Short SGA children

etiological aspects,
metabolic consequences and
effects of GH treatment

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SGA kinderen met een te kleine lengte

etiologische aspecten,
metabole consequenties en
effecten van GH behandeling

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Aan mijn ouders

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Chapter 1 Introduction

Introduction

General introduction and aims of the thesis

Introduction

In 1997 the author of the present thesis started studies relating to short children born small for gestational age (SGA). The present chapter describes definitions of SGA, prevalence and etiology of SGA, factors involving fetal growth and features of short children born SGA. Also an overview of the literature is presented concerning the effects of growth hormone (GH) treatment on growth in these children. Finally, the aims of the study, study design and in- and exclusion criteria of this Dutch multicenter trial are described.

1. Small for gestational age (SGA)

Definitions of SGA

In literature, the term SGA has been applied to all newborns having a birth weight and/or a birth length below the third or tenth percentile (or below –1.88 or –1.29 SDS) for the gestational age. Unfortunately, until recently there has not been consensus among investigators whether birth weight or birth length should be used, whether the cut-off point should be the 3rd or the 10th percentile and which references should be used. Therefore, it has been difficult to compare results between different study groups. In all Dutch multicenter studies, however, SGA was defined as a birth length below the –2.00 SDS using the curves of Usher and McLean (1). Although the definition is somewhat arbitrary, the definition of SGA has been delineated as a birth length and/or birth weight < -2 SD for gestational age.

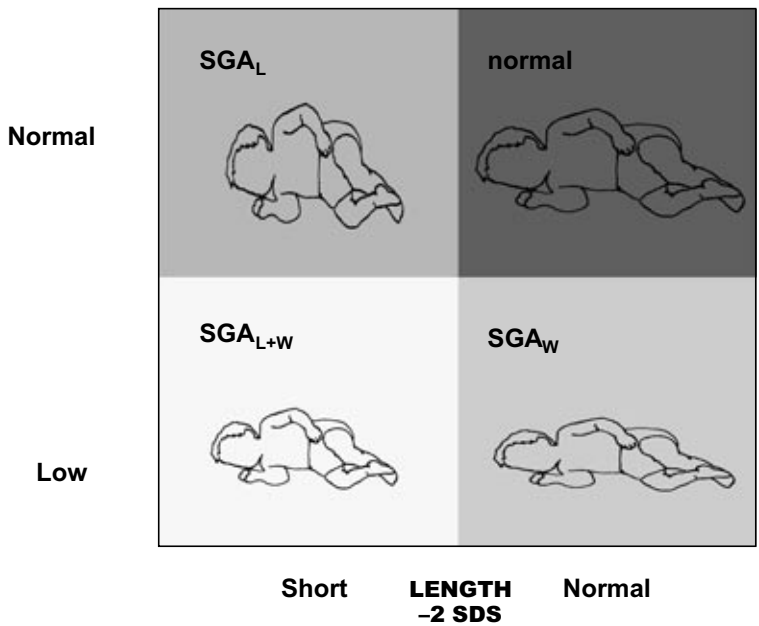
The term intra-uterine growth retardation (IUGR) has also been applied to SGA infants. IUGR, however, refers to fetal growth retardation and can be observed as a deviation of the intra-uterine growth chart. Therefore, IUGR can only be diagnosed when documented by two intrauterine growth assessments. SGA does not refer to fetal growth but refers to body size at birth. Not all SGA infants are intrauterine growth retarded. An SGA infant may have been small from the beginning of fetal life. Also, growth retardation late in gestation does not need to result in an SGA infant.

Different terms are used to subdivide SGA neonates. According to a frequently used classification SGA infants can be divided into 'symmetric' versus 'asymmetric' SGA (2). The term 'symmetric' is applied to SGA neonates having a low birth weight, a small birth length and a small birth head circumference. 'Asymmetric' SGA neonates have a relatively large head since birth weight and birth length are low whereas head circumference at birth is normal. Generally, growth retardation early in fetal life results in symmetrically small neonates whereas growth retardation later in fetal life results in asymmetrical SGA neonates. Another more often used classification divides into 'proportionate' versus 'disproportionate' SGA (3). This subdivision uses birth weight and birth length only. Those SGA neonates having both a low birth weight and

a small birth length are referred to as 'proportionate' SGA neonates whereas those only having a low birth weight are called 'disproportionate' SGA neonates. Infants can also be classified into 4 categories according their birth weight (W) and birth length (L) (4). Light and short SGA neonates are classified as SGA_{L+W}, light neonates as SGA_W and short SGA neonates as SGA_L (see Fig. 1). It is important to describe these classifications since different SGA subsets may have different underlying mechanisms for SGA and may respond differently to therapy.

Fig. 1

Classification of SGA infants Adapted from Albertsson-Wikland K, Karlberg J. Acta Paediatr Suppl. 1994;399:64.



Prevalence and etiology of SGA

Depending on the definition of SGA, about 1.5 to 10 % of all live born neonates is born SGA. The cause of SGA should be identified whenever possible as underlying mechanisms are diverse and may influence prognosis and treatment effects. Impaired fetal growth has multiple causes including a number of maternal, placental and fetal factors (for a detailed list see Table 1) (5).

Fetal factors include chromosomal abnormalities, congenital defects, metabolic diseases and genetic defects. Specialized genetic tests may be helpful to detect the presently known genetic defects.

Table 1

Factors associated with increased incidence of infants who are born SGA and selected examples

Fetal factors	
Karyotypic abnormalities	Trisomy 21 (Down syndrome)
	Trisomy 18 (Edward syndrome)
	Monosomy X (Turner syndrome)
	Trisomy 13 (gonadal dysgenesis)
Other chromosomal abnormalities	Autosomal deletions
	Ring chromosomes
Genetic diseases	Achondroplasia
	Bloom syndrome
Congenital anomalies	Potter syndrome
	Cardiac abnormalities
Maternal factors	
Medical conditions	Hypertension
	Renal disease
	Diabetes mellitus (advanced stages)
	Collagen vascular diseases (eg system lupus erythematosis)
	Maternal hypoxemia (cyanotic heart disease, chronic anemia, chronic pulmonary disease)
Infection	Toxoplasmosis
	Rubella
	Cytomegalovirus
	Herpesvirus
	Malaria
	Trypanosomiasis
	Human immunodeficiency virus
Nutritional status	Low pregnancy weight
	Low pregnancy weight with poor weight gain during pregnancy
Substance use / abuse	Cigarette smoking
	Alcohol
	Illicit drugs
Uterine / placental factors	
Gross structural placental factors	Single umbilical artery
	Velamentous umbilical cord insertion
	Bilobate placenta
	Placental hemangiomas
	Infarcts, focal lesions
Insufficient uteroplacental perfusion	Suboptimal implantation site
Placenta previa	
Low-lying placenta	
Placental abruption	
Demographic factors	
Maternal age	Very young age
	Older age
Maternal height	
Maternal weight	
Maternal and paternal race	
Parity	Nulliparity
	Grand multiparity
Maternal history	Previous delivery of SGA infants
Other	
Multiple gestation	Particularly severe in syndromes associated with shared fetal circulation

Adapted from Bernstein and Divon (58), Pollack and Divon (2), Wollmann (59) and Keller et al (60)

Maternal factors include age, parity, medical conditions such as hypertension, infections (particularly such as toxoplasmosis, rubella, cytomegalovirus and herpes virus), malnutrition, alcohol abuse and cigarette smoking.

Placental factors involve problems in placental perfusion. As the placenta is essential for nutrient and oxygen supply between mother and fetus, it is clear that any placental dysfunction could result in fetal growth retardation. Examples of placental insufficiency are abruption or infarction or other placental vascular abnormalities. Examination of the placenta by a pathologist might discover specific causes.

Demographic factors such as maternal race, obstetric history, age of the mother, multiple gestation might be associated with SGA infants, particularly in case of a shared fetal circulation.

Silver-Russell syndrome

A subgroup of children born SGA consists of children with Silver-Russell syndrome (SRS). Typical features of children with SRS are a severely reduced birth weight and –length, short stature during childhood and adulthood, typical craniofacial abnormalities with a relative large, prominent forehead (frontal bossing), a small, triangular face, asymmetry of head and limbs, clinodactyly of the fifth finger and some other minor abnormalities (6). A mean final height of 151.2 (cm) for boys and 139.7 (cm) for girls has been reported in patients with SRS (6). Chromosome abnormalities (e.g. maternal disomy of chromosome 7) have been reported in only a minority of children (6). The reported genetic abnormalities comprise a heterogeneous group. Maternal uniparental disomy of chromosome 7 is the most frequently observed abnormality and has been described in 7 - 10 % of SRS children (7). Other less frequently observed abnormalities include a ring chromosome 15, deletion of distal q15, translocation of the distal part of chromosome 17q, trisomy 18 mosaicism and deletion of the short arm of chromosome 8q11-13 (8). However many children with genetically proven SRS do not present all characteristic features of SRS whereas on the other hand some children have classical features without proven chromosomal abnormalities thus far. This means that the diagnosis of SRS is primarily based on clinical features, only in few children supported by a chromosomal abnormality. Large varieties exist in the presence of Silver-Russell symptoms. Some children born SGA show only minor features of SRS (e.g. only clinodactily) which makes it sometimes difficult to support the diagnosis of SRS. It seems a certain range exist varying from Silver-Russell like (only one or two minor symptoms) to the classical SRS (all major symptoms). Growth patterns of children with SRS do not differ from short children born SGA (6). Therefore children with SRS were included in most trials investigating growth aspects of short SGA children.

2. Fetal growth

Fetal growth is the result of a very complex metabolic and endocrine process. Several hormones play a role in fetal growth by influencing nutrient supply from mother to fetus and by affecting fetal organogenesis.

Growth hormone (GH)

The role of GH in fetal growth is rather controversial. Initially it was thought that GH did not contribute to fetal growth, since neonates born with congenital GH deficiency (GHD) generally do not have reduced birth weights and birth lengths. Growth retardation in GHD becomes evident only from 3 months after birth when a decline in linear growth starts.

However, it was reported that birth length in these infants was on average 1 standard deviation (SD) lower compared to healthy neonates suggesting GH does have an effect on linear growth in utero (9). GH receptors are already present in fetal tissues although the numbers are lower compared to postnatal tissues (10,11). It has been assumed that the reduced amount of GH receptors may play a role in the moderate effect of GH on fetal growth.

Insulin-like growth factor-I and -II (IGF-I and -II)

IGF's are very important determinants of fetal growth. Insulin-like growth factor-I (IGF-I) plays an important role in both pre- and postnatal growth and its serum levels are regulated by both metabolic and genetic factors. In fetuses and neonates born SGA low circulating IGF-I levels have been observed suggesting a role for IGF-I in fetal growth retardation (12-17). Gene deletion studies in mice clearly present the role of IGF-I (Table 2). IGF-I knock-out mice had a birth weight which was about 60 % of normal (18). Postnatal growth in these mice decreased even further resulting in adult weights of about 30 % of normal mice (19). However, these knock-out mice behaved normally and appeared proportionate in size. In humans, only one homozygous partial deletion of the IGF-I gene has been described in a 15-year old boy (20). This child was born SGA and showed severe postnatal growth failure, sensorineural deafness and mental retardation.

IGF-II plays predominantly a role in prenatal growth. Knockout studies in mice clearly present the role of IGF-II in mice (18). IGF-II knockout mice showed a reduction in birth weight to 60 % of normal mice (Table 1). Also it is clear from these studies that the growth promoting effect of IGF-II acts through the IGF-I receptor. IGF-II was also able to bind to another unidentified receptor. It was shown that IGF-II stimulates growth early in gestation whereas IGF-I is an important growth factor later in gestation.

Table 2
Phenotype of mice mutants leading to growth retardation

Genotype	Birthweight (% of nomal)	Neonatal lethality
IGF-I (-/-)	60	±
IGF-II (p-)	60	-
IGF-IR (-/-)	45	+
IGF-I (-/-) / IGF-II (p-)	30	+
IGF-I (-/-) / IGF-IR (-/-)	45	+
IGF-II (p-) / IGF-IR (-/-)	30	+

IGF-I (-/-) = homozygous null mutation of the IGF-I gene
IGF-II (p-) = heterozygous null mutation of the paternal allele of the IGF-II gene
IGF-IR (-/-) = homozygous null mutation of the IGF-I receptor gene
IGF-I (-/-) / IGF-II (p-) = double mutant of IGF-I and -II
IGF-I (-/-) / IGF-IR (-/-) = double mutant of IGF-I and IGF-I receptor
IGF-II (p-) / IGF-IR (-/-) = double mutant of IGF-II and IGF-I receptor

Adapted from Liu et al, Cell 1993;75:59

Insulin

Initially insulin was thought to be the major growth promoting hormone in fetal life. More recently it is believed that insulin acts via stimulation of cellular nutrient (glucose) uptake and stimulation of IGF-I production (21). Glucose availability and the subsequent increase in fetal insulin are the main regulators of fetal IGF-I production. Fetal pancreatectomy in sheep resulted in low fetal IGF-I levels and caused severe intra-uterine growth retardation (22). Intrafetal infusions of either insulin or glucose increased fetal IGF-I levels. Insulin has also lipogenic effects which are well studied in fetuses from mothers with diabetes gravidarum. Fetal hyperinsulinemia and hyperglycemia due to maternal diabetes results in stimulated fetal growth. The increase in birth weight of these infants mainly consists of fat mass due to the lipogenic effects of insulin. Birth length and lean body mass are only slightly increased which is in turn the effect of IGF-I mediated through the high insulin levels (23).

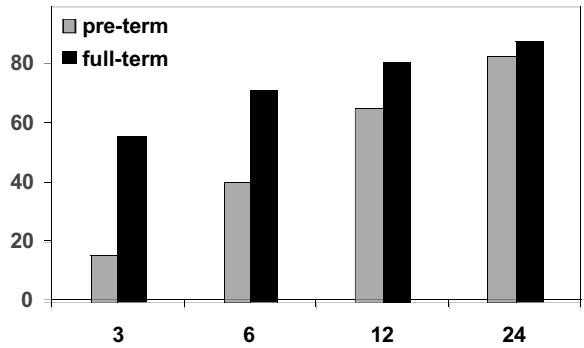
3. Consequences of being born SGA

Perinatal mortality and morbidity is greater in SGA babies compared to babies born appropriate for gestational age (AGA). However, since perinatal care improved rapidly during the last decade, most SGA infants now survive. This enables us to study the consequences of being born SGA during childhood as well as during adulthood.

Postnatal growth

Most of the children born SGA show catch-up growth during the first 2 to 3 years of life. If children have not shown catch-up growth during the first years of life they have a greater risk of remaining short later in life. In a Swedish cohort of 123 infants born SGA, which was defined as a birth weight below –2.0 SDS, 9 % still had a height below –2.0 SDS at the age of 4 years (24). Hokken et al found that 15 % of a group of 724 term and preterm SGA infants, which was defined as a birth length below –1.88 SDS, still had a height below the third percentile at the age of two years (25). Term SGA infants showed a more rapid increase in catch-up growth compared to preterm infants. However, at the age of two years the percentage SGA infants without catch-up growth was the same for term and preterm infants (Figure 2).
Longitudinal studies from birth to final height show that infants born SGA have an increased risk of short stature in adult life. Chausssain et al reported that SGA children born with a birth length < -2 SD for gestational age who remained short during childhood, reached an adult height of 161.9 +/- 8.0 cm (boys) and 147.6 +/- 7.2 cm (girls). These adult heights were significantly lower than the target heights of these patients (26). Also, Karlberg et al found a 7-fold increased risk for short stature at the age of 18 years in those who had been born SGAL and a 5-fold increased risk in those who had been born SGA_W (27).

Fig. 2
Percentage of SGA infants with postnatal catch-up growth to a height ≥ -1.88 SDS
Adapted from Hokken-Koelega et al. Pediatr Res 1995;38:267.



Serum growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels

The mechanism underlying persistent short stature in children born SGA is still not fully understood. Disturbances in the growth hormone (GH)/insulin-like growth factor-I (IGF-I)-axis may play a role. Sixty percent of SGA children with insufficient catch-up growth, defined as a height below –2 SDS, showed a decrease in physiologic 24-hour GH secretion whereas 25 % showed low GH peaks during GH provocation tests (28,29). Also, serum IGF-I levels were significantly lower in short children born SGA compared to normal children (28,29).

Body composition

Short children born SGA have a lean appearance. However, limited data are available on body composition during childhood in these short children. Most studies evaluating body composition in short SGA children have used body mass index (BMI) (30,31). BMI in short SGA children is significantly lower compared to healthy children with the same age and sex. Leger et al measured body composition by magnetic resonance imaging of the thigh and used this as an estimate of total body fat (32). In short SGA children, they found a reduction of both muscle and subcutaneous fat mass measured on cross sectional areas of the thigh. However, a more precise method to investigate total body composition would be by Dual Energy X-ray Absorptiometry (DXA). DXA uses a low radiation dose, is very accurate and is easily performed in children (33,34). DXA measures lean body mass (LBM), fat mass and bone mineral density (BMD). At the start of this study (1997), no data were available of body composition in short SGA children measured by DXA.

Spontaneous bone maturation

Data on bone maturation in short SGA children are very sparse. A French study reported delayed bone ages until the age of 8 years in a group of short children born SGA (35). However, after the age of 8 years bone age accelerated without a concomitant increase in height. This resulted in adult heights which were significantly lower than the predicted adult heights at the age of 8 years. In 1975, Tanner et al already described in a group of short prepubertal children with Silver-Russell syndrome a similar pattern of acceleration of bone maturation from the age of 5 years (36). This suggests that SGA children might experience a different bone maturation over the years compared to their healthy peers. For that reason prediction of adult height based on estimates of bone age are unreliable in these children.

Adult diseases associated with a low birth weight

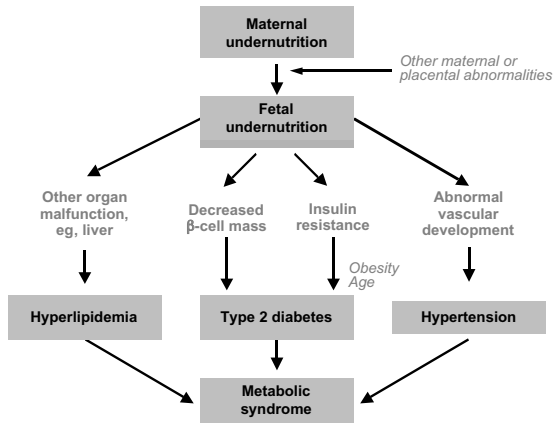
Epidemiological studies have shown that type 2 diabetes mellitus (DM), hypertension and cardiovascular diseases occur more frequently among individuals who were born with a low birth weight (37,38). Also, the combination of type 2 DM, hypertension, dyslipidemia and a high body mass index (BMI), called the Metabolic Syndrome, has

been associated with a low birth weight (38,39). Most evidence is based on large epidemiologic, retrospective studies. The mechanisms underlying these associations are still unknown. Insulin resistance and hyperinsulinism are thought to play a key role in the pathogenesis of both type 2 diabetes mellitus and cardiovascular abnormalities (39-41). Two main hypothesis exist.

Barker's 'fetal origins hypothesis' postulates that these diseases are the result of malnutrition during a critical period in fetal life (Figure 3) (42). Depending on the type and time of poor fetal and early infant growth, a variety of long-term changes in organ function may develop (43). It is not known whether this programming is at the level of cell numbers or organ structure. Malnutrition early in gestation will result in fetal growth retardation as well as growth retardation of several major organs including the fetal pancreas. As one of the consequences the number of β-cells will be reduced resulting in low fetal insulin levels. Since insulin is an important fetal growth factor, low fetal insulin levels will result in a low birth weight and/or birth length. According to this hypothesis, insulin resistance results from fetal malnutrition in order to divert glucose resources to vital structures (brain and heart) at the expense of muscles and other organs. In a post-natal environment of nutritional excess, this programmed insulin resistance becomes permanent and insulin resistance becomes maladaptive which contributes to the development of type 2 diabetes mellitus (44).

The second hypothesis is proposed by Hattersley and is called the 'fetal insulin hypothesis' (45). The fetal insulin hypothesis, postulates that genes involving insulin resistance could effect both birth weight and disease in later life (45-47) (Figure 4). Fetal insulin related growth does not only reflect maternal glycemia but does also reflect fetal genetic factors which regulate the secretion of insulin by the fetal pancreas and the sensitivity of fetal tissues for the effects of insulin.

Fig 3
Representation of the fetal origins of syndrome X
Adapted from Barker DJP et al. Diabetologia. 1993;36:62. Barker DJP. BMJ. 1995;311:171



An example of this hypothesis is the glucokinase gene (Table 3). The enzym glucokinase phosphorylates glucose to glucose-6-phosphate in the pancreas and liver where it is the rate-deter-mining step for glucose metabolism. A heterozygous mutation in the glucokinase gene leads to altered glucose sensing by the β-cells and results in hyperglycemia. If the mother has the mutation and the child has not, the fetal pancreas will sense hyper-glycemia and secrete large amounts of insulin resulting in increased fetal growth. Conversely, if the mother does not have the mutation whereas the child does, the fetal pancreas will sense low serum glucose levels resulting in low insulin levels and reduced fetal growth.

Fig. 4
Simplified representation of fetal insulin hypothesis
Adapted from Hattersley et al. Lancet 1999;353:1789.

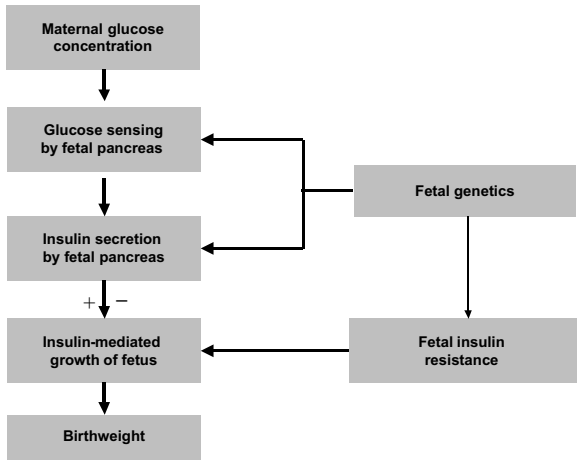


Table 3
Mutations in the glucokinase gene

	Mother + Fetus -	Mother + Fetus +	Mother - Fetus -	Mother - Fetus +	Sign.
N	21	19	8	10	
Birth weight (g)	3957 ± 447	3378 ± 712	3321 ± 463	2889 ± 525	0.0001
Birth weight centile	86 ± 20	53 ± 33	49 ± 35	24 ± 28	<0.0001

Mother + = mutation present in the mother
Mother - = mutation absent in the mother
Fetus + = mutation present in the fetus
Fetus - = mutation absent in the fetus

Adapted from Hattersley et al. Nature Genetics 1998;19:268.

4. Growth hormone (GH) treatment in SGA children

Efficacy

The first Dutch GH trial treating short children born SGA, which started in 1991, was a randomised, double-blind, dose-response multicenter study. After two years of GH treatment, with either a dose of 33 μg/kg/day or 66 μg/kg/day, height increased significantly (30). However, bone maturation accelerated in both groups, especially during the second year of treatment. Since both groups were treated with GH it was unknown whether this acceleration in bone maturation was due to either an effect of GH or an effect of ageing. Therefore the present study, a second multicenter trial with a randomzied control group for 3-years was started.

