Metabolic Health of Children Born SGA

Influence of parental health, prematurity, genetic polymorphisms and growth hormone treatment

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Metabolic Health of Children Born SGA

Influence of parental health, prematurity, genetic polymorphisms and growth hormone treatment

Metabole gezondheid van kinderen die te klein (SGA) geboren zijn

De invloed van familie anamnese, prematuriteit, genetische polymorfismen en groeihormoonbehandeling

Proefschrift

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CHAPTER 1

Introduction

Since 1991 our research group and others have investigated children with short stature who were born small for gestational age (SGA), both before and during treatment with biosynthetic growth hormone (GH). Because of these efforts and the positive results, GH treatment was licensed for short SGA children in 2005. However, many questions remained unanswered, for instance about the association between low birth weight and the risk to develop type 2 diabetes mellitus (DM) or cardiovascular disease (CVD) in adulthood and the effect of GH treatment on such associations.

This doctoral thesis describes studies that evaluated metabolic and cardiovascular risk factors in short children born SGA and the association of these risk factors with family history, preterm birth, GH treatment, serum insulin-like growth factor binding protein-2 (IGFBP-2) levels and genetic polymorphisms. This first chapter describes definitions, prevalence and etiologies of SGA, and clinical and endocrinological aspects associated with SGA. Finally, the aims of the study and outline of this thesis are described.

1. SMALL FOR GESTATIONAL AGE (SGA)

1.1 Definition of SGA

The term “small for gestational age” (SGA) describes the size of an infant at birth. SGA children can be born either preterm or term. In order to determine whether a child is born SGA, one needs accurate information on gestational age, birth weight, birth length and an appropriate reference population to calculate standard deviation scores for birth weight and/or birth length (1). SGA is defined as a birth weight and / or length at least 2 standard deviations (SDS) below the mean for gestational age (2). In the Dutch multicenter studies from which the data in this thesis are derived, the neonatal growth chart from Usher and McLean was used as the reference (3). Segregation of SGA children from their peers is somewhat arbitrary but < - 2 SD was selected because it likely encompasses the majority of patients with disordered fetal growth (2).

SGA birth can be the consequence of intrauterine growth retardation (IUGR) but fetal growth may also have been insufficient from the beginning of gestation without the occurrence of fetal growth deceleration. A child with IUGR late in gestation will not necessarily be born SGA. These different fetal growth patterns are shown in Figure 1. To diagnose IUGR at least two ultrasound measurements are necessary. Because detailed information on fetal growth does often not exist, it is better to refer to an infant with a low birth weight or low birth length for gestational age as SGA rather than IUGR.

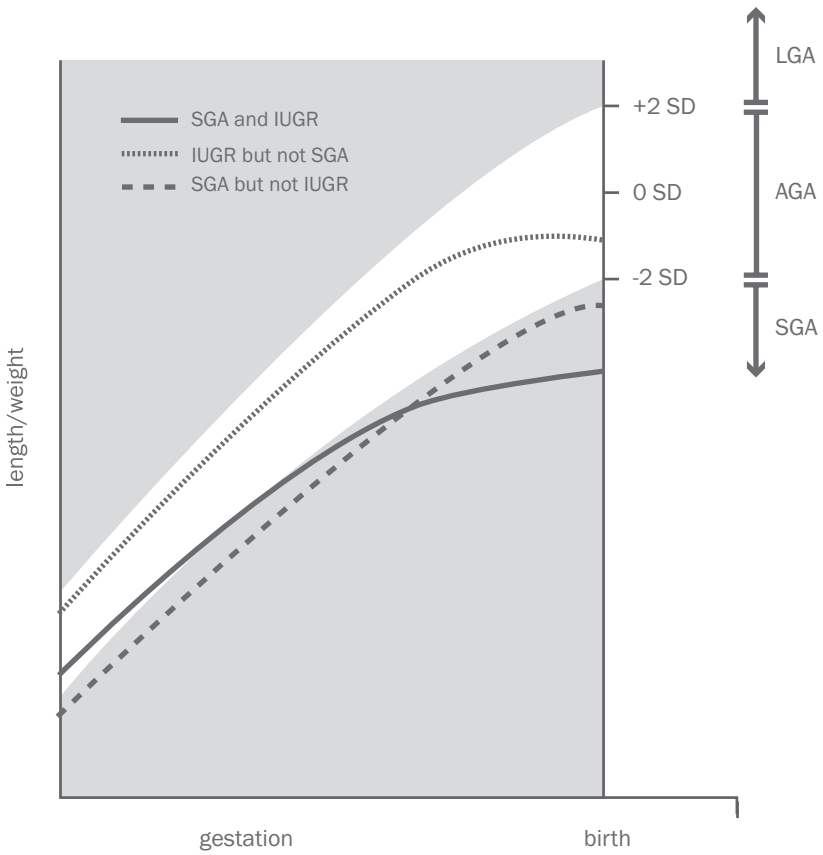

Figure 1. Fetal growth chart showing various intrauterine growth patterns

1.2 Prevalence and etiology of SGA

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.

SGA might be the result of various etiologies but in 40% of the infants born SGA the underlying mechanism remains unclear. Nevertheless it is important to try to identify the underlying cause because this may have consequences for prognosis and treatment. Fetal growth restriction can originate from a number of fetal, maternal, placental, and

demographic factors (4, 5).

Fetal factors include chromosomal disorders (e.g. Down syndrome, Turner syndrome), congenital defects (e.g. cardiac abnormalities, Potter syndrome), metabolic diseases, and genetic disorders (e.g. achondroplasia, Bloom syndrome).

Maternal factors include age (very young or older age), tobacco smoking, alcohol abuse, use of therapeutic (e.g. anticonvulsants, anticoagulants) or illicit drugs (e.g. heroin, cocaine) and maternal medical conditions, such as infection (e.g. Toxoplasmosis, Rubella, Cytomegalovirus, Herpes virus, Malaria, HIV, Trypanosomiasis), hypertension, renal disease, diabetes mellitus, collagen vascular diseases (e.g. systemic lupus erythematosus), cyanotic heart disease, chronic anemia, or chronic pulmonary disease.

Placental factors are associated with problems in placental perfusion resulting in reduced fetal oxygenation. These include structural abnormalities of the placenta (e.g. single umbilical artery, velamentous umbilical cord insertion, bilobate placenta, placental hemangiomas, infarcts or focal lesions), maternal or fetal thrombophilia, and suboptimal implantation site.

Demographic factors include maternal and paternal height and race, parity (e.g. nulliparity or grand multiparity), previous delivery of SGA infants and multiple gestation, particularly in case of shared fetal circulation.

2. CLINICAL AND ENDOCRINOLOGICAL ASPECTS ASSOCIATED WITH SGA

2.1 Short stature

SGA is a common cause of short stature in childhood and adulthood, accounting for 22% of all cases (6). Most children born SGA show spontaneous catch-up growth to a normal height above – 2 SD. However, 10-15% of them do not and remain short (7, 8). Catch-up growth is most pronounced during the first 6 months and is usually completed in the first 2 years of life. In premature infants catch-up growth may take longer (7, 9). The difference between preterm and full-term SGA children in the timing of catch-up growth was explained by differences in the distance between height SDS at the age of 2 years and target height SDS and by differences between height SDS at the age of 2 years and birth length SDS (9). The difference in length of gestation had no effect on the timing of catch-up growth (7, 9). By the age of 8 years, 91% of the SGA born children had reached a height > - 2 SDS (9). The risk of having a short height as an adult (< - 2 SD) is five times higher for children with a low birth weight and seven times higher for those with a low

birth length in comparison with children with a normal birth size (8). Without catch-up growth, SGA born children reached a mean adult height of 161.9 (± 8.0) cm for boys and 147.6 (± 7.0) cm for girls, significantly below the target heights of these children (10). Short children who are born SGA without signs of catch-up growth at the age of 3 years are not likely to catch-up to a normal height later on. These children should be referred to a pediatrician with expertise in endocrinology (2).

2.2 Growth hormone, insulin-like growth factors and IGF-binding proteins

Postnatal growth and development are coordinated by genetic and environmental influences and numerous growth factors (11). The growth hormone-insulin-like growth factor-I (GH-IGF-I) axis (Figure 2) plays an important role in these growth processes. GH is secreted by the pituitary gland under control of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin, as well as ghrelin, a hormone mainly produced in the stomach (12). 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 insulin-like growth factor-I (IGF-I), but GH has also many cellular effects that are independent of IGF-I (11).

The IGF system consists of IGF-I, IGF-II and insulin and three closely related membrane-bound receptors. IGF-I and IGF-II show structural and functional similarity with insulin because they share approximately 50% of their aminoacids, and have important anabolic and metabolic effects. IGF-I is present as circulating IGF-I produced by the liver and as extra-hepatic IGF-I produced by local tissues and acting in a autocrine/paracrine way (13).

The majority of circulating IGF-I is bound to IGF binding proteins (IGFBPs), of which six classes have been identified. IGFBP-3 is the most important carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid-labile subunit (ALS) (14). IGFBP-3 and ALS are both regulated by GH. IGFBP-2 is the second most abundant protein in serum and binds both IGF-I and IGF-II (15). Apart from its role in regulating the amount of free IGF-I, IGFBP-2 is also thought to prevent adipogenesis by impairing adipocyte differentiation (16). In addition, IGFBP-2 is inversely associated with insulin levels (17-19) and HOMA insulin resistance (20) but it is not clear whether the effect of IGFBP-2 on glucose homeostasis is adiposity related or independent from fat mass. Less than 1% of IGF-I is unbound and circulates in its free form. An influence of free IGF-I on short-term metabolic changes and on long-term

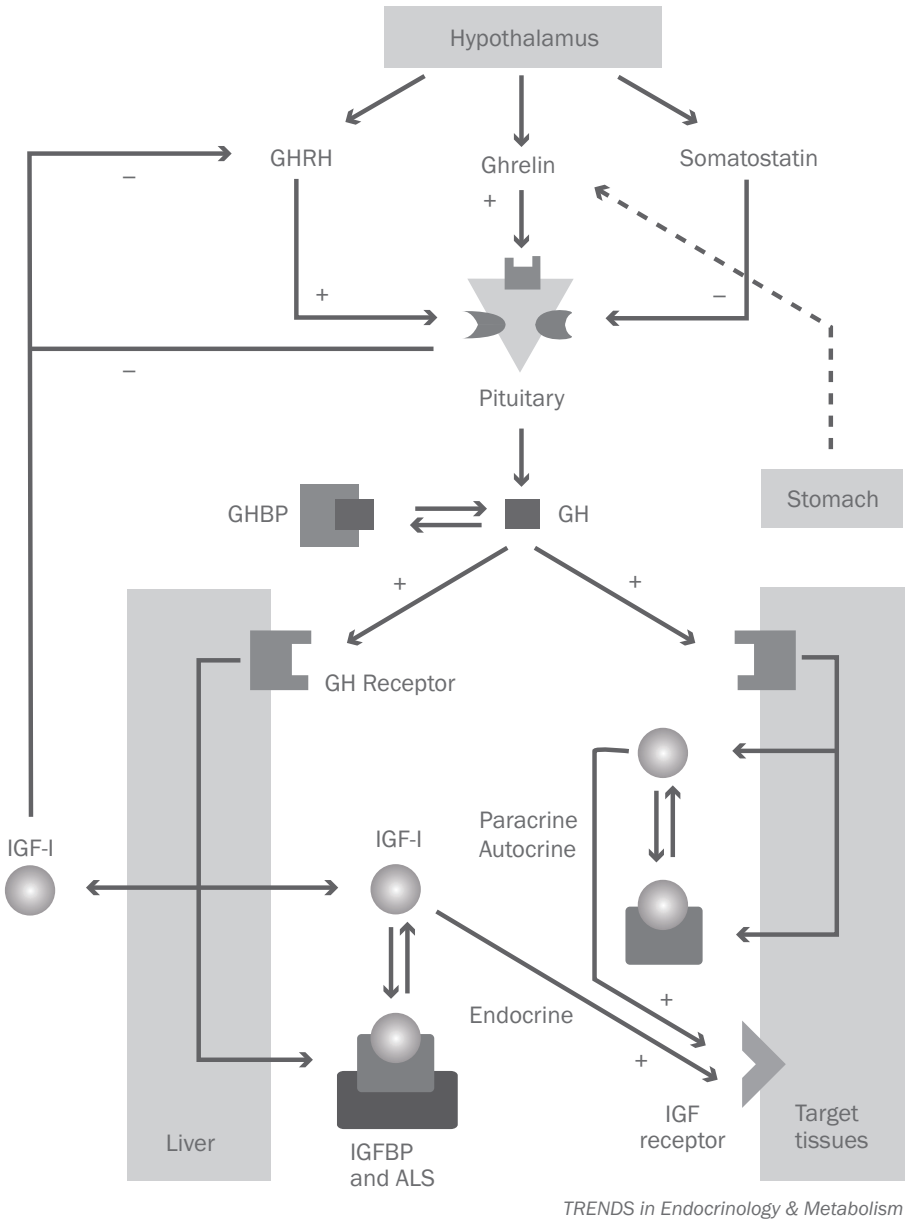

Figure 2. Physiology of the GH-IGF-IGFBP axis. Adapted from Holt (12)

changes like linear growth has been described and therefore free IGF-I is thought to be the biological active form (21, 22).

The mechanism underlying persistent short stature in SGA children is still unknown but disturbances in the GH / insulin-like growth factor-I (IGF-I)-axis may play a role. A significant number of short SGA children show a reduced spontaneous GH secretion during a 24-hour GH-profile and/or low GH peaks during GH provocation tests (23-27). Serum IGF-I and IGFBP-3 levels were also lower in short SGA children than in healthy controls with a normal height (28-30). These observations have led to the first GH studies in short SGA children.

2.3 Thyroid hormones

Studies concerning thyroid hormone levels in SGA children were very limited. Short SGA children were reported to have higher TSH levels compared to SGA children with catch-up growth (31), but the gestational age of these children was not mentioned. In another study in which only SGA children born at term were included, these results could not be replicated (32). One paper suggested that not SGA, but preterm birth is associated with higher TSH levels (33). However, this was studied in SGA children with catch-up growth to a normal height. There were no data on thyroid hormone levels in term and preterm SGA children with persistent short stature.

2.4 Type 2 Diabetes Mellitus and Cardiovascular disease

2.4.1 Historical data and hypotheses

Epidemiological studies reported an inverse association between birth weight and risk for hypertension, cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) in adult life (34-36). Reduced insulin sensitivity plays an important role in the pathogenesis of these disorders (37, 38) but the exact mechanism underlying these associations is still unknown. In the past years several hypotheses have been proposed.

Fetal origins of adult disease hypothesis: In 1989 Barker et al. were the first to report an inverse association between birth weight and death rates from ischaemic heart disease (39). Based on these observations they formulated their fetal origins hypothesis. This hypothesis states that events during pregnancy cause fetal malnutrition and this fetal malnutrition leads to permanent endocrine and metabolic alterations in the fetus (34, 35). This is called re-programming (Figure 3). In utero the fetus benefits from the adaptations but after birth, in the long-term this re-programming results in an increased

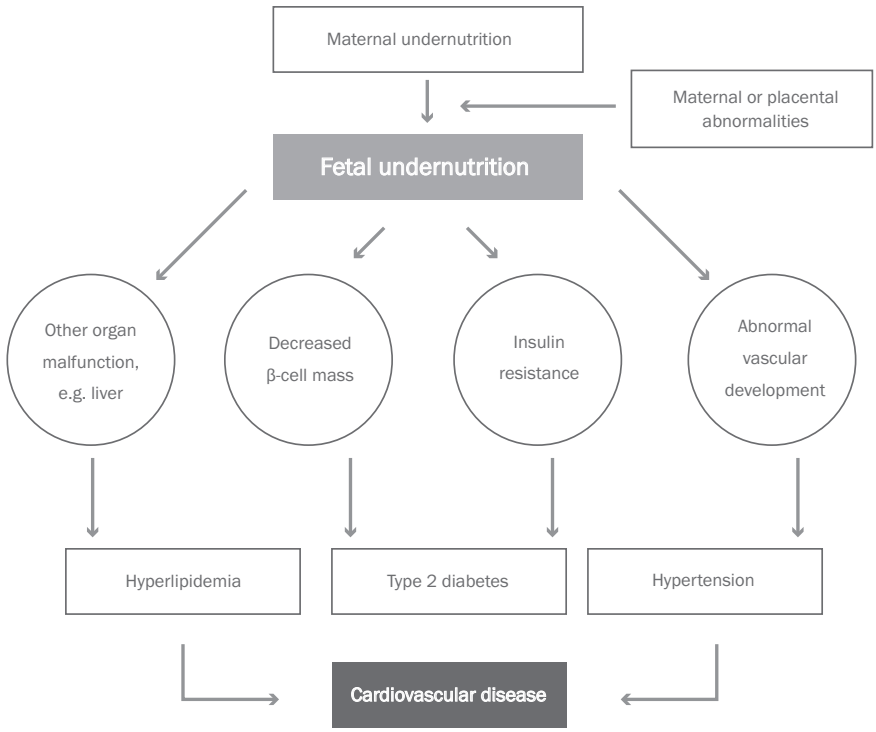

Figure 3. Representation of the fetal origin hypothesis. Adapted from Barker et al. (35, 111)

risk for adult disease.

Fetal insulin hypothesis: The fetal insulin hypothesis was formulated in 1999 by Hattersley et al. This hypothesis states that the association between low birth weight and insulin resistance in adulthood is genetically mediated (40). Insulin is an important growth factor in utero. Therefore, parental genes involved in insulin resistance, which are passed to the fetus, can result in both low-insulin-mediated fetal growth and in insulin resistance in childhood and adulthood (Figure 4).

Growth acceleration hypothesis: In 2004 Singhal and Lucas postulated the hypothesis that not low birth weight per se, but rapid postnatal growth is responsible for the increased risk for CVD in later life (Figure 5) (41). SGA fetuses naturally show faster

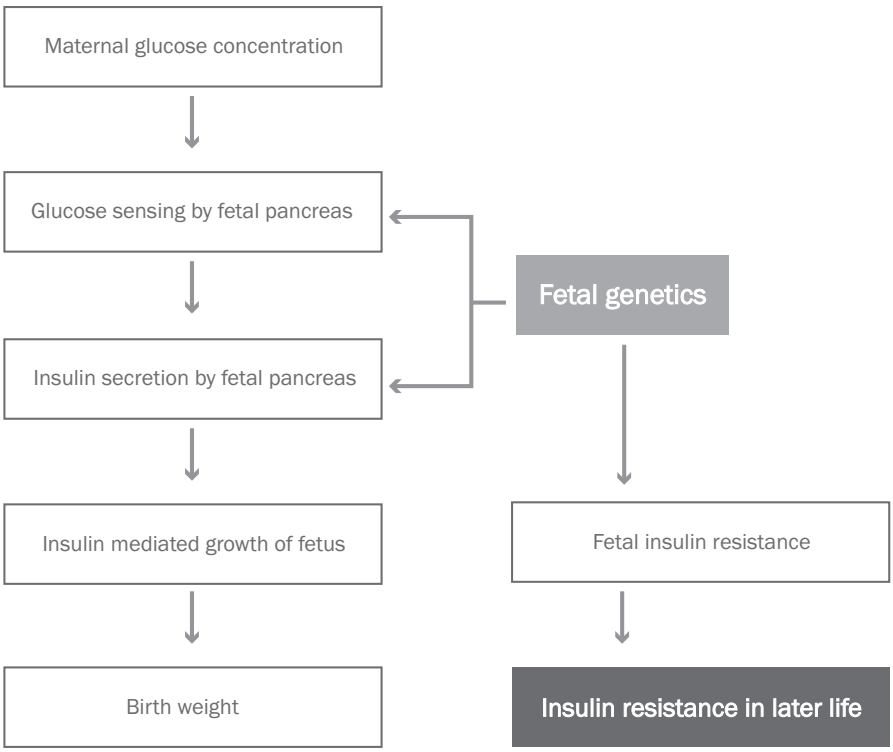

Figure 4. Representation of the fetal insulin hypothesis. Adapted from Hattersley et al. (40)

postnatal growth (while large newborns show growth deceleration (6) because they are small relative to their genetic growth potential. Therefore, associations between low birth weight and later CVD could be a proxy for the adverse effects of early postnatal growth acceleration (41).

Fat accumulation hypothesis: Based on measurement of body composition by dual energy x-ray absorptiometry (DXA), Leunissen et al. further specified growth acceleration into fat accumulation (42). This indicates that small size at birth followed by growth in height and weight as such is not a problem as long as a normal amount of fat is accumulated. Leunissen et al. also demonstrated that especially rapid weight gain during the first 3 months of life is a risk factor for a higher body fat percentage and associated

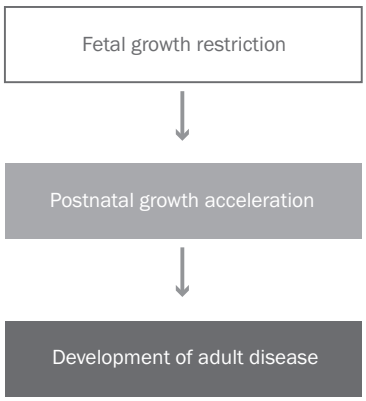

Figure 5. Representation of growth acceleration hypothesis. Adapted from Singhal and Lucas (41)

risk for cardiovascular disease in adulthood (43) (Figure 6).

2.4.2 Assessment of risk for type 2 diabetes mellitus and cardiovascular disease

Reduced insulin sensitivity plays an important role in the pathogenesis of CVD and usually precedes the first symptoms of disease by many years (37, 38). Therefore measurement of insulin sensitivity provides important information on the risk to develop disease in later life. An accurate way to measure insulin sensitivity is by means of the frequent sampling intravenous glucose tolerance test (FSIGT), with Tolbutamide (44, 45). In a group of prepubertal short SGA children with a mean age of 8 years, 8% had an impaired oral glucose tolerance test (46). Further studies indicated that short SGA children were more insulin resistant than children with short stature who were born appropriate for gestational age (AGA) and they had a compensatory higher insulin secretion (47-49). The relationship between insulin sensitivity and insulin secretion is best described by a hyperbolic function. When insulin sensitivity decreases, more insulin is needed to maintain glucose homeostasis (50). If insulin secretion does not change appropriately in response to a change in insulin sensitivity, impaired glucose tolerance and ultimately type 2 DM will develop (51).

SGA children and adolescents had a higher systolic blood pressure than references (48, 52, 53). Also, SGA children were reported to have more often hypercholesterolemia

(54) and more often serum free fatty acids levels above the normal range (48) than AGA children.

It is well known that obesity is an important risk factor for the development of CVD and type 2 DM (55). In children born SGA with spontaneous catch-up in weight, early development of adiposity has been reported (56). Short children born SGA, however, usually have a lean appearance, as indicated by a low body mass index (BMI) SDS and a low sum of skinfolds SDS (52). Further analysis with DXA revealed that body fat percentage in short SGA children is significantly reduced compared with reference values (57).

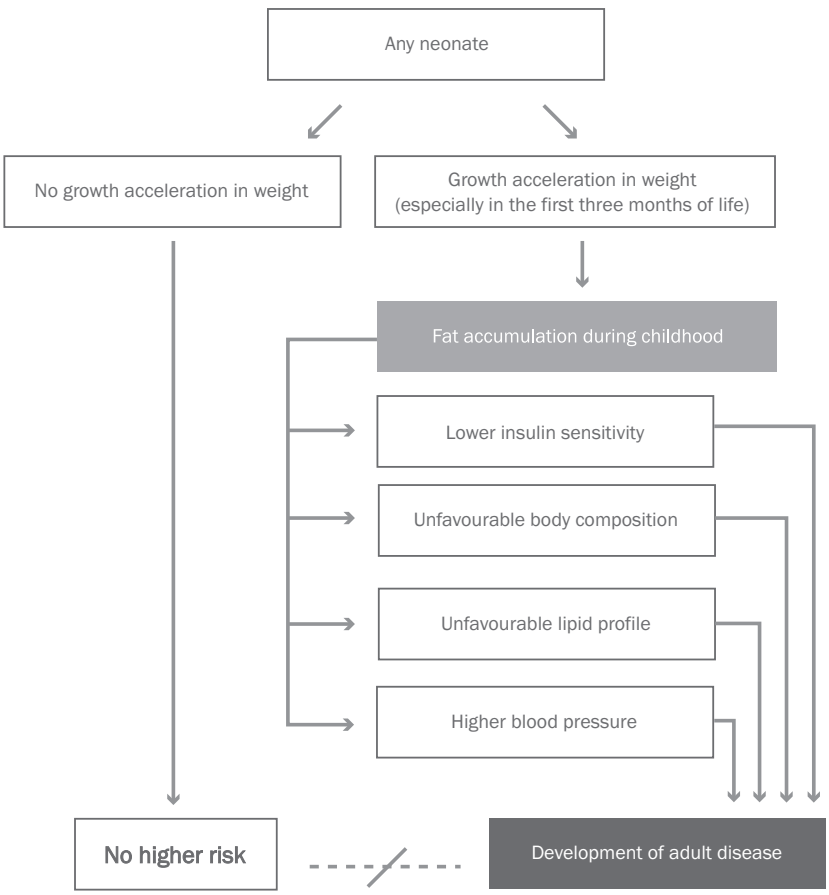

Figure 6. Representation of fat accumulation hypothesis. Adapted from Leunissen et al. (42)

2.4.3 Family history

A family history of CVD could be an explanation for the higher prevalence of cardiovascular risk factors in subjects born with a lower birth weight (58). Clustering of cardiovascular risk factors within families has also been described irrespective of birth weight (59, 60), whereas there is also a relation between birth weight of parents and offspring (61, 62). Some studies described a relation between birth weight of offspring and subsequent cardiovascular mortality of the parents (63, 64). Women who were themselves born SGA are reported to be at increased risk of having an SGA infant and they are also at increased risk of preeclampsia and gestational diabetes (65). It is unknown whether the presence of risk factors for metabolic and CVD in short SGA children can be predicted by the presence of risk factors in their parents.

2.4.4 Single nucleotide polymorphisms and the risk to develop type 2 DM and CVD

A polymorphism is a genetic variant, not necessarily related to disease, which exists in more than 1% of a normal population. A polymorphism that affects only one base is called a Single Nucleotide Polymorphism or SNP. Genome wide association studies identified SNPs in the human genome that associate with the risk to develop type 2 DM (66-71). These SNPs are also candidate genes for explaining the associations between low birth weight and Type 2 DM. Two important examples of such SNPs are found in the peroxisome proliferators-activated receptor γ (PPAR-γ) gene and the Transcription factor 7-like 2 (TCF7L2) gene.

Polymorphisms of *PPAR-γ* are associated with adipocyte differentiation, lipid metabolism and insulin sensitivity (72). Genome-wide association (GWA) studies found that the Ala12 variant of the *PPAR-γ* gene is associated with a lower risk for type 2 DM compared with the Pro12Pro genotype (67, 69-71). However, in young adults born SGA (73), the Ala12 allele was associated with an increased risk for type 2 DM. A higher BMI in Ala12 carriers has also been described (74, 75), while other studies found a lower BMI in Ala12 carriers (76) or no association with BMI (75). The association between the *PPAR-γ* Ala12 allele and glucose homeostasis has been found to interact with size at birth (73). These results regarding associations of the Ala12 allele with insulin resistance and BMI suggest that the allele has a different effect in different environments, and that environmental factors may play a role to increase the genetic effect of *PPAR-γ* on metabolic risk factors.

Another, more recently found polymorphism that associates with the risk to develop type 2 DM is rs7903146 in the *TCF7L2* gene. This is to date the polymorphism with the strongest association with type 2 diabetes mellitus (DM) (66-68), probably because the rs7903146 T-allele associates with decreased insulin secretion (77-81). So far, literature is inconclusive whether the rs7903146 T-allele also associates with insulin sensitivity and disposition index (77-79, 81). Because insulin is an important growth factor in utero, *TCF7L2* might also influence size at birth. One study investigated the association between birth weight and *TCF7L2* genotype and found a positive effect of the rs7903146 T-allele when present in the mother (82). Presence of one or two copies of the T-allele in the fetus seemed not to influence birth weight (82).

3. GROWTH HORMONE (GH) TREATMENT IN SGA CHILDREN

3.1 Effect on linear growth

In 1991, the first Dutch multi-center, randomized, double-blind, dose-response GH trial was started to investigate the efficacy of GH treatment on growth in short SGA children (28, 83). Children were treated with either 1 or 2 mg GH/m^2 /day. In this study, 85% of the children reached a normal adult height above – 2 SDS and 98% reached an adult height within the target height range (83). The catch-up growth in height was accompanied by catch-up growth in BMI (28). Interestingly, adult height SDS was not significantly different between the two GH dosage groups. In 1996, the second Dutch GH trial was started with a randomized control group for 3 years. After 3 years, GH treatment with a dose of 1 mg GH/m^2 /day resulted in a normalization of height, whereas children in the control group, who did not receive GH treatment during those 3 years, remained short (84). Several other studies have demonstrated that GH treatment effectively induces catch-up growth in short SGA children (85-88).

3.2 Effect 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, 28, 89). After one year of GH treatment with a dose of 1 mg/m^2/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 in IGFBP-3 levels up to 0.2 SDS during one year of GH treatment with a dose of 1 mg / m^2 /day (28). Treatment with 2 mg/m^2/day resulted

in mean IGF-I and IGFBP-3 levels of 1.9 SDS and 0.5 SDS, respectively (28). After 3 years of GH treatment, IGF-I and IGFBP-3 levels were similar in both GH dosage groups (28). At discontinuation of GH treatment after attainment of adult height, mean IGF-I SDS was 1.0 in children treated with 1 mg/m^2/day and 1.3 in children treated with 2 mg/m^2/day, both significantly higher than the population mean (83). Mean IGFBP-3 levels were –0.8 SDS in children treated with 1 mg/m^2/day, which is significantly lower than the population mean, and –0.06 in children treated with 2 mg/m^2/day (83). 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 short SGA subjects, indicating that the GH-induced rise in IGF-I and IGFBP-3 levels is completely reversible after discontinuation of GH (45).

During overnight GH profiles in short prepubertal SGA children, mean and maximum GH levels were respectively 34.8 and 104 mU/l when treated with 1 mg/m^2/day, and 64.4 and 161 mU/l when treated with 2 mg/m^2/day (90). For comparison, overnight GH levels in normal prepubertal children are 10.5 mU/l in boys and 10.8 mU/l in girls, and during puberty these levels rise to values of 17.1 mU/l in boys and 20 mU/l in girls (91). Thus, especially short SGA children receiving 2 mg/m^2/day have very high mean serum GH levels (90). Because treatment with a GH dose of 1 mg/m^2/day is as effective as treatment with 2 mg/m^2/day with regard to reach a normal adult height (83) 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/m^2/day.

3.3 Effect on thyroid hormone axis

It has been suggested that GH therapy has an inhibitory effect on the hypothalamo-pituitary-thyroid axis (92) and might increase peripheral conversion of T4 to T3 (93). Because normal thyroid status is necessary for growth and development, it is important to know whether GH treatment alters thyroid function in short SGA children without GH deficiency. This was not yet investigated.

3.4 Effect 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 (94-96). Therefore, concern was expressed regarding the long-term consequences of GH-treatment on risk factors for type 2 DM and associated comorbidities, especially in possibly predisposed

subjects, such as SGA children. Particularly because it was previously shown that short SGA children had reduced insulin sensitivity before receiving GH (47-49) and that GH treatment resulted in a further decline of insulin sensitivity and a compensatory increase in insulin secretion (47, 97). Most studies reported a recovery of insulin sensitivity and insulin levels to pre-treatment levels within 3 to 6 months after withdrawal of GH treatment (45, 97-99). Van Dijk et al. reported that at 6.5 years after discontinuation of GH, insulin sensitivity and insulin secretion were similar in GH-treated SGA subjects and untreated SGA controls (45). Thus, changes in insulin sensitivity and insulin secretion were reversible after discontinuation of GH treatment and, moreover, remained so until at least 6.5 years after discontinuation (45). This suggests that long-term GH treatment of short SGA children does not have permanent effects on glucose homeostasis and does not increase the risk of type 2 DM (45, 98, 99).

GH treatment has also been associated with a reduction in systolic blood pressure as well as a reduction in cholesterol levels in SGA children (45, 98). These are beneficial effects since SGA children were reported to have a higher systolic blood pressure and more often hypercholesterolaemia (52, 54). At a mean age of 22 years, 6.5 years after discontinuation of long-term GH treatment, systolic and diastolic blood pressure and serum cholesterol were significantly lower in previously GH treated SGA subjects than in untreated SGA subjects (45). Thus, GH treatment might have long-lasting beneficial effects on blood pressure and serum cholesterol levels in short SGA subjects (45). 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 last SGA consensus meeting in 2007 (1).

3.5 Effect on body composition

GH has well-documented anabolic effects on muscle mass and lipolytic effects on adipose tissue (100, 101). GH deficiency has been associated with increased fat mass and truncal obesity (102), while GH excess as in acromegaly, has been associated with reduced fat mass and increased lean body mass (103).

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 (MRI) and reported an increase in muscle tissue and a decline in adipose tissue (104). Total body fat and muscle mass were not measured

in this study and only the first 3 years of GH treatment were analyzed. Willemsen et al. investigated the effect of GH treatment on body composition during 6 years of GH treatment and adjusted for catch-up in height (99). They found a significant decline of fat mass SDS adjusted for gender and height and no change in lean mass SDS adjusted for gender and height (99).

Discontinuation of GH treatment was associated with significant changes in body composition 6 months after stop of treatment. Percentage body fat and fat mass SDS adjusted for gender and height increased, whereas lean mass SDS adjusted for gender and height decreased (99). Fat distribution had not changed 6 months after discontinuation of GH treatment. All values remained within the normal range and therefore the clinical relevance of the observed changes is unclear (99). It remains to be elucidated how body composition changes many years after discontinuation of GH treatment.

3.6 Interaction between GH treatment and genetic polymorphisms

As explained in the previous paragraphs, GH treatment of short SGA children generally results in weight gain and a decrease in blood pressure, serum cholesterol and insulin sensitivity. However, not all children respond in the same way. This variation in response to GH treatment might be explained by the genotype of the patients.

The *PPAR-γ* Ala12 allele was associated with BMI and glucose homeostasis (72), so this polymorphism might influence the change of these parameters during GH treatment. The *TCF7L2* T-allele was associated with decreased insulin secretion (77-81) and during GH treatment extra insulin secretion is needed to compensate for the reduced insulin sensitivity. Therefore this SNP might indicate which children are less capable of producing extra insulin. Children with less insulin secretion during GH-treatment might be more at risk to develop DM in later life.

3.7 Safety of GH treatment

The National Cooperative Growth Study (NGCS) monitored the safety of GH treatment from 1984 until 1995 in children with various diagnoses. Reported adverse events included idiopathic intracranial hypertension, edema and lymphedema, carpal tunnel syndrome, slipped capital femoral epiphysis, diabetes mellitus and glucose intolerance (105). The authors concluded that major adverse events in relation to GH treatment were rare and that their frequency might have been affected by preexisting medical conditions.

Concern has been expressed regarding the possible harmful effects of high serum

GH and IGF-I levels for many years (106, 107). Epidemiological studies in adults evaluating the risk for breast (108), prostate (109), and colon (110) cancer , indicated that serum levels of IGF-I in the upper tertile to quintile are associated with an increased risk of cancer, especially when IGFBP-3 levels are low (109, 110). These studies did, however, not establish causality (107) whereas during GH treatment serum levels of IGFBP-3 also increase. It is therefore important to evaluate the serum levels of IGF-I in GH-treated short SGA children and adjust the GH dose when IGF-I levels rise to levels far above the normal range.

Several studies demonstrated that GH treatment was well tolerated by SGA children and that side effects were uncommon (46, 52, 98). Nevertheless, it was concluded that all SGA children receiving GH treatment should be monitored regularly for changes in glucose homeostasis, serum lipid levels, blood pressure and serum IGF-I levels to exclude any possible adverse effect of GH (1).

