentjes, eindeloze gesprekken over van alles en nog wat, uitjes met of zonder de kids erbij. Ik geniet er intens van en hoop dat er nog velen mogen volgen. Mootje, ik bewonder je kracht en je gave om in iemands ziel te kijken. Hopelijk zien we elkaar de komende tijd wat vaker nu ik meerdere keren per week langs afslag Breda-Noord zal rijden. Essie, door de afstand zien we elkaar niet zo heel vaak, maar als we bij elkaar zijn is het altijd goed. Dat is echte vriendschap.

Lieve Marijke, wat had ik je er vandaag graag bij gehad en van je willen horen wat je van mijn 'boekje' (en nog zoveel andere dingen) vindt. Je bent en blijft altijd in mijn gedachten.

Mijn lieve schoonfamilie: Gijs, Ilse, Eline, Richard, Boris, Silke, Willemijn en Stefan. Jullie hebben mij vanaf de aller eerste dag met open armen ontvangen en daar ben ik jullie ontzettend dankbaar voor. Bedankt voor al jullie steun en interesse.

Yvonne, lieve oma van Sven. Je zal altijd mijn 'schoonmoeder' blijven. Dank voor al je hulp, steun en liefde de afgelopen jaren.

Lieve Karin en Francois, lieve zus en zwager, bedankt dat jullie er altijd voor me zijn, voor jullie steun en jullie liefde. Ik gun jullie al het geluk van de wereld en hoop dat al jullie dromen uit mogen komen.

Pap en Mam, jullie zijn mijn rots in de branding geweest de afgelopen jaren. Op de fijne momenten, maar vooral ook op de minder leuke momenten hebben jullie altijd voor mij en Sven klaar gestaan. Dit heeft meer voor mij betekend dan ik uit kan leggen. Dank jullie wel voor jullie rotsvaste vertrouwen in mij.

Berend, wat ben ik blij dat wij elkaar 'gevonden' hebben. Jij hebt me laten zien wat echte liefde is. Samen kunnen we de hele wereld aan.

Lieve Janna en Sofie, wat ben ik ontzettend trots dat jullie onderdeel van mijn leven zijn geworden! Jullie hebben mijn leven verrijkt op meer manieren dan ik kan verwoorden.

Lieve Sven, door jou weet ik wat echt belangrijk is in het leven. Ik hou van je kleine man, tot aan de maan en weer terug! En nog een keer heen en weer...

Curriculum Vitae

Petra E. Breukhoven was born in Rotterdam on December 29th, 1980. She graduated in 1999 from secondary school at the “Emmaus College”, Rotterdam. In that same year she started her medical training at the Medical Faculty of the Erasmus University of Rotterdam. In 2003 she participated in a research project investigating nutritional assessment of critically ill children on the Surgical Intensive Care Unit of the ErasmusMC/Sophia Children's Hospital. After obtaining her medical degree in 2006, she started working as a resident at the Department of Paediatrics at the Sint Fransiscus Hospital and in 2007 at the Neonatal Intensive Care Unit of the Erasmus University Medical Centre/Sophia Children's Hospital. In February 2008, she started as a research fellow at the Department of Paediatrics, Division of Endocrinology of the same hospital (supervisor Prof. Dr. A.C.S. Hokken-Koelega), which resulted in the present thesis. In September 2012, she started her training in General Practice. She lives in Nieuwerkerk aan den IJssel with her son Sven, Drs. Berend Rikken and his two daughters Janna and Sofie.

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Submitted

P.E. Breukhoven, G.F. Kerkhof, A.C.S. Hokken-Koelega. Longitudinal changes in insulin sensitivity and beta (tekenje voor beta)-function after discontinuation of GH treatment: A 5-year follow-up study in subjects born SGA
Submitted

J.S. Renes, J. van Doorn, **P.E. Breukhoven**, A.J. Lem, M.A.J. de Ridder, A.C.S. Hokken-Koelega. Acid-labile subunit: serum levels, polymorphisms and association with response to growth hormone treatment in short children born small for gestational age. Submitted.