At the time the present study was started (1997) several studies had shown beneficial effects of GH treatment on linear growth in short children born SGA (30,48-50). At that time however, all reports contained short-term results. All studies showed a significant increase in height and in height velocity during 2 or 3 years of GH treatment. Since studies evaluating the effects of GH-treatment on growth are longitudinal studies and most patients started treatment at an age of about 6 years, it is clear that it takes at least ten years before most children have reached their adult height. Table 4 presents the summarized results of 4 different studies who were published at the time we started our randomized, controlled GH trial in 1997.

Safety

Short-term studies of treatment with biosynthetic GH showed that major side-effects were very uncommon. Adverse events which have been reported during GH treatment in general were idiopathic intracranial hypertension, edema and lymphedema, carpal tunnel syndrome, slipped capital femoral epiphysis, diabetes mellitus and carbohydrate intolerance (51).

GH treatment is known to decrease insulin sensitivity in normal adults (52,53). Since the known association between a low birth weight and a higher risk of developing type 2 diabetes mellitus in adult life, evaluation of glucose intolerance is recommended during GH treatment in short children born SGA. However, not much is known about possible side-effects of GH treatment on insulin sensitivity in short SGA children. The first Dutch multicenter trial showed that 4 % of the short prepubertal SGA children already had an impaired glucose tolerance before the onset of GH treatment (54). During GH treatment serum glucose levels remained constant while fasting serum insulin levels increased. After 6 years of GH treatment impaired glucose tolerance was found in 4 % of the children suggesting that 6-years of continuous GH treatment has no adverse effects on glucose levels in short SGA children.

Since epidemiological studies also found a relation between low birth weight, hypertension and dyslipidemia, blood pressure and serum lipids need to be monitored during GH treatment of short SGA children. Until now no adverse effects has been described of GH treatment on these parameters (55). During 6 years of continuous

Table 4
Summary of literature data on the results of GH treatment in short children born SGA until 1997

Country	Study	GH-dose (μg/ kg/day)	Duration (in years)	Δ height SDS	Conclusions
France Chatelain et al 1994 (48)	Randomized double blind controlled study	Placebo (for 6 months) 22 vs 66	2	Placebo: no increase Mean (SEM) 0.66 (0.07) vs 1.25 (0.07)	A dose-dependent catch-up growth can be induced by GH treatment
Sweden Boguszewski et al 1996 (49)	One dose	33	2	Mean (SD) 1.2 (0.85)	An increased growth rate in response to GH therapy was found
The Netherlands De Waal et al 1996 (30)	Double-blinded dose-response study	33 vs 66	2	Mean (SD) 1.32 9 (0.65) vs 1.73 (0.82)	GH treatment resulted in a dose-dependent increase in growth
Germany Ranke et al 1996 (50)	KIGS data (Kabi International Growth Study)	33 (mean dose)	3	Median 1.0	GH treatment is effective in increasing final height

GH treatment both serum lipids and diastolic blood pressure remained stable within the normal range. In contrast, before GH treatment systolic blood pressure was higher but decreased significantly after 6 years of GH treatment. Although these results are reassuring, blood pressure and serum lipids need to be checked regularly during GH treatment.

5. Aims of the study

Insulin sensitivity, blood pressure and serum lipids

To assess insulin sensitivity by the Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test, blood pressure and serum lipids in short SGA children. In addition, we investigated the presence of clustering of insulin insensitivity with cardiovascular risk factors in these prepubertal short SGA children since this phenomenon has been described in epidemiological studies of adults born with a low birth weight.

IGF-I gene

To investigate 3 polymorphic markers, located in the IGF-I gene, in children and parents participating in both the present and the first Dutch multicenter study. As IGF-I plays an important role in both fetal and postnatal growth and serum IGF-I levels are reduced in short SGA children we hypothesized that this gene might play a role in the etiology of SGA.

Hypothalamus / pituitary

To study abnormalities in the pituitary region using magnetic resonance imaging (MRI) in short SGA children. Abnormalities in the pituitary region are described in children with either isolated GHD or multiple pituitary hormone deficiencies (MPHD). Since disturbances in the GH/IGF-I axis have been reported in short children born SGA, we hypothesized that these might be related to abnormalities in the pituitary region.

Efficacy and safety of GH treatment versus no treatment in short SGA children

The first Dutch GH trial treating short children born SGA showed a significant increase in height during 2 years of treatment with either a GH dose of 33 μg/kg/day or 66 μg/kg/day. Also a significant acceleration in bone maturation was observed in both groups. This finding was rather confusing since a continuation of this acceleration in bone maturation could negatively influence final height. As both groups were treated with GH it was unknown whether the observed effect was either the result of GH treatment or an effect of ageing. In view of the positive effects of GH treatment on growth on one hand and the acceleration in bone maturation on the other hand, we decided to evaluate the effects of GH treatment in a randomized, controlled trial. The present multicenter study was started with a randomized control group for 3 years, investigating the effects of GH treatment (33 μg/kg/day) versus no treatment during a period of 3 years.

We evaluated the effects of GH treatment versus no treatment in short SGA children on

- Growth and bone maturation.
- Body composition and bone mineral density (BMD). Body composition was measured using DXA, BMI and skinfold thickness. DXA measures fat mass, lean body mass (LBM) and BMD.
- Body proportions.

For the description of the randomized, controlled GH trial see Appendix A.

6. Outline of the thesis

Part I

Being born SGA has, besides an increased risk of being short as adult, also an increased risk of developing type 2 diabetes mellitus (DM), hypertension and cardiovascular diseases later in life. Therefore we investigated insulin sensitivity, lipid metabolism and blood pressure in a group of short SGA children (Chapter 2). Unfortunately, it is still not understood why a small percentage of children born SGA remains short during child- and adulthood. Since IGF-I plays an important role in both pre- and postnatal growth, we investigated the IGF-gene in a large cohort of short SGA children and their parents (Chapter 3). In order to investigate the hypothalamic-pituitary axis MRI's were performed and results were compared to MRI results of children with isolated GHD, multiple pituitary hormone deficiencies and children with normal stature (Chapter 4).

Part II

This part shows results of the randomized, 3-year controlled GH trial. Changes in height, bone age and bone mineral density (BMD) were evaluated, comparing SGA children receiving GH treatment with those remaining untreated. We also investigated the influence of the severity of short stature at start of the study on the gain in height during treatment with different doses of GH (Chapter 5). Before and during GH treatment DXA's were performed in a subgroup of short SGA children and results were compared to skinfold measurements and BMI (Chapter 6). Furthermore head circumference and body proportions were studied at baseline and during 3 years of GH treatment in comparison with results of untreated short SGA children. Also a differentiation was made between those who had been born with both a low birth length and a low birth weight (SGA_{L+W}) and those who had been born with a low birth length only (SGA_{L}) (Chapter 7).

In the general discussion the significance of the presented data and their relationship with the present literature are discussed (Chapter 8). At the end of the thesis an English and a Dutch summary of this thesis can be found (Chapter 9 and 10).

Appendix A

Description of the randomized, 3-year controlled GH trial

Patients

Inclusion criteria:

1. Birth length standard deviation score (SDS) below – 1.88 (that is below 3rd percentile) for gestational age (1)
2. An uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia
3. Chronological age (CA) between 3.00 and 7.99 years at start of the study;
4. Height SDS for age below –1.88 according to Dutch standards (56)
5. Height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (56)
6. Prepubertal, defined as Tanner stage 1 or a testicular volume < 4 (57)
7. Normal liver, kidney and thyroid functions.

Exclusion criteria:

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

Study design

The study design was an open-labelled multicenter study with a randomized control group *(Table 5)*. Before entering the study the GH status was evaluated in all children using GH stimulation tests (arginine and/or clonidine). Children with GH deficiency (GHD) which was defined as a GH peak < 10 μg/l during two GH stimulation tests, were not randomized but started GH treatment at dose of 1 mg/m^2 body surface area/day (~33 μg/kg/day) (GHD-group). The non-GHD children were stratified according to age (3.00-5.50 versus 5.50-7.99) and height of the parents (height of both parents above –1.88 SDS versus height of at least one parent below –1.88 SDS). After stratification the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group started immediately

with GH treatment at a dose of 33 μg/kg/day (~1 mg/m^2 body surface area/day). The control group remained untreated for 3 years and received subsequently the same GH treatment as the GH-group. Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

Table 5
Study design of the randomized, 3-year controlled GH study

Study years	n	1	2	3	further
Randomized study					
GH-group	61	■	■	■	■
Control group	26				■
Comparison cohort					
GHD-group	12	■	■	■	■

References

1. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
2. Pollack RN, Divon MY 1992 Intrauterine growth retardation: definition, classification, and etiology. Clin Obstet Gynecol 35:99-107.
3. Villar J, Belizan J, Smeriglio V 1989 Intrauterine growth retardation. In: J. S (ed.), vol. 18. Raven Press Ltd., New York, pp 261-280.
4. Albertsson-Wikland K, Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71.
5. Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P 2003 International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. Pediatrics 111:1253-61.
6. Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB 1995 Growth and symptoms in Silver-Russell syndrome: review on the basis of 386 patients. Eur J Pediatr 154:958-68.
7. Kotzot D, Balmer D, Baumer A, Chrzanowska K, Hamel BC, Ilyina H, Krajewska-Walasek M, Lurie IW, Otten BJ, Schoenle E, Tariverdian G, Schinzel A 2000 Maternal uniparental disomy 7--review and further delineation of the phenotype. Eur J Pediatr 159:247-56.
8. Hitchins MP, Stanier P, Preece MA, Moore GE 2001 Silver-Russell syndrome: a dissection of the genetic aetiology and candidate chromosomal regions. J Med Genet 38:810-9.
9. Gluckman PD, Gunn AJ, Wray A, Cutfield WS, Chatelain PG, Guilbaud O, Ambler GR, Wilton P, Albertsson-Wikland K 1992 Congenital idiopathic growth hormone deficiency associated with prenatal and early postnatal growth failure. The International Board of the Kabi Pharmacia International Growth Study. J Pediatr 121:920-3.
10. Hill DJ, Riley SC, Bassett NS, Waters MJ 1992 Localization of the growth hormone receptor, identified by immunocytochemistry, in second trimester human fetal tissues and in placenta throughout gestation. J Clin Endocrinol Metab 75:646-50.
11. Klempt M, Bingham B, Breier BH, Baumbach WR, Gluckman PD 1993 Tissue distribution and ontogeny of growth hormone receptor messenger ribonucleic acid and ligand binding to hepatic tissue in the midgestation sheep fetus. Endocrinology 132:1071-7.
12. Foley TP, Jr., DePhilip R, Perricelli A, Miller A 1980 Low somatomedin activity in cord serum from infants with intrauterine growth retardation. J Pediatr 96:605-10.
13. Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL 1983 Levels of insulin-like growth factors I and II in human cord blood. J Clin Endocrinol Metab 57:609-12.
14. Gluckman PD, Johnson-Barrett JJ, Butler JH, Edgar BW, Gunn TR 1983 Studies of insulin-like growth factor -I and -II by specific radioligand assays in umbilical cord blood. Clin Endocrinol (Oxf) 19:405-13.
15. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M 1991 Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 29:219-25.
16. Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, Hintz RL, Rosenfeld RG 1995 Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 80:1548-55.
17. Leger J, Noel M, Limal JM, Czernichow P 1996 Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retar-dation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. Pediatr Res 40:101-7.
18. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59-72.
19. Baker J, Liu JP, Robertson EJ, Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75:73-82.
20. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ 1996 Intrauterine growth retardation and post-natal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335:1363-7.
21. Oliver MH, Harding JE, Breier BH, Gluckman PD 1996 Fetal insulin-like growth factor (IGF)-I and IGF-II are regulated differently by glucose or insulin in the sheep fetus. Reprod Fertil Dev 8:167-72.
22. Gluckman PD, Butler JH, Comline R, Fowden A 1987 The effects of pancreatectomy on the plasma concentrations of insulin-like growth factors 1 and 2 in the sheep fetus. J Dev Physiol 9:79-88.
23. Milner RD, Hill DJ 1984 Fetal growth control: the role of insulin and related peptides. Clin Endocrinol (Oxf) 21:415-33.

24. Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S 1993 Longitudinal follow-up of growth in children born small for gestational age. Acta Paediatr 82:438-43.
25. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71.
26. Chaussain JL, Colle M, Ducret JP 1994 Adult height in children with prepubertal short stature secon-dary to intrauterine growth retardation. Acta Paediatr Suppl 399:72-3.
27. Karlberg J, Albertsson-Wikland K 1995 Growth in full-term small-for-gestational-age infants: from birth to final height. Pediatr Res 38:733-9.
28. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
29. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
30. de Waal W 1996 Influencing the extremes of growth: too tall - too small Thesis. Erasmus MC, Rotterdam.
31. Boguszewski M, Dahlgren J, Bjarnason R, Rosberg S, Carlsson LM, Carlsson B, Albertsson-Wikland K 1997 Serum leptin in short children born small for gestational age: relationship with the growth response to growth hormone treatment. The Swedish Study Group for Growth Hormone Treatment. Eur J Endocrinol 137:387-95.
32. Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. J Clin Endocrinol Metab 78:904-9.
33. Svendsen OL, Haarbo J, Hassager C, Christiansen C 1993 Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. Am J Clin Nutr 57:605-8.
34. Pintauro SJ, Nagy TR, Duthie CM, Goran MI 1996 Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. Am J Clin Nutr 63:293-8.
35. Job JC, Rolland A 1986 [Natural history of intrauterine growth retardation: pubertal growth and adult height]. Arch Fr Pediatr 43:301-6.
36. Tanner JM, Lejarraga H, Cameron N 1975 The natural history of the Silver-Russell syndrome: A longitudinal study of thirty-nine cases. Pediatric Resource 9:611-623.
37. Barker DJ, Bull AR, Osmond C, Simmonds SJ 1990 Fetal and placental size and risk of hypertension in adult life. Bmj 301:259-62.
38. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-7.
39. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-607.
40. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z 1985 Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. J Clin Invest 75:809-17.
41 Ferrannini E, Haffner SM, Mitchell BD, Stern MP 1991 Hyperinsulinaemia: the key feature of a cardio-vascular and metabolic syndrome. Diabetologia 34:416-22.
42. Barker DJP 1994 Mothers, babies and disease in later life. BMJ Publishing Group, London.
43. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS 1993 Fetal nutrition and cardiovascular disease in adult life. Lancet 341:938-41.
44. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82:402-6.
45. Hattersley AT, Tooke JE 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789-92.
46. Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, Golding J, Pembrey ME, Ring S, Bennett ST, Todd JA 1998 Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet 19:98-100.
47. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S 1998 Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 19:268-70.
48. Chatelain P, Job JC, Blanchard J, Ducret JP, Oliver M, Sagnard L, Vanderschueren-Lodeweyckx M 1994 Dose-dependent catch-up growth after 2 years of growth hormone treatment in intrauterine growth-retarded children. Belgian and French Pediatric Clinics and Sanofi-Choay (France). J Clin Endocrinol Metab 78:1454-60.

49. Boguszewski M, Jansson C, Rosberg S, Albertsson-Wikland K 1996 Changes in serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 levels during growth hormone treatment in prepubertal short children born small for gestational age. J Clin Endocrinol Metab 81:3902-8.
50. Ranke MB, Lindberg A 1996 Growth hormone treatment of short children born small for gestational age or with Silver-Russell syndrome: results from KIGS (Kabi International Growth Study), including the first report on final height. Acta Paediatr Suppl 417:18-26.
51. Blethen SL, Allen DB, Graves D, August G, Moshang T, Rosenfeld R 1996 Safety of recombinant deoxyribonucleic acid-derived growth hormone: The National Cooperative Growth Study experience. J Clin Endocrinol Metab 81:1704-10.
52. Bratusch-Marrain PR, Smith D, DeFronzo RA 1982 The effect of growth hormone on glucose metabolism and insulin secretion in man. J Clin Endocrinol Metab 55:973-82.
53. Rizza RA, Mandarino LJ, Gerich JE 1982 Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. Diabetes 31:663-9.
54. Sas T, Mulder P, Aanstoot HJ, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. Clin Endocrinol (Oxf) 54:243-51.
55. Sas T, Mulder P, Hokken-Koelega A 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-92.
56. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23.
57. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-9.
58. Bernstein PS, Divon MY 1997 Etiologies of fetal growth restriction. Clin Obstet Gynecol 40:723-9.
59. Wollmann HA 1998 Intrauterine growth restriction: definition and etiology. Horm Res 49:1-6.
60. Keller C, Keller KR, Shew SB, Plon SE 1999 Growth deficiency and malnutrition in Bloom syndrome. J Pediatr 134:472-9.

Part 1

Clustering of low insulin sensitivity and cardiovascular risk factors in short prepubertal children born small for gestational age (SGA)

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Submitted

Abstract

Epidemiological studies have shown that the metabolic syndrome, a combination of type 2 diabetes mellitus, hypertension, dyslipidemia and a high body mass index (BMI), occurs more frequently among adults who were born with a low birth weight. Since insulin is thought to play a key role in the pathogenesis of this syndrome we investigated insulin sensitivity and possible risk factors for cardiovascular disease in a group of short prepubertal children born small for gestational age (SGA).

Frequently sampled intravenous glucose tolerance tests (FSIGT) were performed in 28 short prepubertal children born SGA. Short stature was defined as a height <-2 SD. SGA was defined as a birth length and/or a birth weight for gestational age <-2 standard deviation (SD). Twelve short children born appropriate for gestational age (AGA) were used as controls for the FSIGT's results only. AGA was defined as a birth weight and/or birth length for gestational age >-2 SD. In short SGA children, blood pressure (BP), fasting levels of serum free fatty acids (FFA), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured and compared to reference values.

Mean insulin sensitivity (Si) level in short SGA children was significantly reduced to 38 % of the mean Si level measured in short AGA controls ($p=0.004$). Mean acute insulin response (AIR) was significantly higher in SGA children compared to short AGA controls ($p<0.001$). Differences in Si and AIR between the 2 groups remained significant after adjusting for age and BMI ($p<0.001$ and $p=0.003$, resp.). The mean systolic BP SDS was 1.3 (1.1), being significantly higher than zero. Mean fasting serum levels of FFA, TC, TG, HDL and LDL were all within the normal range. However, 6 of the 28 SGA children (21 %) had serum FFA levels above the normal range. Clustering was found between insulin *in*sensitivity (1/Si), blood pressure and serum FFA levels.

Although the metabolic syndrome has been described in adulthood, our study showed that risk factors for the development of type 2 diabetes mellitus and cardiovascular disease are already present during childhood in short prepubertal children born SGA, suggesting a pre-type 2 diabetes mellitus phenotype.

Introduction

Epidemiological studies in Europe and the USA have shown that type 2 diabetes mellitus (DM), hypertension and cardiovascular diseases occur more frequently among individuals who were born with a low birth weight (1-3). The combination of DM, hypertension, dyslipidemia and a high body mass index (BMI), called the metabolic syndrome, has also been associated with a low birth weight (4,5). The mechanisms underlying these associations are still unknown. However, insulin resistance and hyperinsulinism are thought to play a key role in the pathogenesis of both type 2 diabetes mellitus and cardiovascular abnormalities (4,6,7). That poor nutrition during a critical period in fetal life may result in later adult disease has lead to the concept of fetal programming (8). According to the fetal programming hypothesis, insulin resistance results from fetal malnutrition in order to divert inadequate glucose resources to vital structures (brain and heart) at the expense of muscle. If insulin resistance becomes permanent (programmed), in a postnatal environment of nutritional excess, insulin resistance becomes mal-adaptive (9). The fetal insulin hypothesis, postulates that genes involving insulin resistance could effect both birth weight and disease in later life (10-12).

Studies in children have revealed that insulin sensitivity and fasting insulin levels were related to blood pressure, serum lipids and obesity (13-15). Epidemiological studies have also shown that clustering of fasting insulin and risk factors for cardiovascular disease was already present during childhood and early adulthood (16,17). Insulin insensitivity and glucose intolerance has been described in short prepubertal children born small for gestational age (SGA) (9,18). However, there are no data on clustering of insulin sensitivity and cardiovascular risk factors in short prepubertal children born SGA. In the present study we investigated insulin sensitivity in a group of prepubertal children born SGA using a frequently sampled intravenous glucose tolerance test (FSIGT). Also, we studied risk factors for cardiovascular disease, i.e. BMI, lipid metabolism and blood pressure and investigated if clustering of insulin sensitivity and cardiovascular risk factors was already present in these young SGA children.

Patients and methods

Patients

The study group comprised 28 prepubertal short children born SGA who were recruited from Sophia Children's Hospital in Rotterdam, The Netherlands. All children fulfilled the same inclusion criteria: 1) birth length and/or birth weight standard deviation score (SDS) below – 2 for gestational age (19), 2) height SDS for age below –2 according to Dutch standards (20), 3) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (20), 4) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys (21), 5) normal GH response to either arginine or clonidine stimulation (defined as GH level ≥ 10 μg/l), 6) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. 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, with the exception of Silver-Russell syndrome. The ethnicity was Caucasian for 25 children, Asian for 1 child and Indo-Mediterranean for 2 children. The study was approved by the Ethics Committee. Written informed consent was obtained from the parents or custodians of each child and from children older than the age of 8 years.

Twelve short normal children born appropriate for gestational age (AGA) served as controls for glucose homeostasis data (FSIGT results) only. They fulfilled the same in- and exclusion criteria as the short SGA children. The short AGA children had a birth length and a birth weight for gestational age above -2 SD, a height below –2 SDS (22), a prepubertal stage and a normal GH response to clonidine stimulation (defined as GH level ≥ 10μg/l). Ethnicity was comparable with the SGA group: Caucasian for 10 children, Asian for 1 child and Polynesian for 1 child.

Both SGA and AGA children were negative for both islet cell antibodies (≤10 Juvenile Diabetes Foundation units) and insulin autoantibodies to exclude type 1 prediabetes. Children with a first degree relative who had type 2 diabetes mellitus, were excluded.

Clinical measurements

In all subjects height and body mass index (BMI) were expressed as SDS adjusting for sex and chronological age (20,22,23). Biceps, triceps, subscapular and suprailiacal skinfold thickness were measured using a Holtain skinfold caliper (24). For skinfold thickness analysis the sum of four measurements, expressed as SDS using references for healthy Dutch children, was used (25). To calculate SDS, data of the reference population were transformed using the LMS method (26). This method transforms the reference data at each age to a normal distribution.

Systolic and diastolic blood pressure (BP) were measured using a Dynamap Critikon 1846SX. BP was expressed as SDS adjusting for height, age and sex (27).

FSIGT

After an overnight fast, a modified frequently sampled iv glucose tolerance test (FSIGT) was performed, as previously described (28). Two iv catheters were inserted, one for sampling and one for drug administration. Three baseline samples were drawn at -20, -10 and 0 min. At time zero, 25% dextrose (0.3 g/kg) was administered intravenously over 30 seconds. Blood samples were taken at 2, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 19 min. At t=20 min., tolbutamide (5 mg/kg) was administered intravenously over 30 seconds. Further blood samples were taken at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80 and 90 min. Blood samples were centrifuged and plasma was frozen for later analysis. Glucose and insulin levels were measured in all samples.

Biochemical measurements

Serum lipids were determined in short SGA children. At start of the FSIGT test, fasting levels of serum free fatty acids (FFA), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) were measured. For all serum lipids reference values were available for children of the same age range as our study group (5 to 12 years) (29).