4. AIMS OF THE STUDY

Familiar clustering of SGA and risk for type 2 DM and CVD in parents and offspring

To evaluate in parents of short SGA children, anthropometry, blood pressure, fasting serum lipids, glucose and insulin levels and compare these values with those of a population-based reference group. We hypothesized that parents of short SGA children have an increased risk for type 2 DM and CVD and that a relative high percentage of parents was born SGA themselves. Also, to evaluate whether anthropometric data and cardiovascular risk factors correlate between parents and children.

Thyroid function

To measure TSH and free T4 levels before and during 2 years of GH treatment in short SGA children who were divided in those born preterm and those born at term. To investigate the effect of preterm birth and the effect of GH treatment on thyroid hormone levels.

Differences between preterm and term born short SGA children

Since both SGA and preterm birth have been associated with increased incidence of adult cardiovascular disease and type 2 DM, the aim was to investigate in short children born SGA whether preterm birth had an independent effect on the response to GH-treatment with regard to the gain in height and weight and the change in risk factors for

CVD and type 2 DM.

Genetic polymorphisms and cardiovascular risk factors

To investigate whether polymorphisms in the *PPAR-γ* and *TCF7L2* genes correlate with longitudinal changes in the metabolic and cardiovascular profile of short SGA children during GH treatment. Because insulin is an important growth factor in utero (40) and *TCF7L2* is associated with insulin secretion (77-81) we aimed to evaluate whether *TCF7L2* associates with fetal and postnatal growth.

IGFBP-2 levels

Since limited data exist on IGFBP-2 levels in children and young adults who were born SGA, to determine IGFBP-2 levels in a large cohort of SGA subjects. Levels were compared to those of age and gender-matched controls with normal stature. In addition, we aimed to investigate the associations between IGFBP-2 levels and various metabolic and cardiovascular risk factors.

5. OUTLINE OF THIS THESIS

Chapter 1 gives an introduction in the topics described in this thesis.
Chapter 2 describes the prevalence of cardiovascular risk factors in short SGA children and their parents and the association of these between parents and children.
Chapter 3 describes the effects of prematurity and GH treatment on thyroid function in short SGA children.
Chapter 4 reports on differences between preterm and term born short SGA children in anthropometry and in response to GH treatment.
Chapter 5 describes differences between preterm and term short SGA children in GH-induced changes in metabolic and cardiovascular risk factors.
Chapter 6 reports the contribution of the *PPARγ* Pro12Ala polymorphism to GH induced changes in determinants of metabolic and cardiovascular disease in short SGA children.
Chapter 7 describes whether *TCF7L2* genotype is associated with growth patterns from fetal life until infancy.
Chapter 8 describes the interaction between *TCF7L2* genotype and growth hormone-induced changes in glucose homeostasis in short SGA children.
Chapter 9 describes the association between IGFBP-2 levels and metabolic and

cardiovascular risk determinants in children and young adults who were born SGA.
Chapter 10 discusses our findings in relation to current literature and comments on the clinical implications and conclusions of our study results.
Chapter 11 summarizes our findings in English.
Chapter 12 summarizes our findings in Dutch.

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CHAPTER 2

Cardiovascular risk factors in parents of short children born small for gestational age (SGA)

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ABSTRACT

SGA children have a higher prevalence of cardiovascular risk factors at a young age. It is not known whether this increased risk is caused by their size at birth, a familial predisposition for cardiovascular disease or smallness at birth or a combination of these factors. The cardiovascular risk profile of parents of SGA children is unknown. We compared anthropometry, blood pressure, fasting serum lipid, glucose and insulin levels of 482 parents (mean age 41 years) and 286 short SGA children with age- and sex-matched references. We also investigated whether these parameters correlated between parents and their offspring. Mothers had higher systolic blood pressure, fathers had a higher body mass index (BMI) and parents had more frequently high fasting glucose levels than age- and sex-matched references. Children had significantly higher systolic and diastolic blood pressure than sex- and height-matched references. Twenty-four percent (mothers) and 10% (fathers) were born SGA but they did not have more cardiovascular risk factors than those born appropriate for gestational age. Cardiovascular risk factors did not correlate between parents and children. In conclusion, parents of short SGA children have a modest increase in some cardiovascular risk factors but risk factors did not correlate between parents and children.

INTRODUCTION

Epidemiological studies reported an inverse association between birth weight and risk for cardiovascular disease (CVD) in adult life (1-3). In addition, some studies described a relation between birth weight of offspring and subsequent cardiovascular mortality of the parents (4, 5). The exact mechanism behind these associations and the relative roles of environmental and genetic factors has not yet been elucidated. A familial component has been proposed (4, 5), but others could not find a family history of CVD as explanation for the higher prevalence of cardiovascular risk factors in subjects born with a lower birth weight (6). Clustering of cardiovascular risk factors within families has also been described irrespective of birth weight (7, 8), whereas there is a relation between birth weight of parents and offspring (9, 10).

Short small for gestational age (SGA) children constitute a special group within the group of low birth weight children because they did not reach a normal height. An increased prevalence of cardiovascular risk factors in short SGA children has been described (11, 12). Some parents of short SGA children were born SGA themselves (13, 14). It is unknown whether an increased risk for CVD in a short SGA child can be predicted by the presence of risk factors in his/her parents. We hypothesized that parents of short SGA children have an increased risk for CVD. In addition, we hypothesized that a relative high percentage of parents was born SGA themselves and that anthropometric data and cardiovascular risk factors correlate between parents and children. We therefore evaluated in parents of short SGA children anthropometry, blood pressure, fasting serum lipids, glucose and insulin levels, which are regarded as predictors of CVD (15). Outcome variables were compared with those of a population-based reference group.

SUBJECTS AND METHODS

Subjects

Five-hundred-and-fifty-one parents (mean age 41 years, 276 (50%) fathers) of 286 Caucasian short SGA children (151 (53%) boys), were eligible for inclusion in the period between December 1999 and September 2006. In 482 (87%) of these parents, anthropometric measurements were performed and 472 (86%) parents filled out a questionnaire. A complete dataset (anthropometry and questionnaire) was available in 410 parents (74%). Two children had the same mother but different fathers. Inclusion criteria for the children have previously been described (16). In short, the children were included when prepubertal, with a birth length or birth weight standard deviation score (SDS) and actual height SDS below –2, without signs of catch-up growth in height and without growth failure caused by other disorders. The study was approved by the Medical Ethics Committees of the participating centres and written informed consent was obtained from all parents.

The reference group consisted of 1699 women (aged 45 (8.5) years) and 1630 men (aged 46 (8.5) years), participating in the year 2001 in a Dutch population-based health survey. The aim of this survey was to monitor risk factors or determinants of chronic disease in the general population (17). This reference group was used to calculate age- and sex-matched SD scores in the following manner: SD score = (value of parent – mean for references of the same age and sex) / SD for references of the same age and sex.

Study design

Parents

Height, weight and waist circumference were measured with the subject standing. BMI was calculated. Systolic and diastolic blood pressure (BP) were measured in the non-dominant arm while in a sitting position and the mean of three measurements was used for analysis. Height and weight for height were expressed as SDS using Dutch standards (18, 19). Waist circumference, BMI and BP were expressed as SDS adjusted for age and sex using the reference group (17). All parents were asked to fill out a questionnaire about birth characteristics, medical history, present health and family history on CVD and DM. CVD was defined as the occurrence of myocardial infarction, cerebrovascular accident, pulmonary embolism or deep vein thrombosis. Small for gestational age (SGA) was defined as a birth length and/ or birth weight standard deviation score (SDS) below –2

for gestational age (20). Fasting blood samples were available for parents of 93 children (92 mothers and 78 fathers). The availability of a fasting blood sample in the parents deviated from the year in which their child was included. Parents with an available fasting blood sample were comparable with parents without an available blood sample with respect to all outcome variables except for age (38.7 versus 42.1 years, $p<0.001$) and systolic blood pressure SDS (0.09 versus 0.33 SDS, $p < 0.05$). Of the 92 mothers with an available blood sample, 44 had birth data and 10 were born SGA. Of the 78 fathers with an available blood sample, 33 had birth data and 4 were born SGA.

Children

Height, weight, head circumference, systolic and diastolic blood pressure (BP) were measured and BMI was calculated. Height, head circumference and BMI were expressed as SD scores adjusting for sex and age according to Dutch reference data (19), whereas BP was adjusted for sex and height (21). Fasting blood samples were available for 191 children.

Biochemical measurements

After centrifugation, all samples were frozen (-80 °C) until assayed. Fasting serum insulin levels were determined by chemoluminescent assay on an Immulite 2000 analyser (Diagnostic Products Corporation, Los Angeles, CA). Fasting glucose was measured on a Hitachi 917 analyser. HOMA-IR was calculated (22). Serum total cholesterol (TC) and triglycerides (TG) were determined enzymatically and LDL-cholesterol and HDL-cholesterol were determined using a homogeneous assay on a Hitachi 917 (Roche Diagnostics, Mannheim, Germany).

Parental fasting TC and HDL-c levels were expressed as SDS adjusted for age and sex using the reference group (17). For children we used reference values of our hospital (Erasmus MC-Sophia) to define high TC, LDL-c and TG levels and low HDL-c levels. Cut-off values for high total cholesterol were ≥5.0 mmol/l for age 0-3 years, ≥5.4 mmol/l for age 4-12 years and ≥6.5 mmol/l for adults (23). Cut-off values for high LDL- cholesterol levels were ≥3.5 mmol/l for age 0-3 years; ≥3.4 mmol/l for age 4-12 years and ≥4.2 mmol/l for adults (23). As TG and HDL-c levels are components of the ATP III criteria, applied cut-off values for these lipids are described in the section about the definition of metabolic syndrome.

Metabolic Syndrome definition

Metabolic syndrome (MS) increases the risk for CVD and DM type 2 (15). According to the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATPIII) criteria, MS is present in adults when 3 or more of the following symptoms are present: central obesity (waist circumference ≥ 102 (males) or 88 cm (females), raised TG levels (TG ≥ 1.7 mmol/L), reduced HDL-c levels (HDL-c < 1.0 (males) or 1.3 (females) mmol/L), high BP (systolic ≥ 130 and/or diastolic bp ≥ 85 mm Hg or current treatment for hypertension), and increased fasting glucose levels (glucose ≥ 5.6 mmol/L) (24).

In children, we used the modified ATP III criteria by Weiss et al. to diagnose MS (25). High BP was defined as a height- and sex-adjusted systolic or diastolic BP >95th percentile (21). MS was diagnosed in children if 3 or more of the following symptoms were present: obesity (BMI ≥ 2 SDS (19)), TG levels >95th percentile (TG ≥ 1.2 mmol/L for age 0-3 years, TG ≥ 1.0 mmol/L for age 4 – 12 years), HDL-c levels <5th percentile (HDL-c < 0.5 mmol/L for age 0-3 years and HDL-c < 0.9 mmol/L for age 4-12 years), high BP and increased fasting glucose levels (glucose ≥ 5.6 mmol/L).

Statistics

Analyses were performed using the statistical package SPSS (version 12.0.1; SPSS Inc., Chicago, IL) for Windows. Results are expressed as means with standard deviations for continuous data and as percentages for dichotomous or categorical variables. Because of a skewed distribution, insulin levels and HOMA-IR are expressed as median (interquartile range). Differences between parents and references were evaluated using the two-tailed one-sample t test and Chi-square test for proportions. Chi-square tests were also used to detect associations between risk factors in children and parents. Independent-sample t tests were used to detect differences between subgroups of parents or children. Correlations within families (child-mother and child-father couples) were analyzed using Spearman's correlation coefficient. All analyses were performed separately for men and women. Because independent samples t-tests demonstrated no significant differences between boys and girls, the children were analyzed as one group.

RESULTS

Clinical characteristics

Clinical characteristics of 482 parents and 286 children are shown in Table 1. Parental height SDS and head circumference SDS were significantly lower than zero SDS ($p<0.001$). Mothers and fathers had a relatively larger head circumference SDS compared with height SDS ($p<0.01$ and $p<0.001$, respectively). BMI SDS of the fathers was significantly higher than zero SDS ($p<0.05$). Systolic BP SDS of the mothers was significantly higher ($p<0.001$) compared with age-matched female references but diastolic BP SDS was not. BP in fathers was comparable with age- and sex-matched references. In the children, systolic and diastolic BP SDS were significantly higher compared with sex- and height-matched references ($p<0.001$).

Table 1: Clinical characteristics of parents (n=482) and children (n=286)

	Mothers		Fathers		Children	
		P value		P value		P value
Age (yr)	39.7 (5.0)	-	42.3 (5.5)	-	6.4 (2.4)	-
Height (cm)	163.4 (6.8)	-	177.0 (7.1)	-	106.1 (13.2)	-
Height SDS	-0.8 (1.1)	<0.001	-0.7 (1.1)	<0.001	-3.0 (0.6)	<0.001
HC (cm)	54.5 (1.6)	-	57.2 (1.6)	-	49.5 (1.9)	-
HC SDS	-0.5 (1.0)	<0.001	-0.3 (0.9)	<0.001	-1.2 (1.0)	<0.001
HC SDS – Ht SDS	0.2 (1.1)	<0.01	0.3 (1.1)	<0.001	1.8 (1.1)	<0.001
BMI	24.9 (4.8)	-	26.4 (3.9)	-	14.1 (1.2)	-
BMI SDS	0.1 (1.2)	0.11	0.2 (1.1)	<0.05	-1.4 (1.0)	<0.001
Waist circ. (cm)	82.8 (11.6)	-	93.1 (12.1)	-	-	-
Waist circ. SDS	0.0 (1.1)	0.74	-0.1 (1.2)	0.14	-	-
Systolic BP (mmHg)	121.5 (16.2)	-	131.1 (16.2)	-	103.5 (12.8)	-
Systolic BP SDS	0.3 (1.1)	<0.001	0.1 (1.2)	0.15	0.9 (1.1)	<0.001
Diastolic BP (mmHg)	79.5 (10.7)	-	83.0 (12.1)	-	59.2 (9.2)	-
Diastolic BP SDS	0.0 (1.1)	0.85	0.0 (1.2)	0.82	0.4 (1.1)	<0.001

SDS = standard deviation score; HC = head circumference; Ht = height; BMI = body mass index; BP = blood pressure. Values are expressed as mean (SD); p values express the difference with age- and sex-matched references

Birth characteristics

Birth characteristics are listed in Table 2. Compared with reference values, mothers had a significantly lower birth weight SDS (p<0.001). Birth length SDS was significantly lower than zero SDS in both mothers (p<0.001) and fathers (p<0.05). Twenty-four mothers (24%) and 9 fathers (10%) with known birth characteristics were born SGA themselves. These percentages are significantly higher than the 2.3%, which is by definition the prevalence of SGA birth in live-born neonates. Even when all parents without known birth characteristics were regarded as born AGA, the prevalence of being born SGA in parents (24/275 (9%) mothers and 9/276 (3%) fathers) was still higher than 2.3%.

Table 2: Birth characteristics

	Mothers			Fathers			Children	
	n			n			n	
Gestational age	121	39.3 (2.1)		104	39.4 (1.7)		286	36.2 (3.7)
Birth weight (kg)	169	3.0 (0.6)		130	3.3 (0.7)		286	1.9 (0.7)
Birth weight SDS	100	-0.6 (1.2)*		87	-0.2 (1.5)		286	-2.3 (1.1)*
Birth length (cm)	112	48.4 (2.8)		68	49.9 (3.4)		209	42.3 (5.0)
Birth length SDS	67	-1.1 (1.4)*		47	-0.7 (1.8)**		209	-3.1 (1.5)*
Born SGA (%)	101	24%		87	10%		286	100%

Data expressed as mean (SD); n = number of persons with available data
Compared with gestational age- and sex- matched references: *p < 0.001; **p < 0.01

Biochemical measurements

TC SD scores were significantly higher than zero SDS, i.e. compared with the median for references (Table 3). Nine percent of the mothers had high TC levels compared with 5% of the female references, but this difference was not significant (p=0.16). Fifteen percent of the fathers had high TC levels compared with 7% of the male references (p<0.05). HDL-c SD scores were significantly higher than zero SDS in both mothers and fathers (p<0.001). Prevalence of high LDL-c was 11% in mothers and 25% in fathers. In the reference population, LDL-c, TG, and insulin were not measured. Compared with references, parents had more frequently fasting glucose levels above the ATP III cutoff level of 5.6 mM (Table 4). Nonetheless, mean parental fasting levels of glucose, insulin, TC, TG, HDL-c, and LDL-c were all within the normal range. Median HOMA-IR was 0.8 (0.5 – 1.1) in mothers, 0.9 (0.5 – 1.7) in fathers and 0.3 (0.2 – 0.4) in children. In the SGA

children, mean fasting serum levels of TC, TG, HDL-c and LDL-c were within the normal range (Table 3). Only one child had a TC level above the normal range.

Table 3: Fasting serum lipid, glucose and insulin levels			
	Mothers	Fathers	Children
TC (mmol/L)	5.3 (0.9)	5.6 (1.0)	4.1 (0.8)
TC SDS	0.5 (1.1)*	0.4 (1.1)**	-
TG (mmol/L)	1.2 (0.7)	1.7 (1.2)	0.8 (0.4)
HDL-c (mmol/L)	1.5 (0.4)	1.2 (0.3)	1.4 (0.4)
HDL-c SDS	0.5 (1.3)*	0.5 (1.2)*	-
TC / HDL-c ratio	3.7 (1.3)	4.8 (1.4)	3.2 (1.1)
LDL-c (mmol/L)	3.1 (0.9)	3.5 (1.0)	2.3 (0.7)
Glucose (mmol/L)	4.7 (1.1)	4.9 (0.7)	4.4 (0.6)
Insulin (pmol/L)	40.8 (27.8-63.2)	45.7 (25.5-89.4)	14.0 (14.0-22.8)
HOMA-IR	0.8 (0.5-1.1)	0.9 (0.5-1.7)	0.3 (0.2-0.4)

Data expressed as mean (SD) and insulin and HOMA-IR as median (interquartile range)
Compared with age- and sex- matched references: *p<0.001; **p<0.01
TC = total cholesterol N: 2.0-5.5 mmol/l (children) or <6.5 mmol/l (adults); TG = triglycerides N: 0.3-1.6 mmol/l (children) or <1.7 mmol/l (adults); HDL-c = high-density lipoprotein cholesterol N: 1.1-2.7 mmol/l (children) or >1.0 mmol/l (male adults) or >1.3 mmol/l (female adults); LDL-c = low-density lipoprotein cholesterol N: 0.0-4.2 mmol/l (children) or <4.2 mmol/l (adults); glucose N: 2.6-6.0 mmol/l; insulin N: < 180 pmol/L

Parents born SGA versus parents born AGA

Mothers born SGA had a significantly smaller head circumference (-1.0 SDS versus –0.3 SDS, p<0.01) and shorter stature (-1.5 SDS versus –0.5 SDS, p<0.001) than mothers born appropriate for gestational age (AGA). Fathers born SGA also had a larger head circumference and shorter stature than AGA fathers but these differences were not significant. Glucose and insulin levels and SD scores for waist circumference, BMI, systolic and diastolic blood pressure, TC and HDL-c of SGA parents were not significantly different from those born AGA.

Metabolic Syndrome (MS)

Table 4 shows the different components of the MS. According to the ATP III criteria, the prevalence of MS was 15% in mothers and 22% in fathers. The number of MS components in parents born SGA was not significantly different from those born AGA. There was one child (age 5 years) with three components of the MS (elevated blood pressure, high

fasting glucose level and a low fasting HDL-c level).

Table 4: Components of metabolic syndrome according to ATP III criteria

	Mothers		Reference			Fathers		Reference			Children	
	n			P value		n			P value			
High sys BP	229	23%	20%	0.32		204	43%	41%	0.70		271	13%
High dia BP	229	29%	27%	0.46		204	38%	37%	0.84		271	6%
Central obesity	225	27%	34%	0.43		199	19%	21%	0.81		286	none
High glucose	92	7%	3%	0.05		78	19%	8%	<0.01		153	5%
Low HDL-c	92	30%	41%	<0.05		78	24%	37%	<0.05		73	6%
High TG	92	17%	-	-		78	35%	-	-		63	18%
≥3 criteria	92	15%	-	-		78	22%	-	-		64	2%

n = number of persons; p values express the difference with the reference group; BP = blood pressure; sys = systolic; dia = diastolic; HDL-c = high-density lipoprotein cholesterol; TG = triglycerides

Cardiovascular disease, DM type 2 and family history

One mother and four fathers had suffered from a non-lethal myocardial infarction (mother at age 44 years; fathers at ages 30, 36, 45 and 52 years, respectively). One father and two mothers had had deep vein thrombosis and one mother pulmonary embolism. Sixty-three of 254 (25%) mothers and 69/249 (28%) fathers had ≥ 1 first-degree relative with CVD. Of these relatives, CVD occurred before the age of 60 in respectively 12 of 63 (19%) and 19 of 69 (28%). Two mothers and one father had DM type 2, whereas 53 (21%) mothers and 49 (20%) fathers had ≥ 1 first-degree relative with DM type 2.

Correlations between parents and children

There was no correlation between birth weight SDS, birth length SDS, systolic and diastolic BP SDS, BMI SDS, fasting levels of lipids and insulin and HOMA-IR within parent-child couples. Glucose levels correlated positively between mothers and children ($r = 0.4$, $p < 0.01$) and fathers and children ($r = 0.3$, $p < 0.05$). Chi-square tests showed no association between parents and children in having a glucose level ≥ 5.6 mmol/l. Head circumference correlated weakly between mothers and children ($r = 0.2$, $p < 0.01$) and fathers and children ($r = 0.3$, $p < 0.001$).

DISCUSSION

This study shows that parents of short SGA children have a modestly higher prevalence of cardiovascular risk factors than age- and sex-matched references. Mothers had higher mean systolic blood pressure SDS, fathers had higher mean BMI SDS and parents had more frequently high fasting glucose levels. On the other hand, parents had less frequently low HDL-c levels than references. Risk factors did not correlate between parents and offspring. To the best of our knowledge, we provide the first data on cardiovascular risk factors in a large cohort of parents of short SGA children.

Our hypothesis was based on reported associations between low birth weight and cardiovascular risk, within subjects and across generations (26). The exact mechanism behind these associations is not completely understood. The fetal origins hypothesis poses that exposure to an adverse in utero environment leads to permanent programming of tissue function and an inceased risk of CVD (27). Alternatively, low birth weight and adult cardiovascular disease might be independent features of a genetic predisposition to CVD (28, 29). There is also evidence of a nongenetic predisposition to low birth weight and adverse cardiovascular risk across a number of generations (26). Because we found only a modest increase of risk factors in parents and no correlation of risk factors between parents and children, we could not demonstrate a familial cause for an increased risk for CVD in short SGA subjects.

Systolic blood pressure of the mothers was significantly higher than that of age-matched female references. Systolic and diastolic blood pressure of the children was also higher than reference values. However, blood pressure in fathers was normal and there was no significant difference in the percentage of parents with elevated blood pressure according to the ATP III criteria (>130 / 85 mm Hg) compared with references. A limitation is that blood pressure was measured thrice within 10 min and not during 24 h. Also, parents were significantly shorter than reference subjects. As blood pressure is known to increase with height (30, 31), parental blood pressure was relatively high for their shorter height.

The percentage of parents with fasting glucose levels above the ATP III cutoff level of 5.6 mmol/L was higher than in age- and sex-matched references. Higher fasting glucose levels might indicate insulin resistance, which in turn might increase the risk to develop DM and subsequently CVD (32). HOMA-IR, on the other hand, was <1, indicating a low level of insulin resistance (22,33). The percentage of parents with a family history of DM

type 2 was comparable with that in a population-based Danish cohort (34).

Parents had higher cholesterol levels than age- and sex-matched references and fathers had significantly more frequent hypercholesterolemia. LDL-c levels were not measured in references but levels were above the normal range of our laboratory in 11% of the mothers and 25% of the fathers. On the other hand, HDL-c levels were also significantly higher in parents than in references. As HDL-c levels contribute to total cholesterol levels, the higher total cholesterol level in parents might thus be explained. This is supported by the total cholesterol/HDL-c ratio of the parents, which was slightly lower than the ratio of the white participants of the community-based Bogalusa Heart Study (35). Higher HDL-c levels are generally known to lower the risk for cardiovascular disease. Therefore , the higher total cholesterol level in the parents might not reflect an increased risk.

Prevalence of MS was 15% in mothers and 22% in fathers. Comparing the prevalence of MS between parents and the age-matched reference group was not possible because triglyceride (TG) levels were not measured in the reference group. No other study described the prevalence of MS in healthy subjects of the same age as our parents. Most reports on the prevalence of MS in Europe comprise older populations or populations with DM or CVD. For example the Dutch Hoorn study (age 50-75 years) reported a prevalence of MS of 19% in females and 26% in males (36). A German study (PROCAM study, age 36-39 years) reported a prevalence of 18% in females and 25% in males (37). In a Danish cohort of women (age 45 years), the prevalence of MS was 15% (34). Compared with these cohorts, the prevalence of MS in parents of short SGA children was comparable or lower.

Power calculations (alpha 0.05 and power 0.8) showed that correlations within parent-child couples with an $r>0.3$ were detectable for birth weight, birth length, systolic and diastolic blood pressure, BMI, head circumference, glucose and insulin levels. Thus, for these parameters there was sufficient power to detect clinically relevant correlations. We only found a correlation between parents and children with regard to glucose levels and head circumference. In contrast to previous population-based studies (9,10,38), there were no correlations for birth weight or blood pressure. Lipid levels were measured in a smaller number of children. As a result, our study did not have enough power to detect correlations in lipid levels between parents and children.

A substantial proportion of parents (24% mothers and 10% fathers) with known birth characteristics, was born SGA themselves but they did not have more cardiovascular risk

factors than the parents who were born AGA. However, the absolute number of parents born SGA was small and even less of them provided a blood sample. This small number limited the power to detect differences between parents born SGA and parents born AGA.

Several other factors may have influenced our results. First, the parent group was relatively young. It is possible that increased risk for cardiovascular disease will be detectable when they are 10 years older. On the other hand, in the children, a higher diastolic and systolic blood pressure was already present at a mean age of 6.4 years. Second, parents with an available blood sample were younger and had a lower systolic blood pressure than parents without an available blood sample. Because analyses were performed after the collection of blood samples, we do not think there was a selection bias. Third, the references were 5 years older than the parents. By transforming all outcome variables to SD scores before analysis, we corrected for the difference in mean age of both populations. Therefore, the difference in mean age cannot explain the absence of markedly increased cardiovascular risks.

In conclusion, parents of short SGA children have a modest increase in some cardiovascular risk factors. Mothers had higher mean systolic blood pressure SDS and fathers had a higher mean BMI SDS, whereas parents had more often high fasting glucose levels than age- and sex-matched references. On the other hand, HOMA-IR was low and parents had less frequently low HDL-c levels than references. Also, the prevalence of MS according to ATP III criteria was 15% in mothers and 22% in fathers, which is not higher than the reported prevalence. Risk factors did not correlate between parents and children and a substantial proportion of parents (24% mothers and 10% fathers) with known birth characteristics, was born SGA themselves.

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CHAPTER 3

Thyroid function in short children born small for gestational age (SGA) before and during Growth hormone treatment

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ABSTRACT

Context: Disturbances in thyroid function have been described in SGA children but the influence of prematurity is unclear. In addition, the effect of growth hormone (GH) treatment on thyroid function has not been studied in short SGA children.

Objectives: To determine whether short SGA children have higher TSH levels compared to age-matched controls and evaluate the influence of gestational age. To investigate whether GH treatment alters thyroid function.

Patients: 264 short SGA children (116 preterm), pre-pubertal and non-GH deficient.

Measurements: Serum FT4 and TSH at baseline and after 6, 12 and 24 months of GH treatment.

Results: Baseline mean TSH was higher in preterm short SGA children than in age-matched controls ($p<0.05$). Mean FT4 was not significantly different between short SGA children and controls. Baseline FT4 or TSH did not correlate with gestational age, or SDS for birth weight, birth length, height, BMI, IGF-1 or IGFBP-3. Mean FT4 decreased significantly during the first 6 months of GH treatment, but remained within the normal range. TSH did not change during treatment. The change in FT4 did not correlate with the change in height SDS during 24 months of GH treatment.

Conclusion: Preterm short SGA children have higher, although within the normal range, TSH levels than controls. The level of TSH does not correlate with gestational age, birth weight SDS or birth length SDS. FT4 decreases during GH treatment, but is not associated with an increase in TSH, nor does it affect the response to GH treatment. Since these mild alterations in thyroid function do not appear clinically relevant, frequent monitoring of thyroid function during GH therapy is not warranted in short SGA children.

INTRODUCTION

Since recombinant human growth hormone (GH) treatment was licensed (Food and Drug Administration, FDA, 2001; European agency for the Evaluation of Medicinal products, EMEA, 2003) for short children born SGA, it has become a frequently applied growth promoting therapy. Because normal thyroid status is necessary for growth and development, it is important to know whether GH treatment alters thyroid function.

Reports about thyroid function in untreated children born SGA are limited. Short SGA children were reported to have higher TSH levels compared to SGA children with catch-up growth (1) but these results could not be replicated in term SGA children (2). A recent paper suggested that not SGA, but preterm birth is associated with higher TSH levels (3). However, this was studied in SGA children with catch-up growth. There are no data on thyroid hormone levels in term and preterm short SGA children. In addition, GH treatment was associated with a decline in serum FT4 without a change in TSH level in other patient groups (4-6). The effect of GH administration on thyroid function in short SGA children has not been studied yet.

In this paper we report FT4 and TSH levels in a large group of short SGA children before and during 2 years of GH treatment. We hypothesized that short SGA children have higher TSH levels compared to reference values. To evaluate whether thyroid hormone levels are related to prematurity or SGA, we evaluated term and preterm short SGA children separately. Our second hypothesis was that FT4 levels would decrease during GH treatment.

METHODS

Subjects

The study group consisted of 264 prepubertal short children born SGA (144 boys; 116 born preterm). Children were included according to the following criteria: 1) birth length and/ or birth weight standard deviation score (SDS) below –2 for gestational age (7); 2) height SDS for age below – 2 according to Dutch standards (8); 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth; 4) prepubertal stage defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys; 5) 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 chronic lung disease. Children with GH deficiency (peak response of serum GH < 20 mU/l to a provocative test (arginine or clonidine)), endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded, with the exception of Silver-Russell syndrome. The Medical Ethics Committees of the participating centers approved the study and written informed consent was obtained from the parents.

Study design

All children were treated with biosynthetic GH at a dose of 1 mg /m2·day. GH was administered subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area. At baseline and after 6, 12 and 24 months of GH treatment, standing height and weight were measured and body mass index (BMI) was calculated. Height, weight for height and BMI were expressed as SD scores adjusting for sex and age according to Dutch reference data (8). At the same time points, blood samples were taken for determination of FT4, TSH, IGF-I and IGFBP-3 levels. Prematurity was defined as a gestational age <37 weeks. Because this was a multicenter study and only measurements performed in one hospital were evaluated, there were missing data at the different time points. The number of patients after 6, 12 and 24 months of GH treatment was 174, 177 and 163, respectively.

Assays

Serum FT4 and TSH levels were measured by Vitros Eci technology (Ortho-Clinical

Diagnostics, Amersham, UK). The within-assay coefficients of variation of Vitros were 3-7% for FT4 and 8-9% for TSH. The between-assay coefficients of variation were 5-10% for FT4 and 5-7% for TSH. Normal TSH reference values were 0.7 – 6.4 mU/l (-2 SD to 2 SD). Normal FT4 reference values were 11.8 – 26.2 pmol/l (-2 SD to 2 SD). Thyroid hormone levels were expressed as age-adjusted SD scores, which were calculated with data of a control group comprising 500 healthy Dutch children (age 1.4-18 years) from the Rotterdam region, The Netherlands, participating in a study to assess reference values. The Medical Ethics Committee of the Erasmus Medical Centre Rotterdam approved this reference study, and written informed consent was obtained from the parents or guardians. All measurements were performed at the same laboratory. Serum levels of total IGF-I and IGFBP-3 were measured in another laboratory using specific RIAs, as previously described (9-11). Serum levels of total IGF-I and IGFBP-3 were also expressed as SDS adjusting for age and sex, using reference values for healthy children with normal stature determined in the same laboratory (12).

Statistics

All analyses were performed using the statistical package SPSS (version 11.1; SPSS Inc., Chicago, IL) for Windows. Differences between term and preterm born children at baseline were evaluated using an independent sample-t-test. We used a one-sample t-test to compare results, expressed as SDS, with 0 SDS (median value for age-matched healthy references). To correct for missing data and multiple testing, changes over time were analyzed with repeated measures of variance (mixed model ANOVA). Firstly, an F test was performed to test whether time had a significant effect. Then, only when $p<0.005$, repeated measures of variance (mixed model ANOVA) was used to test differences between baseline and different time points. Clinical data are presented as mean (SD), model estimates as mean (95% CI). Correlations were calculated using Spearman's correlation coefficient. Multiple linear regression analysis was used to assess multivariable relationships between birth characteristics and FT4 and TSH levels at the start of GH treatment. Statistical significance was defined as $p < 0.05$.

RESULTS

Clinical characteristics at baseline

Mean (SD) age was 7.39 (2.6) years (Table 1). Preterm children had significantly lower birth weight SDS, BMI SDS and head circumference SDS and significantly higher height SDS than term children (Table 2). Mean height SDS was significantly lower compared to age-matched references in all SGA patients. Of 3 adopted children no information was available about their gestational age.

Table 1. Clinical characteristics at baseline	
n	264
Male/female	145/119
Gestational age	36.3 (3.5)
Preterm/ term	116/145
Birth weight SDS	-2.16 (1.2)[a]
Birth length SDS	-2.89 (1.5)[a]
Age	7.39 (2.6)

Data expressed as mean (SDS)
Compared with zero SDS (median for age and sex): [a]p<0.001

Thyroid hormone levels at baseline

Preterm children had significantly higher FT4 SDS than term children but in both groups FT4 SDS was comparable with age-matched references (Table 2). FT4 levels were above the normal range (>2 SDS) in 2% of the term children and in 4% of the preterm children, with values ranging from 26.2 to 41.9 pmol/l. None of the children had a FT4 level < -2 SDS. TSH SDS was significantly higher in preterm short SGA children than in age-matched references ($p<0.05$). TSH levels were above the normal range (>2 SDS) in 2% of the term children and in 4% of the preterm children, with values ranging from 6.4 to 7.6 mU/l. There was no significant relation between prematurity and TSH levels above the upper limit of normality. TSH levels were below the normal range (< -2 SDS) in 2% of the children, in both the term and the preterm group.