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PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Petra Esther Breukhoven Erasmus MC Department: Paediatric Endocrinology Research Schools: Nihes / Molmed	PhD period: 2008-2012 Promotor: Prof. Dr. A.C.S. Hokken-Koelega Supervisor: Prof. Dr. A.C.S. Hokken-Koelega	
1. PhD training		
	Year	Workload (Hours/ECTS)
General courses		
– Biomedical English Writing and Communication	2010	4.0 ECTS
– GCP course (BROK)	2009	1.0 ECTS
– Paediatric Endocrinology Meetings	2008-2012	4.0 ECTS
– Classical Methods for Data-analysis	2008	5.7 ECTS
– Modern Statistical Methods	2010	4.3 ECTS
In-depth courses		
– SNPs and Human Diseases	2008	2.0 ECTS
– Basic and Translational Endocrinology	2011	2.2 ECTS
International conferences		
– ESPE 2008 (Annual Meeting of the European Society for Paediatric Endocrinology). Istanbul, Turkey	2008	1.4 ECTS
– ESPE 2010 (Annual Meeting of the European Society for Paediatric Endocrinology). Praha, Czech Republic (poster presentation)	2010	1.4 ECTS
– ESPE 2011 (Annual Meeting of the European Society for Paediatric Endocrinology). Glasgow, Scotland (poster presentation)	2011	1.4 ECTS

	Year	Workload (Hours/ECTS)
Symposia		
– Genetic and non-genetic causes of growth hormone deficiency and hypopituitarism	2008	0.3 ECTS
– Early origins of diabetes type 2 and cardiovascular disease – relevance for clinical practice	2008	0.3 ECTS
– Recent Genetic Findings in children born small for gestational age	2009	0.3 ECTS
– Nieuwe inzichten in problemen bij kinderen met het Prader-Willi Syndroom, eventuele behandelingen en lange-termijn resultaten van GH-behandeling	2009	0.3 ECTS
– Early growth, infant feeding and long-term risk for metabolic and cardiovascular disease	2009	0.3 ECTS
– Developmental origins of health and disease (DOHaD) - New results and hypothesis (oral presentation)	2012	0.7 ECTS
– Disorders of sex development	2012	0.3 ECTS
Other		
– Annual pediatric research day, ErasmusMC/Sophia	2008	0.3 ECTS
– PhD day, ErasmusMC Rotterdam, The Netherlands	2008	0.3 ECTS
– PhD day. ErasmusMC Rotterdam, The Netherlands	2009	0.3 ECTS
2. Teaching activities		
	Year	Workload (Hours/ECTS)
– Annual SGA Day (SGA platform)	2010	0.5 ECTS
– Educational lectures on Endocrinology, Growth Hormone and SGA (Medical Representatives)	2011	1.4 ECTS
– Educational lecture minor students, Pediatric Endocrinology, Rotterdam	2012	1.0 ECTS

List of abbreviations

AGA	appropriate for gestational age
AH	adult height
AIR	acute insulin response
ApoA-I	apolipoprotein A-I
ApoB	apolipoprotein B
BMI	body mass index
CV	coefficient of variation
CVD	cardiovascular disease
DBP	diastolic blood pressure
DI	disposition index
DM2	type 2 diabetes mellitus
DXA	dual-energy X-ray absorptiometry
FFA	free fatty acids
FM	fat mass
FSIGT	frequently sampled intravenous glucose tolerance test
GH	growth hormone
HDLc	high density lipoprotein cholesterol
HR	heart rate
IGF-I	insulin-like growth factor-I
IGFBP-1	insulin-like growth factor binding protein-1
IGFBP-3	insulin-like growth factor binding protein-3
IR	insulin resistance
ISS	idiopathic short stature
IUGR	intra-uterine growth retardation
LBM	lean body mass
LDLc	low density lipoprotein cholesterol
MR	multiple linear regression
OC-use	usage of an oral contraceptives
SBP	systolic blood pressure
SDS	standard deviation score
SES	socioeconomic status
Sg	glucose effectiveness
SGA	small for gestational age
SGA-CU	subject born SGA with spontaneous catch-up growth
SGA-S	subjects born SGA with short adult stature
Si	insulin sensitivity

TC	total cholesterol
TG	triglycerides
TH	target height