Assays

Glucose and insulin levels in both the SGA and the AGA group were all measured using the same method. Plasma glucose levels were determined on a VITROS analyser 750 (Orthoclinical Diagnostics, Johnson & Johnson Company, Beerse, Belgium). Plasma insulin levels were measured by the same IRMA (Medgenix, Biosource Europe). The intra-assay coefficient of variation (CV) was 2 % to 4.7 % (19 – 405 pmol/l) and the inter-assay CV was 4.2 % to 11.3 % (32 – 375 pmol/l).
TC and TG levels were measured using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit respectively (Roche Diagnostics, Mannheim, Germany) . For HDL cholesterol a homogenous enzymatic colorimetric assay was used (HDL-C, Roche Diagnostics Germany). LDL cholesterol was measured enzymatically (Roche Diagnostics). Non-esterified fatty acids in serum were measured using an enzymatic colorimetric method (WAKO Chemicals, Germany). The total analytical imprecision of the measurements of TG, TC, HDL and LDL cholesterol was 3.3, 2.9, 3.9 and 3.3 %, respectively.

Statistics

The insulin sensitivity index (Si) and glucose effectiveness (Sg) were calculated using Bergman's MINMOD software (30). In the abbreviated FSIGT protocol, the 180 min value was replaced by the time zero value, as described for children by Cutfield et al. (28). SI reflects the ability of insulin to increase net glucose disposal and Sg reflects

glucose ability to increase its own uptake. The acute insulin response (AIR), an estimate of insulin secretory capacity, was measured as the area under the curve from zero to 10 minutes corrected for baseline insulin levels. The glucose disappearance coefficient (Kg) was calculated from the slope of the natural logarithm of glucose concentration between 10 and 19 min (31). Analysis was performed using the statistical package SPSS (version 10.0). Differences between the groups were tested using the independent sample t-test. Differences in time within the SGA group were calculated using a paired t-test. Correlations were analysed using Spearman's correlation coefficient. Level of significance was determined at $p<0.05$.

Since it is known that insulin sensitivity, BMI, serum lipids and blood pressure are related to each other it is too simple to just investigate correlations between two variables. In order to study the interrelationship between insulin insensitivity (1/Si), BMI, blood pressure and serum lipids we performed a cluster analysis. One of the most optimal methods to identify this real data structure is a non-metric principal component analysis (NMPCA) which is an advanced method of clustering variables on the basis of their mutual correlations (32,33). The objective of principal component analysis is to detect the underlying relation between variables in a low-dimensional structure without substantial loss of information. The length and the direction of the vectors (lines) represent the correlations between the variables in two clusters (i.e. dimensions).

Results

The clinical characteristics of the two groups are shown in Table 1. None of the children had Silver-Russell syndrome. FSIGT results of the SGA children were compared to the results of a group of short AGA controls since no other reference data were available. All other measurements were compared with a reference population.

Insulin sensitivity (Si) was significantly lower in the SGA group compared to the AGA control group ($p=0.004$) (Table 2). Mean Si levels in SGA children were 38 % of mean Si levels measured in AGA controls. This difference remained significant after adjusting for BMI and age ($p<0.001$). As expected, there was a compensatory increase in mean acute insulin response (AIR) which was higher in the SGA children ($p<0.001$), even after adjusting for age and BMI ($p=0.003$). Figure 1 shows the hyperbolic relation between Si and AIR for both groups. Mean fasting serum insulin levels were significantly higher in the SGA group ($p=0.01$). However, this difference disappeared after adjusting for BMI and age. Glucose effectiveness (Sg) and the mean fasting serum glucose level were not significantly different between the two groups. Although the glucose disappearance coefficient (Kg) did not differ between the 2 groups, Kg showed a greater variation in the short SGA group. One short SGA child had a Kg in the glucose intolerant range (<1.0).

The SGA group was very lean as indicated by the low BMI SDS and low skinfolds SDS. Both BMI SDS and the sum of skinfold SDS were significantly different from zero (both $p\leq0.001$). Neither BMI nor sum of skinfolds showed a significant correlation with fasting insulin levels or Si in the SGA group.

Systolic BP SDS was significantly higher than zero but values were within the normal range (Table 3). Diastolic BP SDS was not elevated. No significant correlation was found between blood pressure and fasting insulin levels or Si.

Mean fasting serum levels of TC, TG, HDL-cholesterol and LDL-cholesterol in SGA children were all within the normal range (Table 3). Mean serum level of FFA (1.0 mmol/l), however, was in the high-normal range (normal range 0.2 – 1.2) and 6 of the 28 short SGA children (21 %) were above the normal range. None of the children had serum TC, HDL-, LDL-cholesterol and TG levels outside the normal range. No correlations were found between serum lipids and fasting insulin levels or Si.

Cluster analysis was performed to investigate the relationship between insulin insensitivity (1/Si) and cardiovascular risk factors, which were represented in the model by BMI, various serum lipids and blood pressure. In this model two clusters of variables emerged. Interestingly, the first cluster was dominated by insulin insensitivity (1/Si), diastolic and systolic blood pressure and serum FFA (Figure 2). The second cluster comprised TC, LDL, TG and BMI. This means that the variables within one cluster are closely related to each other. However, variables of the first cluster do not relate to the variables of the second cluster. These two clusters of variables explained 69 % of the total variance over all 9 variables, which is considered to be prominent.

Table 1
Baseline characteristics of the short SGA and the AGA controls

	Short SGA n = 28	AGA controls n = 12
Age (yrs)	9.2 (2.2)#	6.9 (1.5)
Boys / girls	17 / 11	8 / 4
Gestational age (weeks)	37.8 (3.0)	37.8 (4.0)
Birth weight SDS	-2.2 (1.1)*	-0.3 (0.7)
Birth length SDS	-3.1 (1.2)	-
Height SDS	-2.8 (0.6)	-2.6 (0.4)
BMI SDS	-1.3 (0.8)†	-0.6 (1.0)

All values are expressed as mean (SD)
* = *significantly different between the groups (p ≤ 0.001)*
\# = *significantly different between the groups (p = 0.002)*
† = *significantly different between the groups (p = 0.02)*

SDS = standard deviation score
BMI SDS = body mass index SDS

Table 2
FSIGT results of the short SGA and the AGA controls

	Short SGA n = 28	AGA controls n = 12
Fasting glucose (mmol/l)	4.8 (0.5)	4.5 (0.6)
Fasting insulin (mU/l)	6.0 (3.2)†	4.4 (0.4)
Si $*10^{-4}$/min-1 (μU/ml)	15.2 (6.6)#	39.8 (23.0)
Sg $*10^{-2}$ (mg/d) min^{-1}	2.3 (1.3)	2.9 (1.1)
AIR (mU/l)	241 (116)*	90 (48)

All values are expressed as mean (SD)
\# = *significantly different between the groups (p = 0.004)*
† = *significantly different between the groups (p = 0.01)*
* = *significantly different between the groups (p < 0.001)*

Si = insulin sensitivity index
Sg = glucose effectiveness
AIR = acute insulin response

Fig. 1.

The relationship between insulin sensitivity (Si) and the acute insulin response (AIR) in short children born SGA (black circles) and in short children born AGA (open circles).

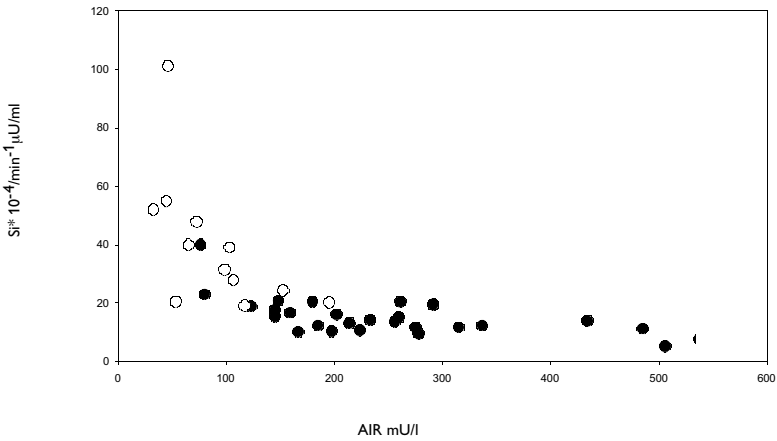

Fig. 2.

Results of the non-metric principal component analysis (NMPCA). The variables consisted of two clusters. Magnitude and direction of correlations are represented by the length and direction of the vectors (lines).

BMI = body mass index BP dia = diastolic blood pressure BP sys = systolic blood pressure

FFA = free fatty acids TG = triglycerides TC = total cholesterol

HDL = high-density lipoprotein LDL = low-density lipoprotein 1/Si = insulin insensitivity

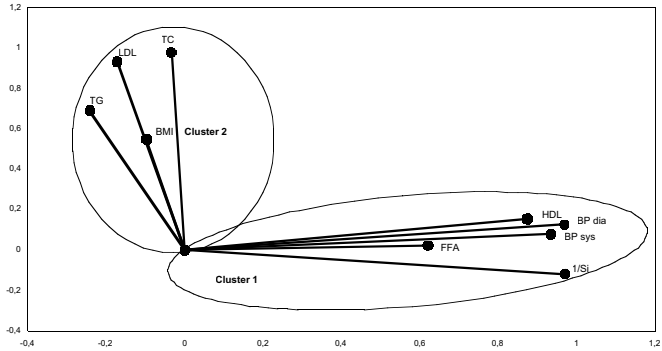

Table 3
Body composition and cardiovascular parameters in short SGA children

	Short SGA
Body composition	
BMI SDS	-1.3 (0.8)*
Skinfolds SDS	-1.1 (0.8)*
Blood pressure	
Systolic BP SDS	1.3 (1.1)*
Diastolic BP SDS	0.2 (0.6)
Serum lipids	
FFA (mmol/l) [N: 0.2-1.2]	0.9 (0.4)
TG (mmol/l) [N: 0.3-1.1]	0.6 (0.2)
TC (mmol/l) [N: 2.8-5.4]	4.1 (0.6)
HDL-cholesterol (mmol/l) [N: 0.8-1.9]	1.5 (0.4)
LDL-cholesterol (mmol/l) [N: 1.3-3.4]	2.3 (0.6)

All values are expressed as mean (SD)
** significantly different from zero ($p \leq 0.001$)*

SDS = standard deviation score
BMI = body mass index
BP = blood pressure
FFA = free fatty acids
TG = triglycerides
TC = total cholesterol
HDL = high-density lipoprotein
LDL = low-density lipoprotein

Discussion

The present study investigates insulin sensitivity in relation to cardiovascular risk factors in a group of young children born SGA. Insulin sensitivity appeared significantly reduced in these SGA children, whereas systolic blood pressure was significantly increased compared to their peers. Although fasting serum lipids were in the normal range, 21 % of the children showed FFA levels above the normal range.

In short SGA children insulin sensitivity (Si) was reduced to only 38 % of the levels found in the short AGA controls. As expected to compensate for this reduction in Si, the acute insulin response (AIR) was nearly three times higher in short SGA children compared to the AGA controls. In normal subjects a reciprocal relationship exists between insulin action and insulin secretion by the pancreatic β-cell (34). If insulin sensitivity decreases, β-cells will secrete larger amounts of insulin in order to maintain a normal glucose metabolism. If the beta-cells fail to adapt or fail to maintain the secretion of large amounts of insulin, the risk of developing type 2 diabetes mellitus increases. A single reduction in either Si, Sg or β-cell function is not enough to cause glucose intolerance. A decrease in two or three of these parameters will, however, increase the risk dramatically (34). Our results show that short prepubertal SGA children maintained a normal glucose tolerance due to a compensatory increase in insulin secretion. Since these abnormalities are found in young children it can be questioned how long it will take before type 2 diabetes will become overt. This important question can only be answered during long-term follow-up. Glucose effectiveness, the ability of glucose to enhance its own peripheral uptake and reduce its endogenous production in the liver independent of insulin, was not significantly different between the short SGA and AGA children. Our findings agree with results described by Hofman et al., who also found a reduced insulin sensitivity in a group of 15 short prepubertal SGA children (9). Our results are also comparable to those found in group of young adults who were born SGA.(35). In the present study, glucose intolerance was already subclinically present in one SGA child (4 %). This finding is comparable to the results described by Sas et al, who found impaired glucose tolerance during an OGTT in 8 % of another group of 79 short prepubertal SGA children (18).

BMI SDS and skinfolds SDS were significantly lower in our short SGA group compared to the control group. Leanness is a typical feature of short prepubertal SGA children (36). It is well known that increasing leanness is associated with a dramatic increase in insulin sensitivity (28). However, we did not find a relation between BMI and insulin sensitivity in these short SGA children. This may be due to a lack of variation in BMI among these children. In contrast to what is to be expected in lean individuals, insulin sensitivity in prepubertal SGA children was significantly reduced. Johnson et al investigated longitudinal (annual) changes in fat mass, measured by Dual Energy X-ray Absorptiometry (DEXA), and insulin sensitivity in a cohort of healthy children. They found that a low insulin sensitivity at a young age was followed

by a higher increase in fat mass over years, independent of sex and pubertal stage (37). This might implicate that lean and short SGA children with a low Si are at an increased risk of becoming obese in early or later adulthood. Since a high BMI is one of the features of the metabolic syndrome, the observed reduction in insulin sensitivity in SGA children may precede the changes in body composition occurring in later life. When these children will develop overweight, it will worsen their underlying reduced Si, thereby strongly increasing the risk of type 2 diabetes mellitus.

Our data showed a significantly higher systolic BP in short SGA children compared to age- and height-matched children (27). In contrast, diastolic BP was not significantly different from age- and height-matched children. These data are consistent with previous data in another group of short SGA children reported by Sas et al., in which systolic blood pressure was elevated to levels in the high-normal range (38). Hypertension in adult life has been associated with a low birth weight (5,39). Also, studies investigating blood pressure during childhood and early adulthood have shown a significant inverse relationship between systolic blood pressure and birth weight (40) (41). Since there is evidence that blood pressure is tracking from childhood to adulthood (42), our results might indicate that short children born SGA are at increased risk of becoming hypertensive in later life. It has been reported that a high systolic BP is more important in the pathogenesis of cardiovascular disease than a high diastolic BP (43). Therefore, short children born SGA need regular evaluation of their blood pressure during childhood and adulthood.

Fasting serum levels of total cholesterol, triglycerides, LDL and HDL-cholesterol were all within the normal range. These findings are in agreement with previous reports (38,44). Our study is the first one investigating serum FFA levels in SGA children. We found that fasting serum FFA levels were in the high-normal range, with 6 children (21 %) having levels above the normal range. Recently, an important role was attributed to FFA in the pathogenesis of type 2 diabetes (45). Two peptides play an important role in the FFA metabolism. Acylation stimulating protein (ASP) stimulates FFA uptake by peripheral cells and esterification into TG (46). If ASP activity is low, serum FFA level will increase. Sniderman et al. found a defect in the ASP pathway leading to an ineffective FFA storage which in turn will result in insulin resistance and obesity (47). Thus, a reduction in ASP activity might play a role in the increased serum FFA levels in these children. Another possibility is an increased activity of hormone sensitive lipase (HSL). HSL is responsible for hydrolysis of TG, the rate limiting step in the breakdown of TG in adipocytes (48). A raised activity of HSL will result in an increased breakdown of TG followed by an increase in serum FFA levels. In normal subjects HSL is deactivated by insulin when energy supply is sufficient (49). If suppression by insulin fails, HSL activity increases which in turn may result in a rise in serum FFA levels. Several studies postulated that the HSL gene is involved in type 2 diabetes (50-52). Further research is required to study FFA metabolism in larger groups of SGA children.

Cluster analysis was used to investigate whether a number of features (i.e. BMI, blood pressure and serum lipids) was related to the insulin insensitivity (1/Si) in these SGA children. We found two important clusters. The first cluster clearly showed that insulin insensitivity (1/Si) was related to higher systolic and diastolic blood pressure and higher serum FFA levels. This is in concordance with previous epidemiological studies in children and adults reporting an association between low insulin sensitivity and cardiovascular risk factors (7,16,53,54). In contrast to previous reports, we found a second cluster, consisting of BMI, total cholesterol, LDL-cholesterol and triglycerides. This second cluster comprised parameters which are known to be closely related to obesity. Since our study group was far from obese it is not surprising that we did not find clustering of BMI with insulin insensitivity (1/Si). Our findings may imply that a reduction in insulin sensitivity together with a slight increase in serum FFA levels and systolic blood pressure are the first abnormalities in the development of the metabolic syndrome. Lean SGA children may be considered as having a pre-type 2 diabetes mellitus which may become overt when they become overweighed. For that reason it seems particularly important to prevent the overweight in children born SGA

In conclusion, low reduced insulin sensitivity is already asymptomatically present in short prepubertal children born SGA. Furthermore, these children had an increased systolic blood pressure whereas 21 % of the children had FFA levels above the normal range. Thus, although the metabolic syndrome is an adult disease, this study shows that risk factors for the development of type 2 diabetes mellitus and cardiovascular diseases are already present during childhood in these lean and short children born SGA.

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References

1. Phillips DI, Barker DJ, Hales CN, Hirst S, Osmond C 1994 Thinness at birth and insulin resistance in adult life. Diabetologia 37:150-4.
2. Leger J, Levy-Marchal C, Bloch J, Pinet A, Chevenne D, Porquet D, Collin D, Czernichow P 1997 Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. Bmj 315:341-7.
3. Valdez R, Athens MA, Thompson GH, Bradshaw BS, Stern MP 1994 Birthweight and adult health outcomes in a biethnic population in the USA. Diabetologia 37:624-31.
4. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-607.
5. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-7.
6. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z 1985 Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. J Clin Invest 75:809-17.
7. Ferrannini E, Haffner SM, Mitchell BD, Stern MP 1991 Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 34:416-22.
8. Barker DJ 1997 The fetal origins of coronary heart disease. Acta Paediatr Suppl 422:78-82.
9. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82:402-6.
10. Hattersley AT, Tooke JE 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353:1789-92.
11. Dunger DB, Ong KK, Huxtable SJ, Sherriff A, Woods KA, Ahmed ML, Golding J, Pembrey ME, Ring S, Bennett ST, Todd JA 1998 Association of the INS VNTR with size at birth. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. Nat Genet 19:98-100.
12. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S 1998 Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 19:268-70.
13. Jiang X, Srinivasan SR, Webber LS, Wattigney WA, Berenson GS 1995 Association of fasting insulin level with serum lipid and lipoprotein levels in children, adolescents, and young adults: the Bogalusa Heart Study. Arch Intern Med 155:190-6.
14. Steinberger J, Moorehead C, Katch V, Rocchini AP 1995 Relationship between insulin resistance and abnormal lipid profile in obese adolescents. J Pediatr 126:690-5.
15. Sinaiko AR, Gomez-Marin O, Prineas RJ 1997 Relation of fasting insulin to blood pressure and lipids in adolescents and parents. Hypertension 30:1554-9.
16. Raitakari OT, Porkka KV, Ronnemaa T, Knip M, Uhari M, Akerblom HK, Viikari JS 1995 The role of insulin in clustering of serum lipids and blood pressure in children and adolescents. The Cardiovascular Risk in Young Finns Study. Diabetologia 38:1042-50.
17. Chen W, Srinivasan SR, Elkasabany A, Berenson GS 1999 Cardiovascular risk factors clustering features of insulin resistance syndrome (Syndrome X) in a biracial (Black-White) population of children, adolescents, and young adults: the Bogalusa Heart Study. Am J Epidemiol 150:667-74.
18. Sas T, Mulder P, Aanstoot HJ, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. Clin Endocrinol (Oxf) 54:243-51.
19. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
20. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23.
21. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-9.
22. Tanner JM, Whitehouse RH, Takaishi M 1966 Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. I. Arch Dis Child 41:454-71.
23. Cole TJ, Freeman JV, Preece MA 1998 British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med 17:407-29.
24. Cameron N 1978 The methods of auxological anthropometry. In: Falkner F, Tanner JM (eds.) Human Growth, vol. 2. Tindall, London, pp 35-87.

25. Gerver WJ, de Bruin R 1996 Body composition in children based on anthropometric data. A presentation of normal values. Eur J Pediatr 155:870-6.
26. Cole TJ 1989 Using the LMS method to measure skewness in the NCHS and Dutch National height standards. Ann Hum Biol 16:407-19.
27. 1987 Task force on blood pressure control in children. 1987 Report of the second task force on blood pressure control in children. Pediatrics 79:1-25.
28. Cutfield WS, Bergman RN, Menon RK, Sperling MA 1990 The modified minimal model: application to measurement of insulin sensitivity in children. J Clin Endocrinol Metab 70:1644-50.
29. Yu HH, Markowitz R, De Ferranti SD, Neufeld EJ, Farrow G, Bernstein HH, Rifai N 2000 Direct measurement of LDL-C in children: performance of two surfactant- based methods in a general pediatric population. Clin Biochem 33:89-95.
30. Bergman RN, Phillips LS, Cobelli C 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Invest 68:1456-67.
31. Lee A, Ader M, Bray GA, Bergman RN 1992 Diurnal variation in glucose tolerance. Cyclic suppression of insulin action and insulin secretion in normal-weight, but not obese, subjects. Diabetes 41:742-9.
32. Gifi A 1990 Nonlinear multivariate analysis. Wiley, Chicester.
33. van Dixhoorn J, Duivenvoorden HJ 1985 Efficacy of Nijmegen Questionnaire in recognition of the hyperventilation syndrome. J Psychosom Res 29:199-206.
34. Bergman RN 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-27.
35. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C 2000 Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 85:1401-6.
36. Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. Journal of Clinical Endocrinology and Metabolism 78:904-909.
37. Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI 2001 Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. J Clin Endocrinol Metab 86:3182-7.
38. Sas T, Mulder P, Hokken-Koelega A 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-92.
39. Barker DJ, Bull AR, Osmond C, Simmonds SJ 1990 Fetal and placental size and risk of hypertension in adult life. Bmj 301:259-62.
40. Law CM, Barker DJ, Bull AR, Osmond C 1991 Maternal and fetal influences on blood pressure. Arch Dis Child 66:1291-5.
41. Lurbe E, Torro I, Rodriguez C, Alvarez V, Redon J 2001 Birth weight influences blood pressure values and variability in children and adolescents. Hypertension 38:389-93.
42. Fuentes RM, Notkola IL, Shemeikka S, Tuomilehto J, Nissinen A 2002 Tracking of systolic blood pressure during childhood: a 15-year follow- up population-based family study in eastern Finland. J Hypertens 20:195-202.
43. Kannel WB 2000 Elevated systolic blood pressure as a cardiovascular risk factor. Am J Cardiol 85:251-5.
44. Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R 2000 Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. Pediatr Res 48:623-8.
45. McGarry JD 2002 Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 51:7-18.
46. Baldo A, Sniderman AD, St-Luce S, Avramoglu RK, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S, Cianflone K 1993 The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. J Clin Invest 92:1543-7.
47. Sniderman AD, Cianflone K, Arner P, Summers LK, Frayn KN 1998 The adipocyte, fatty acid trapping, and atherogenesis. Arterioscler Thromb Vasc Biol 18:147-51.
48. Langin D, Holm C, Lafontan M 1996 Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc Nutr Soc 55:93-109.
49. Holm C, Osterlund T, Laurell H, Contreras JA 2000 Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Annu Rev Nutr 20:365-93.
50. Klannemark M, Orho M, Langin D, Laurell H, Holm C, Reynisdottir S, Arner P, Groop L 1998 The putative role of the hormone-sensitive lipase gene in the pathogenesis of Type II diabetes mellitus and abdominal obesity. Diabetologia 41:1516-22.