Table 2. Preterm vs. term born short SGA children at baseline		
	Preterm (n=116)	Term (n=145)
Birth weight SDS	-2.46 (1.4)[a,d]	-1.92 (0.9)[d]
Birth length SDS	-3.19 (2.1)[d]	-2.71 (1.0)[d]
Age	6.86 (2.4)[b]	7.80 (2.7)
Height SDS	-2.88 (0.5)[b,d]	-3.05 (0.6)[d]
Weight SDS	-1.58 (1.2)[a,d]	-0.92 (1.1)[d]
BMI SDS	-1.58 (1.1)[a,d]	-1.11 (0.9)[d]
Head Circ. SDS	-1.45 (0.9)[c,d]	-1.16 (0.9)[d]
TSH	2.74 (1.5)	2.53 (1.2)
TSH SDS	0.23 (1.0)[e]	0.14 (0.9)
FT4	19.63 (4.1)[c]	18.65 (3.4)
FT4 SDS	0.18 (1.1)[c]	-0.10 (0.9)

Data expressed as mean (SDS)
Preterm short SGA subjects vs term short SGA subjects: [a]p<0.001; [b]p<0.01; [c]p<0.05
Compared with zero SDS (median for age and sex): [d]p<0.001; [e]p<0.05

Table 3 Thyroid hormone levels before and during GH treatment				
	T=0	T=6 mo	T=12 mo	T=24 mo
FT4	19.1 (18.6 to 19.6)	17.6 (17.2 to 18.0)[a]	17.5 (17.1 to 18.0)[a]	17.8 (17.4 to 18.2)[a]
FT4 SDS	0.03 (0.1 to 0.2)	-0.38 (-0.5 to -0.3)[a,c]	-0.40 (-0.5 to -0.3)[a,c]	-0.33 (-0.4 to -0.2)[a,c]
TSH	2.6 (2.5 to 2.8)	2.6 (2.5 to 2.8)	2.5 (2.3 to 2.6)[b]	2.5 (2.4 to 2.7)
TSH SDS	0.17 (0.1 to 0.3)[d]	0.23 (0.1 to 0.3)[c]	0.11 (o.1 to 0.2)[b]	0.17 (0.1 to 0.3)[d]

Data expressed as model estimate (95% CI)
Compared with baseline values: [a]p < 0.001
Compared with previous visit: [b]p<0.05
Compared with zero SDS (median for age): [c]p<0.001; [d]p< 0.01

Thyroid hormone levels during GH treatment

As shown in Figure 1, FT4 SDS decreased significantly during the first 6 months of GH treatment and remained subsequently at the same level until 24 months. Although reduced compared to baseline, the levels remained within the normal range. Mean TSH SDS did not change during treatment (Table 3). Changes in FT4 SDS did not correlate with changes in TSH SDS. None of the children developed symptomatic hypothyroidism during the follow-up period. The effect of GH treatment on FT4 levels was the same for

term and preterm born children (p = 0.99). Table 4 shows the change of anthropometric parameters and serum levels of IGF-I and IGFBP-3 during GH treatment.

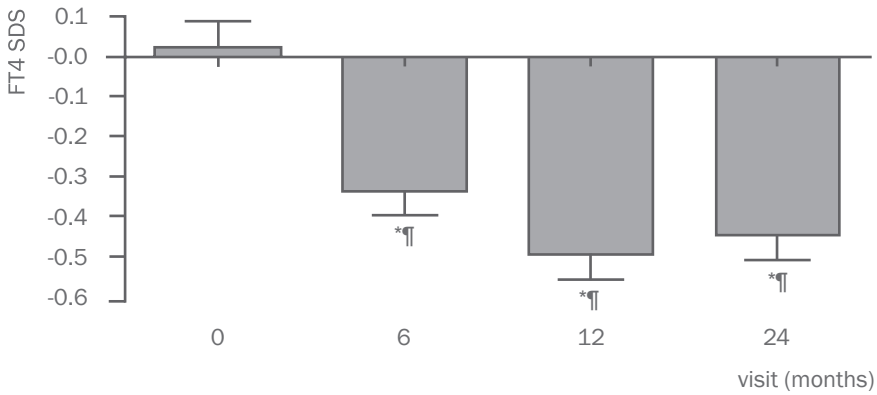

Figure 1: FT4 during GH treatment
Compared with zero SDS (median for age): *p < 0.001
Compared with baseline values: ¶p < 0.001

Correlations

There was no significant correlation between baseline FT4 SDS or TSH SDS and birth weight SDS, birth length SDS or gestational age. Baseline FT4 SDS and baseline TSH SDS did not significantly correlate. There were also no significant correlations between baseline FT4 SDS or TSH SDS and height SDS, BMI SDS, IGF-1 SDS or IGFBP-3 SDS.

The change in height SDS during 24 months GH treatment (Δ height) did not correlate with the 24-months change in FT4 SDS (Δ FT4) or the change in TSH SDS. However, Δ FT4 correlated weakly with the 24-months change in IGF-I SDS (Δ IGF-I) (r = -0.22; p = 0.009). Multiple regression analysis with Δ height as the dependent variable and age at start of treatment, sex, Δ FT4 and Δ IGF-I as independent variables yielded a significant model that explained 60% of the variation of Δ height. However, Δ FT4 did not significantly contribute to the model (β = 0.03, p = 0.246) in contrast to Δ IGF-I (β = 0.09, p < 0.001). This means that corrected for age, sex and the 24-months increase in IGF-1, the decrease of FT4 SDS had no influence on the 24-months change in height SDS during GH treatment.

Using multiple regression, we evaluated whether, corrected for age and sex and

gestational age, birth weight SDS or birth length SDS influenced TSH levels at the age when GH treatment was started. These predictors could, however, not explain the variation in TSH levels, indicating that neither the degree of SGA (birth weight SDS or birth length SDS) nor being born preterm (gestational age) is associated with higher TSH levels.

Table 4. Anthropometric parameters, IGF-1 and IGFBP-3 during GH treatment

	T=0	T=6 months	T=12 months	T=24 months
Height SDS	-1.8 (-1.9 to -1.7)[a,b,d]	-2.2 (-2.3 to -2.2)[a,b,d]	-2.5 (-2.6 to -2.5)[a,d]	-3.0 (-3.1 to -2.9)[d]
Weight SDS	-0.7 (-0.8 to -0.5)[a,b,d]	-0.9 (-1.1 to -0.8)[a,b,d]	-1.1 (-1.2 to -1.0)[a,d]	-1.2 (-1.4 to -1.1)[d]
BMI SDS	-1.3 (-1.5 to -1.2)[d]	-1.3 (-1.4 to -1.2)[d]	-1.2 (-1.3 to -1.1)[a,b,d]	-1.0 (-1.1 to -0.9)[a,b,d]
Head Circ. SDS	-1.3 (-1.4 to -1.2)[d]	-1.1 (-1.2 to -1.0)[a,d]	-1.0 (-1.1 to -0.9)[a,c,d]	-0.9 (-1.0 to -0.8)[a,b,d]
IGF-1 SDS	-1.2 (-1.4 to -1.1)[d]	0.7 (0.6 to 0.9)[a,d]	0.6 (0.4 to 0.8)[a,d]	0.9 (0.7 to 1.1)[a,c,d]
IGFBP-3 SDS	-1.2 (-1.3 to -1.1)[d]	0.1 (0.0 to 0.3)[a]	0.2 (0.0 to 0.3)[a]	0.2 (0.1 to 0.4)[a,e]

Data expressed as model estimate (95% CI)
Compared with baseline values: [a]p<0.001
Compared with previous visit: [b]p<0.001; [c]p<0.01
Compared with zero SDS (median for age and sex): [d]p<0.001; [e]p<0.05

DISCUSSION

We studied thyroid hormone levels in a large population of prepubertal non-GH deficient short SGA children. Baseline mean TSH levels were within the normal range but significantly higher than mean levels of age-matched references. This was more evident in preterm born short SGA children, although gestational age did not correlate with TSH levels. During growth hormone treatment, FT4 levels decreased significantly within 6 months after start, but levels remained within the normal range. The change in FT4 levels was not associated with a change in TSH levels and there was no effect on the 2-year-growth response. None of the children developed symptomatic hypothyroidism.

Our results are not completely comparable to the few reports that have been published about thyroid hormone levels in SGA children because they studied SGA children with catch-up growth to a normal height (1-3, 13). These studies intended to determine the roles played by size at birth and gestational age on thyroid function in childhood. SGA children without catch-up growth had higher TSH levels compared to SGA

children with catch-up growth (1) but these results could not be replicated in term SGA children (2). One study reported no significant difference in FT4 and TSH levels between 82 SGA children and 53 AGA children (1), while another study described significantly higher TSH levels in 40 SGA children compared to 35 term AGA children (13). When 88 SGA children (56 preterm) were compared to 29 preterm AGA children, there was no significant difference in TSH levels (3). TSH correlated negatively with gestational age, independently from birth weight or birth length. (3) In our much larger population of 264 short SGA children, we found no correlation between TSH and gestational age. TSH levels were significantly greater than zero SDS in preterm SGA children and not significantly different from zero SDS in those born at term but there was no significant difference in TSH SDS between term and preterm short SGA children. Also, we found no significant influence of birth length SDS or birth weight SDS on TSH levels. We therefore consider the association between size at birth or gestational age and TSH levels to be weak and not clinically relevant.

To our knowledge, this is the first report about the effect of GH treatment on thyroid function in short SGA children. In GH deficient children and adults and girls with Turner's syndrome, the effect of GH treatment has been studied (5, 6, 14, 15). Similar to our population, a decrease of FT4 levels without a change in TSH levels was described. In most studies, these changes were transitory and disappeared during the second year of GH treatment (5, 6, 14, 15). Others reported a decline in FT4 levels that was maintained during the follow-up period (4), corresponding with our results. The only study performed in short non-GH-deficient children reported no effect of GH treatment on thyroid function (16). However, it is not stated whether the participating subjects had idiopathic short stature (ISS) or were short SGA and the study included only 20 patients receiving GH 3 days a week and in a much lower dose than the children in our study. Therefore, these results are not comparable to ours.

Two mechanisms have been suggested to explain the decline in FT4 levels without a parallel change in TSH levels during GH treatment. The first one points to an inhibition of TSH release mediated by a hypersecretion of somatostatin directly induced by GH (17, 18). The second explains the discrepancy by an increase of peripheral conversion of FT4 to T3 (6, 17, 19). This effect might be mediated by either GH itself or via IGF-I, or both (6, 20).

Because none of the children developed hypothyroidism and there was no effect on the growth response, there seems to be no clinical relevance of the GH-related decline in FT4 levels. This indicates that it is not required to monitor thyroid function more than once

a year in short SGA children during GH therapy, in contrast to children with multiple pituitary hormone deficiencies who may develop true hypothyroidism during GH therapy (4).

We did not measure autoantibodies to thyroglobulin or thyroid peroxidase as FT4 levels remained in the normal range, in combination with completely normal TSH levels. In the study by Radetti et al, none of the SGA children with TSH levels above the upper limit of normal had thyroid antibodies (3).

In conclusion, our study demonstrates that preterm born short SGA children have TSH levels within the normal range, although significantly higher than the average level in healthy controls. The higher TSH levels could not be explained by gestational age, birth weight SDS or birth length SDS. GH treatment coincided with a significant decrease of FT4 during the first 6 months of treatment but mean levels remained in the normal range and there was no compensatory increase in TSH. The decrease in FT4 levels did not affect the 2-year growth response during GH treatment.

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CHAPTER 4

Does preterm birth influence the response to growth hormone treatment in short, small for gestational age children?

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ABSTRACT

Objective: To investigate if prematurity has an independent influence on the response to growth hormone (GH) treatment, in short, small for gestational age (SGA) children.

Design: Longitudinal 3-year GH study

Patients: 392 prepubertal non-GH deficient, short SGA children, divided in 138 preterm (<36 weeks) and 254 term (≥ 36 weeks) children.

Measurements: Height, weight, head circumference, skinfolds and serum insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3) levels were measured before start of GH treatment and after 6 months, 1, 2 and 3 years of treatment.

Results: Preterm short SGA children were significantly lighter and shorter at birth after correction for gestational age than term short SGA children ($p<0.001$). At start of GH treatment, preterm children were significantly shorter than term children when height was corrected for target height (TH). Preterm children were also significantly leaner as shown by a lower BMI SDS and lower sum of 4 skinfolds SDS. Prematurity had no influence on childhood IGF-I and IGFBP-3 levels. The response to GH treatment was similar for preterm and term SGA children.

Conclusions: Within a population of short SGA children prematurity is associated with a smaller size for gestational age, and a shorter height corrected for TH and leaner phenotype in childhood. The response to GH treatment is similar for preterm and term short SGA children.

INTRODUCTION

Since growth hormone (GH) treatment was licensed for short children born small for gestational age (SGA), it has become a frequently applied growth promoting therapy. Short SGA children comprise a heterogeneous group because some are born at term and others are born preterm. Despite this important difference, all SGA children are treated in the same way. SGA is probably caused by a combination of environmental and genetic factors and the contribution of these components is variable (1). The prenatal environment and genetic background might be different when there is a shorter duration of pregnancy. Therefore, preterm birth might be associated with a smaller size for gestational age.

Children who are born SGA have a suboptimal growth pattern in utero. Prematurity often has an additional detrimental effect on spontaneous postnatal growth (2). SGA children with persistent short stature failed to catch-up to a normal height during infancy. When preterm short SGA children suffer from an extra growth impairment during their neonatal period, this may result in a height significantly below the genetic height potential of the child (3). We hypothesized that premature SGA children have a greater height deficit at birth and due to less optimal circumstances in the neonatal period also later on, which would lead to a smaller GH-induced catch-up growth in preterm born short SGA children than in those born at term. To test our hypothesis, we compared preterm short SGA children with short SGA children born at term, with respect to birth characteristics, anthropometry when GH treatment was started and growth during GH treatment.

PATIENTS AND METHODS

Subjects

The study cohort consisted of 392 prepubertal short children born SGA (217 boys). Children were included according to the following criteria: 1) birth length and/ or birth weight standard deviation score (SDS) below –2 for gestational age (4); 2) height SDS for age below – 2 according to Dutch standards (5); 3) height velocity SDS below zero to exclude children with spontaneous catch-up growth; 4) prepubertal stage defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys; 5) 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 chronic lung disease. Children with GH deficiency (defined as an IGF-I level below –2 SDS and a GH peak < 10 µg/l during two GH stimulation tests), other endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded. Children without information on gestational age were excluded as well. In total, 138 children (79 boys) were born preterm, defined as a gestational age < 36 weeks, and 254 children (138 boys) were born at term (≥ 36 weeks). The gestational age of the subjects was determined by ultrasound scans during the first trimester, if available, and otherwise calculated from the date of the last menstruation. The Medical Ethics Committees of the participating centers approved the study and written informed consent was obtained from the parents.

Study design

All children were treated with biosynthetic GH at a dose of 1 mg/m^2 body surface area (~ 0.033 mg/kg), administered subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

At baseline and subsequently every three months after the start of GH treatment, height, weight, head circumference and Tanner stage were recorded, as previously described (6). To exclude the influence of puberty on linear growth and body composition, only measurements in prepubertal children were included in the analyses. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Target height was calculated as TH = (maternal height + paternal height +13) / 2 + 4.5 for boys and TH = (maternal height + paternal height –13) / 2 + 4.5 for girls (7). Height, head circumference,

target height and BMI were expressed in SDS adjusting for age and sex according to Dutch reference data (5). Height corrected for target height was calculated as height SDS – target height SDS. Biceps, triceps, subscapular and suprailiacal skinfold thickness was measured at baseline and subsequently every six months after the start of GH treatment, using a Holtain skinfold caliper (8). For skinfold thickness analysis, the mean of three measurements, expressed as SDS using references for healthy Dutch children, was used (9). Limb skinfold SDS was calculated as biceps SDS + triceps SDS. Trunk skinfold SDS was calculated as subscapular SDS + suprailiacal SDS. Bone age (BA) was determined at start and after 1, 2 and 3 years of GH treatment according to Greulich & Pyle (10). At baseline and after 6 months, 1, 2 and 3 years of GH treatment a blood sample was taken. After centrifugation all samples were frozen (-80 °C) until assayed.

Assays

Serum insulin-like growth factor (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) were measured in one laboratory using specific RIA's, as previously described (11-13). Serum levels of total IGF-I and IGFBP-3 were expressed as SDS adjusting for age and sex, using reference values for healthy children with normal stature determined in the same laboratory (14).

Statistics

A one-sample t-test was used to compare data, expressed as SDS, with those of healthy references (0 SDS). An independent samples t-test was used to compare preterm and term children at baseline. Clinical data are presented as mean (SD). Statistical significance was defined as $p < 0.05$.

The number of prepubertal patients after 6 months, 1, 2, and 3 years of GH treatment was 363, 354, 293, and 191, respectively. To correct for missing data and multiple testing, the changes over time and differences between preterm and term children were analyzed with repeated measures of variance (15). Firstly, we tested whether prematurity had a significant effect on the change of the various parameters during GH treatment. This was done by testing whether the interaction time*prematurity was significant in a model with time and prematurity as covariables. Because all p values were > 0.05, further analyses were not performed. Model estimates are presented in figures as mean (95% CI).

Correlations were calculated using Spearman's rank correlation coefficient. Multiple

linear regression analysis was used to assess multivariable relationships between prematurity and response to GH treatment.

Analyses were performed using the statistical package SPSS (version 15.0; SPSS Inc., Chicago, IL) for Windows. For repeated measures of variance analyses SAS 8.2 (SAS Institute Inc., Cary, /nC, USA) was used.

RESULTS

Size at birth

Birth characteristics of the preterm and term short SGA children are shown in Table 1. Unfortunately, birth length and birth head circumference are not routinely measured by all obstetricians. As auxological data at birth were collected retrospectively we were not able to obtain information on birth length and head circumference from all subjects. Birth weight SDS and birth length SDS were significantly lower in preterm than in term short SGA children. Preterm children also had a smaller head circumference SDS at birth than term children but this difference did not reach statistical significance. A positive value for the difference between birth head circumference SDS and birth length SDS indicates brain sparing and a negative value indicates that head circumference SDS is

Table 1. Clinical characteristics

	Preterm			Term			
	N			N			P value
Male/female	138	79 / 59		254	138 / 116		0.58
Gestational age	138	32.21(2.6)		254	38.6 (1.5)		< 0.001
range		25.7 to 35.9			36.0 to 42.0		
Birth weight SDS	138	-2.8 (1.3)*		254	-2.0 (1.0)*		< 0.001
range		-5.4 to 1.3			-4.7 to 0.4		
Birth length SDS	85	-3.7 (1.9)*		185	-2.9 (1.2)*		< 0.001
range		-8.6 to 1.5			-7.2 to 0.1		
Birth Head Circ. SDS	38	-2.3 (1.3)*		45	-1.7 (1.3)*		0.054
range		-5.6 to 0.5			-4.9 to 1.3		
Birth Head Circ. SDS - length SDS	26	2.1 (1.2)*		36	1.6 (1.8)*		0.28

Data expressed as mean (SD)
Compared with zero (median for gestational age and sex): *p < 0.001

lower than birth length SDS. As shown in Table 1, the degree of brain sparing at birth was not significantly different between preterm and term children.

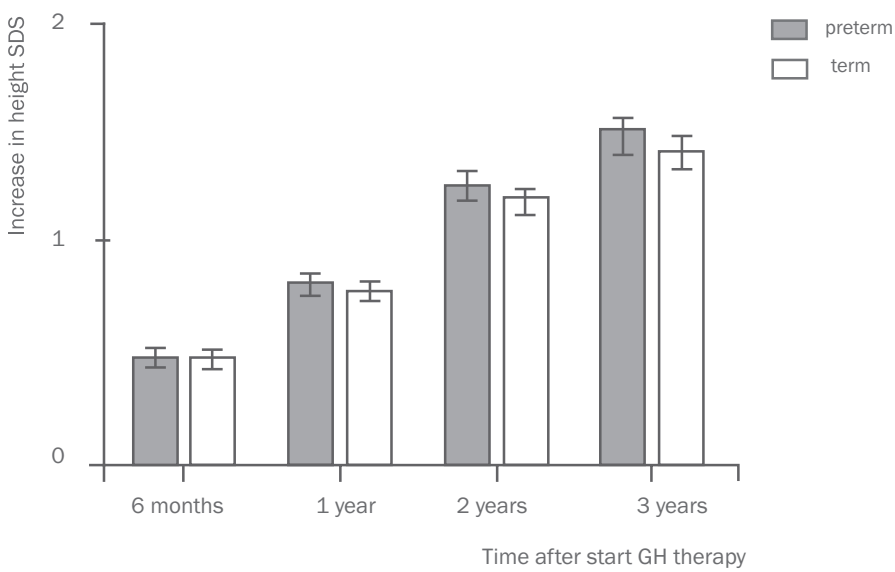
Table 2. Anthropometry and IGF-I and IGFBP-3 levels at start and after 3 years of GH treatment in non-GH-deficient SGA children

	Preterm			Term			
	Baseline	After 3 years		Baseline	After 3 years	P value§	P value¶
n	138	66		254	127		
Age (years)	6.60 (2.3)	8.36 (1.4)		7.19 (2.6)	8.82 (1.6)	**0.02**	
Height SDS	-2.96 (0.6)*	-1.22 (0.5)*		-3.04 (0.6)*	-1.51 (0.7)*	0.21	0.52
Target Height SDS	-0.30 (0.8)*			-0.64 (0.8)*		**<0.001**	
Adjusted Height SDS [1)]	-2.66 (0.9)*	-1.04 (0.9)*		-2.39 (0.8)*	-0.91 (0.9)*	**0.004**	0.60
BMI SDS	-1.68 (1.0)*	-1.18 (1.1)*		-1.19 (1.1)*	-0.77 (1.0)*	**<0.001**	0.20
S4SF SDS	-1.22 (0.9)*	-1.46 (1.1)*		-0.89 (1.2)*	-1.30 (1.0)*	**0.004**	0.36
Limb SF SDS [2)]	-1.65 (1.8)*	-2.71 (1.9)*		-1.03 (2.1)*	-2.44 (1.8)*	**0.004**	0.12
Trunk SF SDS [3)]	-2.38 (1.8)*	-2.00 (2.2)*		-1.90 (2.3)*	-1.66 (2.1)*	**0.02**	0.76
HC SDS	-1.45 (1.0)*	-0.69 (1.0)*		-1.11 (0.9)*	-0.64 (1.0)*	**0.003**	0.11
HC SDS - Height SDS	1.54 (1.1)	0.53 (1.0)		1.90 (1.0)	0.87 (1.0)	**0.001**	0.96
Bone age / age	0.85 (0.2)	0.99 (0.1)		0.85 (0.2)	0.96 (0.1)	0.99	0.34
IGF-I SDS	-1.09 (1.3)*	0.97 (1.6)*		-1.10 (1.3)*	0.92 (1.5)*	0.92	0.66
IGFBP-3 SDS	-1.18 (1.2)*	0.33 (0.7)**		-1.26 (1.1)*	0.38 (0.9)*	0.48	0.05

Data expressed as mean (SD)
[1)] Adjusted height SDS= height SDS – Target height SDS
BMI = body mass index; S4SF = sum of 4 skinfold thicknesses; HC = head circumference
[2)] Limb SF SDS = biceps SF SDS + triceps SF SDS;
[3)] Trunk SF SDS = subscapular SF SDS + suprailiacal SF SDS
Compared with zero SDS (median for age and sex): *p < 0.001; **p < 0.01
P value§ = preterm versus term at start of GH treatment
P value¶ = difference between preterm and term children in overall 3-years-growth response

Anthropometry at start of GH treatment

Before start of GH treatment, height SDS was comparable for preterm and term short SGA children (-2.96 versus –3.04 SDS, p = 0.21) (Table 2). However, adjusted for target height SDS, preterm children were shorter than term children (-2.66 versus –2.39 SDS, p = 0.004). Preterm children had a lower BMI SDS (-1.68 versus –1.19, p < 0.001) and lower sum of 4 skinfolds SDS (-1.22 versus –0.89, p = 0.004). Preterm children had lower skinfolds SDS in the limbs (-1.65 versus –1.03, p = 0.004) and trunk (-2.38 versus –1.90, p = 0.02). The distribution of subcutaneous fat, measured as the difference between limb and trunk skinfolds SDS (limb SF SDS – trunk SF SDS), was comparable in preterm and term children (0.73 versus 0.87, p = 0.36). At a mean age of 7 years, at start of GH treatment, head circumference was smaller in preterm children compared with term children (-1.45 versus –1.11, p = 0.003). The difference between head circumference SDS and height SDS was significantly smaller in preterm children compared with term children (1.54 versus 1.90, p = 0.001), indicating that preterm children also had smaller heads than term children in relation to their height. There were no significant differences between preterm and term children in SD scores of serum levels of IGF-I and IGFBP-3.



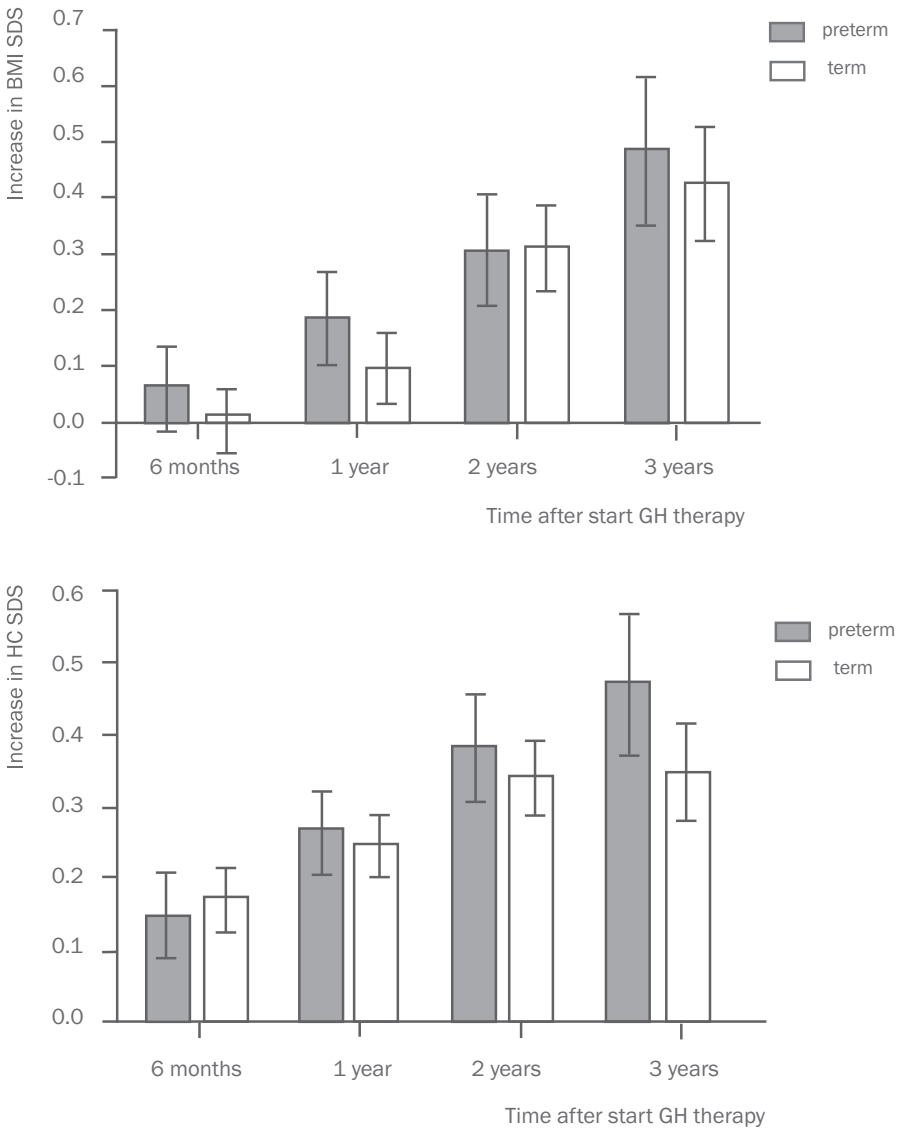
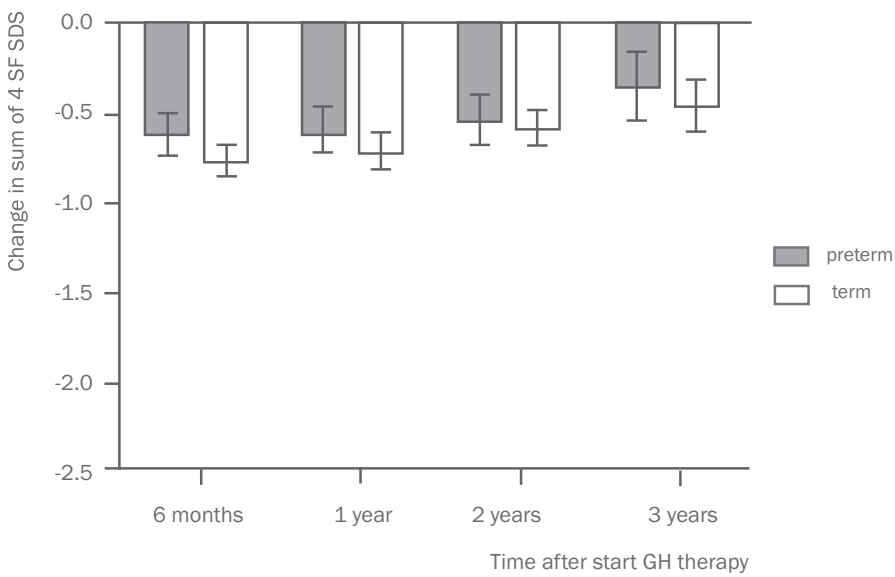


Figure 1. Change in anthropometry during 3 years of GH treatment
Data expressed as model estimate (95% CI)
BMI = body mass index; HC = head circumference
p values for difference in overall growth response between preterm and term children:
Δ height SDS: 0.52, Δ BMI SDS: 0.20 and Δ HC SDS: 0.11

Response to GH treatment

As shown in Figures 1 and 2, there was a parallel change of all anthropometric parameters in preterm and term children. Only measurements in prepubertal children were included in the analyses and a correction for missing data was made. The interaction between time and being born preterm was not significant for any of the parameters indicating that prematurity did not have a significant effect on the change of the parameters during GH treatment, including IGF-I and IGFBP-3 levels. This indicates that preterm birth does not influence the response to GH treatment. The increase in head circumference SDS was greater in preterm children than in term children but the difference did not reach significance ($p = 0.11$). Table 2 shows mean values of the various parameters after 3 years of GH treatment. The increase in height SDS during 3 years of GH treatment was similar for boys and girls. All children responded with an increase in height SDS during the 3-year treatment period (range 0.5 to 2.9 SDS). No side effects were observed during GH treatment. There was no significant correlation between the 1-year or 3-year increase in height SDS during GH treatment and gestational age, birth length SDS or birth weight SDS.



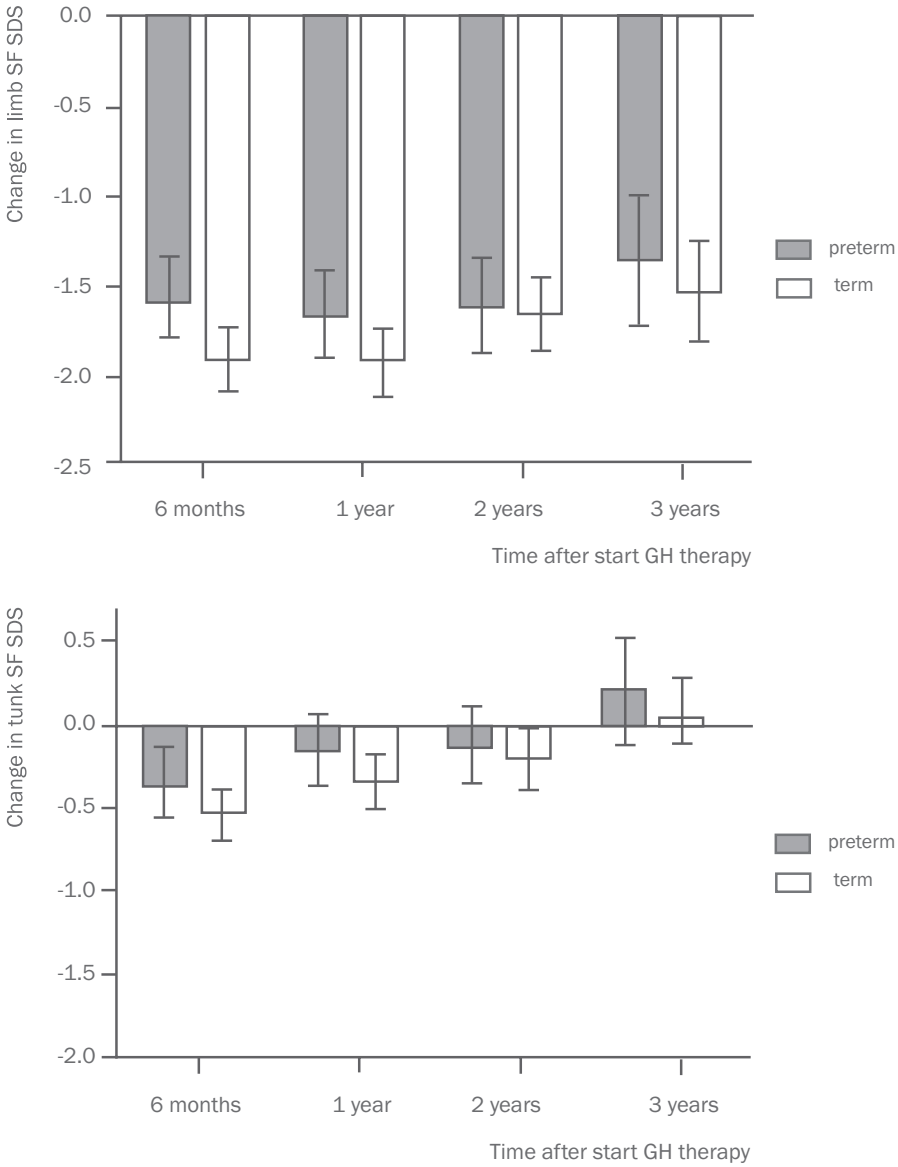


Figure 2. Change in skinfold thickness SDS during 3 years of GH treatment
Data expressed as model estimate (95% CI) SF = skinfold thickness
limb SF = biceps SF SDS + triceps SF SDS; trunk SF = subscapular SF SDS + suprailiacal SF SDS
p values for difference in overall growth response between preterm and term children: Δ sum of 4 SF SDS: 0.36, Δ limb SF SDS: 0.12 and Δ trunk SF SDS: 0.76

Because preterm and term children differed at baseline, we also investigated the influence of preterm birth on the 1-year and 3-year increase in height SDS during GH treatment corrected for possible confounders in a multiple regression analysis (Table 3). In both models, prematurity (yes/no) did not significantly contribute (β = -0.02, p = 0.58 and β = 0.09, p = 0.18, respectively). This suggests that corrected for age, sex, height SDS, target height SDS, IGF-I SDS and IGFBP-3 SDS at start, preterm birth has no influence on the change in height SDS during GH treatment. When we performed the same multiple regression analyses with gestational age instead of prematurity (yes/no), we found similar results.

DISCUSSION

In a large population of 392 short SGA children, we demonstrate that preterm SGA children were significantly smaller for gestational age than those born at term. During childhood, preterm SGA children remained significantly shorter than term SGA children compared to their target height (TH). Preterm SGA children were leaner and had a smaller head circumference. In spite of these differences at birth and at start of GH treatment, the growth response to GH treatment was similar for preterm and term short SGA children.

Birth length, weight and head circumference SD scores were more reduced in preterm SGA children than in term SGA children. Previously, our group demonstrated that the smallest SGA newborns were more often born prematurely and by caesarean section (16). The hypertensive complications of the mother or the severe intra uterine growth retardation might have led to an elective preterm birth to prevent further intrauterine growth deterioration. This might explain why the smallest SGA children were born preterm.

To the best of our knowledge, this is the first study evaluating whether preterm and term SGA children respond differently to GH treatment. We only included children without serious complications during their neonatal period but nonetheless preterm children often experience nutritional problems during the postnatal period (17, 18) which might inhibit postnatal growth (2). Because preterm SGA children have experienced this additional growth inhibition, we hypothesized that they would respond worse to GH treatment than term children. Growth during GH treatment was, however, similar for both groups. Head circumference increased more in preterm children but this difference did not reach significance.

Target height (TH) SDS was significantly greater in preterm children than in term

Table 3. Multiple linear regression analyses				
	Δ Height SDS 0-1 year		Δ Height SDS 0-3 years	
Variables	β	P-value	β	P-value
Prematurity#	-0.02	0.58	0.09	0.18
Age (yr)	-0.08	**<0.001**	-0.10	**<0.001**
Sex*	-0.08	**<0.01**	-0.13	**0.04**
Baseline Height SDS	-0.04	0.12	-0.12	**0.02**
Baseline IGF-I SDS	-0.02	**0.04**	-0.03	0.28
Baseline IGFBP-3 SDS	-0.03	**<0.01**	No effect	
Target height SDS	0.04	**0.03**	0.04	0.32
N		304		167
Overall		< 0.001		<0.001
R^2		0.47		0.24
Adjusted R^2		0.46		0.21

* Coded as male=0 and female=1
# Coded as preterm=1 and term=0
SDS = standard deviation score
Multiple linear regression analysis with gestational age instead of prematurity as independent variable in these models yielded similar results.

children. This indicates that according to their genetic potential, preterm children should have been taller than term children. Thus, preterm children were growing more below their genetic height potential than term children. This is in line with the findings of Cutfield et al. (19). Despite their greater height potential, preterm children did not respond better to GH treatment. In the multiple regression analysis, after correction for several confounders including TH, no significant effect of prematurity on the growth response was found.