51. Magre J, Laurell H, Fizames C, Antoine PJ, Dib C, Vigouroux C, Bourut C, Capeau J, Weissenbach J, Langin D 1998 Human hormone-sensitive lipase: genetic mapping, identification of a new dinucleotide repeat, and association with obesity and NIDDM. Diabetes 47:284-6.

52. Talmud PJ, Palmen J, Luan J, Flavell D, Byrne CD, Waterworth DM, Wareham NJ 2001 Variation in the promoter of the human hormone sensitive lipase gene shows gender specific effects on insulin and lipid levels: results from the Ely study. Biochim Biophys Acta 1537:239-44.

53. Mykkanen L, Haffner SM, Ronnemaa T, Bergman RN, Laakso M 1997 Low insulin sensitivity is associated with clustering of cardiovascular disease risk factors. Am J Epidemiol 146:315-21.

54. Srinivasan SR, Myers L, Berenson GS 2002 Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood: the Bogalusa Heart Study. Diabetes 51:204-9.

Polymorphism in the IGF-I gene: Clinical relevance for short children born small for gestational age (SGA).

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Abstract

Low birth weight is associated with an increased risk in adult life of type 2 diabetes, hypertension and cardiovascular disease (CVD). The fetal insulin hypothesis postulates that genes involving insulin resistance could effect birth weight and disease in later life (Hattersley, 1999). Besides insulin, there is extensive evidence that insulin-like growth factor-I and –II (IGF-I, IGF-II) play an important role in fetal growth. We hypothesized that minor genetic variation in the IGF-I gene could influence pre- and postnatal growth.

Three microsatellite markers located in the IGF-I gene in 124 short children (height <-1.88 SDS) who were born small for gestational age (SGA) and their parents were studied. SGA was defined as both a birth weight and birth length below –1.88 SDS for gestational age.

Two polymorphic markers showed transmission disequili-brium. Allele 191 of the IGF1.PCR1 marker was transmitted more frequently from parent to child (χ^2 = 4.8 and p=0.02) and allele 198 of the 737/738 marker was transmitted less frequently from parent to child (χ^2 = 4.5 and p=0.03) . Children carrying the 191-allele had significantly lower IGF-I levels than children not carrying this allele (-1.1 SDS vs. –0.50 SDS; p=0.03). Also, head circumference SDS remained smaller in children with allele 191 compared to children without allele 191 (-2.1 SDS vs. –0.9 SDS; p=0.003).

Our results show that genetically determined low IGF-I levels may lead to a reduction in birth weight, length and head circumference and to persistent short stature and small head circumference in later life (proportionate small). Since low IGF-I levels are associated with type 2 diabetes and CVD, we propose that the IGF-I gene may provide a link between low birth weight and such diseases in later life.

Introduction

About 10-15 % of children born small for gestational age (SGA) have an increased risk of being short as adults (1, 2). The mechanism underlying persistent short stature in these children is not fully understood. Insulin-like growth factor-I (IGF-I) plays an important role in both pre- and postnatal growth and its serum levels are regulated by both metabolic and genetic factors.

In fetuses and neonates born SGA low circulating IGF-I levels have been observed suggesting a role for IGF-I in fetal growth retardation (3-5). More direct evidence for a role of IGF-I in fetal and postnatal growth comes from gene deletion studies in mice (6). Weight and length at birth were significantly reduced in IGF-I knock-out mice (birth weight about 60% of normal) (6), and postnatally they showed a further deterioration of growth resulting in adult weights of about 30 % of normal mice (7, 8). Until now only one human homozygous partial deletion of the IGF-I gene has been described in a 15-year old boy (9). This child was born SGA and showed severe postnatal growth failure, sensorineural deafness and mental retardation.

Low serum IGF-I levels have been reported in short children born SGA (10, 11). We hypothesized that minor genetic variation in the IGF-I gene might cause a change in serum IGF-I levels resulting in altered fetal and postnatal growth. We therefore investigated the IGF-I gene in a large group of short children born SGA and their parents. Three polymorphic dinucleotide repeat markers were studied and the results were analysed using the transmission disequilibrium test (TDT) (12).

Methods

Subjects

This study included 124 children (66 boys and 58 girls) with short stature born SGA and their parents. All children fulfilled the same inclusion criteria: 1) birth length and birth weight standard deviation score (SDS) below – 1.88 for gestational age (13); 2) height SDS for age below –1.88 according to Dutch standards (14); 3) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth; 4) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. 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, with the exception of Silver-Russell syndrome. The ethnicity was Caucasian for 113 families, Asian for 1 family, Indo-Mediterranean for 4 families and mixed ethnicity for 6 families. The study was approved by the Ethics Committee. Written informed consent was obtained from the parents or custodians of each child and from children older than the age of 8 years.

Clinical and biochemical measurements

In children and parents standing height was measured and expressed as SDS adjusting for sex and chronological age using Dutch standards (14). Body mass index (BMI) was calculated as weight (in kilogram) divided by square of height (in meters) and expressed as SDS for sex and age (15). Serum IGF-I levels were measured in children using a specific RIA (16) and values were transformed into SDS adjusting for sex and age (16).

IGF-I gene

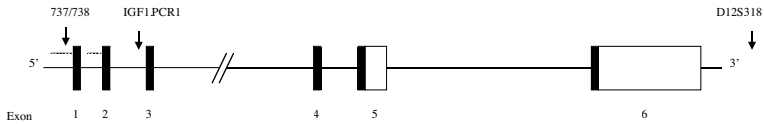
The polymerase chain reaction (PCR) was used to amplify three dinucleotide repeat markers located in the IGF-I gene (Fig. 1)(17). The three markers were 737/738, a cytosine-adenine (CA) repeat in the promoter region of the gene (18), IGF1.PCR1, an intronic cytosine-thymine (CT) repeat lying between exon 2 and 3 (19) and D12S318, a microsatellite marker (CA repeat) lying 3' to the gene (20) (21). All reactions were carried out in a volume of 10 μl in the presence of 0.1 mM dNTP, 1.5 mM $MgCl_2$ and 0.25 units Taq DNA polymerase (Sigma, Poole, UK).

For the IGF1.PCR1 and 737/738markers 100 ng of peripheral leucocyte genomic DNA was amplified using 0.5 nmol/l forward primer (IGF1.PCR1: 5'-TTGTGTCAACTGCTGATATG-3'; 737/738: 5'-GCTAGCCAGCTGGTGTTATT-3') and 0.5 nmol/l reverse primer (IGF1.PCR1: 5'-AACCAAAACATCATTCC-3'; 737/738: 5'-ACCACTCTGGGAGAAGG-3'). Amplification was for 37 cycles of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C. For D12S318 50 ng of genomic DNA was amplified using 0.3 nmol/l forward primer (5'-TGCTTGGGTCATCAATCTGC-3') and 0.3 nmol/l

reverse primer (5'-GGTTATAGACATATAAA-3') for 35 cycles of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C.

Forward primers were labelled with either HEX or FAM. The sizes of PCR products were determined by the ABI 377 DNA Analyser using ROX 500 as a fluorescent size marker. The results were analysed using GENESCAN and GENOTYPER software (Applied Biosystems, UK).

Fig. 1
Schematic organization of the IGF-I gene.



Exons 1 to 6 are indicated by boxes, with coding regions in black and non-coding regions in white. Promoter regions are indicated by a dashed line. Dinucleotide markers are indicated by arrows.

Statistical analysis

The transmission disequilibrium test (TDT) was performed using the TDT/S-TDT program 1.1 of Spielman (12, 22). The TDT test is a valid test for linkage and association, even when the population under study consists of subjects of different ethnic origin. The TDT method evaluates whether the frequency of transmission of alleles from heterozygote parents to their affected children deviates from 50%, the expected Mendelian frequency when there is no linkage.

Statistical tests to analyse genotype-phenotype relationships were performed with use of SPSS package (version 10.0). Independent sample t-testing was used to analyse differences in phenotype between different genotypes. To investigate the possible influence of allele 191 on birth size and growth, a repeated measurement analysis (SAS Proc Mixed) was performed with all length and weight measurements, converted to their SD-score, from birth to the age of 4.0 years. Head circumference measurements were available from birth until the age of 1.5 years in 83 children. As random covariables were used the intercept, age and age-squared, as fixed covariables age, age-squared (if significant), presence of allele 191 and its interaction with age (unadjusted model). If significant, the model was adjusted for sex, gestational age, multiple birth and their interaction with age (adjusted model). Statistical significance was defined as $p < 0.05$.

Results

Baseline characteristics are shown in Table 1. Birth length SDS , birth weight SDS, head circumference SDS and height SDS were all significantly lower compared to –1.88 SDS. BMI SDS, IGF-I SDS, fathers height SDS and mothers height SDS were all significantly lower compared to zero, i.e. compared to the median for normals.

Table 1
Baseline characteristics of 124 short children born SGA

Gestational age (weeks)[1]	37.0 (32.8 – 39)
Birth length SDS[1]	-3.3 (-4.7 - -2.5)*
Birth weight SDS	-3.0 (0.8)*
Birth head circumference SDS	-2.5 (1.1)*
Age (years)	6.7 (2.4)
Height SDS	-3.0 (0.7)*
BMI SDS	-1.4 (1.3)†
IGF-I SDS	-0.6 (1.1)†
Fathers height SDS	-1.1 (1.1)†
Mothers height SDS	-1.3 (1.0)†

Values expressed as mean (SD) unless stated otherwise
BMI SDS = body mass index SDS
1 = Median (interquartile range)
** = significantly lower compared to –1.88 (p<0.001)*
† = significantly lower compared to zero (p<0.001)

TDT results and allele frequencies of the 3 markers are shown in Table 2. Significant evidence of transmission disequilibrium was found in the IGF1.PCR1 and the 737/738 markers.

The wildtype allele of the IGF1.PCR1 marker was allele 189. Significant transmission disequilibrium was found with allele 191 (χ^2 = 4.8 and p=0.02). This allele was transmitted more frequently from parents to children. Interestingly, the mean serum IGF-I level was significantly lower in children carrying allele 191 compared to children without allele 191 (-1.1 SDS vs. –0.50 SDS; p=0.03) suggesting a functional relationship between this polymorphism and the IGF-I gene (Table 3). No significant difference in height SDS was found between children with and without allele 191. Both in the unadjusted and adjusted model, no significant relation was found between the changes in height and weight SDS from birth until 4.0 years and allele 191. However, changes in head circumference SDS during the first 1.5 years were significantly associated with allele 191. At the age of 1.5 years children carrying allele 191 had a significant smaller head circumference SDS than children without allele 191 (p=0.003) (Table 3). Head circumference SDS of the 191-carriers did not

change during the first 1.5 years of life whereas the non-carriers showed a significant increase in head circumference SDS during that period ($p<0.001$). BMI SDS and parental height SDS did not differ significantly between 191-carriers and 191-non-carriers.

Allele 192 of the 737/738 marker was the most common allele and therefore likely to be the wildtype allele. Transmission disequilibrium was found with allele 198 ($\chi^2 = 4.5$ and $p=0.03$). This allele was less likely to be transmitted from parents to children. Since only one child was carrier of the 198 allele it was not possible to analyse phenotypic
differences between the 198-carriers and non-carriers. None of the alleles of the D12S318 marker was in transmission disequilibrium.

Table 3
HC SDS and serum IGF-I SDS in carriers versus non-carriers of allele 191

	Carriers allele 191	Non-carriers allele 191	p-value
HC SDS			
- at birth	-2.0 (0.3)	-2.3 (0.2)	ns
- after 1.3 years	-2.1 (0.4)	-0.9 (0.2)	0.003
IGF-I SDS	-1.1 (0.2)	-0.5 (0.1)	0.03

Values are expressed as mean (SE)
HC SDS = head circumference SDS

Table 2
Allele frequencies of 124 short children born SGA

Allele[1]	Frequency (%)	Transmitted	Non-transmitted	p-value
IGF1.PCR1				
marker				
185	0.8	2	0	
187	0.4	1	1	
189	*65.3*	*61*	*61*	
191	**8.5**	**21**	**9**	**0.02**
193	0.8	2	6	
195	2.4	6	10	
197	4.0	10	14	
199	4.0	8	7	
201	6.5	14	20	
203	1.6	4	3	
205	1.6	3	1	
207	3.6	9	9	
209	0.4	1	0	
211	-	0	1	
737/738				
marker				
176	0.4	1	1	
186	-	0	1	
188	1.2	3	1	
190	4.0	11	7	
192	*71.8*	*62*	*54*	
194	16.5	39	39	
196	5.6	12	19	
198	**0.4**	**1**	**7**	**0.03**
D12S318				
marker				
239	-	-	-	
241	-	0	1	
243	-	0	1	
247	6.0	16	11	
249	10.1	24	22	
251	6.5	17	16	
253	*52.0*	*63*	*60*	
255	8.5	18	25	
257	6.9	15	15	
259	5.2	14	8	
261	4.0	10	17	
263	0.8	2	1	
265	-	0	2	

[1] = allele in number of base pairs
Italics = wildtype allele
Bold = allele with significant transmission disequilibrium

Discussion

Our study provides evidence for an important role of the IGF-I gene in short children born SGA. Allele 191 of the IGF1.PCR1 marker was transmitted more frequently from parent to child suggesting this is a 'causal allele'. The association between head circumference and allele 191 indicates this allele might play a role in SGA children with persistent short stature and small head circumference (proportionate small) . It was to be expected that we could not detect significant differences in changes in height between different genotypes since our study population is a very homogenous group where all children are short (height <-1.88 SDS). Also, children with allele 191 had significantly lower serum IGF-I levels than those without allele 191 suggesting a functional relationship.

Our results are suggestive of the existence of a functional variant of the IGF-I gene located between the promoter region and exon 3 which results in significantly lower serum IGF-I levels. Further research is needed to unravel the exact location and function of this mutation.

The 198 allele of the 737/738 marker was less frequently transmitted from parent to child. Since the frequency of this allele was very low it is difficult to draw conclusions regarding its relation to serum IGF-I levels. An unexpected finding is that parents carrying the 198 allele (n = 8) were significantly shorter than parents without this allele (n = 240) (-2.0 SDS vs. –1.1 SDS; p=0.03). This suggests the allele itself is associated with short stature. Since this allele is less likely to be transmitted to offspring it is unlikely that a causal relation exists between this polymorphism and SGA.

Johnston et al. could not find an association between the IGF-I gene and the SGA phenotype in a cohort of French term singleton SGA subjects and a cohort of adults born appropriate for gestational age (AGA)(23). Although the mean adult height of the SGA cohort was significantly lower compared to the AGA cohort, no distinction was made between the SGA adults who had attained normal height and those who remained short. This might explain why no significant association was found.

Insulin resistance has been reported in short children born SGA (24) and impaired glucose tolerance was described in 8% of our study population (25). Severe insulin resistance has been reported in a child with a homozygous IGF-I gene defect (26). Besides being an important contributor to fetal growth, IGF-I has a stimulatory effect on growth and development of pancreatic beta-cells. A lifetime exposure to low-normal serum IGF-I levels has been reported as a risk factor for developing cardiovascular disease and type 2 diabetes (27-29). Thus, polymorphisms in the IGF-I gene resulting in low serum IGF-I levels may increase the risk of cardiovascular disease and type 2 diabetes. We found an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a specific group of short children born SGA who remained proportionate small. Since low IGF-I levels increase the risk of

developing adult disease we suggest that this IGF-I polymorphism may be a link between a low birth weight and an increased risk of adult disease. This would be consistent with the 'fetal insulin hypothesis' (30). Besides the glucokinase gene (31), the IGF-I gene might not only be involved in fetal growth but also in the pathogenesis of diabetes and cardiovascular disease. Further research is needed to confirm our findings and to explore if other genetic and environmental factors may also contribute to this phenotype.

This is the first study showing an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a group of short children born SGA. Our results suggest that genetically determined low serum IGF-I levels may lead not only to a reduction in birth length, weight and head circumference but also to persistent short stature and small head circumference during childhood and adulthood (proportionate small). As low serum IGF-I levels are associated with adult disease, the IGF-I gene may provide a link between the association of low birth weight and disease in later life in this specific group of small SGA children.

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References

1. Albertsson-Wikland K, Karlberg J. 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399: 64-70; discussion 71.
2. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. 1995 Children born small for gestational age: do they catch up? Pediatr Res 38: 267-271.
3. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M. 1991 Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 29: 219-225.
4. Giudice LC, de Zegher F, Gargosky SE, et al. 1995 Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 80: 1548-1555.
5. Leger J, Noel M, Limal JM, Czernichow P. 1996 Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. Pediatr Res 40: 101-107.
6. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75: 59-72.
7. Baker J, Liu JP, Robertson EJ, Efstratiadis A. 1993 Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75: 73-82.
8. Wang J, Zhou J, Powell-Braxton L, Bondy C. 1999 Effects of Igf1 gene deletion on postnatal growth patterns. Endocrinology 140: 3391-3394.
9. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. 1996 Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335: 1363-1367.
10. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL. 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41: 621-630.
11. Boguszewski M, Rosberg S, Albertsson-Wikland K. 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80: 2599-2606.
12. Spielman RS, McGinnis RE, Ewens WJ. 1993 Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52: 506-516.
13. Usher R, McLean F. 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74: 901-910.
14. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47: 316-323.
15. Roede MJ, van Wieringen JC. 1985 Growth diagrams 1980, Netherlands. Third nation-wide survey. T Soc Gezondheidsz [Suppl] 63: 1-34.
16. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL. 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 71: 688-695.
17. LeRoith D, Roberts CT, Jr. 1993 Insulin-like growth factors. Ann N Y Acad Sci 692: 1-9.
18. Weber JL, May PE. 1989 Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44: 388-396.
19. Polymeropoulos MH, Rath DS, Xiao H, Merril CR. 1991 Dinucleotide repeat polymorphism at the human gene for insulin-like growth factor I (IGFI). Nucleic Acids Res 19: 5797.
20. Gyapay G, Morissette J, Vignal A, et al. 1994 The 1993-94 Genethon human genetic linkage map. Nat Genet 7: 246-339.
21. Krauter K, Montgomery K, Yoon SJ, et al. 1995 A second-generation YAC contig map of human chromosome 12. Nature 377: 321-333.
22. Spielman RS, Ewens WJ. 1998 A sibship test for linkage in the presence of association: the sib transmission/disequilibrium test. Am J Hum Genet 62: 450-458.
23. Johnston LB, Leger J, Savage MO, Clark AJ, Czernichow P. 1999 The insulin-like growth factor-I (IGF-I) gene in individuals born small for gestational age (SGA). Clin Endocrinol (Oxf) 51: 423-427.
24. Hofman PL, Cutfield WS, Robinson EM, et al. 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82: 402-406.

25. Sas T, Mulder P, Aanstoot HJ, et al. 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. Clin Endocrinol (Oxf) 54: 243-251.
26. Woods KA, Camacho-Hubner C, Bergman RN, Barter D, Clark AJ, Savage MO. 2000 Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. J Clin Endocrinol Metab 85: 1407-1411.
27. Spallarossa P, Brunelli C, Minuto F, et al. 1996 Insulin-like growth factor-I and angiographically documented coronary artery disease. Am J Cardiol 77: 200-202.
28. Janssen JA, Jacobs ML, Derkx FH, Weber RF, van der Lely AJ, Lamberts SW. 1997 Free and total insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), and IGFBP-3 and their relationships to the presence of diabetic retinopathy and glomerular hyperfiltration in insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 82: 2809-2815.
29. Vaessen N, Heutink P, Janssen JA, et al. 2001 A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 50: 637-642.
30. Hattersley AT, Tooke JE. 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet 353: 1789-1792.
31. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S. 1998 Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 19: 268-270.

MRI findings of the pituitary gland in short children born small for gestational age (SGA) in comparison with growth hormone-deficient (GHD) children and children with normal stature.

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Abstract

Disturbances in the GH / IGF-I axis are reported in 25 – 60 % of short children born small for gestational age (SGA). We hypothesized that these abnormalities might be related to abnormalities in the pituitary region. Therefore, the results of magnetic resonance imaging (MRI) of short SGA children were compared to MRI results of other groups of short children and to normal controls.

MRI was performed in four groups of short children: SGA children without GH deficiency (SGA group; n=17), SGA children with isolated GH deficiency (SGA + IGHD group; n=10), non-SGA children with isolated GH deficiency (IGHD group; n=24) and non-SGA children with multiple pituitary hormone deficiencies (MPHD group; n=15). MRI was also performed in children with normal stature (control group; n=13). Pituitary height (PH) and thickness of the pituitary stalk (PS) were measured and the relationship with the maximum GH peak during a GH stimulation test, serum IGF-I and IGFBP-3 levels was evaluated.

Short SGA children either with or without IGHD did not show major anatomic abnormalities in the hypothalamic-pituitary region in contrast to 58 % of the non-SGA IGHD children and 87 % of the MPHD children who had anatomic abnormalities. PH in SGA children without GHD was normal whereas it was significantly lower in SGA children with IGHD. The lowest PHs were measured in non-SGA children with MPHD. A moderate decrease in PH was associated with significantly lower maximum serum GH peaks and lower serum IGF-I and IGFBP-3 levels.

Measuring PHs in children with less severe GHD, who underwent MRI as part of the diagnostic process, might support the diagnosis of GHD even in the absence of anatomical abnormalities. Our study demonstrates that there is no indication to perform MRI of the pituitary region in short children born SGA without GHD.

Introduction

Short children born small for gestational age (SGA) constitute a specific group. About 15 % of them fail to show catch-up growth during the first 2 years of life (1-3). If they are still short at the age of 2 or 3 years they have a greater risk of being short as adults (1-3). It is still unknown why a small percentage of SGA children remains short. Disturbances in the growth hormone (GH)-insulin-like growth factor-I (IGF-I)-axis may play a role. Sixty percent of children with insufficient catch-up growth showed a decrease in GH secretion during 24 hours whereas 25 % showed low GH peaks during GH provocation tests (4,5). Also serum IGF-I levels were significantly lower in short children born SGA compared to normal children (4,5).

Several studies in patients with hypopituitarism revealed abnormalities in the pituitary region using magnetic resonance imaging (MRI) (6-8). These abnormalities included ectopia of the neurohypophysis (NH), hypoplasia or interruption of the pituitary stalk (PS) and hypoplasia of the adenohypophysis (AH). Abnormalities were more frequently observed in patients with multiple pituitary hormone deficiency (MPHD) than in patients with isolated GH deficiency (IGHD) (9-11).

No data were available on MRI findings of the pituitary region in short children born SGA. We hypothesized that disturbances in the GH/IGF-I axis, as had been reported in these children, might be related to abnormalities in the pituitary region. Therefore MRI's were performed in short SGA children with and without GHD. Since MRI abnormalities are well described in children with IGHD and MPHD, we used these groups as references. Results were also compared to MRI findings in a group of children with normal stature. In addition, pituitary height (PH) and thickness of the pituitary stalk (PS) were measured and the relationship with different growth parameters was evaluated.

Patients and methods

Patients and controls

The study was performed in 66 children with short stature and in 13 children with normal stature (controls). Short stature was defined as a height for chronological age < –2 SDS according to Dutch references (12). Small for gestational age (SGA) was defined as a birth length for gestational age < – 2 SDS (4,13). GH status was evaluated in all short children using GH stimulation tests (arginine, clonidine or propanolol). Growth hormone deficiency (GHD) was defined as a GH peak < 10 ng/ml during two GH stimulation tests, according to the Dutch Consensus guidelines (14). Multiple pituitary hormone deficiency (MPHD) was defined as GHD associated with at least one deficiency of the other anterior pituitary hormones. Birth data were obtained from all children. Normal controls for this study were children with a height >-2 SDS who underwent an MRI for reasons not related to GHD. Children with evidence of hypothalamo-pituitary disease, neurological disorders and syndromal abnormalities were excluded from the control group. Based on birth length, actual height and GH-status, children were divided into 5 groups:

I a: - SGA without GHD (SGA group; n = 17)
b: - SGA with isolated GHD (SGA+IGHD group; n = 10)
II a: - non-SGA with IGHD (IGHD group; n = 24)
b: - non-SGA with MPHD (MPHD group; n = 15)
III - Controls (control group; n=13)

SGA children with and without GHD participated in a Dutch multicenter study studying the effects of GH treatment. Data of non-SGA children with IGHD and MPHD were evaluated retrospectively. All children, except for the control group, received GH treatment.

The study was approved by the Medical Ethics Committee of the Academic Hospital Rotterdam and the Erasmus University Rotterdam. In the SGA group without GHD written informed consent was obtained from the parents or custodians of each child. In the SGA+IGHD, IGHD and MPHD group an MRI was performed as part of the diagnostic process. All MRI's were performed during adequate hormonal suppletion.

Hormone measurements

Plasma GH was measured by a double antibody RIA using a rabbit anti-GH serum as the first and a goat antirabbit globulin as the second antibody (15). A specific RIA measured IGF-I after acid chromatography, as described previously (15). IGF-I was expressed as SD-scores using reference data from a healthy Dutch population of 600 children (15). IGFBP-3 was isolated from human plasma (16) and determined by a specific RIA using a polyclonal antiserum derived from New Zealand White rabbits. IGFBP-3 levels were expressed as SD-scores using reference data from a healthy Dutch population of 286 children aged 0-14 years provided by the laboratory (17).

Because data from non-SGA children with IGHD and MPHD were collected retrospectively, some serum IGF-I and IGFBP-3 levels were missing.

MRI evaluation

Patients were examined on a 0.5 T Philips Gyroscan (Philips Medical Systems, Best, The Netherlands). Scan sequences included T1 weighted (SE 450-600/TE 21-30) sagittal and coronal 3 mm slices of the hypothalamo-pituitary area and T2 weighted (SE 2000-2500/TE 100) axial 0.5 cm slices of the total brain. Interslice gap was 10 % of slice thickness. The matrix was 205x256, the field of view was 18 cm.

The height of the pituitary gland (PH) was determined by measuring the greatest distance between the base and the top of the gland on a midsagittal image using a slide-rule. The thickness of the PS was measured in the middle on a midsagittal image and both proximal (PS-proximal) and distal (PS-distal) on a coronal image. All measurements were performed blinded to subject classification by the same investigators (N.A. and W.v.d.L.). Since PH is dependent on age and sex, values are expressed in SD scores (18). Pituitary hypoplasia was defined as a PH below –2 SDS.

Statistics

Data are expressed as the mean plus or minus the standard deviation (SD). Differences between groups were tested using independent Student's t-tests. Pearson's correlation coefficient was used for correlations. Statistical significance was defined as $p < 0.05$. Statistical tests were performed with use of SPSS package (version 8.0).

Results

Characteristics of the various groups are shown in Table 1. The mean maximum GH peak was significantly different between groups. Also, the mean IGF-I SDS differed significantly between the groups with the highest values measured in the SGA group and the lowest values in the MPHD group.

Only one out of 17 children of the SGA group (6 %) showed a hypoplastic AH. No other pituitary abnormalities were found in this group. None of these children had a breech delivery, vacuum or forceps extraction.

In the SGA+IGHD group only one out of 10 children (10 %) had a hypoplastic AH. One child was born by breech delivery, but this child did not show MRI abnormalities.

In the IGHD group 58 % of the children showed pituitary abnormalities: 38 % (9/24) had a hypoplastic AH, 42 % (10/24) an ectopic NH and 17 % (4/24) a disrupted/absent PS. One child born by breech delivery had an ectopic NH, a disrupted PS and a normal AH. The children born by vacuum or forceps extraction did not show pituitary abnormalities. Ten out of 24 (42 %) children did not show abnormalities in the pituitary region.

Most severe abnormalities were found in the children with MPHD: 87 % (13/15) had a hypoplastic or absent AH, 87 % (13/15) an ectopic or absent NH and 67 % (10/15) a disrupted/absent PS. Four children were born by breech delivery and one was born with vacuum extraction. All 5 children showed severe abnormalities in the pituitary region. Only 2 children (13 %) had a normal adeno- and neurohypophysis with a normal stalk.

Midline-defects were found in two patients. One non-SGA child with IGHD showed bilateral optic hypoplasia and one non-SGA child with MPHD had bilateral optic hypoplasia and a hypoplastic optic chiasm. No abnormalities were found in the normal controls. None were born by breech delivery, one was delivered by vacuum extraction.

Mean (SD) pituitary height (PH) and pituitary stalk thickness (PS) for the various groups are shown in Table 2. PH SDS was not significantly different between the SGA group and the control group. In the SGA+IGHD group PH SDS was significantly lower compared to the SGA group and the control group ($p=0.003$ and $p=0.004$ respectively). Also, children with IGHD and MPHD showed significantly lower PH SDS compared to the SGA and the control group (all $p<0.001$). Children with MPHD had lower PH SDS than children with IGHD but this difference did not reach the level of significance ($p=0.064$).

PS thickness in SGA and SGA+IGHD children was significantly lower compared to controls ($p=0.037$ and $p=0.046$). PS thickness in children with IGHD did not differ significantly from the controls. Since PS could only be measured in 5 children with MPHD due to an absent or disrupted PS in the remaining 10 children, we could not analyse these data. In all groups, even in the control group, we found the proximal PS

Table 1
Characteristics of the various groups

Groups		n	Sex M/F	GA (wks)	BW SDS	BL SDS	Age (yrs)	Height SDS	BMI SDS	Max. GH (ng/ml)	IGF-I SDS	IGFBP3 SDS
I	SGA	17	10/7	36.5 (2.6)[1]	-2.6 (1.1)[2]	-3.1 (1.3)[3]	6.7 (1.3)	-3.1 (0.6)	-1.0 (1.1)	24.2 (12.1)[5]	-0.3 (1.5) [6]	-1.1 (1.4) [7]
	SGA+IGHD	10	6/4	36.5 (2.9)[1]	-2.4 (1.1)[2]	-3.8 (1.4)[3]	6.1 (1.8)	-3.2 (0.7)	-0.9 (1.3)	8.5 (2.9) [8]	-2.1 (1.0) [9]	-2.6 (1.3)
II	IGHD	24	13/11	39.4 (2.2)	-0.3 (1.1)	-0.7 (0.9)	8.3 (3.5)	-3.1 (0.6)	0.1 (1.3)	5.8 (2.6)[10]	-2.7 (1.8)[10]	-2.7 (1.0)
	MPHD	15	11/4	38.7 (3.7)	-0.1 (1.2)	-0.7 (1.1)	5.3 (3.4)	-3.7 (0.9)	-0.1 (1.5)	2.2 (2.5)	-4.9 (1.2)	-3.9 (3.4)
III	Controls	13	7/6	40 (1.8)	0.1 (1.4)	ND	5.6 (5.0)	0.4 (0.9)[4]	-0.1 (1.4)	ND	ND	ND

All values are expressed in mean (SD)
GA = gestational age; BW SDS = birth weight SD-score; BL SDS = birth length SD-score
ND = not done; for controls no baseline data concerning BL, GH, IGF-I and IGFBP-3 were available

1 = P<0.01 vs. IGHD and controls
2 = P<0.001 vs. IGHD, MPHD and controls
3 = P<0.001 vs. IGHD and MPHD
4 = P<0.001 vs. other groups
5 = P<0.001 vs. SGA+IGHD, IGHD and MPHD
6 = P<0.005 vs. SGA+IGHD, IGHD and MPHD
7 = P<0.05 vs. SGA+IGHD and IGHD
8 = P=0.01 vs. IGHD; P<0.001 vs. MPHD
9 = P<0.001 vs. MPHD
10 = P≤0.001 vs. MPHD

to be significantly thicker than the distal PS (p<0.001). No significant correlation was found between the PH and the PS thickness.

PH SDS showed significant positive correlations with the maximum GH peak (r=0.52; p<0.001), IGF-I SDS (r=0.36; p=0.006) and IGFBP-3 SDS (r=0.56; p<0.001). PH SDS did not show a correlation with height or body mass index. PS thickness did not correlate with the maximum GH peak, IGF-I SDS or IGFBP-3 SDS.

Table 2
Mean (SD) pituitary height (PH) and pituitary stalk (PS) thickness on MRI

	Groups	n	Age (yrs)	PH (mm)	PH SDS	PS (mm)	PS-prox (mm)	PS-distal (mm)
I	SGA	17	8.1 (1.2)	4.9 (0.8)[2]	0.6 (1.4)[1]	1.6 (0.2)	1.9 (0.5)	1.5 (0.2)
	SGA+IGHD	10	7.4 (2.8)	3.7 (0.7)[4]	-1.1 (1.1)[3]	1.6 (0.3)	2.1 (0.4)	1.5 (0.2)
II	IGHD	24	10.5 (4.2)	3.8 (1.0)[6]	-2.5 (2.5)[5]	1.7 (0.8)	2.8 (0.8)[9]	1.6 (0.9)
	MPHD	15	10.6 (4.7)	2.9 (1.0)[7]	-4.4 (3.8)[7]	*	*	*
III	Controls	13	6.3 (4.6)	4.8 (1.0)	0.5 (1.3)	1.8 (0.2) 8	2.0 (0.6)	1.5 (0.2)

** 10 patients missing data due to absent or disrupted PS*
1 = P<0.005 vs. SGA+IGHD, IGHD and MPHD
2 = P ≤0.001 vs. SGA+IGHD, IGHD and MPHD
3 = P=0.099 vs. IGHD; P<0.01 vs. MPHD and controls
4 = P<0.05 vs. MPHD and controls
5 = P=0.064 vs. MPHD; P<0.001 vs. controls
6 = P<0.01 vs. MPHD and controls
7 = P<0.001 vs. controls
8 = P<0.05 vs. SGA and SGA+IGHD
9 = P<0.001 vs. SGA; P<0.01 vs. SGA+IGHD and controls

Discussion

This is the first report describing MRI results in a cohort of short children born SGA with and without IGHD. We found that short children born SGA without GHD (SGA group) did not have lower pituitary heights (PHs) compared to controls with normal stature, except for one child who showed hypoplasia of the AH. None of them had an ectopic NH or a disrupted or an absent PS. Nagel et al. described similar findings in 3 children with short stature after intrauterine growth retardation (IUGR) and found no abnormalities in their pituitary gland (19). Only one out of 10 SGA children with IGHD (SGA+IGHD group) showed a hypoplastic AH (10 %). We did not observe any other anatomical abnormalities in the pituitary region of these children.

Our MRI findings in the non-SGA group with IGHD (IGHD group) were comparable with findings in IGHD children described by others. About 40 % of these children showed a hypoplastic AH and an ectopic NH (38 % and 42 %, respectively) and 4 out of 24 showed PS abnormalities. The observed difference in abnormalities between the SGA+IGHD group and the IGHD group might explain the difference in severity of GHD. The IGHD group had significantly lower maximum GH peaks compared to the SGA+IGHD group. This is in agreement with reports showing that children with severe IGHD had more pituitary abnormalities than children with partial IGHD (10,19).

Most pituitary abnormalities were found in non-SGA children with multiple pituitary hormone deficiencies (MPHD group). In our study 87 % of these children showed a hypoplastic AH and an ectopic NH and 67 % showed PS abnormalities. Children with MPHD showed the lowest maximum GH peaks and serum IGF-I and IGFBP-3 levels. Thus, children with the most severe form of GHD showed the most severe pituitary abnormalities. These findings agree with observations described in previous reports (9-11,19,20).

Evaluating all children we found a significant positive correlation between pituitary height SDS (PH SDS) and the severity of GHD (maximum GH peak, IGF-I and IGFBP-3 levels). This indicates that a child with a low maximum GH peak, low serum IGF-I and low IGFBP-3 levels has a greater risk of having a small pituitary gland. Our results agree with Nagel et al who investigated 91 children with different causes of short stature (19). They also measured PH and found a significant correlation between PH and GH secretion, serum IGF-I and IGFBP-3 levels. We found no difference in PH SDS between the SGA group and the control group. PH SDS in the SGA+IGHD group, however, was significantly lower compared to the SGA group and the control group. This study shows that even a moderate decrease in PH (with PH remaining within the normal range) was associated with significantly lower maximum GH peaks during provocation tests and to significantly lower serum IGF-I and IGFBP-3 levels. Therefore, assessment of PH in children with partial IGHD without MRI abnormalities in the pituitary region, may support the diagnosis of GHD. Our data indicate that MRI is not likely to show anatomic abnormalities of the pituitary in

short children born SGA without GHD. Therefore, in these children, there is no need to perform MRI's as part of a diagnostic process.

Pituitary abnormalities are related to perinatal events such as breech delivery, vacuum or forceps extraction and perinatal asphyxia (7). These pituitary abnormalities may either be caused by birth trauma or may exist before birth and cause a higher risk of complications during delivery (21). As in other reports, we found relatively more breech deliveries in the children with MPHD (7,10,22).

We hypothesized that disturbances in the GH/IGF-I axis in short children born SGA, might be related to abnormalities in the pituitary region. Our findings, however, did not confirm this hypothesis. Subnormal GH secretion and subnormal serum IGF-I and IGFBP-3 levels in these children can not be explained by anatomic abnormalities in the pituitary region. In the SGA+IGHD group pituitary height was significantly lower compared to the SGA group and controls. This suggests that the size of the pituitary plays a role in the GH secretion in SGA children with IGHD. It is not known whether a reduced number or a reduced volume of somatotrophic cells may lead to a smaller pituitary causing a reduction in GH secretion. One report (23) showed that the somatotrophic cells of a patient with IGHD were morphologically identical to normal somatotrophic cells. However, further research is required to answer this question.

In conclusion, short SGA children either with or without IGHD did not show major anatomic abnormalities in the hypothalamic-pituitary region. Pituitary height (PH) in SGA children without GHD was normal whereas it was significantly lower in SGA children with IGHD. This study showed that even a moderate decrease in PH was associated with significantly lower maximum serum GH peaks, serum IGF-I and IGFBP-3 levels.

Therefore, in children with less severe GHD who underwent MRI as part of the diagnostic process, measuring PHs may support the diagnosis of GHD even in the absence of anatomical abnormalities. Our study shows that there is no indication to perform an MRI in short children born SGA without GHD.

References

1. Albertsson-Wikland K, Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71.
2. Chaussain JL, Colle M, Ducret JP 1994 Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. Acta Paediatr Suppl 399:72-3.
3. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71.
4. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
5. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
6. Fujisawa I, Kikuchi K, Nishimura K, Togashi K, Itoh K, Noma S, Minami S, Sagoh T, Hiraoka T, Momoi T, et al. 1987 Transection of the pituitary stalk: development of an ectopic posterior lobe assessed with MR imaging. Radiology 165:487-9.
7. Kikuchi K, Fujisawa I, Momoi T, Yamanaka C, Kaji M, Nakano Y, Konishi J, Mikawa H, Sudo M 1988 Hypothalamic-pituitary function in growth hormone-deficient patients with pituitary stalk transection. J Clin Endocrinol Metab 67:817-23.
8. Pellini C, di Natale B, De Angelis R, Bressani N, Scotti G, Triulzi F, Chiumello G 1990 Growth hormone deficiency in children: role of magnetic resonance imaging in assessing aetiopathogenesis and prognosis in idiopathic hypopituitarism. Eur J Pediatr 149:536-41.
9. Maghnie M, Triulzi F, Larizza D, Preti P, Priora C, Scotti G, Severi F 1991 Hypothalamic-pituitary dysfunction in growth hormone-deficient patients with pituitary abnormalities. J Clin Endocrinol Metab 73:79-83.
10. Ochi M, Morikawa M, Yoshimoto M, Kinoshita E, Hayashi K 1992 Growth retardation due to idiopathic growth hormone deficiencies: MR findings in 24 patients. Pediatr Radiol 22:477-80.
11. Argyropoulou M, Perignon F, Brauner R, Brunelle F 1992 Magnetic resonance imaging in the diagnosis of growth hormone deficiency. J Pediatr 120:886-91.
12. Roede MJ, van Wieringen JC 1985 Growth diagrams 1980, Netherlands. Third nation-wide survey. T Soc Gezondheidsz [Suppl] 63:1-34.
13. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
14. de Muinck Keizer-Schrama SM, Boukes FS, Oostdijk W, Rikken B 1998 Diagnostics of short stature in children; CBO Consensus Meeting. Van Zuiden Communications B.V., The Netherlands.
15. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 71:688-95.
16. Martin JL, Baxter RC 1986 Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J Biol Chem 261:8754-60.
17. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-76.
18. Argyropoulou M, Perignon F, Brunelle F, Brauner R, Rappaport R 1991 Height of normal pituitary gland as a function of age evaluated by magnetic resonance imaging in children. Pediatr Radiol 21:247-9.
19. Nagel BH, Palmbach M, Petersen D, Ranke MB 1997 Magnetic resonance images of 91 children with different causes of short stature: pituitary size reflects growth hormone secretion. Eur J Pediatr 156:758-63.
20. Vannelli S, Avataneo T, Benso L, Potenzoni F, Cirillo S, Mostert M, Bona G 1993 Magnetic resonance and the diagnosis of short stature of hypothalamic- hypophyseal origin. Acta Paediatr 82:155-61.
21. Scotti G, Triulzi F, Chiumello G, Dinatale B 1989 New imaging techniques in endocrinology: magnetic resonance of the pituitary gland and sella turcica. Acta Paediatr Scand Suppl 356:5-14.
22. Maghnie M, Larizza D, Triulzi F, Sampaolo P, Scotti G, Severi F 1991 Hypopituitarism and stalk agenesis: a congenital syndrome worsened by breech delivery? Horm Res 35:104-8.
23. Schechter J, Kovacs K, Rimoin D 1984 Isolated growth hormone deficiency: immunocytochemistry. J Clin Endocrinol Metab 59:798-800.

Part 2

SGA

Growth hormone (GH) treatment and its effect on bone mineral density (BMD), bone maturation and growth in short children born small for gestational age (SGA): Three-year results of a randomized, controlled GH trial.

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Abstract

To investigate in a group of short children born small for gestational age (SGA), the effects of 3 years of GH treatment versus no treatment on bone age (BA), height and bone mineral density (BMD). Also, to evaluate the influence of the severity of growth retardation at start and the GH dose on the gain in height.

The study design was an open-labelled, controlled multicenter GH study for 3 years. Non-growth hormone deficient (GHD) children (n=87) were randomized to either a GH-group (n=61) or an untreated control group (n=26). In addition, 12 SGA children had GHD (GHD-group) and were treated in parallel. Both the GH- and the GHD-group were treated with a GH dose of 33 μg/kg/day. BMD was evaluated using Dual Energy X-ray Absorptiometry (DXA). In addition, data of our first GH trial in which short SGA children were treated with a GH dose of 66 μg/kg/day (n=24) were used for comparison of height gain.

In contrast to the control group, the GH-group showed a significant increase in height ($p<0.001$), as did the parallel GHD-group. Bone maturation (Δ bone age (BA)/ Δ calendar age(CA)) increased significantly during the first two years of GH treatment but slowed-down thereafter. The 3-year ΔBA/ΔCA ratio correlated significantly with the gain in height ($r=0.6$, $p<0.001$). At start, mean BMD SDS and mean BMAD SDS were significantly lower than zero. During GH treatment both increased impressively ($p<0.001$). The gain in height of children with severe short stature at start (≤ -3.00 SDS), did not differ between those receiving either a GH dose of 33 or 66 μg/kg/day.

Three years of GH treatment in short children born SGA results in a normalisation of height during childhood. Also, bone maturation increased proportionately to the height gain. At start, mean values of BMD and BMAD were significantly reduced but normalised during GH treatment. We did not find an indication to treat very short SGA children (H SDS ≤ -3.00) with a higher GH dose. We rather suggest to start GH treatment at an early age in order to achieve a normal height before puberty starts.

Introduction

Short stature is one of the risks of being born small for gestational age (SGA). About 10-15 % of children born SGA fail to show catch-up growth in height above the third percentile during the first years of life and have an increased risk of being short in adult life (1-3). The mechanisms underlying this lack of catch-up growth are still unknown. Disturbances in the growth hormone (GH) / insulin-like growth factor-I (IGF-I) axis have been described (4,5). Sixty percent of these children had abnormalities in GH secretion and/or reduced serum IGF-I levels (4,5).

Several studies have shown beneficial effects of either continuous or discontinuous GH treatment on linear growth in short children born SGA (6-8). In the first Dutch trial, a randomized, double-blind, dose-response multicenter study, normalisation of height during childhood was obtained during 5 years of continuous treatment with either a GH dose of 33 μg/kg/day or 66 μg/kg/day (7). This study showed an acceleration of bone maturation in both groups, which was most pronounced during the first 2 years of treatment. Since both groups were treated with GH it was unknown whether this acceleration in bone maturation was due to either an effect of GH or an effect of ageing. Therefore, the present multicenter study was started with a randomized control group for 3 years, investigating the effects of GH treatment (33 μg/kg/day) versus no treatment on bone maturation and its relation to changes in height during a period of 3 years. We also evaluated the GH-induced growth response in relation to the severity of growth retardation at start of the study. In order to investigate if children with severe short stature would benefit more from treatment with a higher dose of GH, we compared present data with data of our previously performed randomized GH-dose-response trial (33 versus 66 μg/kg/day).

In short children born SGA bone mineral density (BMD) has never been studied in a randomized controlled trial. Low BMD could lead to osteoporosis and would increase the risk of bone fractures in both children and adults (9,10). BMD in adulthood is mainly determined by the peak bone mass achieved during adolescence or early adulthood and by bone resorption during adult life (11). Peak bone mass is the highest level of bone mass achieved as a result of normal growth. Thus, obtaining a high peak bone mass early in life, provides a larger bone reserve later in life (12). Therefore, BMD was studied in a subgroup of short children born SGA using Dual Energy X-ray Absorptiometry (DXA) during this 3-year randomized, controlled GH trial.

Patients and methods

Patients

The study comprised 104 Dutch children (48 boys and 56 girls) with short stature born SGA. All children fulfilled the same inclusion criteria: 1) birth length standard deviation score (SDS) below – 2.00 SDS for gestational age (13); 2) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia; 3) chronological age (CA) between 3.00 and 7.99 years at start of the study; 4) height SDS for age below –2.00 according to Dutch standards (14); 5) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (14); 6) prepubertal, defined as Tanner stage 1 or a testicular volume < 4 (15); 7) normal liver, kidney and thyroid functions. Children with endocrine or metabolic disorders, chromosomal defects and growth failure caused by other syndromes (e.g. emotional deprivation, Turner syndrome, severe chronic illness, chondrodysplasia), with the exception of Silver-Russell syndrome were excluded. The diagnosis Silver-Russell was based on clinical characteristics (16). The study was approved by the Ethics Committees of all nine participating centers. Written informed consent was obtained from the parents or custodians of each child.