Serum IGF-I and IGFBP-3 levels were significantly below age-matched reference values in both groups. This is in agreement with previous reports on short children born SGA (19, 20). We found no significant correlation between IGF-I SDS and IGFBP-3 SDS and gestational age, whereas baseline IGF-1 and IGFBP-3 levels were comparable between term and preterm SGA children. Conversely, Cutfield et al. reported lower plasma IGF-I and IGFBP-3 levels in preterm versus term SGA children and a positive association between gestational age and plasma IGF-I and IGFBP-3 (3). Unfortunately, Cutfield et al. did not mention height as an inclusion criterium which renders it unclear whether all SGA children in their study were short (3). The difference with our results might be explained

by a much larger number of subjects in our study. In addition, we also included children with a gestational age of 32-36 weeks while Cutfield et al. did not. When we performed the analyses without the group born after a gestational age of 32-36 weeks (data not shown), we could not confirm their results either. We therefore conclude that prematurity has no influence on childhood serum IGF-I and IGFBP-3 levels in short SGA children.

In conclusion, within a population of short SGA children prematurity was associated with a smaller size for gestational age (length, weight and head circumference), a shorter height SDS corrected for TH and a leaner phenotype during childhood. Despite the differences in body size, there were no significant differences in age and sex adjusted IGF-I and IGFBP-3 levels between preterm and term short SGA children. Notably, the GH induced catch-up growth and change in serum IGF-I and IGFBP-3 levels were similar for preterm and term short SGA children. Therefore preterm short SGA children should not be excluded from GH treatment.

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CHAPTER 5

The effect of growth hormone treatment on metabolic and cardiovascular risk factors is similar in preterm and term short, small for gestational age children

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ABSTRACT

Context: We reported that short, small for gestational age (SGA) children who were born preterm have a lower body fat percentage and a higher blood pressure, insulin secretion and disposition index than short SGA children born at term. Whether preterm birth also influences these parameters during growth hormone (GH) treatment is unknown.

Objective: To compare blood pressure, insulin sensitivity, beta cell function and body composition during 4 years of GH treatment, between preterm and term short SGA children.

Patients: 404 prepubertal non-GH-deficient short SGA children, divided into 143 preterm (<36 weeks) and 261 term children.

Outcome measures: Height, blood pressure (n=404), body composition measured by Dual energy X-ray Absorptiometry (n=138), and insulin sensitivity and beta cell function calculated from a frequent sampling IV glucose tolerance test (FSIGT) with tolbutamide (n=74) or from HOMA-IR (n=204).

Results: In preterm and term children, GH treatment resulted in a similar decrease of systolic and diastolic blood pressure, body fat percentage, limb fat/total fat ratio and insulin sensitivity, and a similar increase in insulin secretion and disposition index. Lean body mass (LBM) corrected for gender and height increased in term children and did not change in preterm children. Multiple regression analysis revealed that this difference in GH effect on LBM was not associated with gestational age.

Conclusion: The effect of growth hormone treatment on metabolic and cardiovascular risk factors is similar in preterm and term short, small for gestational age children.

INTRODUCTION

A small size at birth for gestational age (SGA) and prematurity have both been associated with adult diseases such as cardiovascular disease and diabetes mellitus type 2 (1-5). Children born SGA with persistent short stature, have a higher prevalence of risk factors for adult diseases during childhood compared to normal references (6-8). Recently, we reported that preterm birth has independent and divergent effects on the cardiovascular risk profile of short SGA children (9). Preterm short SGA children had a higher systolic and diastolic blood pressure than term short SGA children and a lower body fat percentage, a higher insulin secretion and a higher disposition index (9).

Apart from preterm birth, growth hormone (GH) treatment also influences cardiovascular risk factors of short SGA children. During GH treatment, blood pressure (6), insulin sensitivity (10) and fat percentage corrected for age (11) decrease while there is a compensatory increase in insulin secretion (10). However, these studies were performed in mixed populations with subjects born preterm and at term. The independent effect of prematurity was not evaluated and it is therefore unknown whether GH treatment influences cardiovascular risk factors in a different way in preterm than in term SGA children.

The aim of our study was to investigate whether GH-induced changes in cardiovascular risk factors are influenced by preterm birth. Our second aim was to evaluate whether the differences in cardiovascular risk factors between preterm and term short SGA children disappear or increase during GH treatment. To answer our questions, we compared short SGA children born preterm with short SGA children born at term, with respect to the change in blood pressure, insulin sensitivity, beta cell function and body composition during 4 years of GH treatment

SUBJECTS AND METHODS

Subjects

The study cohort consisted of 404 prepubertal short children born SGA (227 boys) who were participating in GH trials with the same inclusion criteria. Children were included if they fulfilled the following criteria: 1) a birth length and/ or birth weight standard deviation score (SDS) of below –2 for gestational age (12); 2) a height SDS for age of below – 2 according to Dutch standards (13); 3) a height velocity SDS of below zero; 4) a Tanner breast stage 1 for girls and a testicular volume of less than 4 ml for boys (14); 5) an uncomplicated neonatal period, with no signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as chronic lung disease; 6) no GH deficiency defined as a GH peak > 10 µg/l during two GH stimulation tests. Children with endocrine or metabolic disorders, chromosomal defects, syndromes and growth failure caused by other conditions (e.g. emotional deprivation, severe chronic illness, chondrodysplasia) were excluded. Children without information on gestational age were also excluded. The Medical Ethics Committees of the participating centers approved the study and written informed consent was obtained from the parents.

Study design

Children were divided into two groups based on their gestational age: 1) preterm (gestational age < 36 weeks), and 2) term (gestational age ≥ 36 weeks). The gestational age of the subjects was determined by ultrasound scans during the first trimester, if available, and otherwise calculated from the date of the last menstruation.

Children were treated with biosynthetic GH at a dose of 1 mg/m^2 body surface area, administered subcutaneously, once daily at bedtime. The GH dosage was adjusted to the calculated body surface area every three months. A subgroup of 35 children was randomized to receive 1 or 2 mg/m^2 GH during the first 6 months and 1 mg/m^2 thereafter (15). The proportion of children receiving 2 mg/m^2 was similar in the preterm group as in the term group (6/13 versus 12/22; p = 0.63). Another subgroup of 57 children was randomized to receive 1 or 2 mg/m^2 GH for the entire follow-up period (16). Also in this subgroup the proportion of children receiving 2 mg/m^2 was similar in the preterm group as in the term group (12/20 versus 17/37; p = 0.31). Because independent samples t-tests demonstrated that the effect of GH treatment on the change in blood pressure,

body composition or insulin sensitivity after 4 years of treatment was not different between the GH dosage groups, the groups were combined to form one GH-group.

Anthropometry

At start and after 6 months, 1, 2, 3 and 4 years of GH treatment, standing height and weight were measured as previously described (16) and expressed as SDS using Dutch reference data (13). Systolic and diastolic blood pressure (BP) was measured twice on the left arm with an automated device and using an appropriate cuff size (Dinamap Critikon, southern Medical Corp., Baton Rouge, LA, USA). The mean of two measurements was used for analysis. As blood pressure is known to increase with height, BP was expressed as SDS adjusted for height and gender (17).

Glucose homeostasis

In a subgroup of 74 children, a modified, frequently sampled intravenous glucose tolerance test (FSIGT) using tolbutamide, was performed before the start of GH treatment and after 6 months, 2 and 4 years, as previously described (18, 19). Serum glucose and insulin levels were measured in one laboratory as previously described (9) and insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILENNIUM software (20). All blood samples were taken after an overnight fast. Insulin sensitivity quantifies the capacity of insulin to promote glucose disposal and glucose effectiveness reflects the capacity of glucose to mediate its own disposal. The acute insulin response, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. Disposition index equals AIR*Si and is an estimate of beta cell function.

In 204 children fasting glucose was measured on a Hitachi 917 analyzer. Also, fasting insulin was measured by chemoluminescent assay on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA) and HOMA insulin resistance index (HOMA-IR) was calculated (21).

Body Composition

Dual-Energy X-ray Absorptiometry scans (DEXA, type Lunar DPX-L, GE Healthcare, Madison, Wisconsin, USA) were performed in a subgroup of 138 children at start and after 6 months, 2 and 4 years of GH treatment. Lean body mass (LBM), fat mass (FM)

and body fat percentage (FM%, % fat mass of total body weight) were determined. FM% and LBM were transformed into SD-scores for gender and chronological age using Dutch reference values for children, which were obtained using the same machine and software (22, 23). Since body composition is strongly related to height, LBM and FM expressed as SDS for age and gender may result in an underestimation in case of short stature. In addition we were interested to know whether GH has an additional effect on LBM and FM beyond the effects due to catch-up in height. For these reasons, LBM and FM were expressed as SDS for height and gender (11).

Statistics

FSIGT parameters, fasting insulin levels, HOMA-IR, the amount of limb fat and trunk fat were logarithmically transformed because of a skewed distribution. A one-sample t-test was used to compare data, expressed as SDS, with those of healthy references (0 SDS). Differences between preterm and term children were tested using an independent samples t-test for continuous variables and the χ^2 test for categorical variables. Clinical data are presented as mean (SD) unless stated otherwise. To correct for missing data and multiple testing, the changes over time and differences between preterm and term children were analyzed using repeated measures of variance (24). Model estimates are presented as mean (95% CI).

Correlations were analyzed using Spearman's correlation coefficient. Multiple linear regression analysis was used to adjust the differences in response to GH treatment between the groups for possible baseline differences. Statistical significance was defined as $p < 0.05$. Analyses at baseline were performed using the statistical package SPSS (version 12.0; SPSS Inc., Chicago, IL) for Windows. For repeated measures of variance analyses SAS 8.2 (SAS Institute Inc., Cary, /nC, USA) was used.

RESULTS

Clinical characteristics

Table 1 lists the clinical data of the preterm and term short SGA children. The preterm group was significantly younger, had a lower birth weight SDS and a lower birth length SDS. Current height SDS was slightly higher in the preterm group while weight for height SDS was lower in the preterm group.

Table 1. Clinical characteristics at birth and at start of GH treatment			
	SGA born preterm (< 36 weeks)	SGA born at term (≥ 36 weeks)	p-value
N	143	261	
Age (yr)	6.7 (2.1)	7.4 (2.6)	**0.01**
Sex (m/f)	84/59	143/118	0.44
Gestational age (wks)	32.2 (2.5)	38.7 (1.5)	**<0.001**
Birth weight SDS	-2.8 (1.3)*	-2.0 (1.0)*	**<0.001**
Birth length SDS	-3.8 (2.0)*	-2.9 (1.2)*	**<0.001**
Birth head circumference SDS	-2.2 (1.3)*	-1.8 (1.3)*	0.19
Height SDS	-2.9 (0.6)*	-3.1 (0.6)*	**0.008**
Weight SDS	-1.7 (1.1)*	-1.1 (1.2)*	**<0.001**

Data expressed as mean (SD)
SDS = standard deviation score; f = female; m = male.
Birth weight and birth length were adjusted for gestational age and sex, height was adjusted for age and sex and weight was adjusted for height and sex.
*p < 0.001 compared with zero SDS, e.g. mean value for references

Blood pressure

Systolic and diastolic blood pressure SDS at start of GH were significantly higher in preterm short SGA children than in short SGA children born at term (p = 0.008 and p < 0.001, respectively, Table 2). In both groups, blood pressure at start was significantly higher than that of gender- and height-matched references (p < 0.001). The decrease in systolic and diastolic blood pressure over 4 years of GH treatment was similar in preterm and term children (p = 0.50 and p = 0.15, respectively, Figure 1). Because age and blood pressure of preterm and term children differed at start, we also compared the decrease in blood pressure between preterm and term children corrected for age and blood pressure at start. Again, systolic and diastolic blood pressure decreased similarly (p = 0.10 and p = 0.30, respectively, Table 2) in the preterm and the term group. After 4 years, systolic blood pressure remained higher than zero SDS, e.g. higher than that of gender-and-height matched references but diastolic blood pressure had completely normalized.

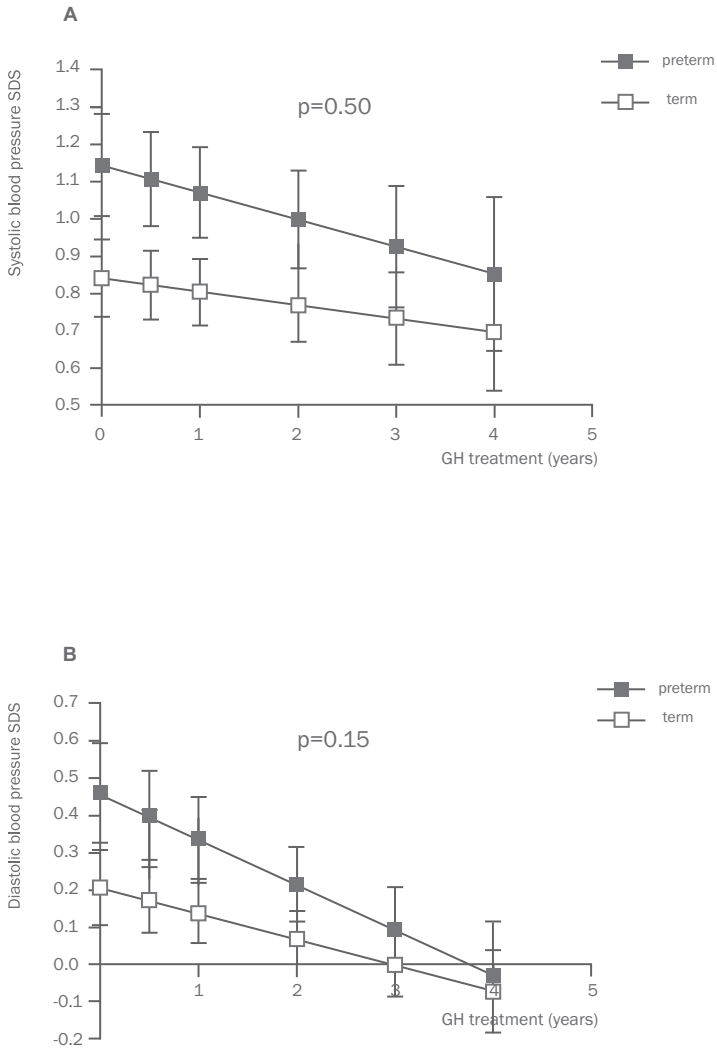


Figure 1: Changes in (A) systolic and (B) diastolic blood pressure during GH treatment. Data are expressed as model estimates (95% CI). P-values express the difference between the groups in overall change in blood pressure during 4 years of GH treatment.

Glucose homeostasis

Table 2 shows the results of the FSIGT tests at start and after 4 years of GH treatment, while Figure 2 shows how the various parameters changed during the 4 years period. The overall decrease in Si during GH treatment was comparable for preterm and term children (p=0.40, Figure 2A), but after 4 years of GH treatment, preterm children had a higher Si than term children. In both groups, there was an increase of insulin secretion (AIR) after 6 months of treatment, which persisted until the end of the 4-year period. DI did not significantly change during GH treatment but the difference between the groups slowly increased over time, resulting in a significantly higher DI in preterm than in term children after 4 years of treatment (Figure 2D). The change in the FSIGT parameters during the 4-year period was, however, comparable between preterm and term children (Figure 2), also after correction for values at start and age at start (Table 2). Fasting glucose and insulin levels and HOMA-IR were comparable for preterm and term children before start and after 4 years of GH treatment (Table 2).

Body composition

Results are shown in Table 3. Body fat percentage (FM%) and FM adjusted for height at start were lower in preterm than in term children but the fat distribution was similar. During 4 years of GH treatment weight adjusted for height increased similarly in both preterm and term children while FM adjusted for height remained at the same level. In both groups the increase in limb fat over time was less pronounced than the increase in trunk fat and therefore the ratio of limb fat/total fat decreased (Figure 3B) while the ratio of trunk fat/total fat increased.

Of children with a DEXA scan, those born preterm were significantly taller than those born at term (Table3 and Figure 4B). LBM adjusted for age and gender was higher in preterm children than in term children, both at start and during GH treatment (Figure 4A). However, when we adjusted LBM for height and gender instead of age and gender, there was no increase in LBM in the preterm children (Figure 4C). In term children, LBM adjusted for height and gender increased, indicating that term children had an additional increase in LBM beyond their increase in LBM due to catch-up in height.

To further investigate the influence of several factors on the change in LBM adjusted for height and gender during GH treatment, we performed multiple regression analysis. In this analysis we only included children who remained prepubertal for 4 years and in which a DEXA scan was performed after 4 years of GH treatment (n=32). The

Table 2. Blood pressure, insulin sensitivity and β-cell function at start and after 4 years of GH treatment

	SGA born preterm <36 weeks				SGA born at term ≥ 36 weeks					
	N	At start	After 4 yr		N	At start	After 4 yt	*p-value	**p-value	***p-value
Systolic bp SDS	143	1.1 (1.0 to 1.3)[a]	0.9 (0.6 to 1.1)a,¶		261	0.8 (0.7 to 0.9)[a]	0.7 (0.5 to 0.9)[a]	**0.008**	**0.04**	0.10
Diastolic bp SDS	143	0.5 (0.3 to 0.6)[a]	0.0 (-0.2 to 0.1)[#]		261	0.2 (0.1 to 0.3)[a]	-0.1 (-0.2 to 0.0)[¶]	**<0.001**	0.24	0.30
Si x 10^{-4}/min^{-1} (μU/ml)	30	14.7 (12.3 to 17.6)	8.7 (6.5 to 11.6)[#]		44	13.9 (12.0 to 16.2)	5.9 (4.7 to 7.6)[#]	0.64	**<0.05**	0.17
Sg x 10^{-2}/min^{-1}	30	1.9 (1.6 to 2.2)	2.0 (1.8 to 2.4)		44	2.3 (2.0 to 2.6)	1.7 (1.5 to 1.9)[§]	0.08	**0.03**	0.07
AIR (mU/L)	30	284 (231 to 348)	581 (423 to 798)[#]		44	231 (195 to 273)	591 (453 to 771)[#]	0.13	0.93	0.72
DI (AIR x Si)	30	4172 (3386 to 5140)	4929 (4006 to 6066)		44	3189 (2684 to 3788)	3393 (2851 to 4037)	0.05	**0.008**	0.18
F Glucose (mmol/L)	69	4.4 (4.2 to 4.5)	4.6 (4.4 to 4.7)[¶]		135	4.5 (4.4 to 4.6)	4.7 (4.6 to 4.8)[§]	0.13	0.28	0.78
F Insulin (pmol/L)	65	21.0 (18.3 to 24.1)	28.9 (21.9 to 38.2)[¶]		124	19.2 (17.4 to 21.3)	34.3 (28.1 to 42.0)[#]	0.31	0.88	0.45
HOMA-IR	57	0.4 (0.3 to 0.5)	0.5 (0.4 to 0.7)[¶]		108	0.4 (0.3 to 0.4)	0.7 (0.6 to 0.8)[#]	0.31	0.85	0.49

Data are expressed as model estimate (95% CI). FSIGT parameters, fasting insulin and HOMA-IR were log-transformed prior to analysis.
Bp = blood pressure; SDS = standard deviation score; F = fasting
Blood pressure was adjusted for height and sex.
*p-value: preterm versus term at start of GH treatment
**p-value: preterm versus term after 4 years
***p-value: change of parameters over 4 years of GH in preterm versus term, corrected for age and value of the parameter at start
[a]p < 0.001; [b]p < 0.01 compared with zero SDS, e.g. mean for sex and height
[#]p<0.001; [§]p<0.01; [¶]p<0.05 compared with start

increase in LBM SDS during 4 years of GH treatment was associated with gender (β = -1.31, p=0.002), birth weight SDS (β = 0.21, p=0.03) and LBM SDS at start (β = -0.65, p<0.001), and not with gestational age and age at start of GH treatment. This model explained 93% of the variance of the change in LBM SDS during 4 years of GH treatment. Thus, children with a higher birth weight SDS had a greater increase of LBM SDS during GH treatment. As preterm children had a lower birth weight SDS than term children, this might explain why the change in LBM SDS was smaller in preterm children.

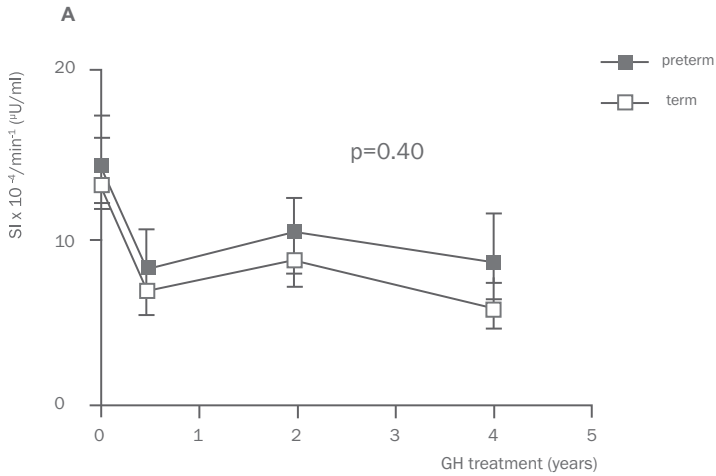
DISCUSSION

Our study shows that GH-induced changes in blood pressure, fat mass, beta cell function and insulin sensitivity are similar in preterm and term born short SGA children. In children born preterm, lean body mass (LBM) adjusted for height and gender remained low during GH treatment whereas in children born at term there was an additional increase in LBM beyond the increase in LBM due to catch-up in height.

Concerns have been expressed that GH treatment may increase any pre-existent risk for cardiovascular disease and type 2 DM of SGA children. Previously, we demonstrated that preterm short SGA children have a higher blood pressure than term SGA children, which suggests a higher risk for cardiovascular disease in later life (9.) On the other hand, they also had a lower fat percentage and a higher disposition index, suggesting a lower risk for later disease (9). The present study is the first one investigating whether GH treatment induces a different cardiovascular risk profile in preterm versus term short SGA children.

Systolic and diastolic blood pressure at start of GH treatment were both significantly higher in preterm children than in term children and also higher than in sex and height matched references. SGA and preterm birth have both been associated with a reduced number of nephrons and a subsequently higher blood pressure (4, 5, 25, 26). We demonstrated that the combination of SGA and preterm birth resulted in a higher blood pressure than SGA alone (9). During GH treatment, blood pressure decreased in both preterm and term children and after 4 years of GH treatment the initial difference in diastolic blood pressure between preterm and term children had disappeared. Thus, although preterm children had a higher blood pressure at start, the GH-induced decrease in blood pressure was similar for preterm and term children.

Insulin sensitivity was not significantly different between the groups at start but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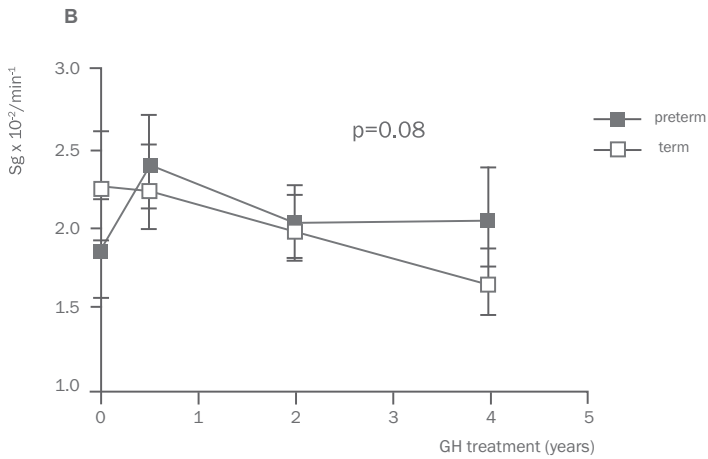
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GH treatment (years)

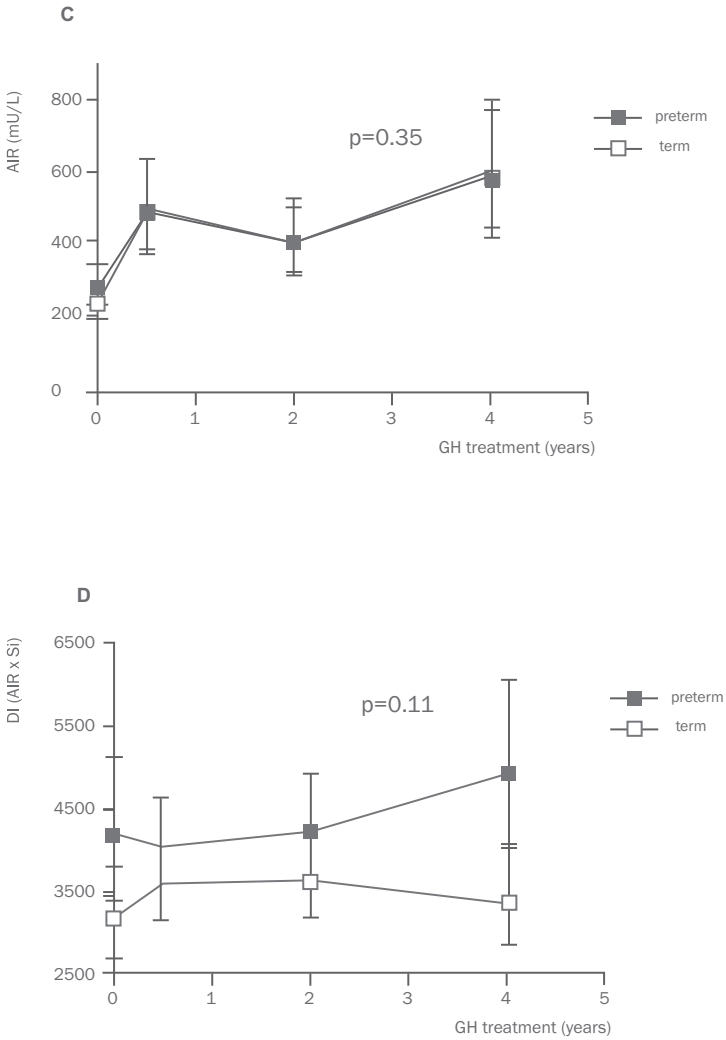


Figure 2. Insulin sensitivity (A), Glucose effectiveness (B), Acute insulin response (C) and Disposition index (D) during GH treatment.

Data expressed as model estimates (95% CI). Data were log transformed for analysis and the exponents of the model estimates were used in the figure.

P-values express the difference between the groups in overall change of the parameter during 4 years of GH treatment.

Table 3. Body composition at start and after 4 years of GH treatment								
	SGA born preterm < 36 weeks (n = 52)			SGA born at term ≥ 36 weeks (n=86)				
	At start	After 4 yr		At start	After 4 yr	*p-value	**p-value	***p-value
Height SDS_{age}	-2.9 (-3.0 to -2.8)[a]	-1.2 (-1.4 to -1.0)[a,#]		-3.2 (-3.3 to -3.1)[a]	-1.8 (-2.0 to -1.6)[a,#]	**<0.001**	**<0.001**	0.24
Weight SDS_{height}	-1.6 (-1.9 to -1.4)[a]	-0.8 (-1.1 to -0.5)[a,#]		-0.6 (-0.8 to -0.4)[a]	0.2 (-0.1 to 0.4)[#]	**<0.001**	**<0.001**	0.49
FM% SDS_{age}	-1.2 (-1.4 to -0.9)[a]	-1.7 (-2.1 to -1.3)[a,§]		-0.6 (-0.7 to -0.4)[a]	-0.5 (-0.9 to -0.2)[b]	**<0.001**	**<0.001**	0.08
FM SDS_{height}	-2.6 (-3.2 to -2.0)[a]	-2.1 (-2.7 to -1.5)[a]		-1.4 (-1.8 to -0.9)[a]	-0.9 (-1.5 to -0.4)[a]	**0.002**	**0.006**	0.22
LBM SDS_{height}	-1.1 (-1.6 to -0.7)[a]	-1.4 (-1.8 to -1.1)[a]		-1.0 (-1.4 to -0.6)[a]	-0.5 (-0.8 to -0.2)[b,¶]	0.69	**<0.001**	**0.01**
Limbs fat (g)	417 (318 to 548)	707 (490 to 1019)[§]		847 (688 to 1043)	1361 (985 to 1882)[§]	**<0.001**	**0.009**	0.55
Trunk fat (g)	268 (205 to 352)	669 (478 to 938)[#]		542 (440 to 666)	1292 (959 to 1741)[#]	**<0.001**	**0.005**	0.28
Limb fat/total fat	0.51 (0.50 to 0.53)	0.46 (0.44 to 0.48)[#]		0.53 (0.52 to 0.54)	0.47 (0.45 to 0.49)[#]	0.14	0.42	**0.44**
Trunk fat/total fat	0.33 (0.32 to 0.35)	0.43 (0.41 to 0.45)[#]		0.34 (0.33 to 0.35)	0.44 (0.41 to 0.46)[#]	0.60	0.72	0.57

Data are expressed as model estimate (95% CI). Limbs and trunk fat were log-transformed prior to analysis.
SDS = standard deviation score; FM% = Body fat percentage; FM = fat mass; LBM = lean body mass
*p-value: preterm versus term at start of GH treatment
**p-value: preterm versus term after 4 years
***p-value: change of parameters over 4 years in preterm versus term, corrected for age and value of parameter at start
[a]$p < 0.001$; [b]$p < 0.01$ compared with zero SDS, e.g. mean for sex and height or sex and age
[#]$p<0.001$; [§]$p<0.01$; [¶]$p<0.05$ compared with start

after 4 years of GH treatment, preterm children had significantly higher insulin sensitivity than term children. A reduction in insulin sensitivity is a known phenomenon during GH treatment and it appears to be reversible after withdrawal of GH treatment (27, 28). When insulin sensitivity decreases, glucose homeostasis is maintained by increasing insulin secretion (AIR). Because term children had a lower insulin sensitivity than preterm children, we expected to find a compensatory stronger increase in insulin secretion (AIR) in the term group. However, after 4 years of GH treatment, the AIR did not significantly differ between the groups. As a result the disposition index (DI) – the product of insulin sensitivity and AIR – was higher in preterm children than in term children. DI reflects the capacity of pancreatic islets to compensate for insulin resistance. It has been shown that particularly a reduction in DI relates to an increased risk for type 2 DM (29, 30). There was no significant change in DI during GH treatment, neither in preterm nor in term children. This might indicate that in both groups glucose homeostasis remained unaffected during GH treatment.

Preterm SGA children had a considerably lower body fat percentage (FM%) than age-and- sex-matched references but also than term SGA children. As the third trimester of pregnancy is a period of rapid adipose tissue deposition, preterm infants have often less adipose tissue (31). Also, the postnatal course of preterm infants is often marked by nutritional problems and poor growth. These are plausible reasons for reduced adipose tissue deposition in preterm children. FM% is known to strongly correlate with insulin sensitivity (32). At start, insulin sensitivity was comparable between the groups despite the significant difference in FM%. GH treatment induced a further reduction in FM% in preterm children but not in term children. The lipolytic effects of GH treatment have been well documented (33, 34) but it is unknown why these effects are greater in the adipose tissue of preterm children. The reduction in FM% in preterm children resulted in a greater difference in FM% between preterm and term children after 4 years of GH which might explain why at that time insulin sensitivity was indeed higher in preterm children than in term children.

During GH treatment, we observed a decrease of limb fat/total fat ratio in both groups. The increase in trunk fat/total fat ratio demonstrated that this was due to a relatively lower amount of limb fat and not due to a relatively higher amount of trunk fat. Recently De Schepper et al. suggested that GH treatment of short SGA children is accompanied by a centripetal shift in fat distribution (35). However, they showed that the increase in trunk fat was not significantly different between 11 GH treated and 14

untreated short SGA children while the increase in limb fat was (35). Therefore, the data of De Schepper et al. in fact demonstrate that there is not a centripetal shift of fat mass but that GH treated SGA children loose fat mass in their limbs while the amount of trunk fat remains stable. This is in line with our results.

Children born at term had an increase in LBM beyond the increase associated with catch-up in height while preterm children had not. The absence of an additional increase in LBM in preterm children might be explained by several factors. Firstly, preterm children had a significantly lower birth weight and birth length SDS, indicating a more severe degree of growth retardation, which might adversely affect the development of LBM. A lower LBM at birth might limit the increase in LBM during stimulation by GH. This explanation is supported by the multiple regression analysis, which showed that birth weight SDS was positively associated with the increase in LBM. Secondly, the neonatal period of preterm infants is often characterized by nutritional and other problems (36, 37) which might result in a different development of LBM.

The clinical relevance of a change in LBM during GH treatment is unknown. Skeletal muscle plays a major role in insulin-mediated glucose uptake (38) and decreased muscle mass may therefore lead to more insulin resistance (39). Others, however, did not find an association between LBM and insulin sensitivity (32). In our cohort, preterm children had a lower LBM after 4 years of GH treatment but their insulin sensitivity was higher than that of term children. The latter does not support an increased risk for type 2 DM for preterm SGA children during GH treatment.

In conclusion, within a population of short SGA children, preterm birth does not have an independent influence on the effect of GH on blood pressure, insulin sensitivity, beta cell function and body composition. Children born at term had a higher birth weight for gestational age, which was associated with an increase in LBM even after adjustment for height and gender during GH treatment. We conclude that GH treatment has the same effect on cardiovascular risk factors in preterm as in term short SGA children.

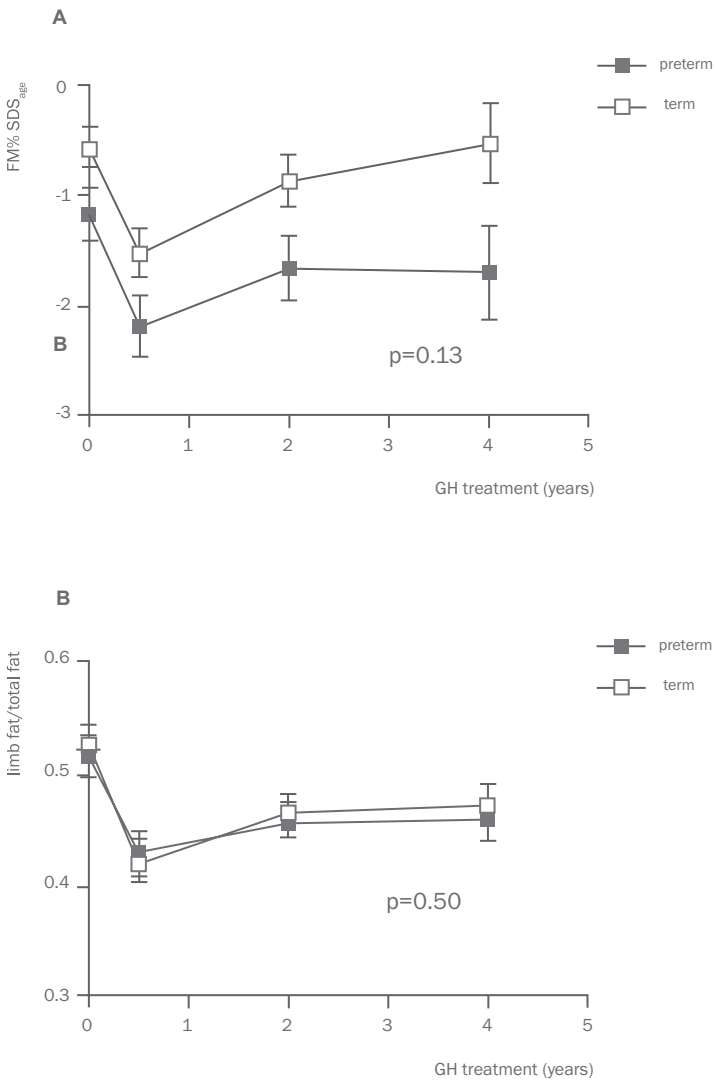
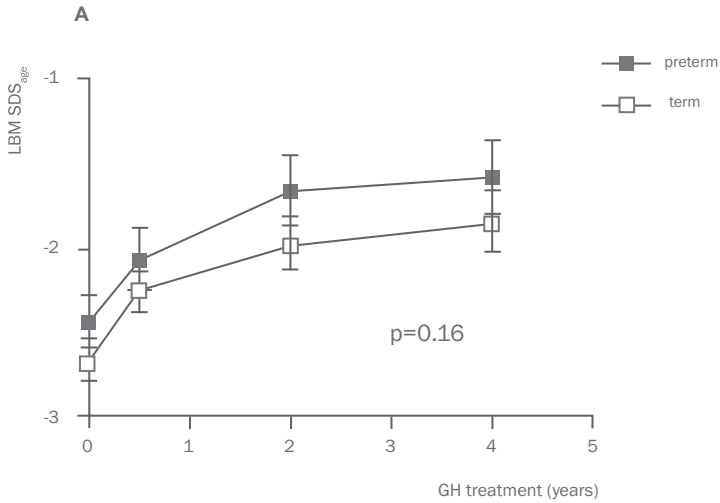
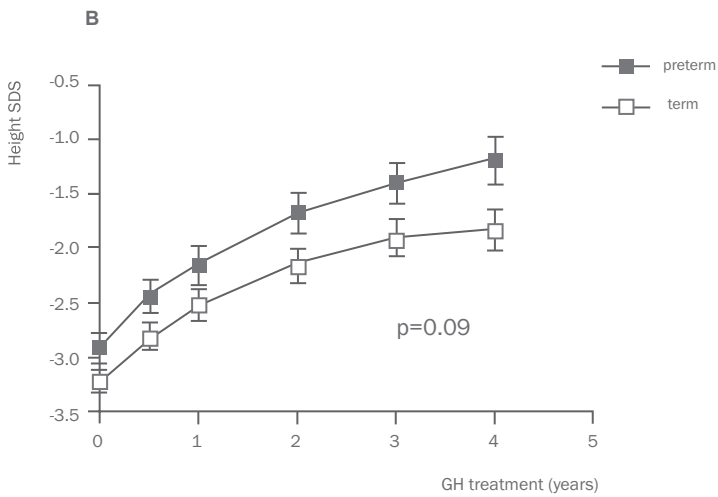


Figure 3. Percentage body fat (FM%) and limb fat / total fat ratio during GH treatment
Data are expressed as model estimates (95% CI)
P-values express the difference between the groups in overall change of the parameter during 4 years of GH treatment.


A
LBM SDS age
-1
-2
-3
preterm
term
p=0.16
0
1
2
3
4
5
GH treatment (years)


B
Height SDS
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
preterm
term
p=0.09
0
1
2
3
4
5
GH treatment (years)

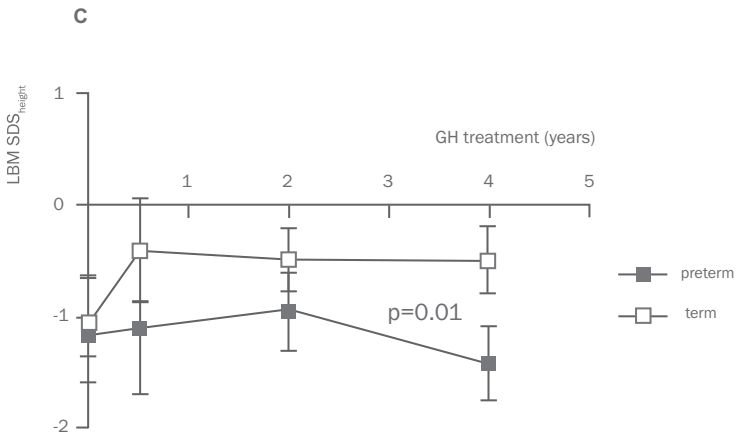


Figure 4. Lean body mass (LBM) and height SDS of the children with a DEXA scan, during GH treatment. A) LBM adjusted for age and gender; B) Height adjusted for age and gender; C) LBM adjusted for height and gender. Data are expressed as model estimates (95% CI)
P-values express the difference between the groups in overall change of the parameter during 4 years of GH treatment.

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CHAPTER 6

The *PPAR-□* Pro12Ala polymorphism associates with weight gain during GH-treatment in short children born small for gestational age

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Submitted

ABSTRACT

Context: Short children born small for gestational age (SGA) have a lean phenotype with a lower insulin sensitivity and higher blood pressure. Growth hormone (GH) treatment results in weight gain, and a decrease in blood pressure and insulin sensitivity. However, not all children respond in the same way. The Pro12Ala polymorphism of the peroxisome proliferator-activated receptor (PPARG) gene is inversely associated with BMI, changes in BMI and the risk to develop type 2 diabetes mellitus (DM).

Objective: To analyze the contribution of the *PPAR*□ Pro12Ala polymorphism to GH induced changes in determinants of metabolic and cardiovascular disease in short SGA children.

Methods: *PPAR*□ was genotyped in 238 Caucasian short SGA children (mean age 7.5 years). Height, weight, blood pressure and serum lipids were measured before start and during 4 years of GH treatment. In addition glucose homeostasis by HOMA-IR (n = 148) and by frequently sampled intravenous glucose tolerance test (FSIGT) (n = 51), and body composition by DXA (n = 79) were measured.

Results: At baseline, the Ala12 allele was not associated with any determinant of metabolic and cardiovascular disease. After 4 years of GH treatment, the increase in weight for height SDS and BMI SDS was significantly greater in carriers of an Ala12 allele than in non-carriers. The change in all other parameters was not associated with Pro12Ala genotype.

Conclusion: The Ala12 variant of the *PPAR*□ gene is associated with higher weight gain during GH treatment but not with changes in determinants of metabolic and cardiovascular diseases in Caucasian subjects born SGA.

INTRODUCTION

Growth hormone (GH) treatment may be a modifier of the association between polymorphisms in the peroxisome proliferators-activated receptor γ (PPAR-γ) gene and glucose homeostasis and the risk of type 2 diabetes mellitus (DM) in later life. PPAR-γ is a nuclear hormone receptor that controls genes involved in adipogenesis and lipid and glucose metabolism (1). The Ala12 variant of the *PPAR-γ* gene has been associated with improved insulin sensitivity and a lower risk for type 2 DM compared with the Pro12Pro genotype (2-5). In contrast, in a study in overweight individuals with impaired glucose tolerance (6) and in young adults born small for gestational age (SGA) (7), the Ala12 allele was associated with an increased risk for type 2 DM.

Results about the association of the Ala12 variant with BMI are conflicting. The Ala12 variant has been associated with a higher BMI (8, 9) and a tendency to gain weight over time (10). However, in subjects with a normal body weight the Ala12Ala genotype was associated with a lower BMI and a lower increase in BMI at follow-up (11) and in a meta-analysis of subjects with a mean BMI < 27 kg/m^2, there was no significant difference in BMI between genotype groups (9). Size at birth also interacts with the effect of the Ala12 allele. In young adults who were born SGA, increased BMI amplified the effect of *PPAR-γ* polymorphism on glucose homeostasis while in those born appropriate for gestational age (AGA) there was not such an association (7). These conflicting results regarding associations of the Ala12 allele with insulin resistance and BMI demonstrate that the allele has a different effect in different environments, and that environmental factors may play a role to increase the genetic effect of *PPAR-γ* on metabolic risk factors.

Subjects born SGA might have an increased risk to develop type 2 DM in later life (12, 13), particularly those with catch-up in weight (14). It is, however, unknown whether this increased risk is genetically or environmentally determined or by a combination of both. Most children with short stature who were born SGA are nowadays treated with GH. GH treatment generally results in weight gain, a decrease in blood pressure, serum lipid levels and insulin sensitivity (15-17). Also, there is a compensatory increase in insulin secretion (17). However, not all children respond in the same way. Since the *PPAR-γ* gene was associated with BMI, glucose homeostasis, and atherosclerosis (1), genotyping might indicate which children are less capable to compensate for the effects of GH-treatment on metabolic and cardiovascular risk factors and are thus more at risk to develop adult diseases.

The present study aimed to investigate whether *PPAR-γ* polymorphisms correlate with changes in the metabolic and cardiovascular profile during GH treatment. To investigate this question, we performed genotyping of the *PPAR-γ* gene, measurements of anthropometry, blood pressure, serum lipids, FSIGTs and calculations of HOMA-IR in 238 short children who were born SGA and treated with GH.

SUBJECTS AND METHODS

Subjects

The study group comprised 238 children born SGA. Children were included when they were SGA at birth, had short stature (height standard deviation score (SDS) for age and gender of below – 2 (18)), did not show catch-up growth to a height > -2 SDS, and had no growth failure caused by other disorders. These inclusion criteria have previously been described (19). SGA was defined as a birth length and/or birth weight SDS of below –2.0 for gestational age (20). The Medical Ethics Committees of Erasmus Medical Center, Rotterdam, and the other participating centers approved all studies and written informed consent was obtained from all participants and their parents. Birth characteristics were collected from hospital registries. The gestational age of the subjects was determined by ultrasound in the first trimester, if available, and otherwise calculated from the date of the last menstruation.

Study design

Children were treated with biosynthetic GH at a dose of 1 mg/m^2 body surface area, administered subcutaneously, once daily at bedtime. The GH dosage was adjusted to the calculated body surface area every three months. At start and after 6 months, 1, 2, 3 and 4 years of GH treatment, standing height and weight were measured as previously described (19) and expressed as SDS using Dutch reference data (18). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and was expressed as SDS adjusting for age and gender (18). Systolic and diastolic blood pressure (BP) was measured twice on the left arm with an automated device and using an appropriate cuff size (Dinamap Critikon, southern Medical Corp., Baton Rouge, LA, USA). The mean of two measurements was used for analysis. As blood pressure is known to increase with height, BP was expressed as SDS adjusted for height and gender (21). The pubertal stage was determined according to Tanner (22) and coded as prepubertal or pubertal.

Glucose homeostasis

In 148 children, fasting glucose was measured on a Hitachi 917 analyzer and fasting insulin was measured by chemoluminescent assay on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA). HOMA insulin resistance index (HOMA-IR) was calculated (23).

In a subgroup of 51 children (determined by the date of inclusion), a modified, frequently sampled intravenous glucose tolerance test (FSIGT) using tolbutamide, was performed before the start of GH treatment and after 6 months, 2 and 4 years, as previously described (16, 24). Serum glucose and insulin levels were measured in one laboratory as previously described (25) and insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILENNIUM software (26). All blood samples were taken after an overnight fast. Insulin sensitivity quantifies the capacity of insulin to promote glucose disposal and glucose effectiveness reflects the capacity of glucose to mediate its own disposal. The acute insulin response, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. The Disposition index (DI) is an overall measure of the ability of the beta cells to secrete insulin in response to the degree of insulin resistance. DI is the product of AIR and Si (DI = AIRg * SI) (26).

Serum lipids

All blood samples were taken after an overnight fast. Serum cholesterol levels were determined as previously described (27). Triglycerides were measured on the Chem-I analyzer according to the manufacturer's instructions (Technicon Instruments, Tarrytown, New York) and after 1998 on the Hitachi 917 analyser according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Both methods were comparable ($y = x - 0.030$).

Body Composition

Dual-Energy X-ray Absorptiometry scans (DXA, type Lunar DPX-L, GE Healthcare, Madison, Wisconsin, USA) were performed in a random subgroup of 79 children at start and after 6 months, 2 and 4 years of GH treatment. The date of inclusion determined in which subjects a DXA scan was performed. Lean body mass (LBM), fat mass (FM) and body fat percentage (FM%, % fat mass of total body weight) were determined. FM% and LBM

were transformed into SD-scores for gender and chronological age using Dutch reference values for children, which were obtained using the same machine and software (28, 29). Since body composition is strongly related to height, LBM and FM expressed as SDS for age and gender may result in an underestimation in case of short stature. Therefore, LBM and body fat percentage were expressed as SDS for height and gender (15).

Genotyping

Genomic DNA was extracted from samples of peripheral venous blood according to salting out procedure (30). Genotyping of the *PPARγ* gene Pro12ala polymorphism (rs1801282) was performed using the Taqman allelic discrimination assay. PCR was performed in 384 wells PCR plates in an ABI 9700 PCR system (Applied Biosystems Inc., Foster City, CA, USA) and consisted of initial denaturation for 10 minutes at 95o and 40 cycles with denaturation of 15 seconds at 92o and annealing and extension for 60 seconds at 60o. Results were analysed by ABI Taqman 7900HT using the sequence detection system 2.22 software (Applied Biosystems Inc., Foster City, CA, USA).

Statistics

Fasting insulin levels, FSIGT results and HOMA-IR were logarithmically transformed because of a skewed distribution. Because the number of subjects carrying the rare homozygous genotype was low, we used the dominant genetic model in our analyses. Homozygosity for the Pro12 allele was coded as 0, and the heterozygous state and homozygosity for the Ala12 allele were coded as 1. Genotype distributions for significant departure from the Hardy-Weinberg equilibrium were calculated using the $\chi 2$ test. Differences between genotype groups were evaluated using independent sample t-tests. A retrospective power analysis with a level of significance (α) of 0.05 and a chosen power of 80% showed that with our number of subjects per Pro12Ala genotype-subgroup we could detect differences in weight for height SDS of 0.48 SDS, in systolic and diastolic blood pressure of 0.41 SDS (25), in HOMA IR of 0.22 (25), in insulin sensitivity (Si) of $6.1*10^{-4}$ µU/ml, in AIR of 107 mU/l (31) and in cholesterol levels of 0.38 mmol/l (25).

To assess longitudinally measured growth and metabolic characteristics from the start of GH treatment to 4 years after start of treatment, we performed repeated measures regression analysis with weight for height SDS, body fat percentage, body fat percentage SDS, LBM SDS, blood pressure SDS, fasting insulin and fasting glucose levels, HOMA-IR, insulin sensitivity, insulin secretion and disposition index as outcomes. This

regression technique takes the correlation of multiple measurements within one subject into account, assesses both the time-independent and time-dependent effect of *PPAR-γ* genotype on the outcome variable, and allows for incomplete outcome data (32). In these models, genotype was included as both independent variable and as interaction with the study duration. All models were corrected for age, gender and puberty. The models can be written as:

Outcome variable = ß0 + ß1*age + ß2*gender + ß3*puberty + ß4* study duration + ß5* genotype + ß6*genotype*study duration.

In this model, the term including 'ß0' reflects the intercept, the terms including 'ß1, ß2 and ß3' reflect the corrections for age, gender and puberty, respectively and the term including 'ß4' reflects the slope of change (in weight SDS, body fat %, body fat % SDS, LBM SDS, systolic blood pressure SDS, diastolic blood pressure SDS, glucose, insulin, HOMA-IR, Si, AIR or DI) per month GH therapy for the reference group (Pro12Pro genotype). The term including 'ß5' reflects the difference in outcome variable between the genotype groups independent of age, gender, puberty and study duration. The term including 'ß6' reflects the difference in change of the outcome variable between the genotype groups independent of age, gender, puberty and study duration (33).

Clinical data are presented as mean (SD) unless stated otherwise. Statistical significance was defined as $p < 0.05$. Analyses were performed using the statistical package SPSS (version 15.0; SPSS inc., Chicago, IL) for Windows. SAS 8.2 (SAS Institute Inc., Cary, /nC, USA) was used for repeated measures of variance analyses.

RESULTS

The genotype distribution was Pro12Pro 70.6%, Pro12Ala 26.9%, and Ala12Ala 2.5% and was not different from the Hardy-Weinberg expectations ($\chi^2 = 0.001$, $p = 0.97$). The frequency of the Ala12 allele was 16%, as reported in literature (6, 34). No significant differences in age, gender distribution, size at birth, anthropometry, or body composition were observed between the Ala12 carriers and non-carriers (Table 1). Blood pressure, glucose homeostasis and serum lipid levels were also not significantly different between Ala12 carriers and non-carriers (Table 2). There were 23 (9.7%) children in puberty at start of the study. The proportion of pubertal subjects was similar in both genotype

groups (p = 0.91) and all results were similar when pubertal subjects were excluded.

During 4 years of GH treatment the increase in weight for height SDS and in BMI SDS was greater in carriers of Ala12 than in non-carriers (Table 3). The change in all other evaluated parameters was not significantly different between carriers and non-carriers of the Ala12 allele. The genotype distribution of the Pro12Ala polymorphism was not significantly different for children with a change in weight for height SDS (n = 186) or BMI SDS (n = 172) greater than zero and children with a change in weight for height SDS (χ^2 = 2.23, p = 0.14) or BMI SDS (χ^2 = 1.79, p = 0.18) of zero or less. In the subgroup of 79 children with a DXA scan, the Pro12Ala polymorphism was not associated with the change in body fat % SDS or the change in lean body mass SDS. However, in this much smaller group, weight for height SDS and BMI SDS were also not associated with the Pro12Ala polymorphism, which might indicate a power problem in the relatively small DXA group.

Table 1. Clinical characteristics before the start of GH treatment

	All	Pro/Pro	Pro/Ala or Ala/Ala	p-value
N	238	168	70	
Age	7.51 (2.9)	7.56 (3.0)	7.40 (2.5)	0.68
Gender (m/f)	124/114	90/78	34/36	0.48
Birth weight SDS	-2.27 (1.1)	-2.28 (1.0)	-2.25 (1.2)	0.86
Birth length SDS	-3.15 (1.4)	-3.15 (1.4)	-3.15 (1.4)	0.97
Birth head circumference SDS	-1.42 (1.3)	-1.46 (1.4)	-1.34 (1.1)	0.60
Height SDS	-2.98 (0.7)	-2.97 (0.7)	-3.01 (0.7)	0.72
Weight SDS_{height}	-1.14 (1.2)	-1.16 (1.3)	-1.09 (1.0)	0.68
BMI SDS_{age}	-1.25 (1.0)	-1.29 (1.0)	-1.16 (0.9)	0.36
Body fat %*	12.68 (7.0)	12.83 (7.7)	12.32 (5.0)	0.77
Body fat % SDS_{age}*	-0.76 (1.0)	-0.76 (1.1)	-0.76 (1.0)	1.00
Body fat % SDS_{height}*	-0.77 (1.3)	-0.79 (1.4)	-0.73 (1.2)	0.87
LBM SDS_{age}*	-2.49 (0.7)	-2.54 (0.8)	-2.39 (0.4)	0.38
LBM SDS_{height}*	-0.87 (1.9)	-1.03 (1.9)	-0.50 (1.8)	0.25

Values are expressed as mean (SD)
* n = 79

Table 2. Association of *PPAR-γ* Pro12Ala genotype with metabolic and cardiovascular risk factors at baseline				
	All	**Pro/Pro**	**Pro/Ala or Ala/Ala**	**p-value**
Systolic bp SDS	1.0 (1.1)	1.0 (1.1)	1.2 (1.1)	0.24
Diastolic bp SDS	0.3 (1.0)	0.2 (1.0)	0.4 (1.1)	0.36
F Glucose (mmol/L)	4.7 (0.6)	4.7 (0.6)	4.7 (0.6)	0.73
F Insulin (pmol/L)	24.0 (14.0 to 36.0)	25.0 (14.0 to 40.0)	30.0 (15.0 to 48.0)	0.39
HOMA-IR	0.4 (0.3 to 0.7)	0.5 (0.3 to 0.8)	0.6 (0.3 to 0.9)	0.31
$Si*10^{-4}/min^{-1}$ (μU/ml)	13.4 (9.9 to 19.0)	12.2 (7.7 to 18.4)	12.1 (8.9 to 16.0)	0.76
AIR (mU/L)	267 (180 to 352)	214 (170 to 355)	317 (266 to 440)	0.11
DI (AIR*Si)	3139 (2575 to 4715)	2880 (2190 to 4600)	3115 (2671 to 4671)	0.32
Cholesterol (mmol/L)	4.3 (0.8)	4.3 (0.8)	4.2 (0.7)	0.69
HDL (mmol/L)	1.5 (0.4)	1.4 (0.4)	1.5 (0.4)	0.91
LDL (mmol/L)	2.4 (0.7)	2.4 (0.7)	2.4 (0.6)	0.69
Triglycerides (mmol/L)	0.8 (0.4)	0.8 (0.3)	0.8 (0.5)	0.69

Values are expressed as mean (SD) or as median (interquartile range). Data with a skewed distribution were log transformed before analysis with t-tests.
SDS = standard deviation score; F = fasting; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index
N = 225 for blood pressure; N = 148 for F glucose, F insulin and HOMA-IR; N = 136 for lipid levels; N = 51 for Si, AIR and DI

DISCUSSION

In this study we investigated the impact of the *PPAR-γ* Pro12Ala polymorphism on determinants of metabolic and cardiovascular disease in short children who were born SGA and treated with GH. We demonstrated that the Pro12Ala polymorphism was not associated with baseline weight SDS, BMI SDS or other determinants of metabolic and cardiovascular disease. During GH treatment the Ala12 allele was associated with a greater increase in weight for height SDS and in BMI for age SDS. The Ala12 allele was not associated with the change in determinants of metabolic and cardiovascular disease during GH treatment.

No other study evaluated whether *PPAR-γ* polymorphisms associate with GH-induced changes in body composition and determinants of DM and cardiovascular disease (CVD). GH treatment changes the environment by inducing insulin resistance and a compensatory increase in insulin secretion (17). Also, GH treatment reduces blood pressure in short SGA children (16) but the mechanism behind this effect is not yet known. Because of the effect of GH-treatment on determinants of DM and CVD and the variation between patients in this GH-effect, our unique cohort of short SGA children provided an ideal opportunity to investigate whether there is an interaction between polymorphisms of the *PPAR-γ* gene and GH treatment on the metabolic risk profile of short SGA subjects. We found no indication that the *PPAR-γ* Pro12Ala variant explains the variation in GH-induced changes in determinants of DM and CVD.

During GH treatment some SGA children remain lean while others demonstrate catch-up in BMI SDS. As the *PPAR-γ* gene is involved in lipid metabolism and adipocyte differentiation (1) it might be a response modifier to a triggering factor such as GH treatment. Other studies indicated that the effect of *PPAR-γ* on BMI is different in various phenotypes, which suggests gene-environment interactions (1, 11). We found a significantly higher increase in BMI SDS in carriers of the Ala12 allele but the frequency of the Ala12 allele was not different in subjects with catch-up in BMI SDS and subjects without catch-up in BMI SDS. Also, in the subgroup with DXA scans there was no significant effect of genotype on the increase in body fat percentage. Thus, the Pro12Ala polymorphism possibly predicts weight gain during GH treatment but further research is needed to confirm and extend our findings.

The association between *PPAR-γ* polymorphisms and measures of glucose homeostasis was not yet studied in a population of short children born SGA. Most SGA

born individuals have catch-up growth to a normal height and catch-up growth in weight is associated with insulin resistance (35). Among the risk factors that potentiate the insulin resistance associated with low birth weight, obesity is known to play a key role (14). Short SGA subjects are usually lean. As the association of *PPAR-γ* Pro12Ala with insulin

Table 3. Association of *PPARγ* with changes in body size and determinants of metabolic and cardiovascular disease during 4-years of GH-treatment

	All	Pro/Pro	Pro/Ala or Ala/Ala	p-value *
Change of Weight SDS_{height}	0.7 (0.6 to 0.9)	0.6 (0.4 to 0.8)	0.9 (0.6 to 1.3)	**0.02**
Change of BMI SDS_{age}	0.5 (0.3 to 0.6)	0.4 (0.2 to 0.6)	0.6 (0.3 to 0.9)	**0.03**
Change of systolic bp SDS	-0.2 (-0.3 to 0.0)	-0.2 (-0.4 to 0.0)	-0.1 (-0.4 to 0.2)	0.32
Change of diastolic bp SDS	-0.2 (-0.3 to 0.0)	-0.1 (-0.3 to 0.1)	-0.3 (-0.5 to 0.0)	0.79
Change of F Glucose (mmol/L)	1.1 (-0.4 to 2.7)	1.4 (-0.4 to 3.2)	-0.3 (-0.5 to 0.0)	0.22
Change of F Insulin (pmol/L)	21.5 (11.6 to 31.3)	23.2 (12.0 to 34.4)	15.5 (-5.2 to 36.2)	0.38
Change of HOMA-IR	0.4 (0.2 to 0.6)	0.4 (0.2 to 0.6)	0.3 (-0.1 to 0.6)	0.18
Change of $Si*10^{-4}/min^{-1}$ (μU/ml)	-7.0 (-9.8 to -4.3)	-6.1 (-9.4 to -2.9)	-9.8 (-15.4 to -4.2)	0.83
Change of AIR (mU/L)	423 (271 to 575)	411 (229 to 592)	470 (154 to 786)	0.40
Change of DI (AIR*Si)	453 (6 to 899)	577 (62 to 1093)	84 (-825 to 993)	0.31

Values are expressed as model estimates (95% CI). SDS = standard deviation score; F = fasting; HOMA %B = beta cell function; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index
* p-values are derived from repeated measures regression analyses under dominant model adjusted for gender, age and puberty. Parameters with a skewed distribution were log-transformed for analysis.

sensitivity might be mediated through body weight (3), it is important to study SGA born subjects with short stature separately.

In SGA subjects, the Ala12 variant of *PPAR-γ* was associated with insulin resistance (7) while in various other clinical situations, such as in subjects with type 2 DM and obesity, the Ala12 variant was associated with an increased insulin sensitivity (36-38). Other studies in SGA subjects found the opposite and reported that the Ala12 allele protects against the insulin resistance and higher systolic blood pressure that is associated with a small size at birth (34, 39). Notably, these studies comprised SGA subjects with catch-up growth to a normal height and weight and therefore these results are not comparable

with our results. In our study in short SGA subjects, there was no association between *PPAR-γ* Pro12Ala genotype and glucose homeostasis or blood pressure.

We found no association between the Pro12Ala polymorphism and serum lipid levels before the start of GH treatment. In obese children, the Ala12 variant of *PPAR-γ* was also not associated with serum lipid levels (38). One study in adults found no differences between Ala12 carriers and non-carriers in serum lipid concentrations (34) while another study found higher HDL cholesterol and lower triglycerids levels in subjects homozygous for the Ala12 allele (3). In a study that took birth weight into account, the Ala12 allele was associated with higher concentrations of total and LDL cholesterol but only in people with a birth weight below 3000 grams (40). It was not specified whether these birth weights were low due to prematurity or due to SGA. Based on our study, it appears that the Pro12Ala polymorphism of the *PPAR-γ* gene does not associate with serum lipid levels but a larger sample size would allow more definitive conclusions.

We previously demonstrated that blood pressure in short SGA children is higher than blood pressure of height and gender matched references at a mean age of 7 years (41). A study in elderly hypertensive subjects found that in carriers of an Ala12 allele, low birth weight was not associated with adult blood pressure while in subjects with the Pro12Pro genotype this association was present (39). The authors suggested that the insulin resistance associated with the Pro12Pro genotype enhanced the regulatory responses of the renin-angiotensin system and thus induced raised blood pressure levels (39). Our subjects were much younger and we found no association between *PPAR-γ* genotype and blood pressure adjusted for height and gender.

In conclusion, our data demonstrate the possible involvement of the *PPAR-γ* Pro12Ala polymorphism in weight gain during GH treatment in short SGA children. The polymorphism was not associated with determinants of metabolic and cardiovascular disease at baseline and during GH treatment.

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CHAPTER 7

Type 2 diabetes gene *TCF7L2* polymorphism is not associated with fetal and postnatal growth in two birth cohort studies

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ABSTRACT

Background:
An inverse association between birth weight and the risk of developing type 2 diabetes (T2D) in adulthood has been reported. This association may be explained by common genetic variants related to insulin secretion and resistance, since insulin is the most important growth factor in fetal life. The objective of this study was to examine whether T2D gene polymorphism *TCF7L2* rs7903146 is associated with growth patterns from fetal life until infancy.

Design and Methods:
This study was performed in two independent birth cohort studies, one prospective population-based (Generation R), and one of subjects born small-for-gestational-age (SGA cohort). Fetal growth was assessed by ultrasounds in second and third trimesters of pregnancy in Generation R. Growth in infancy was assessed in both cohorts at birth and at 6, 12 and 24 months postnatally. *TCF7L2* genotype was determined in 3,419 subjects in Generation R and in 566 subjects in the SGA cohort.

Results:
Minor allele frequency did not differ significantly (p=0.47) between Generation R (T-allele: 28.7%) and the SGA cohort (T-allele: 29.8%). No differences at birth were found in gestational age or size (head circumference, length, weight) between the genotypes in either cohort. *TCF7L2* genotype was also not associated with any pre- or postnatal growth characteristic in either Generation R or the SGA cohort.

Discussion:
We found no evidence for an association between *TCF7L2* genotype and fetal and early postnatal growth. Furthermore, this *TCF7L2* polymorphism was not associated with an increased risk of SGA.

INTRODUCTION

Several epidemiological studies have shown inverse associations between birth weight and metabolic diseases, including type 2 diabetes (T2D) in adulthood (1, 2). These associations may be influenced by common genetic variants (2). Insulin is the most important fetal growth factor and insulin-mediated fetal growth might be affected by genetic polymorphisms that regulate fetal insulin secretion or insulin sensitivity (2). Therefore, gene variants associated with T2D have been suggested as candidate genes for influencing early growth (2).

Genome-wide association (GWA) studies have consistently shown that the C>T substitution in *TCF7L2* gene (rs7903146) increases the risk of T2D approximately 2-fold when two risk allele copies (TT) are present (3-5). The T-allele of this *TCF7L2* polymorphisms has been suggest to reduce proinsulin to insulin conversion (6), though the exact mechanism has not been elucidated yet. Other single nucleotide polymorphisms (SNPs) of the *TCF7L2* gene have been shown to be associated with type 2 diabetes, although less strongly (7). The T-allele of rs7903146, which according to HapMap has an allele frequency amongst Caucasians (CEU) of 28% (8), has been shown to be associated with reduced insulin response and secretion in both diabetic and non-diabetic individuals (9-11), though results in non-diabetics are not consistent (12). This polymorphism may also lead to an increased risk of gestational diabetes (13). Such findings make *TCF7L2* one of the most important candidate genes for explaining the associations between low birth weight and T2D.

Freathy *et al.* were the first to investigate the association between *TCF7L2* genotype and birth weight, and they found an association with maternal *TCF7L2* genotype (14). Each maternal copy of the risk allele was associated with a 30 grams increase in offspring birth weight, probably as a result of higher maternal glucose levels stimulating fetal insulin production (14). After adjustment for maternal genotype, fetal *TCF7L2* genotype did not influence fetal birth weight (14). This finding was replicated in the Helsinki birth cohort (15). In another study, no association was found between fetal *TCF7L2* genotype and the risk of small size for gestational age (16). Birth weight might be an inappropriate measure of the individual growth potential since different fetal growth rates may lead to the same birth weight (17). Furthermore, rapid postnatal weight gain, especially in fat mass, has also been shown to be associated an increase risk of obesity and type 2 diabetes in later life, independent of birth weight (18, 19).

Therefore we hypothesized that longitudinally measured fetal and postnatal growth are better parameters in the investigation of the possible effect of *TCF7L2* on growth than specific growth characteristic such as birth weight. We first assessed the associations of *TCF7L2* rs7903146 with fetal and postnatal growth characteristics in a population-based prospective cohort study among 3,419 subjects followed from early fetal life onwards. Second, we assessed associations of this genotype with birth weight and postnatal growth in 566 small-for-gestational-age (SGA) children participating in an independent cohort study.

MATERIALS AND METHODS

Cohort Descriptions

The Generation R Study

The Generation R Study is a population based prospective cohort study from early fetal life onwards. The study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood. It has been described previously in detail (20, 21). Fetal and postnatal growth and their main determinants were repeatedly measured by physical examinations, fetal ultrasounds, biological samples and questionnaires. We have previously shown that of all eligible children born in the study area 61% participated in the study (21). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants or their parents.

Fetal growth and birth characteristics

Fetal ultrasound examinations were carried out during visits to one of the research centers. These fetal ultrasounds were used for establishing gestational age in the first trimester of pregnancy (conception to 12 weeks of gestational age), as well as for assessing fetal growth characteristics in second (17-25 weeks of gestational age) and third trimesters (>25 weeks of gestational age) of pregnancy (22). Fetal growth measurements used in the present study included head circumference (HC), abdominal circumference (AC) and femur length (FL) measured in second and third trimesters to the nearest mm using standardized ultrasound procedures (23). Estimated fetal weight (EFW) was calculated by means of the formula from Hadlock using head circumference, abdominal circumference and femur length (log10 EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) +

0.00034 (HC)2 – 0.003685 (AC * FL)) (24). First trimester ultrasound measures were not included for assessing growth characteristics because these ultrasound examinations were primarily performed to establish gestational age.

Birth and postnatal growth

Birth weight, date of birth and gender were obtained from community midwife and hospital registries. Information on head circumference or length at birth was not available, but many children were measured during the first two months of life. Well-trained staff in community health centers obtained postnatal growth characteristics using standardized procedures. Based on the routine health care program, the visits at which these growth characteristics were measured were grouped into three age periods: 6 months (range 5 to 8.99); 12 months (range 9 to 12.99); and 24 months (range 23 to 34.99 months). Postnatally, head circumference was not measured at the age of 24 months.

Population for analysis

Analyses were restricted to singletons from whom DNA was available for *TCF7L2* genotyping and who also had Dutch or other Caucasian ethnicity as defined by having both parents born in the Netherlands or another European country (n = 3,419)(Figure 1). Fetal growth measurements were available for 3,320 and 3,384 children in second and third trimesters, respectively. Of these children, those living outside the study area postnatally (10%) were not followed up in infancy and a further 12% were lost during postnatal follow-up, leaving 2,675 subjects eligible for the postnatal analyses (Figure 1).

The SGA Cohort

The SGA cohort was designed for the purpose of assessing growth and development of subjects born SGA. Subjects were included at childhood age (n = 367) or at young adult age (n = 252). Children were included in the SGA cohort when they were SGA at birth, had short stature (height standard deviation score (SDS) for age and gender of below – 2 (25)), did not show catch-up growth in height, and had no growth failure caused by any other identified disorder. These inclusion criteria have previously been described (26). Young adults included in the SGA cohort were randomly selected from hospitals in the Netherlands, where they had been registered because of being SGA. Only those young adults born at 36 weeks or more of gestation, being singleton and Caucasian and not

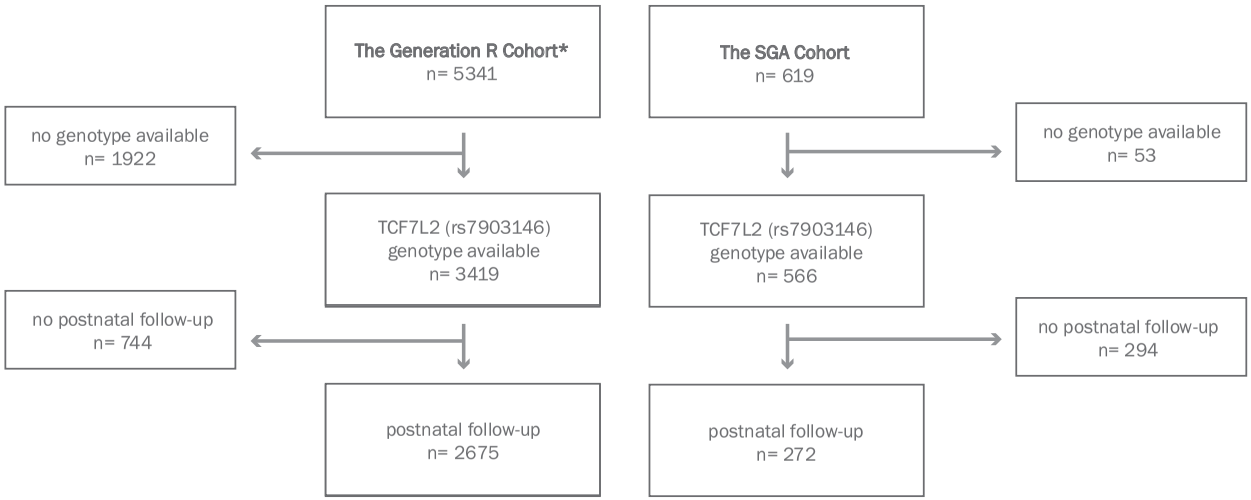

Figure 1. Flow diagram indicating number of subjects in the two cohorts
* All live-born, Caucasian, singleton subjects within Generation R

suffering from conditions or receiving treatment known to interfere with growth, were invited to participate. SGA was defined as a birth length and/ or birth weight SDS of below –2.0 for gestational age (27). The Medical Ethics Committees of Erasmus Medical Center, Rotterdam, and of the participating centers approved all studies and written informed consent was obtained from all participants or their parents.