Study design

The study design was a multicenter GH trial with a randomized control group for 3 years. Before entering the study the GH status was evaluated in all children using GH stimulation tests. Children with growth hormone deficiency (GHD), which was defined as a GH peak < 10 μg/l during two GH stimulation tests, were not randomized but started GH treatment at dose of 33 μg/kg/day (~1 mg/m^2 body surface area/day) (GHD-group; n=12; 5 boys and 7 girls). The non-GHD children (n=87) were stratified according to age (3.00-5.50 versus 5.50-7.99) and height of the parents (height of both parents above –2.00 SDS versus height of at least one parent below –2.00 SDS). After stratification the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group (n=61; 25 boys and 36 girls) started with GH treatment at a dose of 33 μg/kg/day. The control group (n=26; 16 boys and 10 girls) remained untreated for 3 years and subsequently received the same GH treatment as the GH-group.

Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

In order to evaluate the GH-induced effect on growth in relation to the severity of growth retardation at start, we compared the results of the present study with those of children receiving GH at a dose of 66 μg/kg/day in our first randomized GH-dose-response trial (7). These children fulfilled exactly the same in- and exclusion criteria as described above.

Anthropometric measurements

Standing height (H) was measured 3-monthly by two trained investigators (NA and later on VB) using a Harpenden stadiometer. The mean of 4 measurements was used for analysis. Height was expressed as SD-score for sex and chronological age (HSDS-CA) using Dutch references (14). Target height was calculated using Dutch reference data according to the formula: $1/2 * (H_{father} + H_{mother} + 13) + 4.5$ for boys and $1/2 * (H_{father} + H_{mother} - 13) + 4.5$ for girls, where the addition of 4.5 cm represents the secular trend. TH was expressed as SD-score using Dutch references (14). Bone age (BA) was determined blindly by one investigator (NA) according to the Tanner & Whitehouse method (17). Bone maturation was expressed as the ratio between the change in BA and the change in CA ($\Delta BA/\Delta CA$).

Bone mineral density measured by DXA

In a subgroup of 38 SGA children (20 of the GH group, 10 of the control group and 8 of the GHD group), bone mineral density (BMD) was measured in one center by Dual Energy X-ray Absorptiometry (DXA) type Lunar DPX-L PED using the pediatric medium scan mode. Since both BMD at start and during GH treatment did not differ between the GH- and the GHD group, these results were analysed together. BMD of the total body (BMD_{TB} in gram/cm^2) and of the lumbar spine (BMDLS in gram/cm^2) was measured. The coefficients of variation for BMD_{TB} and BMD_{LS} have been reported to be 0.64 % and 1.04 %, respectively (18). To adjust for differences in bone size, bone mineral apparent density (BMAD) was calculated according to the formula: $BMD_{LS} * [4/(\pi * width)]$ (19). Width stands for the mean width of the second to fourth lumbar vertebral body. Since all parameters of BMD were dependent on age and sex, the values were transformed into SD-scores using Dutch reference values for children older than 4 years (20,21).

Biochemical measurements

Blood samples were taken at the start of the study from all children and subsequently every 6 months from GH-treated children (GH-group) and every 12 months from non-treated children (control group) for determination of serum levels of IGF-I and IGFBP-3. After centrifugation, all samples were frozen (-20 C) until assayed.

Hormone assays

A specific RIA measured IGF-I and IGF-II after acid chromatography as described previously (22). Both growth factors were expressed as SD-scores using reference data from a healthy Dutch population of 600 children (22).

IGFBP-3 was isolated from human plasma according to the method developed by Martin and Baxter (23) and determined by a specific RIA using a polyclonal antiserum derived from New Zealand White rabbits. IGFBP-3 levels were expressed as SD-scores using reference data from a healthy Dutch population of 286 children aged 0-14 years provided by the laboratory (24).

Statistics

Of 104 children 5 children dropped out of the study for the following reasons: one child was very disappointed she was randomized into the control group, 3 children had psychological problems with the daily GH injections and in 1 child coeliac disease was diagnosed. Since these children dropped out either at start or during the first year of the study, these children were excluded from baseline and 3-year analysis. Therefore, 99 children (46 boys and 53 girls) were eligible for statistical analysis. Data are expressed as the mean plus or minus the standard deviation (SD). SD-scores were compared with zero using Student's one sample t-test. Differences between groups were tested using independent Student's t-tests. Differences in 1-year changes between the groups were tested using analysis of covariance and differences between points in time within the groups were tested by paired Student's t-tests. Pearson's correlation coefficient was used for correlations. Stepwise multiple regression analysis was used to assess multivariate relationships. Factors showing a linear correlation with the 3-year change in H SDS were entered into the model. Only results of the most significant model are shown. Statistical significance was defined as $p < 0.05$. Statistical tests were performed with use of SPSS package (version 10.0).

Results

At baseline

The baseline characteristics of the 3 groups, GH-, control- and GHD group, are shown in Table 1. Thirteen children had Silver-Russell syndrome. At the start of the study no significant differences were found between the 3 groups. During the 3-year study period, puberty started in 4 children of the GH-group, in 2 children of the control group and in 2 children of the GHD group. Since most children remained prepubertal during the study period, analysis was performed in prepubertal children only. As soon as a child entered puberty he or she was excluded from further analysis. Results did not change when data of these children were excluded from the start of the study (data not shown). Results of children with SRS did not differ from results of children without signs of SRS (data not shown).

Table 1
Baseline characteristics

		SGA	
	Randomized controlled trial		GHD group
	GH group	Control group	
	n=61	n=26	n=12
Boys / girls	25 / 36	16 / 10	5 / 7
Gestational age (wks)	36.1 (3.9)	36.0 (3.6)	35.9 (3.6)
Birth length SDS	-3.4 (1.5)	-3.1 (1.3)	-3.5 (2.0)
Birth weight SDS	-2.3 (1.2)	-2.7 (1.0)	-2.5 (1.2)
Chronological age (yr)	6.0 (1.6)	5.9 (1.5)	5.2 (1.4)
Bone age (RUS) (yr)	4.9 (1.6)	4.8 (1.7)	4.2 (1.9)
Height SDS_{CA}	-3.0 (0.6)	-3.2 (0.5)	-3.4 (0.8)
TH SDS	-0.5 (0.8)	-0.6 (0.7)	-0.5 (0.7)
Silver Russell syndrome (n)	6	5	2

All values are expressed as mean (SD) or number

Changes in height

In the GH-group H SDS increased significantly from –3.0 SDS at start to –1.3 SDS after 3 years of GH treatment ($p<0.001$) (Figure 1). The GHD-group showed similar growth as height increased significantly from –3.4 SDS to –1.2 SDS after 3 years ($p<0.001$). The control group, however, showed a very small increase in H SDS from –3.2 to –2.9 SDS ($p<0.001$).

Figure 1

Changes in H SDS of the GH- (black circles), the GHD- (grey circles) and the untreated control group (open squares).

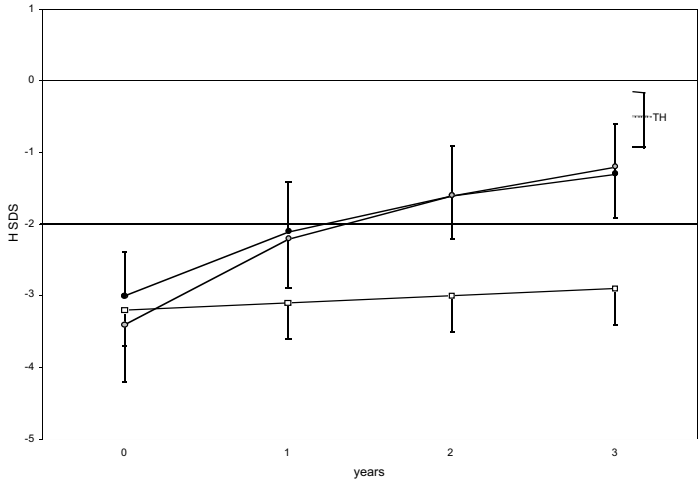


Table 2

Mean serum IGF-I and IGFBP-3 levels at start and during the 3-year study period

	Randomized controlled trial	SGA	
	GH group	Control group	GHD group
	n=61	n=26	n=12
IGF-I SDS			
Baseline	-0.3 (1.1)[2]	-0.3 (1.0)	-1.7 (0.9)[1†]
3 years	1.2 (1.4)[1]	-0.9 (1.3)[1*]	1.4 (1.3)[2]
IGFBP-3 SDS			
Baseline	-1.4 (1.1)[1]	-1.2 (1.1)[1]	-3.3 (1.3)[1†]
3 years	0.3 (0.7)[1]	-1.0 (1.0)[1*]	0.3 (0.6)

All values are expressed as mean (SD)

[1] *= significantly different compared to 0 SDS (p<0.005)*

[2] *= significantly different compared to 0 SDS (p<0.05)*

* *= significantly different compared to the GH and the GHD group (p ≤ 0.001)*

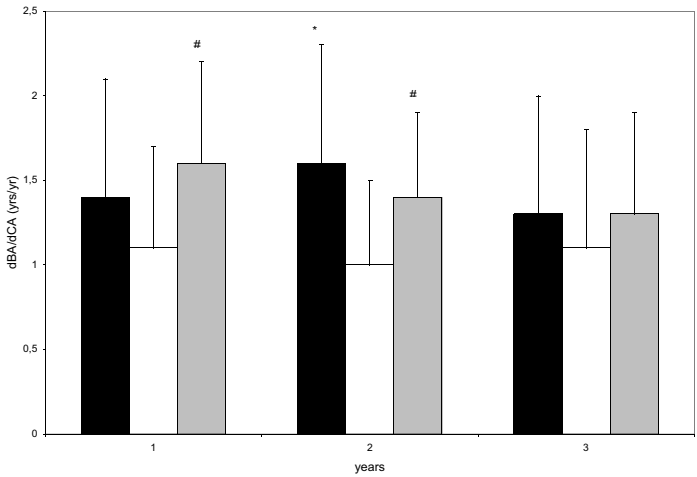
† *= significantly different compared to the control and the GH group (p ≤ 0.001)*

Changes in levels of growth factors

Serum IGF-I and IGFBP-3 levels at start and during the study are shown in Table 2. At baseline serum IGF-I and IGFBP-3 levels of the GHD group were significantly lower compared to the non-GHD children (both p<0.001). During GH treatment both serum IGF-I and IGFBP-3 levels increased significantly in the GH treated groups. In the total group, the 3-year change in both IGF-I SDS and IGFBP-3 SDS correlated significantly with the 3-year change in H SDS (both r=0.6 and p<0.001). In the GH-treated groups, this correlation was weaker but still significant (both r=0.3 and p=0.02).

Figure 2

Changes in bone maturation (ΔBA/ΔCA ratio) during the first, second and third study year of the GH- (black bars), the GHD- (grey bars) and the untreated control group (open bars).



* = *significantly different compared to the control group (p<0.001)*

\# = *significantly different compared to the control group (p<0.05)*

Changes in BA

At start of the study the mean BA delay of 1.0 (0.9) year was comparable between the 3 groups. During the 3-year study period the delay in bone maturation of the control group remained unchanged. In contrast, the GH- and GHD-group showed a significant increase in bone maturation. Figure 2 shows ΔBA/ΔCA ratio per year for the 3 groups. The highest ΔBA/ΔCA ratio for the GH-group was observed during the second year of GH treatment (1.6 (0.70) yrs/yr) and for the GHD-group during the first year of GH treatment (1.6 (0.6) yrs/yr). During the third year, however, the ΔBA/ΔCA ratio was comparable for the 3 groups. During the entire 3-year period the mean ΔBA/ΔCA ratio was 4.3 (0.8) yrs / 3.0 (0.0) yr in the GH-group and 3.2 (0.8) yrs / 3.0 (0.0) yr in the control group (p<0.001).

No significant correlation was found between age and the 3-year ΔBA/ΔCA ratio. In the total group the 3-year ΔBA/ΔCA ratio correlated significantly with the 3-year change in H SDS (r=0.6, p<0.001) and with serum IGF-I levels after 1, 2 and 3 years. Also, within each subgroup the 3-year ΔBA/ΔCA ratio correlated significantly with the 3-year change in H SDS (GH- and GHD group: r=0.3, p=0.006; control group r=0.5, p=0.007). However, within each subgroup, the 3-year ΔBA/ΔCA ratio did not correlate with changes in serum IGF-I levels.

Table 3
Changes in BMD_{TB} SDS, BMD_{LS} SDS and BMAD SDS during the 3-year study period in the GH-treated and the control group

	BMD_{TB} SDS	BMD_{LS} SDS	BMAD SDS
GH- and GHD group (n=28)			
Baseline	-0.9 (1.1)[1]	-1.7 (1.0)[1]	-0.6 (1.1)[2]
1 year	-0.6 (0.8)[1]	-0.9 (0.9)[1]	-0.3 (1.1)
2 years	0.1 (0.6)*	-0.4 (0.8)[2,#]	-0.1 (1.0)
3 years	0.2 (0.7)*	0.1 (0.6)*	0.3 (0.9)
Control group (n=10)			
Baseline	-0.9 (1.2)[1]	-1.6 (0.8)[1]	-0.7 (0.6)[2]
1 year	-1.0 (1.2)[2]	-1.4 (1.0)[1]	-0.6 (0.8)[2]
2 years	-0.8 (1.0)[2]	-1.3 (0.9)[1]	-0.3 (1.0)
3 years	-1.0 (0.8)[2]	-1.3 (0.6)[1]	-0.3 (0.7)

All values are expressed as mean (SD)
[1] = significantly different compared to 0 SDS (p<0.005)
[2] = significantly different compared to 0 SDS (p<0.05)
** = significantly different compared to the control group (p ≤ 0.005)*
 # = significantly different compared to the control group (p ≤ 0.05)

Changes in BMD

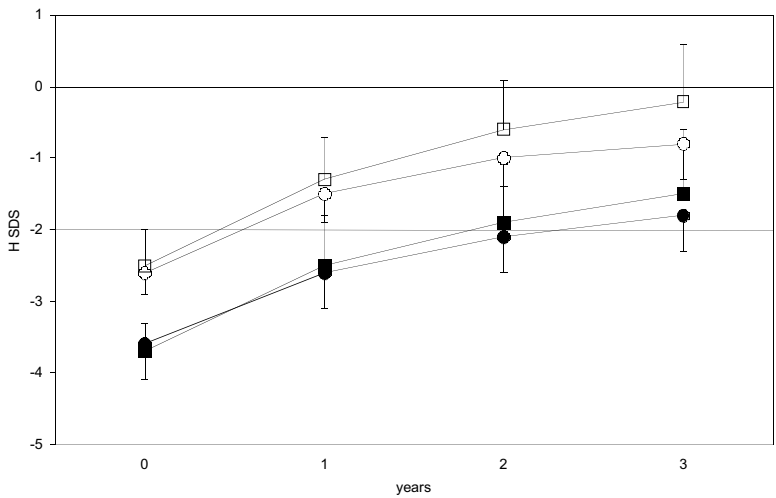
No difference was found in mean BMD_{TB}, BMD_{LS}, and BMAD SDS at start and during GH treatment between the GH- and the GHD-group (data not shown). Therefore, BMD of these 2 groups are presented together. At baseline mean BMD_{TB}, BMD_{LS}, and BMAD SDS in both the GH-treated group (GH- and GHD group) and the control group were significantly lower than zero (Table 3). At start of the study 10 out of 38 children (26 %) had a BMD SDS below –2.00 SDS and 3 out of 38 children (8 %) had a BMAD SDS below –2.00 SDS. During 3 years of GH treatment mean BMD_{TB}, BMD_{LS}, and BMAD SDS showed an impressive increase (all p<0.001). In contrast, the control group showed only a slight increase in parameters of bone density. After 2 and 3 years of GH treatment, respectively, all children had a BMD_{TB} SDS, BMD_{LS} SDS and BMAD SDS in the normal range (above -2.00 SDS). The 3 year change in BMD_{TB} SDS did neither correlate with changes in H SDS nor with changes in IGF-I SDS. However, changes in BMD_{LS} SDS and BMAD SDS correlated significantly with changes in H SDS (r=0.8, p<0.001; r=0.5, p=0.003) and with changes in IGF-I SDS (r=0.6, p<0.001; r=0.3, p=0.05).

Multiple regression analysis

Using multiple regression, we found age at start of the study (β=-0.1, p=0.001), 1 year ΔBA/ΔCA ratio (β=0.2, p=0.01) and the 1-year change in IGF-I SDS (β=0.1, p=0.04) to be the best predictors of the 3-year increase in H SDS during GH treatment. These three variables explained 31 % of the variation in the 3-year change in H SDS. TH SDS, the maximum GH peak during stimulation tests and baseline IGF-I levels did not significantly contribute to this model.

Figure 3

Changes in H SDS in A) children with a baseline H SDS between –2.00 and –3.00 receiving a GH dose of either 33 (n=33, open circles) or 66 μg/kg/day (n=8, open squares) and B) children with a baseline H SDS ≤ –3.00 receiving a GH dose of either 33 (n=34, black circles) or 66 μg/kg/day (n=16, black squares).



Growth response in relation to height at start and GH dose

An analysis was performed investigating the effect of GH dose in children with either a H SDS at start between –2.00 and –3.00 or a H SDS at start ≤ -3.00. For this analysis we used data of the present randomized, controlled GH trial (33 μg/kg/day vs control) and data of our previously performed randomized GH-dose-response trial (33 vs 66 μg/kg/day). Analysis was performed in prepubertal children only. TH SDS of children receiving either 33 or 66 μg/kg/day did not differ (-0.5 (0.8) and –0.5 (0.9), respectively). Also, age at start of the study was not significantly different between the groups. Figure 3 shows that in children with a H SDS at start between –2.00 and –3.00, the mean H SDS had normalised (> –2.00 SDS) after 1 year of treatment with either a normal or a double GH dose. After 2 years, all of these children (100 %) had a H SDS in the normal range (above –2.00). The 3-year change in H SDS was signifi-

cantly higher in children treated with a double dose versus children treated with a normal dose (2.3 (0.6) versus 1.8 (0.4); p=0.003).

In children with a baseline H SDS ≤ -3.00, mean H SDS showed a normalisation after 2 years in the double dose group and after 3 years in normal dose group. However, after 3 years of GH treatment, still 31 % of the double dose group and 41 % of the normal dose group had a H SDS below –2.00 (difference between the groups not significant). Remarkably, in these very short children the 3-year change in H SDS did not significantly differ between the two GH-dosage groups.

Safety

GH injections were well tolerated and no adverse events were reported during treatment that could be attributed to GH. Thyroid function and hemoglobin A_{1c} levels remained normal during the study period.

Discussion

This study reports the effects of GH treatment in a randomized, 3-year controlled study design with a separate group of GHD children born SGA, treated parallel to the randomized trial. GH treatment with a dose of 33 μg/kg/day in short SGA children either with or without GHD resulted in normalisation of their height during childhood. After two years of GH treatment, the mean H SDS of both GH treated groups had normalised (i.e. above –2.00), while the H SDS of the untreated control group remained far below –2.00. These results are comparable to those reported previously by us and others (6-8).

Serum IGF-I and IGFBP-3 levels, expressed as SDS, were significantly lower than zero in short non-GHD children born SGA. In short SGA children with GHD serum IGF-I and IGFBP-3 levels were even more severely reduced. Remarkably, during GH treatment a similar increase in both IGF-I and IGFBP-3 levels was found in children with and without GHD. It has been suggested that disturbances in the GH/IGF-I axis play a role in the mechanisms underlying poor catch-up growth of short children born SGA (4,25). The 3-year increment in IGF-I and IGFBP-3 SDS correlated well with the 3-year gain in H SDS suggesting that the good response to GH treatment was mediated through increased serum IGF-I levels.

At start of the study all children had a 1-year delay in bone age, regardless of their GH-status. During the 3-year study period, bone maturation in untreated short children born SGA did neither accelerate nor decelerate. So, after the 3-year study period they still had a bone age delay of one year. In contrast, bone maturation accelerated significantly in the GH-treated groups. In the GHD group this acceleration was most obvious during the first year of treatment while in the GH group the maximum acceleration was observed during the second year of treatment. After the first two years of treatment this acceleration slowed-down and during the third year no significant difference was found compared with the untreated control group. Data describing bone maturation during GH treatment of short SGA children are rather confusing. It has been reported that bone maturation accelerated spontaneously in untreated short SGA children (26-28). De Zegher et al., however, found a slower bone maturation in both untreated and GH-treated SGA children with a relatively young age at start of the study compared to SGA children with a relatively old age at start of the study (29). In contrast, during a 3-year follow-up period, we did not observe any difference in bone maturation between younger and older (until the age of 8 years) SGA children, either untreated or GH-treated (data not shown). Interestingly, our study showed that the acceleration in bone maturation is associated with a strong catch-up growth during the first 3 years of GH treatment.

The 1-year $\Delta BA/\Delta CA$ ratio appeared a very good predictor of the 3-year gain in height. As has been previously reported by us in another trial also, age at start of the study showed a significant inverse correlation with the 3 year growth response during GH treatment (7). Neither target height nor baseline parameters of GH status

did significantly contribute to the prediction model.

Our study evaluated bone mineral density (BMD) in short prepubertal SGA children. We found that before GH treatment the mean BMD level was significantly lower compared to normal children. However, since DXA measures an areal density (g/cm^2), BMD is underestimated in short children (30). For that reason we calculated bone mineral apparent density (BMAD) which is a widely used validated method to correct for short stature (19). At baseline, mean BMAD SDS was reduced to a lesser degree than mean BMD_{TB} and mean BMD_{LS} SDS levels. However, mean BMAD SDS differed significantly from zero, suggesting bone mineral density in these children is not only reduced because of their short stature. During GH treatment BMAD and BMD_{TB} normalised after 1 and 2 years, respectively, whereas BMD_{LS} normalised after 3 years of GH treatment. These results are comparable to results observed in GHD children (31,32).

It has been questioned if SGA children with a H SDS below –3.00 should be treated with a higher dose of GH in order to achieve a more rapid normalisation of their height. To answer this question we compared the results of the present randomized, controlled GH trial with the results of our first randomized GH-dose-response trial, in which comparable SGA children received GH treatment at a higher dose of 66 μg/kg/day (double dose) (7). All SGA children with a H SDS at start between –2.00 and –3.00, who received either a double or normal dose of GH, showed a normalisation of H SDS (i.e. > -2.00) within 2 years of treatment. None of these children had a H SDS below –2.00 after 2 years of treatment. In SGA children with severe short stature at start of the study (≤ –3.00), mean H SDS normalised after 2 years of GH treatment in children receiving the double GH dose and after 3 years in children receiving a normal dose. We did, however, not find a significant effect of GH dose on the 3-year gain in H SDS in children with a H SDS at start ≤ –3.00. After three years of GH treatment 31 % of the double dose group and 41 % of the normal dose group still had a H SDS below the normal range. The difference in H SDS at start between the two height groups was 1 SD. Therefore, it is not surprising that children with a very low baseline H SDS need more time to catch-up in height into the normal range. Our results, however, show that treatment with a higher GH dose does not result in a more rapid catch-up growth. Since it is important to achieve a height within the normal range before a child enters puberty, we suggest that in children with a very low H SDS, GH treatment should start at a relatively young age. So, if a child born SGA is still very growth-retarded (H SDS ≤ -3.00) at the age of 3 years without showing any signs of spontaneous catch-up growth, we suggest to start treatment with a normal dose of GH at that young age. Obviously, other causes for short stature (e.g. Turner syndrome, coeliac disease) should be excluded before starting GH treatment.