Birth and postnatal growth

Birth characteristics of the SGA cohort were collected from hospital registries. The gestational age of the subjects was determined by ultrasound in the first trimester, if available, and otherwise calculated from the date of the last menstruation. Growth data (head circumference, height and weight) measured during the first two years of life were collected from records of hospitals, community health services and general practitioners. Longitudinal growth data were available in 272 participants in the SGA cohort (Figure 1).

Genotyping

DNA was collected from cord blood samples in the Generation R cohort and from peripheral venous blood samples in the SGA cohort. Cord blood for DNA isolation was available for 59% of all participating children of the Generation R cohort. When cord blood samples were missing, this result was mainly due to logistical constraints at the delivery. Venous blood samples were available in the complete SGA cohort. Genotyping of the C>T substitution in *TCF7L2* (rs7903146) gene was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg Germany). The genotyping reaction was amplified using the GeneAmp® PCR system 9600 (95° C (15 minutes), with 40 cycles of 94° C (15 seconds) and 60° C (1 minute)). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems). Genotyping was successful in 98% and 91% of the samples in the Generation R and SGA cohort, respectively. To confirm the accuracy of the genotyping results, 276 randomly selected samples from the Generation R Study were genotyped for a second time with the same method. The error rate was less than 1%. The frequency distribution in Generation R did not deviate from the Hardy-Weinberg equilibrium in subjects with Dutch ethnicity nor did it deviate in the SGA cohort.

Data analysis

With sample sizes in the Generation R Study of 3,419 and 2,675 subjects for fetal and postnatal analyses respectively, and assuming a statistical power level (1 – β) of 0.80, a level of significance (α) of 0.05 and a variance of 1.0, we were able to detect differences in growth characteristics of 0.048 SDS and 0.054 SDS respectively. First, differences in allele distribution between children born SGA (from the SGA cohort) and non-SGA subjects (from Generation R) were assessed. Differences were calculated using the Chi-square test. Second, we examined the differences in birth characteristics between genotype groups with linear regression analyses assuming an additive model. Weight, length and head circumference at birth and at different ages were analyzed using gender and age adjusted standard deviation scores (SDS) (27, 28). Standard deviation scores were obtained using Dutch reference growth curves (Growth Analyser 3.0, Dutch Growth Research Foundation). For Generation R, we used the first length SDS and head circumference SDS measured after birth and before the second month of life, since these measurements were not available at birth. Third, we compared fetal (only Generation R) and postnatal characteristics between the genotypes with linear regression analyses. Finally, to assess longitudinally measured weight and length patterns from fetal life to infancy, we performed repeated measures regression analysis in both cohorts with weight and length from birth to 24 months as outcome variables. This regression technique takes the correlation of multiple measurements within one subject into account, assesses both the time-independent and time-dependent effect of *TCF7L2* genotype on growth, and allows for incomplete outcome data (29). In these models, genotype was included as both intercept and interaction with age. To account for (gestational) age at each specific measurement, these analyses were conducted with age-adjusted standard deviation scores. The models can be written as:

Height (SDS) or weight (SDS) = ß0 + ß1*age + ß2* TCF7L2 genotype + ß3* TCF7L2 genotype*age.

In this model, the term including 'ß0' reflects the intercept and the term including 'ß1' reflects the slope of growth (weight or length) per week for the reference group (CC genotype). The terms including 'ß2' and 'ß3' reflect the age independent growth differences in weight (and length) between the different categories of the *TCF7L2* genotype respectively (30). All models were unadjusted (all growth characteristics are

age and gender adjusted SD scores) since population genotype distribution is assumed to be unrelated to covariates and the effect estimates were not materially affected by adjusting for maternal age, pre-pregnancy body mass index or parity (31). The occurrence of gestational diabetes in the entire cohort was 0.6% and did not affect the effect estimates. Therefore, occurrence of gestational diabetes was not included in the analyses.

All effect estimates are presented with their 95% confidence interval (95% CI). Statistical analyses were performed using the Statistical Analysis System version 9.1.3 (SAS, Stata corporation, College Station, TX, USA), including the PROC MIXED module for unbalanced repeated measurements as well as the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).

RESULTS

Subject characteristics of Generation R and SGA cohort are presented in Table 1. The minor allele frequency distributions did not differ significantly (p=0.47) between non-SGA subjects (from Generation R) (T-allele: 28.7%) and the SGA cohort (T-allele: 29.8%) (Table 2).

No significant differences between genotype groups were observed in fetal growth characteristics in Generation R (Table 3). No differences in birth characteristics (head circumference, length and weight), between genotype groups were observed in either cohort (Table 4). Postnatal growth characteristics for both cohorts are shown in Table 5. No significant differences were found in either cohort for head circumference, weight or height at any age.

Finally, no differences were found in weight growth rate (SDS/year) from birth until the age of 2 years in either Generation R or the SGA cohort. Compared to the CC genotype, differences were -0.014 (95% confidence interval (CI): -0.064, 0.036) SDS/year and -0.028 (95% CI: -0.057, 0.002) SDS/year, for the CT and TT genotype, respectively, in Generation R. In the SGA cohort, differences were -0.134 (95% CI: -0.376, 0.108) SDS/year and 0.002 (95% CI: -0.125, 0.129) SDS/year, for the CT and TT genotype, respectively, using the CC genotype as a reference. Similarly, no differences were found in height growth rate from birth to 2 years in either cohort (data not shown).

Table 1. Subject characteristics by cohort.		
Characteristics	**Generation R**	**The SGA cohort**
Gender (% boys)	50.8%	47.2%
Gestational age (weeks)	40.1 (36.7 – 42.4)	38.0 (29.9 – 41.0)
Birth weight (grams)	3513 (511)	1819 (716)
Premature (gestational age < 37 weeks) (%)	2.9%	44.9%
Birth weight < 2500 grams (%)	2.5%	82.7%
Small for gestational age (weight < -2 SDS) (%)	0.9%	100%
Gestational diabetes (%)	0.6%	N/A

Values are means (SD), medians (95% range) or percentages. N/A = not available

Table 2. Distribution of *TCF7L2* rs7903146 minor allele frequency according to cohort.			
	Allele frequency		
	C-Allele	**T-Allele**	**p-value**
Non-SGA (Generation R) (%)	4828 (71.3)	1948 (28.7)	
SGA (SGA cohort) (%)	795 (70.2)	337 (29.8)	0.49

Non-SGA = All subjects from Generation R, excluding SGA subjects
SGA = birth weight SDS and/or birth length SDS < -2
p-value express differences in distribution between SGA and General population tested with Chi-square test.

Table 3. Fetal characteristics according to fetal *TCF7L2* rs7903146 genotype in the Generation R study.				
	CC (n = 1736)	**CT (n = 1329)**	**TT (n = 301)**	**p- value#**
Fetal characteristics second trimester				
Head circumference (SDS)	0.04 (1.0)	0.02 (1.0)	0.05 (0.9)	0.88
Femur length (SDS)	-0.01 (1.0)	-0.01 (1.0)	0.05 (0.9)	0.64
Estimated fetal weight (SDS)	-0.06 (1.0)	-0.07 (1.0)	0.00 (1.0)	0.57
Fetal characteristics third trimester				
Head circumference (SDS)	0.11 (1.0)	0.13 (1.0)	0.15 (0.9)	0.45
Femur length (SDS)	0.01 (1.0)	-0.02 (1.0)	-0.04 (1.0)	0.34
Estimated fetal weight (SDS)	0.12 (1.0)	0.14 (1.0)	0.11 (0.9)	0.99

Values are means (SD). SDS = standard deviation score for age and gender.
#p-values for additive models. Differences were tested using linear regression analyses.

Table 4. Birth characteristics in both cohorts according to *TCF7L2* rs7903146 genotype of child.					
Generation R	**N**	**CC (n = 1762)**	**CT (n = 1351)**	**TT (n = 306)**	**p-value#**
Gestational age (weeks)	3419	40.3 (36.7 – 42.3)	40.3 (36.6 – 42.4)	40.1 (37.1 – 42.6)	0.83
Birth head circumference (SDS)*	2314	0.22 (0.9)	0.24 (0.9)	0.26 (0.9)	0.55
Birth length (SDS)*	1959	-0.07 (1.0)	-0.08 (1.0)	0.00 (1.1)	0.66
Birth weight (SDS)	3419	0.21 (1.0)	0.22 (1.0)	0.20 (1.0)	0.97
SGA Cohort		**CC (n = 270)**	**CT (n = 255)**	**TT (n = 41)**	**p-value#**
Gestational age (weeks)	566	38.0 (28.6 – 42)	38.0 (28.6 – 41.0)	38.0 (29.0 – 42.0)	0.57
Birth head circumference (SDS)	203	-1.51 (1.4)	-1.20 (1.6)	-1.31 (1.4)	0.32
Birth length (SDS)	491	-3.11 (1.4)	-3.27 (1.5)	-3.02 (1.5)	0.41
Birth weight (SDS)	566	-2.40 (1.0)	-2.46 (0.9)	-2.32 (0.9)	0.58

* Length and head circumference were measured in the first two months of after birth.
Values are means (SD) or medians (95% range). SDS = standard deviation score for age and gender.
p-values for additive models. Differences were tested using linear regression analyses.

DISCUSSION

In the current study, we found that T2D gene polymorphism *TCF7L2* rs7903146 is not associated with growth in fetal life in the general population or with growth in early postnatal life in either the general population or in a cohort of subjects born SGA. We also confirmed previous suggestions that this variant of *TCF7L2* is not associated with birth weight and, more importantly, demonstrated that it does not influence the fetal development using direct fetal measurements. Finally, we showed that this polymorphism does not appear to be associated with the risk of being born SGA.

To our knowledge, this study is the first to examine the association of *TCF7L2* with longitudinally measured growth patterns in fetal and early postnatal life in two independent birth cohorts. In the Generation R Study, DNA for genotyping was available in 59% of all subjects and was isolated from cord-blood. Missing cord-blood was mainly caused by logistical restraints at delivery. Children who were not genotyped had a shorter gestational age ($p < 0.001$) and were lighter at birth ($p < 0.001$) than subjects who were genotyped. Of all genotyped eligible subjects at baseline, 22% did not participate in follow-up measurements. In the SGA cohort, genotyping was successful in 91% of the subjects and longitudinally growth data were available in 48% of the cohort. Our effect estimates could

Table 5. Postnatal characteristics at 6, 12, and 24 months according to *TCF7L2* rs7903146 genotype.					
Generation R		**CC (n = 1375)**	**CT (n = 1063)**	**TT (n = 237)**	**p-value[#]**
6 months	Head circumference (SDS)	-0.02 (0.93)	-0.03 (0.89)	-0.06 (0.91)	0.83
n = 2675	Height (SDS)	0.03 (0.91)	0.03 (0.90)	0.07 (0.93)	0.81
	Weight (SDS)	0.41 (0.96)	0.44 (0.95)	0.54 (0.99)	0.14
12 months	Head circumference (SDS)	0.00 (0.89)	-0.04 (0.94)	-0.03 (1.12)	0.54
n = 2559	Height (SDS)	-0.01 (0.90)	-0.05 (0.90)	-0.01 (0.90)	0.70
	Weight (SDS)	0.18 (0.98)	0.18 (0.99)	0.24 (1.00)	0.63
24 months	Height (SDS)	-0.19 (0.93)	-0.21 (0.89)	-0.18 (0.87)	0.82
n = 2445	Weight (SDS)	-0.11 (0.99)	-0.13 (1.00)	-0.09 (0.96)	0.87
SGA cohort		**CC (n = 143)**	**CT (n = 107)**	**TT (n = 22)**	
6 months	Head circumference (SDS)	-1.38 (0.92)	-1.36 (0.90)	-1.74 (1.04)	0.41
n = 272	Height (SDS)	-2.39 (1.37)	-2.43 (1.26)	-2.51 (1.55)	0.93
	Weight (SDS)	-2.18 (1.40)	-2.22 (1.26)	-2.37 (2.05)	0.86
12 months	Head circumference (SDS)	-1.21 (0.83)	-1.24 (0.88)	-1.72 (1.06)	0.16
n = 268	Height (SDS)	-2.25 (1.25)	-2.30 (1.06)	-2.30 (1.47)	0.94
	Weight (SDS)	-2.14 (1.41)	-2.25 (1.15)	-2.17 (1.89)	0.82
24 months	Head circumference (SDS)	-1.10 (0.82)	-1.13 (0.87)	-1.60 (1.06)	0.20
n = 244	Height (SDS)	-2.39 (1.24)	-2.47 (1.05)	-2.94 (1.04)	0.20
	Weight (SDS)	-2.19 (1.33)	-2.31 (1.21)	-3.06 (1.65)	0.04

Values expressed as mean (SD). SDS = standard deviation score for age and gender.
[#] p-values for additive models. Differences were tested using linear regression.

be biased if the associations between genotypes and growth characteristics differed between those with and without postnatal growth data available. In the Generation R cohort, no differences were observed between children with and without postnatal growth measurements. In the SGA cohort the T-allele was slightly more frequent in subjects with postnatal growth measurements than in subjects without these measurements ($p < 0.05$). Finally, it could be possible that there is differential effect of genotype on growth according to availability of follow-up data. This bias would affect our estimates, though such a bias seems unlikely.

Several studies have investigated the effect of common genetic variants related to insulin action and secretion on early growth (14, 15, 32, 33). Of the initially identified T2D gene polymorphisms identified by the GWA, fetal *CDKAL1* (rs7754840) and *HHEX*

(rs1111875) genotype, and maternal *TCF7L2* (rs7903146) genotype have been shown to affect birth weight. Pulizzi *et al.* demonstrated in the Helsinki Birth Cohort that fetal *TCF7L2* genotype did not interact with birth weight to increase the risk of T2D in adulthood (15). *TCF7L2* rs7903146 has been shown to have the strongest genetic effect on T2D and this result has been replicated in several studies (3-5). Therefore, *TCF7L2* is a very important candidate gene for explaining the association between low birth weight and T2D risk. Our study is the first to investigate the effect of *TCF7L2* rs7903146 on longitudinal growth in early life. Longitudinal assessment of growth provides more information than just measurements at birth as we have demonstrated earlier that different fetal growth patterns may result in a similar birth weight (17). Furthermore, most SGA born children have catch-up growth during the first months of life but 15% remain small (34). Thus, to investigate whether *TCF7L2* rs7903146 influences fetal and postnatal growth, longitudinal growth data provide more information than birth weight alone.

Freathy *et al.* found an increase of birth weight for each fetal and maternal risk allele (14). They concluded that the most likely mechanism for this association was that maternal genotype was associated with a reduction of maternal insulin secretion, leading to increased fetal glucose and insulin levels and subsequently increased birth weight, rather than a direct effect of the fetal genotype on birth weight. Pulizzi *et al.* found no effect of the fetal genotype of this polymorphism on birth weight. Since fetal and maternal genotypes are 50% correlated, it cannot be excluded that, when the risk allele is present in both mother and child, small effects of fetal genotype that reduce fetal growth could be masked by opposing effects of maternal genotype. Since maternal genotype was not available in our study, we were not able to test this hypothesis. However, we did not find any effect of fetal genotype on birth weight in the general population nor in a specific population of children with insufficient fetal growth resulting in small size for gestational age at birth. Our findings are therefore in line with the conclusions of these previous studies. Furthermore, we found no effect of fetal genotype on estimated fetal weight or weight during infancy, indicating that there is no evidence for any association between this fetal genotype and weight or change in weight during early life either. The effect of this polymorphism on the metabolic phenotype found in adults would therefore appear to develop after early childhood. Nonetheless, our results also could be explained by a lack of power and we cannot rule out that we were unable to detect smaller effects of this variant on early growth.

Regarding intra-uterine growth retardation, an earlier study examined the effect of *TCF7L2* rs7903146 genotype on SGA. Cauchi et al. found no association between this genotype and SGA, using family-based association analyses in over 3,000 subjects of which 627 subjects were SGA (16). In this analyses, the SGA group was slightly larger than in our current study and included parents, but postnatal growth data were not analyzed longitudinally. In our study, we did not find a difference in minor allele frequency between the general population (Generation R) and the SGA cohort. On the basis of two independent and negative studies, one may conclude that there is no association between this genetic polymorphism and risk of SGA.

In summary, our results suggest that *TCF7L2* rs7903146 does not influence growth from early fetal life to infancy. Furthermore, minor allele frequency was not different in SGA subjects than in non-SGA subjects, indicating that it is unlikely that this polymorphism is associated with the risk of being born SGA. Systematic searches for common genetic variants by means of genome-wide association studies will enable us to obtain a more complete understanding of which genes are involved in growth in fetal life and infancy.

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CHAPTER 8

Interactions between *TCF7L2* genotype and growth hormone-induced changes in glucose homeostasis in small for gestational age children

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ABSTRACT

Context: The *TCF7L2* rs7903146 gene polymorphism has been associated with the risk of developing type 2 DM, possibly by decreasing insulin secretion. Small for gestational age (SGA) birth has been associated with type 2 DM in later life. Growth hormone (GH) treatment reduces insulin sensitivity and increases insulin secretion. Therefore, GH-treated SGA children are an ideal group to investigate whether *TCF7L2* rs7903146 genotype associates with changes in glucose homeostasis.

Objective: To determine the impact of the *TCF7L2* rs7903146 polymorphism on changes in insulin secretion and insulin sensitivity during 4 years of GH treatment in children born SGA.

Subjects: 246 Caucasian short children born SGA, with a median age of 7.8 years.

Outcome measures: Insulin sensitivity and insulin secretion measured by FSIGTs (n = 68) and HOMA calculations (all).

Results: There was no association between rs7903146 genotype and insulin sensitivity or insulin secretion at baseline but after adjustment for possible confounders, insulin secretion was higher in the CT/TT group than in the CC group. During GH treatment, carriers of the rs7903146 T-allele had a similar increase in insulin secretion as carriers of the CC genotype. The decrease in insulin sensitivity was only significant in the CT/TT group but the difference in decrease between genotype groups did not reach significance (p = 0.06). The disposition index (insulin secretion * insulin sensitivity), which is an estimate of beta cell function was not associated with genotype and did not change during GH treatment.

Conclusion: The *TCF7L2* rs7903146 polymorphism is not associated with the change in insulin secretion during GH treatment in short SGA children.

INTRODUCTION

Transcription factor 7-like 2 (TCF7L2 [MIM 602228]) rs7903146 is to date the polymorphism with the strongest association with type 2 diabetes mellitus (DM) (1-3), probably because the rs7903146 T-allele associates with decreased insulin secretion (4-8). So far, literature is inconclusive whether the rs7903146 T-allele also associates with insulin sensitivity and disposition index (4-6, 8).

Subjects born SGA are considered to have an increased risk to develop type 2 DM in later life (9, 10), but it is unknown whether this increased risk is genetically or environmentally determined or by a combination of both. In a population of young adults born SGA, there was no association between *TCF7L2* genotype and insulin sensitivity assessed by HOMA-IR (11). A more accurate method than HOMA calculations to assess beta cell function and insulin sensitivity is the frequently sampled intravenous glucose tolerance test (FSIGT). FSIGT results were previously assessed in relation to *TCF7L2* genotype (8, 12) but it was inconclusive whether they were associated with rs7903146 genotype. FSIGT results in association with *TCF7L2* rs7903146 genotype were not yet evaluated in a population born SGA.

Nowadays, most children with short stature who were born SGA are treated with growth hormone (GH). GH treatment is known to decrease insulin sensitivity and to induce a compensatory increase in insulin secretion (13). Concerns were expressed that GH may increase the risk to develop DM in adulthood. Since the *TCF7L2* T-allele was associated with decreased insulin secretion, this SNP might indicate which children are less capable of producing extra insulin to compensate for the reduced insulin sensitivity induced by GH-treatment. Children with less insulin secretion during GH-treatment might be more at risk to develop DM in later life.

We have detailed measurements of insulin sensitivity and insulin secretion during long-term GH treatment in a cohort of short children born SGA. Because of the effect of GH-treatment on insulin sensitivity and insulin secretion, this unique cohort provides an ideal opportunity to investigate whether there is an interaction between the *TCF7L2* genotype and GH treatment on the metabolic risk profile of short SGA subjects.

The present study aimed to investigate whether *TCF7L2* rs7903146 genotype correlates with changes in insulin sensitivity and insulin secretion during GH treatment. To investigate this question, we performed *TCF7L2* genotyping, FSIGTs and HOMA calculations in 246 short children who were born SGA and treated with GH.

SUBJECTS AND METHODS

Subjects

The study cohort consisted of 246 Caucasian short SGA children who were participating in GH trials with the same inclusion criteria (14). SGA was defined as birth weight and/or birth length adjusted for gender and gestational age (15) < -2 SD. The inclusion criteria were previously described (14). In short, the children were included when prepubertal, with a birth length or birth weight standard deviation score (SDS) and actual height SDS below –2 (16), without growth failure caused by other disorders. The gestational age of the subjects was determined by ultrasound scans during the first trimester, if available, and otherwise calculated from the date of the last menstrual period. The date of inclusion determined whether an FSIGT was performed. The Medical Ethics Committees of the participating centers approved the study and written informed consent was obtained from the parents.

Genotyping and quality control

DNA was isolated from peripheral blood leucocytes by standard method from all patients. Genotyping of the *TCF7L2* (rs7903146) gene was performed using TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg Germany). The genotyping reaction was amplified using the GeneAmp® PCR system 9600 (95° C (15 minutes), with 40 cycles of 94° C (15 seconds) and 60° C (1 minute)). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were determined using SDS software (version 2.3, Applied Biosystems).

GH treatment

Short SGA children were treated with biosynthetic GH with a dose of 1 mg/m^2 body surface area, subcutaneously, once daily at bedtime. The GH dosage was adjusted to the calculated body surface area every three months. A subgroup of 19 children was randomized to receive 1 or 2 mg/m^2 GH during the first 6 months and 1 mg/m^2 thereafter (17). Because independent samples t-tests demonstrated that the effect of GH treatment on the change in insulin sensitivity or HOMA values after 4 years of treatment was not different between the children who received 1 or 2 mg/m^2 during the first 6 months, these children were added to the large GH-group.

Glucose homeostasis

Fasting glucose was measured on a Hitachi 917 analyzer. Fasting insulin was measured by chemoluminescent assay on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA) and HOMA insulin resistance index (HOMA-IR) and beta cell function (HOMA %B) were calculated (18). Fasting glucose and insulin levels were measured again after 6 months, 1, 2 and 4 years of GH treatment.

In a subgroup of 68 subjects (determined by the date of inclusion), a modified, frequently sampled intravenous glucose tolerance test (FSIGT) using tolbutamide, was performed, as previously described (19, 20). The FSIGT was repeated after 6 months, 2 years and 4 years of treatment. Serum glucose and insulin levels were measured in one laboratory as previously described (14) and insulin sensitivity (Si), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILENNIUM software (21). All blood samples were taken after an overnight fast. Insulin sensitivity quantifies the capacity of insulin to promote glucose disposal. The acute insulin response, an estimate of insulin secretory capacity, was measured as the area under the curve from zero to ten minutes corrected for baseline insulin levels. The Disposition index (DI) is an overall measure of the ability of the beta cells to secrete insulin in response to the degree of insulin resistance. DI is the product of AIR and Si (DI = AIRg * SI) (21).

Body Composition

Dual-Energy X-ray Absorptiometry scans (DEXA, type Lunar DPX-L, GE Healthcare, Madison, Wisconsin, USA) were performed in 83 subjects. The date of inclusion determined in which subjects a DEXA scan was performed. Fat mass (FM) and body fat percentage (% fat mass of total body weight) were determined.

Statistical Analysis

FSIGT parameters and calculated HOMA values were logarithmically transformed because of a skewed distribution. Associations were tested under a dominant genetic model: homozygosity for the C allele was coded as 0, and the heterozygous state and homozygosity for the T allele were coded as 1. Genotype distributions for significant departure from the Hardy-Weinberg equilibrium were calculated using the $\chi 2$ test. A post-hoc power analysis with a level of significance (α) of 0.05 and a chosen power of 80% showed that with a number of 110 subjects per genotype-subgroup we could detect differences in fasting glucose levels of 0.27 mmol/l, differences in fasting insulin of 1.1

pmol/l, and differences in HOMA IR of 0.15 (14). A similar power analysis showed that with 30 subjects per genotype-subgroup we could detect differences in insulin sensitivity (Si) of $4.9*10^{-4}$ µU/ml and differences in AIR of 85 mU/l (22). Clinical data are presented as mean (SD) unless stated otherwise. Statistical significance was defined as $p < 0.05$.

Differences between genotype groups were evaluated using independent sample t-tests. Multiple linear regression analysis was used to compare FSIGT parameters and calculated HOMA values between the CC and the CT/TT genotype adjusted for possible confounders. Known covariables for each parameter of glucose homeostasis were taken into account and genotype was added to the model as a covariable. To test whether a certain amount of body fat percentage was needed for the T-allele to influence parameters of glucose homeostasis we added an interaction term to the model: body fat percentage*T-allele.

FSIGT parameters and calculated HOMA values during GH treatment were analyzed using repeated measures of variance (23). Model estimates are presented as mean (95% CI). To compare longitudinally measured FSIGT and HOMA parameters during 4 years of GH treatment for genotype groups, we performed repeated measures regression analysis with Si, AIR, DI, HOMA %B and HOMA IR from start of treatment to 4 years later as outcome. In these models, genotype was included as both independent variable and as interaction with the study duration. All models were corrected for puberty. The models can be written as:

Outcome variable = $ß_0 + ß_1$*puberty + $ß_2$* study duration + $ß_3$* *TCF7L2* genotype + $ß_4$* *TCF7L2* genotype*study duration.

In this model, the term including '$ß^0$' reflects the intercept, the term including '$ß^1$' reflects the correction for puberty and the term including '$ß^2$' reflects the slope of change (in Si, AIR, DI, HOMA %B or HOMA IR) per month GH therapy for the reference group (CC genotype). The term including '$ß^3$' reflects the difference in Si, AIR, DI, HOMA %B or HOMA IR between the CC and CT/TT variant of *TCF7L2* rs7903146 independent of puberty and study duration. The term including '$ß^4$' reflects the difference in change of Si, AIR, DI, HOMA %B or HOMA IR between the CC and CT/TT variant of *TCF7L2* rs7903146 independent of puberty and study duration (24).

Analyses were performed using the statistical package SPSS (version 15.0; SPSS inc., Chicago, IL) for Windows. SAS 8.2 (SAS Institute Inc., Cary, /nC, USA) was used for

repeated measures of variance analyses.

RESULTS

Clinical characteristics of the participants are presented in Table 1. Genotype distribution was in Hardy-Weinberg equilibrium ($\chi 2 = 2.17$; $p = 0.14$). The minor allele frequency (MAF) was 30 % and genotype frequencies for CC, CT, and TT were 46%, 46% and 7%, respectively. There was no significant association between the *TCF7L2* rs7903146 SNP and insulin sensitivity (Si), insulin secretion (AIR and HOMA %B), disposition index or insulin resistance (HOMA IR) (Table 2). After adjustment for age, puberty, gender and body fat percentage insulin secretion (AIR) was higher in carriers of a T-allele (Table 2). There were no significant differences between genotype groups in other measures of glucose homeostasis (adjusted P-values Table 2). The linear regression model for Si explained 38% of the variance in Si and the model for AIR explained 30% of the variance in AIR. There was no significant interaction between body fat percentage and genotype for *TCF7L2* rs7903146 in any of the regression models, indicating that the influence of the T-allele on measures of glucose homeostasis did not depend on body fat percentage.

During 4 years of GH treatment, there was a significant decrease in Si in the CT/TT genotype group (from 13.2 to 7.2 μU/ml; $p < 0.001$) and a significant increase in DI

Table 1. Clinical characteristics (n = 246)	
Age	7.8 (5.3 to 10.8)
Gender (% males)	56%
Gestational Age (weeks)	38.0 (33.9 to 40)
Birth weight SDS	-2.2 (1.1)
Birth length SDS	-3.1 (1.5)
Height SDS	-2.9 (0.7)
Weight SDS*	-1.1 (1.2)
BMI SDS	-1.3 (1.0)
Genotype (% carriers T-allele)	54%
Body fat %**	12.4 (9.5 to 17.6)

Values are expressed as mean (SD), percentage, or as median (interquartile range) in case of a skewed distribution.
*Weight for height SDS
**n = 83

Table 2. Insulin sensitivity and insulin secretion according to *TCF7L2* rs7903146 genotype at baseline					
	All	**CC**	**CT/TT**	**P-value**	**Adjusted P-value***
n	246	113	133		
Fasting glucose (mmol/l)	4.7 (4.1 to 5.0)	4.7 (4.2 to 5.0)	4.7 (4.1 to 5.1)	0.99	0.96
Fasting insulin (pmol/l)	25.0 (14.0 to 41.0)	24.0 (14.0 to 36.0)	26.0 (14.0 to 43.5)	0.38	0.85
HOMA %B	68.1 (52.7 to 90.4)	64.7 (52.7 to 87.6)	71.1 (52.5 to 91.8)	0.38	0.85
HOMA IR	0.5 (0.3 to 0.8)	0.4 (0.3 to 0.7)	0.5 (0.3 to 0.8)	0.43	0.88
n	68	31	37		
$Si*10^{-4}/min^{-1}$ (μU/ml)	12.1 (7.8 to 17.0)	11.3 (7.5 to 15.1)	12.6 (8.6 to 17.7)	0.44	0.11
AIR (mU/L)	283 (189 to 395)	255 (187 to 335)	296 (195 to 496)	0.20	**0.02**
DI (AIR*Si)	3028 (2377 to 4647)	2873 (1846 to 3851)	3329 (2637 to 4771)	0.15	0.06

Values are expressed as median (interquartile range) because of a skewed distribution
Data were log transformed before analysis with t-tests and linear regression.
HOMA %B = beta cell function; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index. Significant p-values are in bold.
*Adjusted p-values are derived from linear regression analyses under dominant model (CC versus CT/TT) adjusted for gender, age, puberty (coded as yes/no) and body fat percentage. Si was adjusted for gender, age, and puberty (coded as yes/no) because a linear regression models including body fat percentage was not significant. DI was only adjusted for age for the same reason.

in the CC genotype group (from 2841 to 4189; $p < 0.01$) compared to values at start but the difference between genotype groups in overall change in Si and DI did not reach significance (Table 3). All other estimates of glucose homeostasis showed a similar change during GH therapy in the CC genotype and the CT/TT genotype group.

DISCUSSION

We investigated whether there is an interaction between *TCF7L2* rs7903146 genotype and the change in insulin sensitivity and insulin secretion during GH treatment in Caucasian subjects with short stature, born small for gestational age. We demonstrated that presence of the rs7903146 T allele was not associated with the increase in insulin secretion during GH treatment. The difference between genotype groups in change in insulin sensitivity did not reach significance but tended to be larger in carriers of a T-allele.

Table 3 Insulin sensitivity and insulin secretion during GH treatment in short SGA children by *TCF7L2* rs7903146 genotype						
		At start	6 months	2 years	4 years	*p-value
All	F glucose (mmol/L)	4.5 (4.4 to 4.5)	4.7 (4.6 to 4.8)†††	4.6 (4.4 to 4.8)	4.8 (4.4 to 5.1)	
	F insulin (pmol/L)	23.6 (22.0 to 25.4)	29.1 (26.1 to 32.5)†††	33.6 (30.0 to 37.6)†††	30.9 (26.3 to 36.3)††	
	HOMA %B	68.9 (65.4 to 72.4)	71.1 (66.8 to 75.8)	83.0 (77.4 to 89.0)†††	78.3 (70.4 to 87.0)†	
	HOMA IR	0.43 (0.40 to 0.46)	0.54 (0.48 to 0.60)†††	0.62 (0.55 to 0.69)†††	0.57 (0.49 to 0.67)†††	
	Si*10-4/min-1 (µU/ml)	11.8 (10.1 to 13.7)	7.0 (5.8 to 8.5)†††	9.5 (8.2 to 10.9)†	6.6 (5.6 to 7.8)†††	
	AIR (mU/L)	275 (236 to 320)	532 (430 to 659)†††	455 (376 to 551)†††	596 (491 to 724)†††	
	DI (AIR*Si)	3240 (2814 to 3731)	3771 (3368 to 4221)	4036 (3593 to 4532)††	3872 (3353 to 4472)†	
CC	F glucose (mmol/L)	4.5 (4.3 to 4.6)	4.6 (4.5 to 4.7)	4.7 (4.4 to 4.9)	4.9 (4.4 to 5.3)	
	F insulin (pmol/L)	22.2 (20.0 to 24.7)	28.8 (24.6 to 33.8)††	33.0 (28.1 to 38.7)†††	27.0 (21.4 to 34.1)	
	HOMA %B	66.1 (61.2 to 71.5)	74.2 (67.8 to 81.2)†	83.4 (75.7 to 92.0)†††	72.0 (61.7 to 84.0)	
	HOMA IR	0.40 (0.36 to 0.45)	0.53 (0.45 to 0.62)†	0.60 (0.51 to 0.70)†††	0.49 (0.39 to 0.61)	
	Si*10-4/min-1 (µU/ml)	11.1 (8.7 to 14.1)	8.8 (6.8 to 11.5)	12.0 (10.1 to 14.3)	9.0 (7.2 to 11.2)	
	AIR (mU/L)	234 (186 to 295)	471 (342 to 647)†††	336 (277 to 409)†††	468 (357 to 613)†††	
	DI (AIR*Si)	2841 (2380 to 3392)	4082 (3257 to 5115)††	3755 (3029 to 4655)†	4189 (3239 to 5419)††	
CT/TT	F glucose (mmol/L)	4.4 (4.3 to 4.6)	4.7 (4.5 to 4.8)††	4.6 (4.3 to 4.8)	4.6 (4.1 to 5.0)	0.45
	F insulin (pmol/L)	23.5 (21.3 to 26.0)	28.0 (23.9 to 32.8)†	31.7 (27.1 to 37.2)†††	30.5 (23.9 to 38.9)†	0.65
	HOMA %B	69.0 (64.2 to 74.2)	68.5 (62.5 to 75.0)	78.2 (71.0 to 86.2)†	78.0 (66.3 to 91.8)	0.88
	HOMA IR	0.43 (0.38 to 0.47)	0.52 (0.44 to 0.61)	0.59 (0.50 to 0.70)†††	0.58 (0.46 to 0.74)††	0.36
	Si*10-4/min-1 (µU/ml)	13.2 (10.5 to 16.5)	6.8 (4.9 to 9.5)†††	9.2 (7.5 to 11.2)†	7.2 (5.6 to 9.3)†††	0.06
	AIR (mU/L)	282 (227 to 350)	497 (342 to 721)†††	462 (375 to 569)†††	529 (390 to 718)†††	0.98
	DI (AIR*Si)	3577 (3014 to 4245)	3475 (2567 to 4703)	3917 (3082 to 4979)	3555 (2575 to 4906)	0.18

Values are expressed as model estimates (95% CI) and were adjusted for puberty (n = 33 for glucose, insulin and HOMA calculations and n = 14 for FSIGT results). Parameters were log-transformed for analysis.
F = fasting; HOMA %B = beta cell function; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index
*p-value = Change of parameter during 4 years of GH treatment in carriers T allelle (CT or TT) versus wild type (CC)
†††p<0.001; ††p<0.01; †p<0.05 compared with start

No other study evaluated the effect of the rs7903146 T allele on GH-induced changes in glucose homeostasis. GH treatment changes the environment by inducing insulin resistance and a compensatory increase in insulin secretion (13). Several studies demonstrated that the *TCF7L2* rs7903146 T-allele is associated with decreased insulin secretion (4-8). Therefore, our cohort with detailed longitudinal information on glucose homeostasis of GH-treated short SGA children is very valuable to investigate whether there is a gene-environment interaction, e.g. whether *TCF7L2* rs7903146 genotype predicts how well the beta-cells are capable to produce extra insulin in response to the GH-induced insulin resistance. We found no association between *TCF7L2* genotype and the increase in insulin secretion. The decrease in insulin sensitivity compared to values at start was significant in the CT/TT group and not in the CC group, but the difference between the groups in decrease of insulin sensitivity was not significant. Despite the larger decrease in insulin sensitivity in carriers of a T-allele, both groups had a similar increase in insulin secretion during GH treatment. We conclude that *TCF7L2* rs7903146 genotype does not predict how well a short SGA child can produce extra insulin during GH treatment. With regard to the effect of rs7903146 on the decrease in insulin sensitivity a larger sample size would allow more definitive conclusions.

One other study analyzed whether the rs7903146 polymorphism in *TCF7L2* has an effect on changes in glucose homeostasis (25). In overweight children and adolescents, aged 6-16 years, who participated in a lifestyle intervention program to reduce BMI, a positive association between the T allele of rs7903146 and the increase in insulin levels was found (25). The T allele at rs7903146 was associated with a negative effect on the improvement of HOMA-IR and a positive effect on the decrease in HOMA-%B after the lifestyle intervention (25). That study and ours have in common that there is a change in the environment (GH-treatment or weight reduction) that changes beta cell function. During GH-treatment the beta cells are stimulated to produce more insulin while during weight reduction insulin sensitivity increases and less insulin is needed. This different effect on the beta cells might explain why we did not find significant associations between rs7903146 genotype and the change in glucose homeostasis while the other study did find such an association.

Another important difference between that study and ours is that we studied lean SGA children. Studies in adults did not find an association of *TCF7L2* variants and obesity or BMI (5, 26). We did not find a significant interaction between rs7903146 genotype and body fat percentage either. Therefore, we do not think that the absence

of a significant association between the rs7903146 T-allele and changes in measures of insulin sensitivity and insulin secretion in our population was caused by studying lean SGA children instead of obese children.

We also investigated whether the *TCF7L2* rs7903146 genotype correlated with estimates of glucose homeostasis at baseline. We found no associations between rs7903146 genotype and fasting levels of glucose and insulin, HOMA %B and HOMA-IR, which is in line with others (11). However, as we examined SGA subjects without catch-up growth to a normal height and Cauchi et al. did not specify the type of SGA participants (with or without catch-up growth to a normal height), our study is not completely comparable with that study. In addition, our study provides more detailed results because we also studied the association between *TCF7L2* genotype and insulin secretion by means of FSIGT whereas others only used fasting glucose and insulin levels and HOMA calculations (11). After adjustment for possible confounders we did find a higher insulin secretion (AIR) measured by FSIGT in carriers of a T-allele. Most studies that found associations between genotype and glucose homeostasis were performed in large cohorts of adults (4-8). Our study and the study by Cauchi et al. have the limitation that they can only detect large genotypic influences on glucose homeostasis because of their sample size. Population studies demonstrated that *TCF7L2* does not have a large effect size (1).

Insulin is an important growth factor in utero, so *TCF7L2* might influence size at birth. The minor allele frequency (MAF) in our population was 30% which is similar as the 29% in SGA subjects and 31% in appropriate for gestational age (AGA) subjects reported in literature (11). Thus, there seems to be no association between being born SGA and the *TCF7L2* genotype. As far as we know only one study investigated the association between birth weight and *TCF7L2* genotype and found a positive effect of the rs7903146 T-allele when present in the mother (27). Presence of one or two copies of the T-allele in the fetus seemed not to influence birth weight (27).

In conclusion, our study indicates that in short children who were born SGA, *TCF7L2* rs7903146 genotype is not associated with the change in insulin secretion during GH treatment. The rs7903146 polymorphism does not predict which children are less capable of producing extra insulin to compensate for the reduced insulin sensitivity induced by GH-treatment and as a result have an increased risk to develop type 2 DM in later life. With regard to the effect of rs7903146 on the decrease in insulin sensitivity during GH treatment, a larger sample size would allow more definitive conclusions.

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CHAPTER 9

Serum Insulin-Like Growth Factor-Binding Protein-2 levels and metabolic and cardiovascular risk factors in young adults and children born small for gestational age

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ABSTRACT

Background: IGF binding protein (IGFBP)-2 might protect against cardiovascular disease (CVD). Small for gestational age (SGA) birth could be associated with a higher risk for type 2 diabetes mellitus and CVD in later life. No data are available on the relationship between serum IGFBP-2 levels and cardiovascular risk factors in young adults and children born SGA.

Objective: To determine circulating IGFBP-2 levels in subjects born SGA, and to investigate the association with cardiovascular risk factors.

Methods: IGFBP-2 levels were measured in sera from 151 young adults born SGA and 147 short SGA children. Age and gender adjusted standard deviation scores (SDS) were calculated. Blood pressure, serum lipids, body composition by DXA and glucose homeostasis by HOMA-IR or frequently sampled intravenous glucose tolerance test (FSIGT) were determined.

Results: Serum IGFBP-2 SDS was significantly reduced in SGA young adults (with normal or short stature). Fat mass SDS was relatively high in SGA young adults and reduced in short SGA children. Serum IGFBP-2 SDS in SGA young adults correlated positively with insulin sensitivity (Si) and negatively with fat mass SDS, insulin secretion (AIR), fasting insulin, HOMA-IR, total cholesterol, triglycerides and blood pressure SDS. The association between serum IGFBP-2 SDS and Si, blood pressure, total cholesterol and triglycerides levels persisted after adjustment for known covariates including fat mass SDS. In short SGA children, IGFBP-2 SDS did not correlate with any of the cardiovascular risk factors.

Conclusion: Serum IGFBP-2 SDS is an independent cardiovascular risk marker in young adults who were born SGA.

INTRODUCTION

Plasma insulin like growth factor binding protein 2 (IGFBP-2) belongs to the family of IGFBPs that is present in extracellular fluids and binds both IGF-I and IGF-II (1). Serum levels of IGFBP-2 show little diurnal variation and do not vary in response to meals or glucose infusions (1, 2). IGFBP-2 levels do increase after a prolonged period of fasting (1), indicating that IGFBP-2 concentrations are metabolically regulated and might reflect long-term alterations in hepatic exposure to insulin (3). It has been reported that IGFBP-2 possibly alters the activity of intracellular kinases which modulate insulin signalling in metabolically active tissues by both IGF-dependent and IGF-independent mechanisms, thereby modulating insulin sensitivity (3).

In middle aged adults, elderly and cancer patients, serum concentrations of IGFBP-2 were inversely associated with insulin levels (4-7) and HOMA insulin resistance (5, 8). Also, IGFBP-2 was inversely associated with body mass index (BMI) (4, 6-8) and fat mass (6, 8). Studies in mice demonstrated that overexpression of IGFBP-2 results in diminished postnatal weight gain and reduced fasting glucose and insulin levels (9). Transgenic mice were protected from age-related development of glucose intolerance, insulin resistance and high blood pressure (10). Possibly, IGFBP-2 limits adipocyte expansion by inhibiting adipocyte differentiation (3, 10). IGFBP-2 knockout mice were heavier than controls but were not insulin resistant (11). This suggests that the effect of IGFBP-2 on glucose homeostasis is independent of its effect on BMI.

In subjects born small for gestational age (SGA), the association between IGFBP-2 levels and metabolic and cardiovascular risk factors has not yet been studied. Epidemiological studies have shown that low birth weight and postnatal catch-up in weight are associated with the development of type 2 DM and associated disorders such as hypertension, dyslipidemia, and cardiovascular disease in adults (12-14). Most children who were born SGA show spontaneous catch-up growth in height and weight but about 10% remains short through adult life. SGA infants were reported to have elevated IGFBP-2 levels (15, 16) and the only study that measured IGFBP-2 levels in short SGA children found also high levels (17). These studies, however, measured IGFBP-2 levels with another purpose and did not study the association with cardiovascular risk factors. For SGA young adults, serum IGFBP-2 levels have not been documented.

In this study, we measured serum IGFBP-2 levels in a cohort of young adults and children who were born SGA, and compared these levels to those found in age and

gender-matched controls with normal stature. We hypothesize that serum IGFBP-2 levels are a marker of metabolic and cardiovascular risk in subjects born SGA.

SUBJECTS AND METHODS

Subjects

The group of young adults born SGA (i.e. birth length < -2 SDS) (18) comprised 151 subjects with either short stature (adult height adjusted for age and gender < - 2 SDS; n = 37) or a normal height (> - 2 SDS; n = 114) (19). Catch-up growth was defined as a height > -1 SDS (n = 67). They were recruited as part of the PROgramming factors for GRowth And Metabolism (PROGRAM) study (20). In addition, 147 children born SGA with short stature were studied. Inclusion and exclusion criteria for the short SGA children have been previously described (21). In brief: children were included when they were SGA at birth (birth length and/or birth weight SDS of below –2.0 for gestational age (18)), had short stature (height SDS for age and gender < – 2) (19), did not show catch-up growth to a height > -2 SDS, and had no growth failure caused by other disorders. Birth characteristics were collected from hospital registries. The gestational age of the subjects was determined by ultrasound in the first trimester or otherwise calculated from the date of the last menstruation. None of the young adults and children studied was treated with growth hormone (GH). The Medical Ethics Committees of Erasmus Medical Center, Rotterdam, and of the participating centers approved all studies and written informed consent was obtained from all participants or their parents.

Study design

Standing height and weight were measured as previously described (21) and expressed as SDS using Dutch reference data (19). Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2) and was expressed as SDS adjusting for age and gender (19). Systolic and diastolic blood pressure (BP) was measured twice on the left arm with an automated device and using an appropriate cuff size (Dinamap Critikon, southern Medical Corp., Baton Rouge, LA, USA). The mean of two measurements was used for analysis. As blood pressure is known to increase with height, BP was expressed as SDS adjusted for height and gender (22). Dual-energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) was performed in all young adults and in 24 children. In which children a DXA was performed was determined by the date

of their inclusion in the study. The intra-instrument reliability for lean and fat tissue was 1.57-4.49% and 0.41-0.88%, respectively (23). Lean body mass (LBM) and fat mass (FM) were determined. FM and LBM were transformed into SD-scores for gender and chronological age using Dutch reference values, which were obtained using the same machine and software (24, 25). Since body composition is strongly related to height, LBM and FM expressed as SDS for age and gender may result in an underestimation in case of short stature. Therefore, LBM and FM were expressed as SDS for height and gender (26).

Glucose homeostasis

A modified, frequently sampled intravenous glucose tolerance test (FSIGT) with tolbutamide, was performed in a subgroup of 67 young adults and 22 children, as previously described (27, 28). In which children an FSIGT was performed was determined by the date of their inclusion in the study. Serum glucose and insulin levels were measured in one laboratory as previously described (29) and insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR) and disposition index (DI) were calculated using Bergman's MINMOD MILENNIUM software (30). Si is defined as the capacity of insulin to promote glucose disposal. Sg reflects the capacity of glucose to mediate its own disposal. AIR is an estimate of the insulin secretory capacity, being represented by the area under the curve from zero to ten minutes corrected for baseline insulin levels. DI is an overall measure of the ability of the beta cells to secrete insulin in response to the degree of insulin resistance, i.e. the product of AIR and Si (DI = AIRg * SI) (30).

Laboratory parameters

All blood samples were taken after an overnight fast. Serum IGFBP-2 levels were determined by a specific RIA, using an antiserum (WKZ35920) that was raised in a New Zealand rabbit against recombinant bovine IGFBP-2 (rbIGFBP-2; GroPep, Adelaide, Australia), in principle as described previously (31) with some slight modifications. In brief: the assay buffer was composed of 50 mM sodium phosphate (pH 7.4), 10 mM ethyleen-diamino-tetra-acetaat (EDTA), 0.05% (w/v) Tween-20, 0.2% BSA and 0.02% NaN3. Recombinant hIGFBP-2 (GroPep, Adelaide, Australia) was used as a standard (range 0.02-15 ng/tube) and [125I]-rhIGFBP-2 as tracer. The RIA incubation mixture consisted of 100 µL standard or diluted sample, 100 µL antiserum (final dilution in assay buffer: 1:80.000), and 100

µL tracer (≈10.000 cpm). After equilibrium incubation for 17-20 hrs at room temperature in polystyrene tubes, 100 µL Sac-Cel solid phase anti-rabbit IgG- coated cellulose suspension (Immunodiagnostic Systems, Boldon, UK) was added. Complexation was complete after 30 min at room temperature, and 0.5 mL distilled phosphate buffered saline was added to the samples, which were subsequently centrifuged at 10,000 x g for 4 min. Pellets were counted in a t-counter (Packard Instrument Co.,Inc., Downers Grove, IL).). Intra-assay variations (10 replicates) were 8.3, 4.9 and 4.5 % at mean serum levels of 73, 256, and 1205 µg/L, respectively. Inter-assay variations (n=8) were 12.1 %, 8.9 % and 8.1 % at mean serum levels of 77, 246, 1268 µg/L, respectively. The sensitivity of the assay was 0.1 µg/L (absolute concentration). Serum levels of IGFBP-2 were expressed as SDS, adjusting for age and gender, based on blood samples of 831 healthy individuals being derived from sources described previously (32).

Fasting glucose was measured on a Hitachi 917 analyzer and fasting insulin was measured by chemoluminescent assay on an Immulite 2000 analyzer (Diagnostic Products Corporation, Los Angeles, CA). HOMA insulin resistance index (HOMA-IR) was calculated (33). Serum cholesterol levels were measured as previously described (34). Triglycerides were measured on the Chem-I analyzer according to the manufacturer's instructions (Technicon Instruments, Tarrytown, New York) and after 1998 on the Hitachi 917 analyser according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Both methods were comparable (y = x – 0.030).

Statistics

Due to a skewed distribution, serum values of IGFBP-2 SDS, glucose, insulin, and lipids, and HOMA-IR, Si, AIR, DI, were log-transformed before analyses. These data are presented as median (interquartile range). The various other parameters investigated are expressed as mean (SD). SD scores were compared with reference population means (zero SDS) using one-sample t tests. Results in young adults with short stature and young adults with catch-up growth to a height > -1 SDS were compared using independent samples t tests. After log-transformation, Pearson's correlation coefficient was used for correlations. Associations of IGFBP-2 with metabolic and cardiovascular risk factors were investigated by multiple linear regression analysis. We entered age, gender, height SDS, birth length SDS, birth weight SDS, lean body mass SDS_{height}, and fat mass SDS_{height} to the model because these are determinants of evaluated cardiovascular risk (20). The interaction term birth length SDS * adult height SDS was added to all models because

the young adults were selected on birth length and adult height, in order to ensure that the effect of these variables was modelled correctly. IGFBP-2 SDS was added to the model in order to determine the effect of IGFBP-2 SDS on the outcome variable adjusted for other determinants. A p value < 0.05 was considered significant. Analyses were performed using the computer statistical package SPSS version 15 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Clinical data

Clinical characteristics are shown in Table 1. Young adults had a normal fat mass (FM) for age. However, on average, they exhibited a shorter stature than the population mean. As a consequence their FM for height SDS was significantly higher than zero SDS. In contrast, short SGA children, showed SDS values of both FM for age and FM for height that were significantly reduced (< -2 SDS).

Differences between young adults with a height SDS < -2 and young adults with a height SDS > -1 in BMI SDS, FM SDS_{age}, FM SDS_{height}, Si, AIR, DI, and serum levels of total cholesterol and triglycerides did not reach significance.

When the total group of SGA young adults was considered, diastolic blood pressure was elevated when compared to values of gender and height matched references. No differences were observed between short young adults and those with catch-up growth to a normal height. In the SGA children, both systolic and diastolic blood pressure appeared to be elevated.

Serum IGFBP-2 levels

Serum IGFBP-2 levels in SGA young adults were significantly lower than in gender and age matched references. There was no significant difference in IGFBP-2 SDS between young adults with short stature and young adults with catch-up growth to a normal height (p = 0.95). Short SGA children had similar IGFBP-2 levels as their peers (Table 1). In both young adults and children, IGFBP-2 SDS did not significantly correlate with birth weight SDS, birth length SDS, gestational age, or height SDS.

Table 1. Clinical and laboratory characteristics in two cohorts of SGA born subjects					
	SGA young adults			**SGA children**	
		n			n
Male/female	64/87	151		81/66	147
Age	20.9 (1.7)	151		7.1 (2.8)	147
Gestational age (wk)	39.0 (38.0 to 40.0)	151		37.3 (32.3 to 39.3)	147
Birth weight SDS	-2.3 (0.7)*	151		-2.4 (1.0)*	147
Birth length SDS	-2.7 (1.0)*	151		-3.6 (1.5)*	110
Height SDS	-1.2 (1.2)*	151		-3.0 (0.6)*	147
BMI	23.1 (4.1)	151		14.2 (1.5)	147
BMI SDS	0.2 (1.3)	151		-1.4 (1.0)*	147
Fat mass SDS_{age}	0.2 (1.1)	147		-2.7 (1.7)*	24
Fat mass SDS_{height}	0.5 (1.0)*	147		-2.6 (1.8)*	24
Lean mass SDS_{age}	-1.0 (1.0)*	147		-2.6 (0.5)*	24
Lean mass SDS_{height}	0.3 (1.4)***	147		-1.3 (1.7)**	24
Systolic bp SDS	0.0 (0.8)	99		0.9 (1.1)*	145
Diastolic bp SDS	0.3 (0.5)*	99		0.4 (1.0)*	145
IGFBP-2 (ng/ml)	122 (64 to 227)	151		262 (188 to 331)	147
IGFBP-2 SDS	-0.86 (1.8)*	151		-0.16 (1.2)	147
F Insulin (pmol/l)	58.2 (45.9 to 85.8)	74		19.5 (14.0 to 36.0)	126
F Glucose (mmol/l)	4.9 (4.5 to 5.1)	135		4.4 (4.0 to 4.8)	131
HOMA-IR	1.1 (0.9 to 1.6)	74		0.3 (0.2 to 0.6)	124
$Si*10^{-4}/min^{-1}$ (µU/ml)	4.7 (2.9 to 8.3)	67		12.4 (7.1 to 18.3)	22
AIR (mU/L)	441 (302 to 774)	67		312 (205 to 515)	22
DI (AIR*Si)	2466 (1595 to 4015)	67		3130 (2362 to 4911)	22
Cholesterol (mmol/l)	4.5 (3.9 to 5.4)	145		4.3 (3.7 to 5.0)	77
Triglycerides (mmol/l)	0.9 (0.7 to 1.2)	145		0.8 (0.6 to 1.3)	59

Values are expressed as mean (SD) or as median (interquartile range).
Gestational age was compared with a nonparametric test. Data with a skewed distribution were log transformed before analysis with t-tests.
SDS = standard deviation score F = fasting; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index
Compared with zero SDS: *p < 0.001; **p < 0.01; ***p < 0.05

Table 2. Correlations between IGFBP-2 SDS and various metabolic and cardiovascular risk factors					
	SGA young adults (n = 151)			SGA children (n = 147)	
	R	p-value		R	p-value
BMI SDS	-0.31	< 0.001		-0.04	0.66
Fat mass SDS_{age}	-0.48	< 0.001		-0.16	0.57
Fat mass SDS_{height}	-0.45	< 0.001		0.12	0.58
Lean mass SDS_{age}	-0.15	0.08		-0.07	0.76
Lean mass SDS_{height}	0.05	0.52		0.04	0.86
Systolic bp SDS	-0.27	0.007		0.04	0.60
Diastolic bp SDS	-0.30	0.002		0.16	0.06
F insulin (pmol/l)	-0.55	< 0.001		-0.15	0.09
F Glucose (mmol/l)	0.07	0.46		-0.01	0.88
HOMA-IR	-0.55	< 0.001		-0.13	0.14
$Si*10^{-4}/min^{-1}$ (µU/ml)	0.54	< 0.001		0.18	0.44
AIR (mU/L)	-0.45	< 0.001		-0.22	0.32
DI (AIR*Si)	0.03	0.82		0.02	0.95
Cholesterol (mmol/l)	-0.40	< 0.001		-0.21	0.07
Triglycerides (mmol/l)	-0.40	< 0.001		-0.05	0.73

R = Pearson's correlation coefficient; SDS = standard deviation score F = fasting; HOMA IR = insulin resistance index; Si = insulin sensitivity; AIR = acute insulin response; DI = disposition index
F insulin, HOMA-IR, Si, cholesterol and triglycerids were log-transformed for analysis.

Associations between IGFBP-2 SDS and metabolic and cardiovascular risk factors

In the SGA young adults, IGFBP-2 SDS correlated negatively with BMI SDS, FM SDS, blood pressure SDS, fasting insulin, HOMA-IR, insulin secretion (AIR), cholesterol, and triglycerides (Table 2). There was a positive correlation between IGFBP-2 SDS and insulin sensitivity (Si). In the short SGA children, IGFBP-2 SDS did not correlate with any of the metabolic or cardiovascular risk factors studied.

In addition, we evaluated the association of serum IGFBP-2 SDS with several metabolic and cardiovascular risk factors in the SGA young adults with multiple regression (MR) analyses (Table 3). These analyses revealed that IGFBP-2 SDS associated with Si, also after adjustment for known determinants of Si, including FM SDS_{height}. Notably, there was a significant association between Si and FM SDS_{height} (β -0.39, $p < 0.001$) but when we added IGFBP-2 SDS to the model this association was no longer significant

Table 3. Multiple regression analyses for the independent influence of IGFBP-2 on Si, blood pressure, total cholesterol and triglycerides in SGA young adults										
	Insulin sensitivity (Si)		Systolic bp SDS		Diastolic bp SDS		Total cholesterol		Triglycerides	
Variables	**β***	**p-value**	**β**	**p-value**	**β**	**p-value**	**β***	**p-value**	**β***	**p-value**
Age (yrs)	0.05	0.34	0.11	0.02	0.09	0.005	0.01	0.29	0.01	0.51
Gender	-0.04	0.85	-0.24	0.17	-0.25	0.03	0.13	< 0.001	0.06	0.49
Height SDS	-0.54	0.07	-0.06	0.74	-0.08	0.50	0.002	0.95	0.06	0.47
Birth length SDS	-0.43	0.03	0.12	0.29	0.08	0.29	-0.006	0.80	-0.05	0.34
BL*AH (SDS)	-0.18	0.09	0.03	0.62	-0.02	0.55	-0.003	0.80	-0.01	0.85
Birth weight SDS	0.20	0.19	-0.007	0.95	0.01	0.86	0.02	0.53	-0.03	0.64
Fat mass SDS_{height}	-0.19	0.14	0.10	0.28	0.05	0.44	0.02	0.35	0.03	0.50
LBM SDS_{height}	0.03	0.72	0.12	< 0.05	0.05	0.18	0.01	0.33	0.06	< 0.05
IGFBP-2 SDS	**0.15**	**0.01**	**-0.11**	**0.03**	**-0.11**	**0.001**	**-0.03**	**0.02**	**-0.09**	**< 0.001**
Overall	< 0.001		0.001		< 0.001		< 0.001		< 0.001	
N	67		95		95		142		142	
R2	0.39		0.28		0.30		0.26		0.23	
R2 adjusted	0.29		0.20		0.23		0.21		0.18	

BL: Birth length SDS; AH: Adult height SDS

* Si, cholesterol and triglycerides were log-transformed for analysis.

(Table 3). Thus, the association between Si and IGFBP-2 SDS was stronger than the association between Si and FM SDS_{height}. When instead of Si, systolic and diastolic blood pressure, total cholesterol and triglycerides levels were analyzed as dependent variable, similar results were found. After adjustment for age, gender, size at birth, height SDS, LBM SDS_{height} and FM SDS_{height}, IGFBP-2 SDS was not associated with HOMA-IR (β -0.04, p = 0.17). However, when we removed FM SDS_{height} from the model, there was a negative association between IGFBP-2 SDS and HOMA-IR (β -0.11, p < 0.001). This indicates that the association between IGFBP-2 and HOMA-IR is probably mediated through fat mass. When instead of HOMA-IR, fasting insulin levels and insulin secretion (AIR) were analyzed as dependent variable, similar results were found.

DISCUSSION

In this study we show that serum IGFBP-2 levels in SGA young adults were significantly reduced, when compared to age and gender-matched references. To our knowledge, so far data on serum IGFBP-2 levels for SGA young adults did not exist. We found no association between size at birth or adult height SDS and serum IGFBP-2 SDS. In a study on disease-free men (50-70 yrs of age), Gunnell et al. could also not find an association between circulating levels of IGFBP-2 and height (35). Our study shows that lower serum IGFBP-2 levels in SGA young adults were neither associated with their relatively small size at birth nor with height SDS, but appeared to be related to their increased fat mass SDS.

The inverse correlation between serum IGFBP-2 levels and BMI has been consistently reported (4, 6-8), but only a few studies also examined body composition. We performed DXA scans in 147 young adults and found a significant inverse correlation between IGFBP-2 SDS and fat mass SDS. Two studies in elderly subjects yielded a similar result (6, 8). However, in contrast to these previous reports (6, 8), we did not find a significant relationship between serum IGFBP-2 SDS and lean mass SDS. There are several possible explanations for this apparent discrepancy. Besides the fact that we studied much younger individuals, in one study (8) body composition was determined by leg-to-leg bioimpedance which is less accurate than DXA. Furthermore, we adjusted lean body mass for height and gender while this was not performed in the other two studies referred to above.

Data on the association between serum IGFBP-2 and metabolic and cardiovascular risk factors were not available yet for subjects born SGA. In this study we found that serum

IGFBP-2 SDS is an independent cardiovascular risk marker in SGA young adults. Studies in subjects with other conditions found correlations between IGFBP-2 and cardiovascular risk factors in line with our results. In older adults with type 2 DM, IGFBP-2 correlated independently and negatively with fasting glucose, triglycerides and LDL cholesterol and positively with insulin sensitivity (36). In adults with a mean age of 55 years, IGFBP-2 correlated inversely with insulin, triglycerides, fasting glucose levels, systolic and diastolic blood pressure (4) and in non-diabetic patients with chronic heart failure, IGFBP-2 correlated inversely with insulin and HOMA-IR but not with insulin sensitivity measured by hyperinsulinemic-euglycemic clamp (5). As there is no marked prandial regulation of IGFBP-2, this IGF-binding protein is considered a robust biomarker for cardiovascular risk (36).

In our cohort of short SGA children, with a mean age of seven years, serum IGFBP-2 levels were comparable to reference values. Only one other study measured circulating IGFBP-2 levels in a much smaller cohort of 17 short SGA children (mean age 6 years) and reported higher IGFBP-2 levels than in age-matched references (17). Infants with intrauterine growth restriction are reported to exhibit decreased levels of IGF-I, IGF-2, IGFBP-3, and insulin, and elevated levels of IGFBP-I and IGFBP-2 (15, 16). It is, however, not known whether the increased IGFBP-2 levels in SGA infants do normalize during childhood.

In contrast to the SGA young adults, serum IGFBP-2 levels in short SGA children did not correlate with any of the metabolic and cardiovascular risk parameters investigated. This may be explained by the difference in BMI SDS between SGA young adults and children, more precisely, the difference in fat mass adjusted for height and gender. SGA children had a low fat mass for height SDS while young adults had a significantly higher fat mass for height SDS compared to the population mean. Since serum IGFBP-2 SDS negatively associate with fat mass SDS, a certain threshold level of fat mass may be required before serum IGFBP-2 levels correlate with other cardiovascular risk factors. However, it must be emphasized that the association of serum IGFBP-2 SDS with insulin sensitivity, blood pressure, cholesterol and triglycerides persisted after adjustment for fat mass SDS. Longitudinal studies, in which the same subjects are evaluated in childhood and at young adult age, will allow more definite conclusions.

For children, data on the relationships between serum IGFBP-2 levels and metabolic and cardiovascular risk factors are very scarce. Three studies on obese children reported decreased circulating IGFBP-2 levels compared to those in controls (37-39). One of

these studies also reported an inverse association between serum levels of IGFBP-2 and insulin (37). Other metabolic risk factors were not investigated.

In conclusion, SGA birth per se is not associated with altered IGFBP-2 levels. In contrast to short SGA children, young adults born SGA have significantly lower serum IGFBP-2 levels than references, and these correlated inversely with systolic and diastolic blood pressure, total cholesterol levels and triglycerides and positively with insulin sensitivity, also after correction for possible confounders including fat mass SDS. Hence, for this particular group of subjects, serum IGFBP-2 levels could serve as an independent cardiovascular risk marker.

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CHAPTER 10

General discussion

The present thesis describes the studies investigating the influence of family history, preterm birth, genetic polymorphisms, and growth hormone treatment on metabolic and cardiovascular risk factors in SGA children with short stature. In addition, thyroid hormone levels during GH treatment and the association between serum IGFBP-2 levels and cardiovascular risk factors were studied.

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.

FAMILY HISTORY

Concern has been expressed regarding the possible detrimental effects of GH treatment in children with a possible increased risk to develop type 2 DM or CVD in later life (1-3). Therefore, it would be very informative to know which children have the highest risk to develop such diseases. As determinants of metabolic and cardiovascular disease are known to cluster in families (4, 5), family history could be a valuable indicator of the risk of a child to develop disease.

In a group of 286 short SGA children and 482 parents, we compared anthropometry, blood pressure, fasting serum lipid, glucose and insulin levels with age- and sex-matched references. We also investigated whether these parameters correlated between parents and their offspring. Children had significantly higher systolic and diastolic blood pressure than sex- and height-matched references. Mothers had higher systolic blood pressure, fathers a higher body mass index (BMI) and parents had more often high fasting glucose levels than age- and sex-matched references. Twenty-four percent of mothers and 10% of fathers were born SGA but these parents did not have more cardiovascular risk factors than those born appropriate for gestational age. Cardiovascular risk factors did not correlate between parents and children. We concluded that parents of short SGA children have a modest increase in some cardiovascular risk factors but that there is no correlation between risk factors in parents and in children.

This is the first study evaluating anthropometry and cardiovascular risk factors in a large cohort of short SGA children and their parents. Previous studies regarding intergenerational associations focused either on cardiovascular risk factors (4, 5) or on low birth weight or SGA (6) but not on the combination.

Clinical implications and conclusions

Our data indicate that there is no association between cardiovascular risk factors in parents and short SGA children. However, the children were young at the time of the study (mean age 6.4 years). Possibly the association does not develop before the offspring has reached adult age. Therefore it is mandatory to keep monitoring GH-treated and untreated SGA children into adulthood. This is the best way to investigate whether a family history of DM or CVD will predict which SGA children have the highest risk to develop disease.

THYROID HORMONE LEVELS

The secretion and action of thyroid hormones and those of the GH/IGF-I axis are interdependent (7). Disturbances in serum thyroid hormone levels were reported for SGA children but the association with spontaneous catch-up growth and prematurity was unclear (8-10). In addition, the effect of growth hormone (GH) treatment on thyroid function had not been studied in short SGA children. To clarify these issues we evaluated serum thyroid hormone levels in short SGA children, in comparison with those of age-matched controls, and evaluated the influence of gestational age and GH treatment.

In our population of 264 short SGA children, we found similar free T4 (FT4) levels as those in age-matched references. TSH levels were within the normal range but significantly higher than in age-matched references. When preterm and term born short SGA children were analyzed separately, FT4 adjusted for age was significantly higher in preterm children than in term children but both groups had similar FT4 levels as age-matched references. TSH was significantly higher compared to controls in preterm born children but not in term children. Notably, TSH levels were all within the normal range. There was no correlation between TSH and gestational age, birth length SDS or birth weight SDS. Other studies in smaller cohorts, reported increased (11) or normal (8, 10) TSH levels in SGA children compared with AGA children. One study investigated the influence of gestational age and reported a negative correlation between TSH and gestational age, independently from birth weight or birth length (10). Since we included much more SGA children and found no correlation between TSH levels and gestational age or size at birth, we consider the association between size at birth or gestational age and TSH levels to be weak and not clinically relevant.

During GH treatment we observed a significant decrease in FT4 levels during the

first 6 months of GH, but levels remained within the normal range. The change in FT4 levels was not associated with a change in TSH levels and there was no effect on the 2-year-growth response. None of the children developed hypothyroidism. In other patient groups, GH treatment was also associated with a decline in serum FT4 without a change in TSH level (12-14).

Clinical implications and conclusions

We demonstrated that alterations in serum thyroid hormone levels in preterm and term born short SGA children are very subtle, and within the normal range. During GH treatment FT4 levels declined but remained within the normal range and the decline in FT4 was not associated with a change in TSH levels. We therefore conclude that frequent monitoring of thyroid function during GH therapy is not warranted in short SGA children. This is in contrast to the monitoring of children with multiple pituitary hormone deficiencies who may develop true hypothyroidism during GH therapy (12).

PREMATURITY

Short SGA children comprise a heterogeneous group also with respect to their gestational age, as some are born at term and others are born preterm. Despite this important difference, GH treatment is the same for all SGA children. The prenatal environment and genetic background might be different when there is a shorter duration of pregnancy. Therefore, preterm birth might be associated with a smaller size for gestational age, a different post-natal growth pattern and a different cardiovascular risk profile. To investigate whether this is the case we performed two studies within our large population of short SGA children. In the first study we evaluated growth and in the second study we evaluated cardiovascular risk factors.

In our population of 392 short SGA children prematurity was indeed associated with a smaller size for gestational age (more severe SGA), and a shorter height corrected for target height and leaner phenotype in childhood. However, the response to GH treatment was similar for preterm and term short SGA children. Therefore preterm short SGA children should not be excluded from GH treatment. Ours was the first study evaluating whether the response to GH treatment is different for preterm and term born SGA children.

Both prematurity and SGA birth have been associated with increased metabolic and cardiovascular risk factors (1, 2, 15-18), but the combination of SGA and preterm birth

probably does not result in an additional increase of all risk factors (19). The independent effect of prematurity on GH-induced changes in metabolic and cardiovascular risk factors had not been evaluated. We compared short SGA children born preterm with short SGA children born at term, with respect to the change in cardiovascular risk factors during 4 years of GH treatment. We found that preterm birth did not have an independent influence on the effect of GH on blood pressure, insulin sensitivity, beta cell function and body composition. Therefore, we concluded that GH treatment has the same effect on cardiovascular risk factors in preterm as in term short SGA children.

Willemsen et al. investigated whether preterm birth is associated with an independent adverse risk profile in short SGA children and reported a lower body fat percentage, a higher systolic and diastolic blood pressure and a higher insulin secretion in preterm children compared with term children, whereas insulin sensitivity and lipid levels were not different (19). Other studies investigated the influence of birth weight SDS and gestational age on metabolic and cardiovascular risk factors in the general population (17, 20) or in populations of preterm born or low-birth-weight subjects (21-25), of which only a small proportion was born SGA. As a result, these studies were not suitable to investigate whether preterm birth has an additional detrimental effect on the cardiovascular risk profile of short SGA children.

Clinical implications and conclusions

We have shown that it is justified to make no difference between short SGA children born preterm and short SGA children born at term when treating them with GH. The GH-induced catch-up growth and change in serum IGF-I and IGFBP-3 levels were similar for preterm and term short SGA children and GH treatment had the same effect on cardiovascular risk factors in preterm and term short SGA children.

GENETIC POLYMORPHISMS

Recent genome-wide association studies found various single nucleotide polymorphisms (SNPs) that associated with the risk to develop type 2 DM including *PPAR-γ* Pro12Ala and *TCF7L2* rs7903146 (26-30). We studied associations between these genetic polymorphisms and metabolic and cardiovascular risk factors and growth in short SGA children.