In conclusion, three years of GH treatment results in normalisation of height during childhood. During GH treatment, bone maturation increased proportionately to the gain in height. The mean level of bone mineral density in short SGA children is

significantly reduced compared to normal children but normalises during GH treatment. The height gain of children with severe short stature at start of the study (≤-3.00 SDS) does not differ between those receiving a normal versus those receiving a double GH dose. We therefore conclude that there is no indication to treat these very short SGA children with a higher GH dose. We want to emphasize, however, that severely growth retarded SGA children should start GH treatment at a young age to enable sufficient catch-up growth before onset of puberty.

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References

1. Albertsson-Wikland K, Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71.
2. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71.
3. Chaussain JL, Colle M, Ducret JP 1994 Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. Acta Paediatr Suppl 399:72-3.
4. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
5. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
6. Boguszewski M, Albertsson-Wikland K, Aronsson S, Gustafsson J, Hagenas L, Westgren U, Westphal O, Lipsanen-Nyman M, Sipila I, Gellert P, Muller J, Madsen B 1998 Growth hormone treatment of short children born small-for-gestational- age: the Nordic Multicentre Trial. Acta Paediatr 87:257-63.
7. Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose- response trial. J Clin Endocrinol Metab 84:3064-70.
8. de Zegher F, Du Caju MV, Heinrichs C, Maes M, De Schepper J, Craen M, Vanweser K, Malvaux P, Rosenfeld RG 1999 Early, discontinuous, high dose growth hormone treatment to normalize height and weight of short children born small for gestational age: results over 6 years. J Clin Endocrinol Metab 84:1558-61.
9. Cummings SR, Black DM, Nevitt MC, Browner W, Cauley J, Ensrud K, Genant HK, Palermo L, Scott J, Vogt TM 1993 Bone density at various sites for prediction of hip fractures. The Study of Osteoporotic Fractures Research Group. Lancet 341:72-5.
10. Goulding A, Jones IE, Taylor RW, Manning PJ, Williams SM 2000 More broken bones: a 4-year double cohort study of young girls with and without distal forearm fractures. J Bone Miner Res 15:2011-8.
11. Hansen MA, Overgaard K, Riis BJ, Christiansen C 1991 Role of peak bone mass and bone loss in post-menopausal osteoporosis: 12 year study. Bmj 303:961-4.
12. Ribot C, Tremollieres F, Pouilles JM 1995 Late consequences of a low peak bone mass. Acta Paediatr Suppl 411:31-5; discussion 36.
13. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
14. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23.
15. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-9.
16. Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB 1995 Growth and symptoms in Silver-Russell syndrome: review on the basis of 386 patients. European Journal of Pediatrics 154:958-968.
17. Tanner JM, Whitehouse RH, Cameron N, Marshall WA, Healy MJR, Goldstein H 1983 Assessment of skeletal maturity and prediction of adult height (TW2-method), 2nd ed. Academic Press, London.
18. Johnson J, Dawson-Hughes B 1991 Precision and stability of dual-energy X-ray absorptiometry measurements. Calcif Tissue Int 49:174-8.
19. Kroger H, Vainio P, Nieminen J, Kotaniemi A 1995 Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 17:157-9.
20. Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama SM 1997 Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. J Clin Endocrinol Metab 82:57-62.
21. Boot AM, Bouquet J, de Ridder MA, Krenning EP, de Muinck Keizer-Schrama SM 1997 Determinants of body composition measured by dual-energy X-ray absorptiometry in Dutch children and adolescents. Am J Clin Nutr 66:232-8.
22. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 71:688-95.

23. Martin JL, Baxter RC 1986 Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J Biol Chem 261:8754-60.
24. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-76.
25. Boguszewski M, Jansson C, Rosberg S, Albertsson-Wikland K 1996 Changes in serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 levels during growth hormone treatment in prepubertal short children born small for gestational age. J Clin Endocrinol Metab 81:3902-8.
26. Job JC, Rolland A 1986 [Natural history of intrauterine growth retardation: pubertal growth and adult height]. Arch Fr Pediatr 43:301-6.
27. Davies PS, Valley R, Preece MA 1988 Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 63:130-5.
28. Ranke MB, Lindberg A 1996 Growth hormone treatment of short children born small for gestational age or with Silver-Russell syndrome: results from KIGS (Kabi International Growth Study), including the first report on final height. Acta Paediatr Suppl 417:18-26.
29. de Zegher F, Butenandt O, Chatelain P, Albertsson-Wikland K, Jonsson B, Lofstrom A, Chaussain JL 1997 Growth hormone treatment of short children born small for gestational age: reappraisal of the rate of bone maturation over 2 years and metanalysis of height gain over 4 years. Acta Paediatr Suppl 423:207-12.
30. Mazess RB, Barden H, Mautalen C, Vega E 1994 Normalization of spine densitometry. J Bone Miner Res 9:541-8.
31. Saggese G, Baroncelli GI, Bertelloni S, Barsanti S 1996 The effect of long-term growth hormone (GH) treatment on bone mineral density in children with GH deficiency. Role of GH in the attainment of peak bone mass. J Clin Endocrinol Metab 81:3077-83.
32. Boot AM, Engels MA, Boerma GJ, Krenning EP, De Muinck Keizer-Schrama SM 1997 Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. J Clin Endocrinol Metab 82:2423-8.

Body composition by Dual Energy X-ray Absorptiometry (DXA) in short children born small for gestational age (SGA) before and during growth hormone (GH) treatment: Results of a randomized, controlled GH treatment study.

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Abstract

The aim of this randomized, controlled GH treatment study is to investigate lean body mass (LBM), fat mass and bone mineral density (BMD) using Dual Energy X-ray Absorptiometry (DXA), skinfold thickness and BMI in a group of short children born SGA before and during GH treatment.

All children participated in a 3-year randomized, controlled GH treatment study. Non-GHD SGA children (n=87) were randomized to receive GH (GH-group, n=61) or remained untreated (control group, n=26). Twelve SGA children with GH-deficiency served as a comparison cohort (GHD-group, n=12). All GH treated children were treated with 33 μg GH/kg/day. In a subgroup BMD, LBM and fat mass were measured using DXA. The sum of 4 skinfolds (skinfolds), BMI, and serum IGF-I levels were measured in all children. Measurements were expressed as standard deviation scores (SDS), adjusting for sex and age.

At start of the study, SGA children showed a significant reduction of LBM, fat mass and BMD measured by DXA. Also, skinfolds and BMI were significantly reduced. Skinfolds correlated strongly with fat mass measured by DXA ($r=0.8$, $p<0.001$), whereas BMI correlated with both fat mass and LBM ($r=0.6$, $p<0.001$; $r=0.5$, $p=0.008$). During 3 years of GH treatment, both LBM and BMD increased impressively ($p<0.001$), in contrast to the control group. Fat mass decreased significantly during the first year of GH treatment ($p<0.001$) followed by an increase to levels comparable to baseline. Changes in fat mass correlated with changes in skinfolds ($r=0.4$, $p=0.05$). Changes in BMI did not correlate with either changes in fat mass or LBM.

In short children born SGA, leanness is the result of a significant reduction in LBM and to a lesser extent of a reduction in total body fat. In addition, BMD is significantly reduced in these children. During GH treatment LBM and BMD normalised. Also, our study indicates that the sum of 4 skinfolds is a better tool than BMI for estimating total body fat in short SGA children before and during GH treatment.

Introduction

Children who were born small for gestational age (SGA) have a higher risk of being short in adult life. During the first 2 years of life, ten to fifteen percent of all children born SGA fail to show catch-up growth in height above the third percentile (1,2). Disturbances in the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis, which have been reported in 25 % of the short SGA children during a GH provocative test and in 60 % during a GH profile test, might be involved (3,4).

GH is known to influence linear growth, bone density and body composition. Children with untreated classical GH deficiency (GHD) have reduced bone mineral content, reduced muscle mass and increased percentage body fat (5). Not much is known about the body composition in short children born SGA without GHD (6). Since disturbances in the GH/IGF-I axis are present in SGA children, it can be hypothesised that their body composition is also altered. In contrast to classical GHD children who have truncal obesity, short children born SGA either with or without GHD have a lean appearance. This finding is supported by calculations of body mass index (BMI) as well as measurements of skinfold thickness (7). However, Dual Energy X-ray Absorptiometry (DXA) is a more precise method to investigate body composition. DXA uses a low radiation dose, is very accurate and can easily be performed in children (8,9).

This is the first controlled GH treatment study investigating fat mass, lean body mass (LBM) and bone mineral density (BMD) using DXA in a group of short children born SGA without GHD. All children participated in a 3-year randomized, controlled GH treatment study. DXA was performed at start of the study and subsequently every year in both GH-treated and untreated SGA children. DXA results were compared to two commonly used estimates of body composition, i.e. BMI and skinfold thickness. Results of a non-randomized group of SGA children with GHD served as a comparison cohort.

Patients and methods

Subjects

The study comprised 104 Dutch children (48 boys and 56 girls) with short stature born SGA. All children fulfilled the same inclusion criteria: 1) birth length standard deviation score (SDS) below – 2.00 for gestational age (10); 2) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia; 3) chronological age (CA) between 3.00 and 7.99 years at start of the study; 4) height SDS for age below –2.00 according to Dutch standards (11); 5) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth (11); 6) prepubertal, defined as Tanner stage 1 or a testicular volume < 4 (12); 7) normal liver, kidney and thyroid functions. Children with endocrine or metabolic disorders, chromosomal defects and growth failure caused by other syndromes (e.g. emotional deprivation, Turner syndrome, severe chronic illness, chondrodysplasia), with the exception of Silver-Russell syndrome, were excluded.
The study was approved by the Ethics Committees of all nine participating centers. Written informed consent was obtained from the parents or custodians of each child.

Study design

The study design was an open-labelled multicenter study with a randomized control group. Before entering the study the GH status was evaluated in all children using GH stimulation tests (arginine and/or clonidine). Children with GH deficiency (GHD) which was defined as a GH peak < 10 μg/l during two GH stimulation tests, were not randomized but started GH treatment at dose of 1 mg/m^2 body surface area/day (~33 μg/kg/day) (GHD-group). The non-GHD children were stratified according to age (3.00-5.50 versus 5.50-7.99) and height of the parents (height of both parents above –2.00 SDS versus height of at least one parent below –2.00 SDS). After stratification the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group started immediately with GH treatment at a dose of 33 μg/kg/day (~1 mg/m^2 body surface area/day). The control group remained untreated for 3 years and received subsequently the same GH treatment as the GH-group.
Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

Anthropometric measurements

Every 3 months height (H) and weight (W) were measured by two trained investigators (N. Arends and later on V. Boonstra). The mean of 4 measurements was used for analysis. Both height and weight were expressed as SD-score for sex and chronological age ($HSDS_{CA}$ and $WSDS_{CA}$) using Dutch references (11). Body mass index (BMI)

was calculated as weight (in kilogram) divided by square of height (in meters) and was expressed as SD-score for sex and age (11). Skinfold measurements (SF) of biceps, triceps, subscapular and suprailiacal were measured in all children using a Holtain skinfold calliper by the same two investigators (NA and VB) (13). The mean value of two measurements was calculated. For analysis we used the sum of the four measurements, expressed as SD-score using references for healthy Dutch children (14). To calculate SD-scores, data of the reference population were transformed using the LMS method (15). This method transforms the reference data at each age to a normal distribution.

Body composition by DXA

In a subgroup of 38 SGA children living near Rotterdam (20 of the GH group, 8 of the GHD group and 10 of the control group), bone mineral content (BMC in gram), fat mass (in gram), % body fat and lean body mass (LBM in gram) were measured by DXA type Lunar DPX-L PED using the pediatric medium scan mode. The coefficients of variation for BMC, fat mass and LBM has been reported to be 1.8 %, 4.1 % and 1.0 % respectively (9). Bone mineral density (BMD in g/cm^2) measured by DXA is an area density obtained by dividing BMC (in gram) by the projected bone image (area, cm^2). This method adjust for smaller body sizes since the total bone area is less in shorter individuals. The coefficient of variation for BMD has been reported to be 0.64 % (16). Since all parameters of LBM, fat mass, % body fat and BMD were dependent on age and sex, the values were transformed into SD-scores using Dutch reference values for children older than 4 years (17,18). Reference values were obtained using the same instrumentation and software.

Biochemical measurements

Blood samples from all children were taken at the start of the study and subsequently every 12 months for determination of serum IGF-I levels. After centrifugation, all samples were frozen (-20 C) until assayed.

Hormone assays

A specific RIA measured IGF-I after acid chromatography as described previously (19,20). Serum IGF-I levels were expressed as SD-scores using reference data from healthy Dutch children (19,21).

Statistics

Of 104 children 5 children dropped out of the study for the following reasons: one child was very disappointed she was randomized into the control group, 3 children had psychological problems with the daily GH injections and in 1 child coeliac disease was diagnosed. Since these children dropped out either at start or during the first year of the study, these children were excluded from baseline and 3-year analysis. Therefore, 99 children (46 boys and 53 girls) were eligible for statistical analysis. Data

are expressed as the mean plus or minus the standard deviation (SD). SD-scores were compared with zero using Student's one sample t-test. Differences between groups were tested using independent Student's t-tests. Differences in 1-year changes between the groups were tested using analysis of covariance and differences between points in time within the groups were tested by paired Student's t-tests. Pearson's correlation coefficient was used for correlations. Multiple regression analysis was used to assess multivariate relationships. Statistical significance was defined as $p < 0.05$. Statistical tests were performed with use of SPSS package (version 10.0).

Results

At baseline

Table 1 shows the baseline characteristics of all children in the GH-, control and GHD-group. Since DXA's were performed in a subgroup of children, their baseline characteristics are shown as well. At the start of the study no significant differences were found between the 3 groups in either the total or the subgroup. Also, the DXA subgroup did not differ significantly from the total group. Results of children with SRS did not differ from results of children without signs of SRS (data not shown).
During the 3-year study period, puberty started in 4 children of the GH-group, in 2 children of the control group and in 2 children of the GHD group. Analyses were performed in prepubertal children only.

Table 1
Baseline characteristics

	Randomized study				Comparison cohort	
	GH group		Control group		GHD group	
	Total group	Subgroup DXA	Total group	Subgroup DXA	Total group	Subgroup DXA
	n=61	n=20	n=26	n=10	n=12	n=8
Boys / girls	25 / 36	7 / 13	16 / 10	6 / 4	5 / 7	4 / 4
Gestational age (wks)	36.1 (3.9)	35.4 (3.2)	36.0 (3.6)	36.3 (2.7)	35.9 (3.6)	36.5 (2.6)
Birth length SDS	-3.4 (1.5)	-3.3 (1.6)	-3.1 (1.3)	-3.2 (1.4)	-3.5 (2.0)	-4.0 (2.5)
Birth weight SDS	-2.3 (1.2)	-2.3 (1.4)	-2.7 (1.0)	-2.7 (1.0)	-2.5 (1.2)	-2.8 (1.0)
Chronological age (yr)	6.0 (1.6)	6.0 (1.4)	5.9 (1.5)	6.0 (1.7)	5.2 (1.4)	5.6 (1.5)
Height SDSCA	-3.0 (0.6)	-3.0 (0.6)	-3.2 (0.5)	-3.2 (0.5)	-3.4 (0.8)	-3.4 (0.8)
Weight SDSCA	-3.1 (0.9)	-2.9 (0.7)	-3.0 (0.8)	-3.1 (0.8)	-3.1 (1.3)	-3.2 (1.5)
TH SDS -0.5 (0.8)	-0.5 (0.7)	-0.6 (0.7)	-0.7 (0.6)	-0.5 (0.7)	-0.5 (0.7)	
Silver Russell syndrome (n)	6	2	5	2	2	2

All values are expressed as mean (SD) or number

Height, height velocity and weight

GH treatment resulted in a normalisation of height in both the GH- and the GHD-group. In the GH-group height SDS increased from –3.0 SDS at start to –1.3 SDS after 3 years ($p<0.001$) and in the GHD-group height SDS increased from –3.4 SDS to –1.2 SDS ($p<0.001$). Changes in height SDS did not differ between the GH- and the GHD-group. The untreated control group showed only a slight increase in height SDS during the 3 year study period (–3.2 at start to –2.9 SDS; $p<0.001$).
Height velocity (HV), expressed as cm/year, during the first study year, was significantly higher in the GH- and the GHD-group compared to the control group (10.3, 11.6 and 5.9 cm/yr respectively; both $p<0.001$). During the third year, HV decreased to 6.9 in the GH-group, 7.4 in GHD-group and 5.0 in the untreated control group. However, HV was still significantly higher in the GH-treated groups versus the control group (both $p<0.001$).

In the GH- and the GHD-group weight SDS increased significantly during 3 years of GH treatment, from –3.1 SDS to –1.4 SDS (p<0.001) and from –3.1 SDS to –1.1 SDS (p<0.001), respectively. Weight SDS in the control group increased only slightly from –3.0 SDS at start to –2.8 SDS after 3 years (p=0.04).

Table 2

Three year changes in LBM SDS, fat mass SDS, % body fat SDS and BMD SDS of the GH-, the control and the GHD group.

	Randomized study GH-group n=20	Control group n=10	Comparison cohort GHD-group n=8
LBM SDS			
At start	-2.7 (0.5)[1]	-2.7 (0.5)[1]	-3.0 (1.2)[1]
1 year	-1.8 (0.5)[1]	-2.6 (0.4)[1,*]	-2.0 (1.0)[1]
2 years	-1.3 (0.5)[1]	-2.8 (0.4)[1,*]	-1.4 (0.9)[2]
3 years	-1.1 (0.6)[1]	-3.0 (0.3)[1,*]	-1.1 (0.9)[2]
Fat mass SDS			
At start	-1.4 (0.5)[1]	-1.3 (0.7)[1]	-1.5 (0.6)[1]
1 year	-1.6 (0.5)[1]	-1.2 (0.5)[1,#]	-1.6 (0.2)[1]
2 years	-1.3 (0.5)[1]	-1.0 (0.8)[1]	-1.4 (0.6)[1]
3 years	-1.2 (0.4)[1]	-1.1 (0.8)[1]	-1.4 (0.7)[2]
% Body fat SDS			
At start	-1.1 (0.8)[1]	-1.1 (1.0)[2]	-1.2 (0.9)[2]
1 year	-1.7 (0.7)[1]	-0.9 (0.9)[2,#]	-1.8 (0.3)[1]
2 years	-1.3 (0.7)[1]	-0.6 (1.3)	-1.6 (0.8)[1]
3 years	-1.2 (0.6)[1]	-0.6 (1.4)	-1.5 (0.8)[2]
BMD SDS			
At start	-0.8 (1.0)[1]	-0.9 (1.1)[1]	-1.3 (1.2)[2]
1 year	-0.5 (0.9)[2]	-1.0 (1.2)[2]	-0.8 (0.6)[2]
2 years	0.2 (0.7)	-0.8 (1.0)[2,*]	-0.2 (0.3)
3 years	0.2 (0.8)	-0.9 (0.8)[2,*]	0.1 (0.5)
IGF-I SDS			
At start	-0.3 (1.0)	-0.2 (1.1)	-1.3 (1.7)[*]
3 year	1.7 (1.2)[1]	-0.3 (1.3)[*]	0.5 (1.0)

All values are expressed as mean (SD)

[1] = significantly different compared to zero (p<0.005)

[2] = significantly different compared to zero (p<0.05)

[*] = significantly different compared to the GH-group (p ≤ 0.005)

[#] = significantly different compared to the GH-group (p ≤ 0.05)

Fat mass and lean body mass (LBM) by DXA

We compared the GH-group versus the untreated control group and used the non-randomized GHD-group as a comparison-cohort only. Remarkably, at start of the study no differences in LBM and fat mass were observed between SGA children with and without GHD.

At start of the study, the SD-scores of lean body mass (LBM), fat mass and % body fat were significantly lower than zero in all 3 groups (Table 2). LBM was most severely reduced, with a mean level being significantly lower than –2.0 SDS (p<0.001).

LBM increased impressively during GH treatment ($p<0.001$) but remained unchanged in the control group (Figure 1). Before GH treatment 19 of 20 children (95 %) showed a LBM SDS below –2.0, whereas after 3 year of treatment only 1 of the 20 children (5 %) had a LBM SDS lower than –2.0.

Both fat mass and % body fat showed a decrease after 1 year of GH treatment ($p=0.09$ and $p=0.002$ respectively) followed by an increase to levels which were not different from baseline levels. Before GH treatment fat mass SDS was in 1 of the 20 children (5 %) lower than –2.0 SDS and % body fat SDS was also in only 1 child (5 %) below –2.0. After 3 years of GH treatment none of the children had a fat mass below –2.0 SDS, whereas two out of 20 children (20) had a % body fat SDS of –2.0 SDS. The control group showed a moderate increase in % body fat during 3 year ($p=0.04$), whereas the amount of fat mass remained the same.

Bone mineral density (BMD) by DXA

For DXA analysis we compared the GH-group versus the untreated control group. Since the GHD-group was not randomized and rather small, this group was used as a comparison-cohort only. However, at start of the study no differences in BMD SDS were found between the SGA children with and without GHD.

Mean BMD SDS at start of the study was significantly lower compared to zero in all 3 groups (Table 2). GH-treatment resulted in a significant increase in BMD SDS ($p=0.001$). After 2 years of GH treatment mean BMD had normalized in both the GH- and the GHD- group. Mean BMD SDS of the control group remained unchanged. At baseline, 3 out of 20 children (15%) had a BMD SDS below –2 SDS whereas after 3 years all children had a BMD SDS in the normal range.

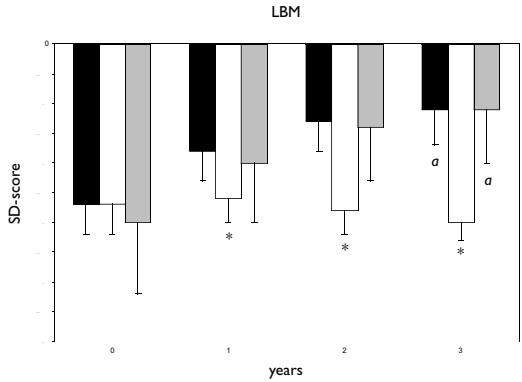
Body composition by skinfold thickness and BMI

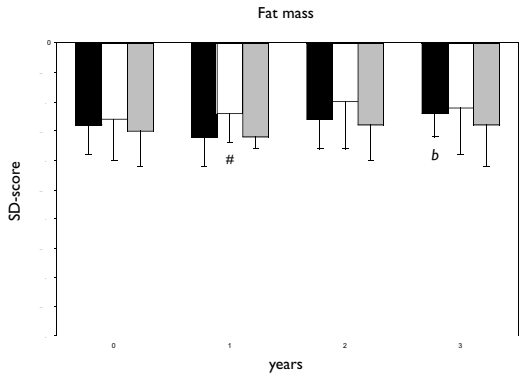
At start of the study, the sum of 4 skinfolds expressed as SD-score ('skinfolds'), was significantly lower compared to zero in all groups (Table 3). Skinfolds showed a significant decrease during the first year of GH treatment ($p<0.001$) which stabilised during the second and third year of treatment. After 3 years of GH treatment skinfolds of the GH-group and the GHD-group were lower compared to the skinfolds at start ($p=0.04$ and $p=0.07$ respectively). Skinfolds of the control group did not change during the 3 year study period.