Short SGA children usually have a lean phenotype with a relatively low insulin

sensitivity and high blood pressure (1, 2, 15). GH treatment of short SGA children results in a decrease in blood pressure and insulin sensitivity, and an increase in BMI and insulin secretion (15, 31). However, not all GH-treated short SGA children demonstrate the same change in these determinants of cardiovascular disease, suggesting additional influencing factors such as genetic variations. When genetic variations would predict the change of important determinants of the risk for type 2 DM and cardiovascular disease during GH treatment, we might be able to predict how children would respond to GH treatment. Children who are less able to compensate for the GH-induced reduction in insulin sensitivity because they have a certain genetic variant, could then be monitored more closely during and after GH treatment.

During GH treatment some SGA children remain lean while others demonstrate catch-up in BMI SDS. As the *PPAR-γ* gene is involved in lipid metabolism and adipocyte differentiation (32) it might be a response modifier to a triggering factor such as GH treatment. Other studies indicated that the effect of *PPAR-γ* on BMI is different in various phenotypes, which suggests gene-environment interactions (32, 33). We found a significantly higher increase in BMI SDS in carriers of the Ala12 allele but the frequency of the Ala12 allele was not different in subjects with catch-up in BMI SDS and subjects without catch-up in BMI SDS. The Ala12 allele was not associated with changes in determinants of metabolic and cardiovascular diseases.

In large populations of adults the T-variant of *TCF7L2* rs7903146 was associated with reduced insulin secretion and a higher risk for type 2 DM (34-39). Insulin is the most important fetal growth factor and insulin-mediated fetal growth might therefore be affected by genetic factors that regulate fetal insulin sensitivity or insulin secretion before and after birth (40). In line with two other studies (41, 42) we found, however, no association between fetal *TCF7L2* genotype and birth size. We extended the research on the association between *TCF7L2* genotype and early growth by demonstrating that longitudinal growth patterns were also not associated with *TCF7L2* genotype. We therefore concluded that, despite its supposed effect on insulin secretion, *TCF7L2* does not influence fetal and postnatal growth.

As the *TCF7L2* rs7903146 SNP was associated with insulin sensitivity and insulin secretion (34-38), it could be a marker predicting how well short SGA children are able to increase their insulin production during GH treatment. Children with less insulin secretion during GH-treatment might be more at risk to develop DM in later life. We demonstrated that presence of the *TCF7L2* rs7903146 T allele was not associated with the increase

in insulin secretion during GH treatment. The decrease in insulin sensitivity compared to values at start was significant in the CT/TT group and not in the CC group, but the difference between the groups in decrease of insulin sensitivity was not significant. This merits further study. We concluded that *TCF7L2* rs7903146 genotype does not predict how well a short SGA child can produce extra insulin during GH treatment.

Clinical implications and conclusions

Our data show that the *PPARγ* Pro12Ala polymorphism was not associated with changes in the metabolic and cardiovascular risk profile and thus does not predict the response to GH treatment with regard to determinants for DM and CVD. Although we did not find an association with cardiovascular risk factors, there was a positive association of the Ala12 allele with weight gain during GH treatment. As this might be clinically relevant this finding warrants further investigation. The *TCF7L2* rs7903146 T-variant was neither associated with spontaneous growth during the first years of life nor with the GH-induced changes in insulin secretion. The *TCF7L2* rs7903146 T-variant was associated with a larger GH-induced decrease in insulin sensitivity but not significantly. When the T-variant indeed predicts a stronger decrease in insulin sensitivity it would be very interesting to investigate what happens with insulin sensitivity after discontinuation of GH treatment in subjects carrying this variant.

IGFBP-2 LEVELS AND CARDIOVASCULAR RISK FACTORS

In a study population of 147 SGA children with short stature, we found similar IGFBP-2 levels as in age and gender matched references. However in 151 SGA young adults with various heights (37 with a height SDS < -2), we found significantly lower IGFBP-2 levels compared to age and gender matched references. Further analysis revealed that IGFBP-2 levels did not correlate with height SDS, birth weight SDS or birth length SDS but that there was a strong negative correlation with fat mass SDS. Since short SGA children had a low fat mass and SGA young adults had a higher fat mass, this might explain the observed difference in IGFBP-2 levels. There was no significant correlation between IGFBP-2 levels in short SGA children and metabolic and cardiovascular risk factors. In contrast, in the SGA young adults there were strong correlations between IGFBP-2 SDS and blood pressure, serum lipids and insulin sensitivity. These correlations persisted after adjustment for other covariates including fat mass SDS.

In SGA infants, IGFBP-2 levels have been reported to be higher than in references (43, 44) but data about IGFBP-2 levels in childhood are very scarce. Only one study compared IGFBP-2 levels in a small cohort of short SGA children with age-matched references and found high levels (45). IGFBP-2 levels in SGA young adults were unknown. IGFBP-2 levels are particularly interesting in subjects born SGA because they associate inversely with several cardiovascular risk factors in middle aged and older adults and in cancer patients (46-49). Subjects who were SGA at birth might have an increased risk to develop type 2 DM or CVD in adulthood, especially when there is catch-up in weight and fat accumulation (50-53). The association between IGFBP-2 levels and metabolic and cardiovascular risk factors had not yet been studied in subjects born SGA.

The direction and degree of the associations between IGFBP-2 levels and estimates of glucose homeostasis, blood pressure and serum lipids in our study was in line with the associations found in other populations (46-49, 54, 55). IGFBP-2 levels adjusted for age and gender did not correlate with size at birth or height SDS. Serum levels of IGFBP-2 show little diurnal variation and do not vary in response to meals or glucose infusions (56, 57). We therefore hypothesize that IGFBP-2 levels might be an independent marker of cardiovascular risk in all patients. To confirm this hypothesis, DXA scans, FSIGTs, serum lipids, blood pressure and IGFBP-2 levels should be measured in a large population of healthy young adults.

Clinical implications and conclusions

Our data show that IGFBP-2 levels are not associated with SGA birth or height SDS but strongly associate with fat mass. In short SGA children with a low fat mass SDS, there is no association between IGFBP-2 levels and blood pressure, glucose homeostasis and serum lipids but in SGA young adults with a higher fat mass IGFBP-2 is inversely associated with the metabolic risk profile. Higher IGFBP-2 levels are independently associated with a lower blood pressure, better insulin sensitivity and lower serum lipids. Serum IGFBP-2 level is an independent cardiovascular risk marker in young adults who were born SGA and possibly also in all adults.

GENERAL CONCLUSIONS

The observation by Barker et al. that men with the lowest birth weights had the highest risk to die from a myocardial infarction, has led to the initiation of many studies investigating

the association between low birth weight and metabolic and cardiovascular disease in later life. Since GH treatment was approved for short SGA children, they comprise a large group of GH-treated children. Because of the metabolic effects of GH treatment, it is important to know which SGA children have the highest risk to develop type 2 DM or CVD in adulthood and to predict the effect of GH treatment on this risk. In this thesis we investigated the influence of family history, prematurity, genetic polymorphisms, serum IGFBP-2 levels, and GH treatment on several metabolic risk determinants in short SGA children. Table 1 summarizes the most important recent findings from trials in SGA subjects performed by our research group.

In summary, our results do not provide evidence that determinants of metabolic and cardiovascular disease or the change in those determinants during long-term GH treatment associates with a family history of type 2 DM or CVD, preterm birth or *TCF7L2* genotype. *PPARγ* genotype possibly predicts weight gain during GH treatment but this needs to be confirmed in other cohorts. Serum IGFBP-2 levels associate with several determinants of metabolic and cardiovascular disease but only in SGA young adults and not in childhood.

DIRECTIONS FOR FUTURE RESEARCH

Growth hormone has well-known anti-insulinemic effects, such as a reduction in insulin sensitivity. *TCF7L2* has repeatedly been found to associate with insulin secretion (34-38). We had the opportunity to accurately measure insulin sensitivity and insulin secretion in a sample of short SGA children by means of FSIGT. We found no association between *TCF7L2* genotype and the change in insulin secretion but there seemed to be an association with the decrease in insulin sensitivity. Because of our sample size we could only detect large effects. It would be very interesting to perform FSIGT tests during GH treatment in a larger group of short SGA children to provide a definitive answer to the question whether the decrease in insulin sensitivity associates with *TCF7L2* rs7903146 genotype.

As the *PPAR-γ* Pro12Ala polymorphism is reported to associate with BMI and weight gain (33, 58-60) and we also observed an association with weight gain during GH treatment, it would be very interesting to investigate whether this polymorphism also associates with postnatal spontaneous catch-up growth in weight in SGA children.

Serum IGFBP-2 levels were not only associated with determinants of the risk for

cardiovascular disease and DM (54) but also with bone growth and metabolism (61) (62). Size at birth and during childhood may affect bone mineral density (BMD) and the risk of osteoporotic fracture in adulthood (63). In short SGA children, lumbar spine BMD adjusted for height was reported to be significantly lower than normal (64). Therefore it will be interesting to investigate IGFBP-2 levels in subjects born SGA in association with BMD.

In addition it remains important to perform long-term follow-up studies at regular intervals in SGA cohorts after discontinuation of GH treatment. When we know which subjects may eventually develop adult diseases we could investigate whether GH treatment improves or worsens this risk. It will be very interesting to investigate previously GH treated SGA subjects at an age when type 2 DM and CVD develop in the general population. The majority of the children benefits from GH treatment with an increase in height but since GH treatment was only licensed in 2001 long-term follow up data are not yet available. These are important research question for future studies.

Table 1. What have we learned about cardiovascular risk factors in Dutch children and young adults born SGA?	
	Name author/researcher
Catch-up in weight - Fat accumulation during childhood is associated with reduced insulin sensitivity and increased serum lipids and blood pressure in early adulthood, independent of size at birth. - Weight gain relative to height gain in the first three months of life is associated with an unfavourable cardiovascular and metabolic profile in early adulthood.	R.W.J. Leunissen (53, 65, 66)
Family history - Parents of short SGA children have a modest increase in some cardiovascular risk factors but there is no correlation between blood pressure, lipid levels and glucose homeostasis in parents and their children.	S.W.K. de Kort (this thesis)(67)
Prematurity - preterm short SGA children have a lower body fat percentage, higher blood pressure and higher insulin secretion than term short SGA children, whereas insulin sensitivity and lipid levels are comparable. - GH treatment has the same effect on cardiovascular risk factors in preterm as in term short SGA children.	R.H. Willemsen (19) S.W.K. de Kort (this thesis)(68)
Genetic polymorphisms - The rs7903146 polymorphism of *TCF7L2* is not associated with the increase in insulin secretion during GH treatment. - The Pro12Ala polymorphism of *PPAR-γ* positively associates with weight gain during GH treatment but not with the change in other cardiovascular risk factors.	S.W.K. de Kort (this thesis)(69)
IGFBPs - Both IGFBP-1 and IGFBP-2 were similar to controls in short SGA children with a low fat mass and lower than controls in young SGA adults with a normal or increased fat mass. - IGFBP-1 correlated inversely with systolic blood pressure, insulin and triglycerides and positively with HDL-cholesterol. - In young SGA adults, IGFBP-2 levels associated independently and inversely with blood pressure, cholesterol and triglycerides and positively with insulin sensitivity. - IGFBP-1 and IGFBP-2 might serve as markers to identify subjects with an adverse metabolic outcome.	D.C.M. van der Kaay (70) S.W.K. de Kort (this thesis)
Growth hormone treatment - GH treatment in short SGA children did not negatively influence glucose levels. - GH treatment induced higher insulin levels and lower insulin sensitivity, but these effects were completely reversible after discontinuation of GH treatment. - GH treatment associated with a decrease of blood pressure and serum cholesterol levels and these effects remained until 6.5 years after discontinuation of GH. - GH treatment induced a decrease in body fat percentage. - After stop of GH treatment, body fat percentage increases and lean body mass decreases but values remain well within the normal range.	M.van Dijk (71) R.H. Willemsen (72)

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CHAPTER 11

Summary

SUMMARY

Chapter 1

This chapter provides an introduction in the definitions, prevalence and possible causes of SGA birth. Furthermore, clinical and endocrinological aspects associated with SGA are described, such as short stature, the GH-IGF-IGFBP axis, the limited available data on thyroid hormones, the association with cardiovascular disease (CVD) and type 2 diabetes mellitus (type 2 DM), family history for CVD and type 2 DM and genetic polymorphisms. This chapter also gives a summary of previously reported effects of GH treatment on growth and the GH-IGF-IGFBP axis in short SGA children. Finally, the aims and outline of this thesis are presented.

Chapter 2

An increased prevalence of cardiovascular risk factors at a young age had been described in SGA children with short stature. It was not known whether this increased risk is caused by their size at birth, a familial predisposition for cardiovascular disease or smallness at birth or a combination of these factors. The cardiovascular risk profile of parents of SGA children had never been investigated. We compared anthropometry, blood pressure, fasting serum lipid, glucose, and insulin levels of 482 parents and 286 short SGA children with age-and gender-matched references. In addition, we investigated whether these parameters correlated between parents and their children. We found a higher blood pressure in mothers, a higher BMI in fathers and more frequently high fasting glucose levels than age- and gender matched references in both mothers and fathers. The short SGA children had significantly higher systolic and diastolic blood pressure than gender- and height-matched references. Twenty-four percent (mothers) and 10% (fathers) were born SGA but they did not have more cardiovascular risk factors than parents born AGA. Cardiovascular risk factors did not correlate between parents and children. In conclusion, our study shows that parents of short SGA children have a modest increase in some cardiovascular risk factors but there is no correlation between risk factors in parents and children.

Chapter 3

Disturbances in thyroid function had been described in SGA children but the influence of prematurity was unclear. As normal thyroid function is necessary for growth and

development, it is important to know whether GH treatment alters thyroid function. Studies concerning thyroid hormone levels in SGA children were very limited and the effect of GH administration on thyroid function in these children had not been studied yet. We measured TSH and free T4 (FT4) levels before and during 2 years of GH treatment in 264 short SGA children who were divided in those born preterm and those born at term. We found higher, though normal TSH levels in preterm born short SGA children compared with age-matched controls. TSH levels in term short SGA children were not different than in controls. Mean FT4 at baseline was not different between short SGA children and controls. TSH and FT4 levels did not correlate with gestational age, birth weight SDS or birth length SDS. During GH treatment, FT4 decreased but was neither associated with an increase in TSH nor did it affect the growth response to GH treatment. Because these mild alterations in thyroid function do not appear clinically relevant, we conclude that frequent monitoring of thyroid function during GH therapy is not warranted in short SGA children.

Chapter 4

Short SGA children comprise a heterogeneous group, also because some are born at term and others are born preterm. Despite this important difference, all SGA children are treated with the same dose of growth hormone. Prematurity might have a detrimental effect on spontaneous postnatal growth. When preterm short SGA children suffer from extra growth impairment during their neonatal period, this may result in a height significantly below the genetic height potential of the child. This could lead to a smaller GH-induced catch-up growth in preterm born short SGA children than in those born at term. We investigated in a longitudinal 3-year GH study, whether prematurity has an independent influence on the response to GH treatment in short SGA children. Adjusted for gestational age, preterm short SGA children were lighter and smaller at birth than term short SGA children. Preterm children also had a shorter height adjusted for target height and a leaner phenotype than children born at term. Despite the differences in body size, there were no significant differences in age- and sex-adjusted IGF-I and IGFBP-3 levels. The response to GH treatment was similar for preterm and term born short SGA children. Therefore, preterm short SGA children should not be excluded from GH treatment.

Chapter 5

Both SGA and preterm birth had been associated with increased incidence of adult

cardiovascular disease and type 2 DM. Short SGA children who were born preterm have a lower body fat percentage and a higher blood pressure, insulin secretion and disposition index than short SGA children born at term. It was unknown whether preterm birth also influences these parameters during GH treatment. We compared blood pressure, insulin sensitivity, beta-cell function and body composition during 4 years of GH treatment between 143 preterm and 261 term short SGA children. We demonstrate that GH treatment resulted in a similar decrease in systolic and diastolic blood pressure, body fat percentage, limb fat/total fat ratio and insulin sensitivity, and a similar increase in insulin secretion and disposition index. Lean body mass adjusted for gender and height increased in term children while it did not change in preterm children but multiple regression analysis revealed that this difference in change of lean body mass was not associated with gestational age. We conclude that the effect of GH treatment on metabolic and cardiovascular risk factors is similar in preterm and term short SGA children.

Chapter 6

The Pro12Ala polymorphism of the *PPAR-γ* gene had been inversely associated with BMI, changes in BMI and the risk to develop type 2 DM. Short SGA children are lean and have a lower insulin sensitivity and higher blood pressure. GH treatment results in weight gain, and a decrease in blood pressure and insulin sensitivity. However, not all children respond in the same way. We analyzed the contribution of the *PPARγ* Pro12Ala polymorphism to GH induced changes in determinants of metabolic and cardiovascular disease in 238 Caucasian short SGA children. We found no association between the Ala12 allele and baseline anthropometry, blood pressure, serum lipids, insulin sensitivity, insulin secretion and disposition index. However, after 4 years of GH-treatment, the increase in weight for height SDS and BMI SDS was significantly greater in carriers of an Ala12 allele than in non-carriers. The change in all other parameters was not associated with Pro12Ala genotype. In conclusion, the Ala12 variant of *PPARγ* might be associated with higher weight gain during GH-treatment but this variant is not associated with changes in determinants of metabolic and cardiovascular diseases in subjects born SGA.

Chapter 7

Since insulin is the most important growth factor in fetal life, the reported inverse association between birth weight and the risk to develop type 2 DM in adulthood might be explained by common genetic variants related to insulin secretion and resistance.

TCF7L2 rs7903146 had been shown to have a genetic effect on the risk to develop type 2 DM and this result had been replicated in several studies. In this study we investigated whether the *TCF7L2* rs7903146 polymorphism is associated with growth patterns from fetal life until infancy in a population-based cohort and in a cohort of subjects born SGA. We found a similar minor allele frequency in the SGA cohort as in the general population, so the polymorphism was not associated with an increased risk of SGA birth. In both the SGA cohort and the general population, there were no differences between the genotypes in gestational age or size at birth and the *TCF7L2* rs7903146 polymorphism was also not associated with growth in fetal and early postnatal life.

Chapter 8

The *TCF7L2* rs7903146 polymorphism might increase the risk to develop type 2 DM by decreasing insulin secretion. SGA birth has been associated with type 2 DM in later life while GH treatment reduces insulin sensitivity and increases insulin secretion. This makes GH-treated SGA children an ideal group to investigate whether *TCF7L2* rs7903146 genotype is associated with changes in glucose homeostasis. In a cohort of 246 Caucasian short SGA children, we found no association between *TCF7L2* rs7903146 genotype and insulin sensitivity or insulin secretion at baseline. After adjustment for possible confounders, insulin secretion was higher in carriers of a T-allele than in non-carriers. During 4 years of GH treatment, the increase in insulin secretion was, however, not associated with *TCF7L2* rs7903146 genotype. The decrease in insulin sensitivity was only significant in carriers of the T-allele, but the difference between genotype groups did not reach significance. In conclusion, the *TCF7L2* rs7903146 polymorphism does not predict how well a child is capable of producing extra insulin to compensate for the reduced insulin sensitivity induced by GH treatment. With regard to the effect of this polymorphism on the decrease in insulin sensitivity a larger sample size will allow more definite conclusions.

Chapter 9

IGFBP-2 levels might protect against cardiovascular disease. In several adult populations, serum concentrations of IGFBP-2 were inversely associated with insulin levels, HOMA insulin resistance, BMI, and fat mass. In subjects born SGA, the association between IGFBP-2 levels and metabolic and cardiovascular risk factors had not yet been studied. Our aim was to determine circulating IGFBP-2 levels in subjects born SGA, compare

these levels to those found in age and gender-matched controls with normal stature, and to investigate the association with metabolic and cardiovascular risk factors. We found significantly decreased IGFBP-2 levels in SGA young adults only. Fat mass SDS was higher than average in young adults and reduced (< -2 SDS) in short children. Serum IGFBP-2 SDS values for SGA young adults correlated positively with insulin sensitivity (Si), and negatively with fat mass SDS, insulin secretion (AIR), fasting insulin, HOMA-IR, blood pressure SDS, total cholesterol, and triglycerides. The association between serum IGFBP-2 SDS and Si, blood pressure, total cholesterol and triglycerides levels remained significant after adjustment for fat mass SDS and other known covariates. In short SGA children, IGFBP-2 SDS did not correlate with any of the cardiovascular risk factors. In conclusion, serum IGFBP-2 SDS is an independent cardiovascular risk marker in young adults who were born SGA but not in short SGA children.

Chapter 10

In the general discussion, we discuss our findings in a broader context. The chapter ends with general conclusions and suggestions for further research.

CHAPTER 12

Summary in Dutch

Acknowledgments

Curriculum Vitae

List of publications

SAMENVATTING

Hoofdstuk 1

Dit hoofdstuk beschrijft de definities, prevalentie en verschillende oorzaken van SGA. Ook wordt er een overzicht gegeven van klinische en endocrinologische aspecten van SGA, zoals een te kleine lichaamslengte, de GH-IGF-IGFBP-as, mogelijke veranderingen in schildklierhormoon concentraties, de gerapporteerde associatie met het krijgen van hart- en vaatziekten en type 2 Diabetes Mellitus (type 2 DM) op latere leeftijd, prevalentie van hart- en vaatziekten in de familie en variaties in het DNA. Dit hoofdstuk geeft ook een samenvatting van eerder gerapporteerde effecten van groeihormoon (GH)-behandeling op de groei en GH-IGF-IGFBP-as van te kleine SGA kinderen. Aan het eind van dit hoofdstuk worden de doelstellingen van de studies en de indeling van dit proefschrift besproken.

Hoofdstuk 2

Te kleine SGA kinderen hebben mogelijk al op vrij jonge leeftijd risicofactoren voor het op latere leeftijd krijgen van hart- en vaatziekten of type 2 DM. Het was echter nog niet bekend of het hebben van risicofactoren voor deze ziekten veroorzaakt wordt doordat deze kinderen te klein waren bij de geboorte (SGA), door het vaker voorkomen van hart- en vaatziekten, type 2 DM of SGA in de familie, of door een combinatie van deze factoren. Er was nog nooit onderzocht of de ouders van te kleine SGA kinderen cardiovasculaire risicofactoren hebben. Wij vergeleken lichaamsmetingen, bloeddruk, nuchtere vetspiegels, glucoseconcentraties en insulineconcentraties van 482 ouders en 286 te kleine SGA kinderen met een controlegroep met dezelfde leeftijd en geslacht. Ook onderzochten we of er een verband was tussen deze parameters bij ouders en hun kinderen. Vergeleken met de controlegroep hadden de moeders een hogere bloeddruk, de vaders een hogere BMI en moeders en vaders vaker hoge nuchtere glucose spiegels. De te kleine SGA kinderen hadden een significant hogere systolische en diastolische bloeddruk dan andere kinderen met dezelfde lengte en van hetzelfde geslacht. Vierentwintig procent van de moeders en 10% van de vaders was zelf SGA bij de geboorte maar deze ouders hadden niet meer cardiovasculaire risicofactoren dan ouders met een normale grootte (AGA) bij de geboorte. Er was geen verband tussen de aanwezigheid van risicofactoren bij ouders en hun kinderen. Wij concluderen daarom dat ouders van te kleine SGA kinderen iets meer risicofactoren voor hart- en vaatziekten

en type 2 DM hebben maar dat er geen verband is in het voorkomen van risicofactoren tussen ouders en kinderen.

Hoofdstuk 3

Het zou kunnen dat SGA geboorte geassocieerd is met een veranderde schildklierfunctie maar of prematuriteit hier iets mee te maken heeft was nog niet duidelijk. Voor groei en ontwikkeling is een goed werkende schildklier nodig en daarom is het belangrijk om te weten of GH-behandeling de werking van de schildklier beïnvloedt. Er was echter nog maar heel weinig onderzoek gedaan naar de schildklierhormoon spiegels bij SGA kinderen en het effect van GH-behandeling was helemaal nog niet onderzocht. Wij hebben bij 264 te kleine SGA kinderen TSH en vrij T4 spiegels gemeten voor aanvang van de GH-behandeling en gedurende de twee jaar daarna. Een deel van de kinderen was prematuur geboren en een deel aterm waardoor we ook het effect van vroeggeboorte konden onderzoeken. We vonden hoger dan gemiddelde (maar wel normale) TSH spiegels bij prematuur geboren SGA kinderen en gemiddelde TSH spiegels bij aterm geboren SGA kinderen. Voor aanvang van de GH-behandeling hadden te kleine SGA kinderen een normale vrij T4 spiegel. Er was geen verband tussen de TSH en vrij T4 spiegels en zwangerschapsduur, geboortegewicht SDS of geboortelengte SDS. Tijdens de GH-behandeling daalde de vrij T4 concentratie maar deze daling ging niet samen met een stijging van de TSH concentratie en had ook geen invloed op de groei. Omdat deze milde veranderingen in schildklierfunctie geen klinische relevantie lijken te hebben, concluderen wij dat het bij GH-behandeling van te kleine SGA kinderen voldoende is om één keer per jaar de schildklierfunctie te controleren.

Hoofdstuk 4

De groep te kleine SGA kinderen is heterogeen, ook omdat sommige kinderen te vroeg geboren zijn en andere aterm. Ondanks dit belangrijke verschil worden alle SGA kinderen met dezelfde dosis groeihormoon behandeld. Prematuriteit kan een negatieve invloed hebben op de spontane groei na de geboorte. Als te vroeg geboren SGA kinderen een extra groeiafbuiging na de geboorte hebben zou het kunnen dat hun lengte nog verder onder de voor hun genetisch normale lengte uitkomt. Hierdoor zou de inhaalgroei van prematuur geboren SGA kinderen tijdens GH-behandeling minder kunnen zijn dan bij aterme SGA kinderen. Wij onderzochten of prematuriteit een onafhankelijke invloed heeft op het effect van 3 jaar GH-behandeling bij te kleine SGA kinderen. Na correctie

voor de zwangerschapsduur, waren de premature kleine SGA kinderen lichter en kleiner bij de geboorte dan de aterme kinderen. Bij aanvang van de GH-behandeling hadden de premature kinderen na correctie voor de lengte van de ouders, een kleinere lengte en slankere lichaamsbouw dan aterme kinderen. Ondanks deze verschillen in lichaamsgrootte was er geen verschil in voor leeftijd en geslacht gecorrigeerde IGF-I en IGFBP-3 spiegels. Ook de veranderingen in lichaamsgrootte en –samenstelling en in IGF-I en IGFBP-3 spiegels waren vergelijkbaar voor premature en aterme SGA kinderen. De conclusie is daarom dat prematuur geboren SGA kinderen met een te kleine lengte baat hebben bij GH-behandeling, vergelijkbaar met aterm geboren SGA kinderen.

Hoofdstuk 5

In sommige studies werd gevonden dat zowel SGA als prematuriteit geassocieerd zijn met een grotere kans om op latere leeftijd hart- en vaatziekten of type 2 DM te krijgen. Premature SGA kinderen hebben een lager vetpercentage en een hogere bloeddruk, insuline secretie en dispositie index dan aterme SGA kinderen. Het was echter nog niet bekend of prematuriteit ook een onafhankelijk effect heeft op de verandering van deze parameters tijdens GH-behandeling. Wij hebben gedurende 4 jaar GH-behandeling 143 premature SGA kinderen vergeleken met 261 aterme SGA kinderen wat betreft hun bloeddruk, insuline gevoeligheid, bèta cel functie en lichaamssamenstelling. In dit onderzoek tonen we aan dat tijdens GH-behandeling de systolische en diastolische bloeddruk, het percentage lichaamsvet, de verhouding perifeer vet / totaal vet en de insulinegevoeligheid evenveel daalt bij premature als aterme SGA kinderen. Ook de toename in insuline secretie en dispositie index was vergelijkbaar. De spiermassa gecorrigeerd voor lengte en geslacht nam toe bij de aterme kinderen en bleef onveranderd laag bij de prematuur geborenen. Dit verschil in verandering van spiermassa bleek echter niet samen te hangen met het verschil in zwangerschapsduur. De conclusie is daarom dat het effect van GH-behandeling op risicofactoren voor hart- en vaatziekten en type 2 DM hetzelfde is bij prematuur geboren SGA kinderen als bij aterm geboren SGA kinderen.

Hoofdstuk 6

Het Pro12Ala polymorfisme van het *PPAR-γ* gen vertoont een negatieve associatie met BMI, veranderingen in BMI en de kans op het ontwikkelen van type 2 DM. Te kleine SGA kinderen zijn slank en hebben vergeleken met het gemiddelde een lagere

insulinegevoeligheid en hogere bloeddruk. GH-behandeling zorgt voor een toename in gewicht en een afname van de insulinegevoeligheid en bloeddruk. Deze veranderingen zijn echter niet bij alle kinderen even groot. Wij hebben bij 238 te kleine SGA kinderen onderzocht wat de bijdrage is van het *PPARγ* Pro12Ala polymorfisme aan de veranderingen in risicofactoren voor hart- en vaatziekten en type 2 DM tijdens GH-behandeling. Voor aanvang van de GH-behandeling was er geen verband tussen het hebben van het Ala12 allel en lichaamsafmetingen, bloeddruk, serumspiegels lipiden, insulinegevoeligheid, insulinesecretie en dispositie index. Na vier jaar GH-behandeling was de toename in gewicht SDS en in BMI SDS significant groter bij dragers van een Ala12 allel dan bij niet-dragers. De verandering in alle andere risicofactoren tijdens de 4 jaar behandeling was niet geassocieerd met het Pro12Ala genotype. Wij concluderen dat bij SGA geboren kinderen de Ala12 variant van PPARγ mogelijk geassocieerd is met gewichtstoename tijdens GH-behandeling, maar dat deze variant bij SGA geborenen niet geassocieerd is met risicofactoren voor hart- en vaatziekten of type 2 DM.

Hoofdstuk 7

Voor een foetus is insuline de belangrijkste groeifactor. Het omgekeerde verband tussen geboortegewicht en het risico om als volwassene type 2 DM te krijgen zou daarom verklaard kunnen worden door variaties in het DNA die iets te maken hebben met insulinesecretie en insulineresistentie. Meerdere studies hebben aangetoond dat de T-variant van *TCF7L2* rs7903146 effect heeft op de kans om type 2 DM te krijgen. In dit onderzoek hebben wij onderzocht of het *TCF7L2* rs7903146 T-allel geassocieerd is met groeipatronen van de foetale periode tot de leeftijd van 2 jaar. Wij hebben dit onderzocht in een cohort dat een afspiegeling is van de algemene populatie en in een cohort van SGA geboren kinderen. Het T-allel bleek even vaak voor te komen in het SGA cohort als in de algemene populatie dus deze variant leek niet geassocieerd te zijn met een grotere kans om SGA geboren te worden. In beide populaties was er geen verschil in zwangerschapsduur of grootte bij de geboorte tussen dragers van het T-allel en niet-dragers. Het T-allel was ook niet geassocieerd met groei in de foetale periode en in de eerste twee levensjaren.

Hoofdstuk 8

Mogelijk verhoogt het *TCF7L2* rs7903146 polymorfisme de kans om type 2 DM te krijgen doordat het de insulinesecretie doet afnemen. SGA geboorte is geassocieerd

met een grotere kans op het ontstaan van type 2 DM. Van GH-behandeling is bekend dat het de insulinegevoeligheid verlaagt en de insulinesecretie doet toenemen. SGA kinderen die met GH behandeld worden zijn daarom een zeer geschikte groep om te onderzoeken of het *TCF7L2* rs7903146 genotype geassocieerd is met veranderingen in de glucosehuishouding. Wij vonden, in een groep van 246 te kleine SGA kinderen, geen verband tussen het *TCF7L2* rs7903146 genotype en insulinegevoeligheid of insulinesecretie. Wanneer er gecorrigeerd werd voor mogelijk beïnvloedende factoren dan was de insulinesecretie hoger bij kinderen met een *TCF7L2* rs7903146 T-allel dan bij kinderen zonder T-allel. Tijdens 4 jaar GH-behandeling was de toename in insulinesecretie vergelijkbaar bij kinderen met en kinderen zonder T-allel. Er was alleen een significante afname van de insulinegevoeligheid bij kinderen met een T-allel, maar het verschil in daling van de insulinesecretie tussen de groep met een T-allel en de groep zonder T-allel was niet significant. De conclusie is daarom dat variatie in *TCF7L2* rs7903146 niet voorspelt hoe goed een kind zijn insulinesecretie kan verhogen om te compenseren voor de afgenomen insulinegevoeligheid als gevolg van GH-behandeling. Een grotere groep kinderen zal onderzocht moeten worden om erachter te komen of dit polymorfisme invloed heeft op de afname in insulinegevoeligheid.

Hoofdstuk 9

Het zou kunnen dat hogere IGFBP-2 spiegels beschermen tegen het krijgen van hart- en vaatziekten. Bij volwassenen is de IGFBP-2 concentratie omgekeerd geassocieerd met insulinespiegels, HOMA insulineresistentie, BMI en vetmassa. Bij mensen die SGA geboren zijn was het verband tussen IGFBP-2 spiegels en risicofactoren voor hart- en vaatziekten en type 2 DM nog niet onderzocht. Wij hebben de IGFBP-2 concentratie gemeten bij kinderen met een te kleine lengte en jongvolwassenen met een normale of te kleine lengte die SGA waren bij de geboorte. Wij hebben deze concentraties vergeleken met die van controlepersonen met dezelfde leeftijd en geslacht en een normale lengte. Ook onderzochten we het verband tussen de IGFBP-2 concentratie en cardiovasculaire risicofactoren. Alleen bij de jongvolwassenen was de IGFBP-2 concentratie significant lager dan in de controlegroep. De vetmassa was hoger dan gemiddeld bij de jongvolwassenen en lager dan gemiddeld bij de kinderen. Wij vonden een positieve relatie tussen IGFBP-2 en insulinegevoeligheid en een negatieve relatie tussen IGFBP-2 en vetmassa, insulinesecretie, nuchtere insuline spiegels, HOMA-IR, bloeddruk, totale cholesterol concentratie en triglyceriden concentratie. Het verband tussen IGFBP-2

en insulinegevoeligheid, bloeddruk, cholesterol en triglyceriden bleef ook bestaan als we corrigeerden voor vetmassa en andere beïnvloedende factoren. Bij de te kleine SGA kinderen was er geen verband tussen IGFBP-2 en cardiovasculaire risicofactoren. Onze resultaten laten zien dat laag IGFBP-2 bij SGA geboren jongvolwassenen een onafhankelijke risicofactor voor het krijgen van hart- en vaatziekten is.

Hoofdstuk 10

In de algemene discussie worden de resultaten van de verschillende studies besproken en vergeleken met de literatuur. Wij sluiten dit hoofdstuk af met algemene overwegingen en suggesties voor toekomstig onderzoek.

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CURRICULUM VITAE

Sandra de Kort was born in Dordrecht on October 19th, 1977. She passed (cum laude) her secondary school exam at the "Sint Oelbert Gymnasium" in Oosterhout in 1996. In the same year she started her medical training at the Medical Faculty of the Catholic University of Leuven (Belgium). In 1997 she switched to the Medical Faculty of the University of Leiden (1997-2004). During her study she performed a research project on Juvenile Idiopatic arthritis at the pediatric rheumatology department of the Leiden University Medical Center (supervisors: Dr. M.A.J. van Rossum and Dr. R. Ten Cate). Also, she spent 6 months in Spain at the university of Valencia where she did optional internships at the departments of pediatrics, obstetrics and gynaecology, ophthalmology and oto-rhino-laryngology. Her final internship of 3 months was spent in the Juliana Children's hospital in The Hague. After obtaining her medical degree (cum laude) in March 2004, she worked as a resident at the department of Pediatrics at the Amphia Hospital Breda (Dr. A. Vaessen-Verberne) and at the department of Pediatrics at the Leiden University Medical Center (Prof.dr. J.M. Wit). In October 2005 she started a research fellowship at the department of Pediatric Endocrinology of the Erasmus MC – Sophia Children's Hospital (supervisor Prof.dr. A.C.S. Hokken-Koelega). The research performed during this period is presented in this thesis.

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