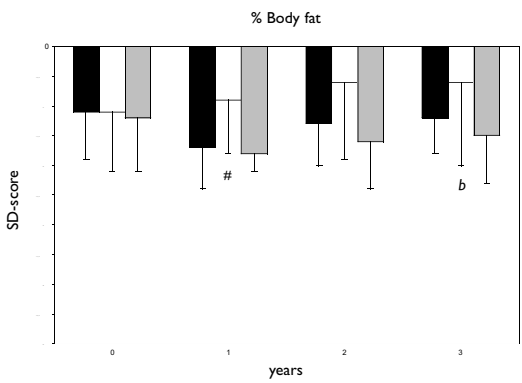
BMI SD-score at start of the study was also significantly lower compared to zero in all 3 groups. BMI of both the GH-group and the GHD group showed a significant increase after 3 years ($p<0.001$ and $p=0.04$ respectively) whereas BMI of the untreated control group remained unchanged.

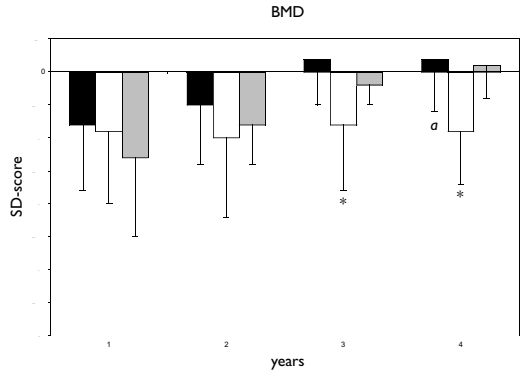
Figure 1.

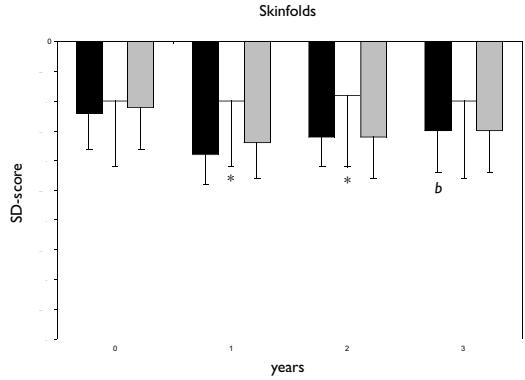
Changes in LBM (a), Fat mass (b), % body fat (c), BMD (d), skinfolds (e) and BMI (f) of the GH-group (black bars), the untreated control group (open bars) and the GHD-group (grey bars).

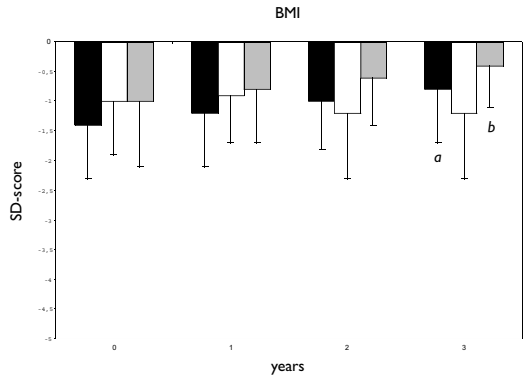












* = *significantly different compared to the GH-group ($p \leq 0.005$)*

\# = *significantly different compared to the GH-group ($p \leq 0.05$)*

^{a} = *significantly different compared to baseline ($p \leq 0.005$)*

^{b} = *significantly different compared to baseline ($p \leq 0.05$)*

Correlations

For investigating relationships between different parameters of body composition and serum IGF-I levels, all correlations were corrected for sex and age.
At start of the study fat mass and LBM correlated positively with serum IGF-I levels (r=0.4, p=0.04 and r=0.3, p=0.05, respectively). No correlation was found between BMD and IGF-I.

In order to investigate if skinfolds and BMI are accurate estimates of body composition in these children, we investigated their relation to DXA which is considered to be the 'gold standard'. Skinfolds correlated strongly with fat mass (p=0.8; p<0.001) but not with LBM. BMI, however, correlated with both fat mass and LBM (r=0.6, p<0.001; r=0.5, p=0.008). Neither skinfolds nor BMI correlated with serum IGF-I levels.

The increase in LBM correlated strongly with changes in IGF-I (r=0.6, p=0.002) but not with changes in skinfolds or BMI. The 3-year change in fat mass correlated moderately with changes in skinfolds (r=0.4, p=0.05) but not with changes in BMI. The 3-year change in BMD SDS did not correlate with the change in IGF-I SDS.

Table 3

Three year changes in skinfold thickness and BMI of the GH-, the GHD and the control group.

	Randomized study				Comparison cohort	
	GH-group		Control group		GHD-group	
	Total group	Subgroup DXA	Total group	Subgroup DXA	Total group	Subgroup DXA
	n=56	n=20	n=24	n=10	n=10	n=8
Skinfolds SDS						
At start	-1.2 (0.6)[1]	-1.3 (0.6)	-1.0 (1.1)[1]	-1.4 (1.1)	-1.1 (0.7)1	-0.6 (1.5)
1 year	-1.9 (0.5)[1]	-2.0 (0.6)	-1.0 (1.1)[1,*]	-1.4 (0.8)	-1.7 (0.6)1	-1.5 (1.0)
2 years	-1.6 (0.5)[1]	-1.7 (0.5)	-0.9 (1.2)[1,*]	-1.1 (1.0)	-1.6 (0.7)1	-1.4 (1.2)
3 years	-1.5 (0.7)[1]	-1.4 (0.6)	-1.0 (1.3)[1]	-1.3 (0.9)	-1.5 (0.7)1	-1.0 (1.3)
BMI SDS						
At start	-1.4 (0.9)[1]	-1.1 (0.9)	-1.0 (0.9)[1]	-1.1 (0.9)	-1.0 (1.1)2	-1.0 (1.3)
1 year	-1.2 (0.9)[1]	-0.9 (0.7)	-0.9 (0.8)[1]	-1.0 (0.8)	-0.8 (0.9)2	-0.9 (1.1)
2 years	-1.0 (0.8)[1]	-0.8 (0.7)	-1.2 (1.1)[1]	-1.5 (1.1)	-0.6 (0.8)2	-0.7 (1.0)
3 years	-0.8 (0.9)[1]	-0.7 (0.7)	-1.2 (1.1)[1]	-1.3 (1.3)	-0.5 (0.7)	-0.3 (0.9)

All values are expressed as mean (SD)

[1] = significantly different compared to zero (p<0.005)

[2] = significantly different compared to zero (p<0.05)

[*] = significantly different compared to the GH-group (p ≤ 0.005)

Discussion

This is the first controlled GH treatment study investigating body composition by DXA in a group of short children born SGA before and during GH treatment. We demonstrated that at start of the study lean body mass (LBM), total body fat and bone mineral density (BMD) of short SGA children were significantly lower compared to normal children.

'Leanness' is a very broad term often used in short SGA children indicating they are very thin. However, until now, this characteristic was mainly based on either a low BMI or a reduction in skinfold thickness (7). Leger et al investigated body composition of these children measuring fat and muscle mass on a cross-sectional area of the thigh by Magnetic Resonance Imaging (MRI) and found a reduction in both fat and muscle mass (6). However, this method does not measure total body muscle and total body fat. DXA has been described as a very precise method to investigate lean body mass (LBM), fat mass and bone mineral density (BMD) of the whole body using a low radiation dose (22). The present study, therefore, enables us to better specify the leanness of these children.

We found that at start of the study both LBM and total body fat were significantly lower compared to children of the same age and sex. Surprisingly, we did not detect any differences in either total body fat or LBM between short SGA children with or without GHD. This confirms the clinical observation that short SGA children with GHD do not show the typical GHD appearance of truncal obesity which is a well known feature of children with GHD. Apparently, in short SGA children who are GHD, SGA related leanness dominates their typical GHD appearance. These data also demonstrate that in SGA children the diagnosis of GHD based on GH provocative tests alone is not reliable to predict body composition typical for GHD.

Another interesting finding was the fact that at start of the study all short SGA children either with or without GHD, showed a more severe reduction in LBM than in total body fat. So, leanness in these short SGA children can be specified into a marked reduction in muscle mass and to a lesser extent a reduction in total body fat. Since our results were compared to healthy age- and sex matched children with a normal height, part of the reduction in LBM and fat mass in these short SGA children can be explained by their short stature.

Epidemiological studies have found that adults, who were born with a low birth weight, have an increased risk of developing type 2 diabetes mellitus, hypertension and cardiovascular diseases later in life (23-25). Although the precise mechanism underlying this association is not known, it is thought that hyperinsulinism and insulin resistance play an important role in the pathogenesis of type 2 diabetes mellitus and cardiovascular disease (26,27). The fetal program hypothesis supposes insulin resistance occurs as a result of undernutrition during a critical period in fetal life (28). During fetal malnutrition glucose is only available for growth of vital organs, such as the brain and the heart, at the expense of muscle mass. This could be an explanation for the

reduction in muscle mass, observed in short SGA children. In turn, the severely reduced muscle mass may play a role in the reduced insulin sensitivity which was found in a comparable group of short prepubertal children born SGA (29,30).

By calculating BMD an area density is obtained by dividing BMC (in gram) by the projected bone image (area, cm^2). This adjust for smaller body sizes and therefore partly corrects for the short stature of these children. At baseline we found a significant reduction in mean bone mineral density. This suggests that other factors besides height might also play an important role. It is well known that muscles form a large part of the load on bone and therefore have a stimulatory effect on bone mass. Indeed, we found a severe reduction in LBM at start of the study. We suggest that part of the reduction in BMD in short SGA children may be explained by a reduced muscle mass. Other possible causes may comprise low serum IGF-I levels and a low nutritional intake since most parents report a poor dietary intake.

GH treatment resulted in an impressive increase of LBM. In contrast, total body fat reduced during the first year of GH treatment and remained stable thereafter. These findings are comparable to the effects of GH treatment in GHD children and adults (5,31,32).

During GH treatment BMD increased significantly. This observed increment in BMD could be either a result of the increase in muscle mass, taller stature or a more direct effect of GH on bone. Furthermore, a better nutritional intake and increased physical activity during GH treatment, as was reported by parents, might also have played a role in the increase of BMD.

Both skinfold thickness and BMI are generally used tools for estimating body composition. In our study, we compared these methods to results obtained by DXA, which is considered as the 'gold standard'. Skinfold measurements assess subcutaneous fat only, in contrast to BMI, which estimates both total body fat and LBM. At start of the study the sum of 4 skinfolds correlated strongly with total body fat measured by DXA whereas BMI did not differentiate as it correlated both with total body fat and LBM. During GH treatment changes in skinfolds did correlate with changes in total body fat measured by DXA but changes in BMI neither correlated with changes in LBM nor with changes in total body fat. Our findings implicate that measuring skinfold thickness before and during GH treatment gives a good estimate of total body fat in short SGA children. Moreover, measuring skinfolds is cheap and easy to perform. Conversely, BMI can not distinguish between total body fat and LBM. For that reason BMI is not an accurate estimate of body composition, at least in short SGA children. In addition, BMI is not a useful tool to investigate changes in body composition during GH treatment.

In conclusion, leanness in short children born SGA can now be specified as a reduction in LBM and to a lesser extent in a reduction of total body fat. BMD was significantly reduced in these children. During GH treatment LBM and BMD increased significantly, whereas total body fat decreased during the first year and remained stable thereafter. Our study also indicates that, in contrast to BMI, measuring skinfold

thickness gives a reliable estimate of total body fat in short SGA children both before and during GH treatment.

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References

1. Albertsson-Wikland K, Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71.
2. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71.
3. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
4. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
5. Boot AM, Engels MA, Boerma GJ, Krenning EP, De Muinck Keizer-Schrama SM 1997 Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. J Clin Endocrinol Metab 82:2423-8.
6. Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. Journal of Clinical Endocrinology and Metabolism 78:904-909.
7. Sas T, Mulder P, Hokken-Koelega A 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-92.
8. Svendsen OL, Haarbo J, Hassager C, Christiansen C 1993 Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. Am J Clin Nutr 57:605-8.
9. Pintauro SJ, Nagy TR, Duthie CM, Goran MI 1996 Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. Am J Clin Nutr 63:293-8.
10. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
11. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23.
12. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-9.
13. Cameron N 1978 The methods of auxological anthropometry. In: Falkner F, Tanner JM (eds.) Human Growth, vol. 2. Tindall, London, pp 35-87.
14. Gerver WJ, de Bruin R 1996 Body composition in children based on anthropometric data. A presentation of normal values. Eur J Pediatr 155:870-6.
15. Cole TJ 1989 Using the LMS method to measure skewness in the NCHS and Dutch National height standards. Ann Hum Biol 16:407-19.
16. Johnson J, Dawson-Hughes B 1991 Precision and stability of dual-energy X-ray absorptiometry measurements. Calcif Tissue Int 49:174-8.
17. Boot AM, Bouquet J, de Ridder MA, Krenning EP, de Muinck Keizer-Schrama SM 1997 Determinants of body composition measured by dual-energy X-ray absorptiometry in Dutch children and adolescents. Am J Clin Nutr 66:232-8.
18. Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama SM 1997 Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. J Clin Endocrinol Metab 82:57-62.
19. Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. J Clin Endocrinol Metab 71:688-95.
20. Martin JL, Baxter RC 1986 Insulin-like growth factor-binding protein from human plasma. Purification and characterization. J Biol Chem 261:8754-60.
21. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-76.

22. Favus MJ, editor 1996 Primer on the metabolic bone disease and disorders of mineral metabolism., 3 ed. Lippincott-Raven, Philadelphia.
23. Barker DJ 1997 Intrauterine programming of coronary heart disease and stroke. Acta Paediatr Suppl 423:178-82; discussion 183.
24. Barker DJ, Bull AR, Osmond C, Simmonds SJ 1990 Fetal and placental size and risk of hypertension in adult life. Bmj 301:259-62.
25. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-7.
26. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-607.
27. Ferrannini E, Haffner SM, Mitchell BD, Stern MP 1991 Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 34:416-22.
28. Barker DJ 1997 The fetal origins of coronary heart disease. Acta Paediatr Suppl 422:78-82.
29. Arends NJT, Boonstra VH, Cutfield WS, Hokken-Koelega ACS 2002 Insulin sensitivity and other risk factors for syndrome X in short prepubertal children born small for gestational age (SGA). Clin Endocrinol (in press).
30. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82:402-6.
31. Baum HB, Biller BM, Finkelstein JS, Cannistraro KB, Oppenhein DS, Schoenfeld DA, Michel TH, Wittink H, Klibanski A 1996 Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. Ann Intern Med 125:883-90.
32. Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3- year treatment period and after 1 year's withdrawal. J Clin Endocrinol Metab 83:3512-6.

Head circumference and body proportions before and during GH treatment in short children born small for gestational age (SGA)

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Abstract

Although short SGA children appear to have normal body proportions, objective data both before and during GH treatment are very limited. Therefore we investigated in a large group of short children born small for gestational age (SGA), the effects of GH treatment versus no treatment on head circumference and body proportions. Furthermore, we studied differences in linear growth and head circumference between SGA children born with a low birth length and birth weight (SGA_{L+W}) and SGA children born with a low birth length only (SGA_L).

The present study is an open-labelled, GH-controlled, multicenter study for 3 years. Non-growth hormone deficient short SGA children (n=87), with a mean (SD) age of 5.9 (1.5) years, were randomised to either a GH-group (n=61), receiving GH in a dose of 33 μg/kg/day, or an untreated control group (n=26). Height, weight, HC, sitting height, armspan and hand, tibial and foot size were measured and expressed as SDS adjusting for sex and age.

At baseline all anthropometric measurements, except HC SDS, were significantly lower than –2 SDS. During GH treatment all anthropometric measurements normalised in accordance to the normalisation of height SDS. At start of the study, mean HC SDS was significantly lower in SGA_{L+W} children compared to SGA_L children (p=0.006). Interestingly, most children (14 out of 16) with a HC SDS below –2.00 had been born SGA_{L+W}. During GH treatment, the 3-year increase in height, HC and other anthropometric measurements was comparable between SGA_{L+W} and SGA_L children. In both SGA_{L+W} and SGA_L controls, no changes in SD-scores of height, HC and other anthropometric measurements were found during the 3-year follow-up period.

Untreated short SGA children have normal body proportions with the exception of HC which is relatively large in many of these children. SGA_{L+W} children still had a smaller HC at the age of 5.9 years compared to SGA_L children. Three years of GH treatment induced a proportionate growth resulting in a normalization of height and other anthropometric measurements, including HC, in contrast to untreated SGA controls.

Introduction

Short stature occurs in 10-15 % of the children who were born small for gestational age (SGA) (1,2). The pathophysiology underlying this insufficient catch-up growth is still unknown but disturbances in the growth hormone (GH)/insulin-like growth factor-I (IGF-I) axis may play a role (3,4).

Several studies have shown beneficial effects of growth hormone (GH) treatment on height in these short children (5-7). GH treatment resulted in a significant increase in height to values within the normal range followed by growth along the target height (TH) percentile.

Although short children born SGA appear to have normal body proportions, objective data are limited. Sas et al reported in a group of short SGA children a normalisation of body proportions during GH treatment with either a dose of 1 or 2 mg/m^2 body surface area/day (8). Since this study did not comprise an untreated control group, a causal effect of GH treatment on the normalisation of body proportions could not be proven. There are no data on longitudinal changes of body proportions in short untreated SGA children.

In most GH-trials in short SGA children SGA is defined on either birth length and birth weight alone or both. None of the GH trials have investigated differential effects of GH on growth and hormonal changes in those SGA children who have been either born with both a low birth length and a low birth weight (SGA_{L+W}) or those born with a low birth length only (SGA_L). It is generally thought that in those born SGA_{L+W} growth restriction occurred early in gestation while in SGA_L newborns this occurred later in gestation. It has been reported that during childhood SGA children have, in general, a smaller head circumference compared to AGA children (9,10). No data are available on head circumference in short SGA children before and during GH treatment.

We present the results of a randomised, 3-year controlled GH-trial studying head circumference and body proportions in short SGA children at baseline and during 3 years of GH treatment in comparison with results of untreated short SGA children. Furthermore, we studied linear growth and head circumference in SGA_{L+W} versus SGA_L children. Our study is the first one differentiating between SGA children who had been proportionately (SGA_{L+W}) or disproportionately (SGA_L) small at birth.

Patients and methods

Subjects

The study comprised 91 Dutch children (43 boys and 48 girls) with short stature born SGA. All children fulfilled the same inclusion criteria: 1) birth length standard deviation score (SDS) below – 2.00 for gestational age (11); 2) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia; 3) chronological age (CA) between 3.00 and 7.99 years at start of the study; 4) height SDS for age below –2.00 according to Dutch standards (12); 5) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth; 6) prepubertal, defined as Tanner stage 1 or a testicular volume < 4 (13); 7) sufficient GH response to a GH-stimulation test with either clonidine or arginine, which was defined as a GH peak above 10 μg/l; 8) normal liver, kidney and thyroid functions. Children with endocrine or metabolic disorders, chromosomal defects and growth failure caused by other syndromes (e.g. emotional deprivation, Turner syndrome, severe chronic illness, chondrodysplasia), with the exception of Silver-Russell syndrome were excluded.

The study was approved by the Ethics Committees of all nine participating centers. Written informed consent was obtained from the parents or custodians of each child.

Study design

The study design is an open-labelled multicenter study with a randomised control group. All children (n=91) were stratified according to age (3.00-5.50 versus 5.50-7.99) and height of the parents (height of both parents above –2.00 SDS versus height of at least one parent below –2.00 SDS). After stratification the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group (n=64; 27 boys and 37 girls) started immediately with GH treatment at a dose of 1 mg/m^2 body surface area/day (~33 μg/kg/day). The control group (n=27; 16 boys and 11 girls) remained untreated for 3 years and subsequently received the same GH treatment as the GH-group. Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

Anthropometric measurements

Standing height was measured 3-monthly by two trained investigators (NA, later VB) using a Harpenden stadiometer and values were expressed as SD-score for sex and chronological age (height SDS) using Dutch references (12). Target height (TH) was calculated using Dutch reference data according to the formula:1/2 * (H_{father} + H_{mother} + 13) + 4.5 for boys and 1/2 * (H_{father} + H_{mother} - 13) + 4.5 for girls, where the addition of 4.5 cm represents the secular trend. TH was expressed as SD-

score using Dutch references (12). Weight for height was used as a measure of body composition since body mass index (BMI) is not an accurate estimate of body composition during GH treatment in short SGA children (Arends et al, submitted). WH was expressed as SD-score for sex and age (12). Head circumference (HC) was measured 3-monthly and expressed as SD-score for sex and age (12).
Sitting height (SH) was measured every 6 months using a Harpenden sitting height table. Every 6 months armspan (armspan), the length of the left hand (hand), left foot (foot) and left tibia (tibia) were measured by the same investigators using a Harpenden anthropometer. All measurements were expressed as SD-score (SDS) adjusting for sex and age. Reference data were available from the Dutch Oosterwolde study which consisted of 1240 healthy boys and 1093 healthy girls (14).

Definition of SGA_{L+W} and SGA_{L}

SGA_{L+W} was defined as a birth length and a birth weight ≤ -2.00 SDS for gestational age (1,11). SGA_{L} was defined as a birth length ≤ -2.00 and a birth weight > -2.00 SDS for gestational age.

Statistics

Of 91 children 4 children dropped out of the study for the following reasons: one child was very disappointed she was randomized into the control group, 2 children had psychological problems with the daily GH injections and in 1 child coeliac disease was diagnosed. Since these children dropped out either at start or during the first year of the study, these children were excluded from baseline and 3-year analysis. Therefore, 87 children were included for statistical analysis.
During the 3-year study period, puberty started in 4 children of the GH-group and in 2 children of the control group. Analysis was performed in prepubertal children only. As soon as a child entered puberty he or she was excluded from further analysis. Data were expressed as the mean plus or minus the standard deviation (SD). SD-scores were compared with zero using Student's one sample t-test. Differences between groups were tested using independent Student's t-tests. Differences in 1-year changes between the groups were tested using analysis of covariance and differences between points in time within the groups were tested by paired Student's t-tests. Spearman's correlation coefficient was used for correlations. Statistical significance was defined as $p < 0.05$. Statistical tests were performed with use of SPSS package (version 10.0).

Results

Table 1 shows the baseline characteristics of the total, the GH- and the control group. At baseline no significant differences were found between the 2 groups. Thirteen children had Silver-Russell syndrome. Results of children with SRS did not differ from results of children without signs of SRS (data not shown).

At baseline no significant differences in anthropometric measurements were found between the 2 groups (Table 2). All anthropometric measurements, except HC SDS, were significantly lower compared to –2 SDS. Remarkably, HC SDS was the least affected part of the body and was not significantly lower than –2.00 SDS. Sixteen of 87 children (18 %) had a HC SDS ≤ -2.00. Height SDS, SH SDS and foot SDS were most severely reduced. Height SDS correlated significantly with SH ($r=0.6$; $p<0.001$), foot ($r=0.6$; $p<0.001$), tibia ($r=0.7$; $p<0.001$) and armspan ($r=0.5$; $p<0.001$). However, height SDS did neither correlate with hand nor with HC SDS.

During GH treatment the size of the measured body parts expressed as SDS showed an increment towards zero (Figure 1). After 2 years of GH treatment, the mean height, SH and foot SDS had normalised (i.e. >-2.00 SDS), whereas the mean hand, tibial and armspan had normalised after 1 year of GH treatment. In contrast, anthropometric measurements of the untreated control group remained unchanged or showed only a slight increase during the study period. In the GH-group, 3-year changes in height correlated significantly with changes in SH ($r=0.5$; $p<0.001$), foot ($r=0.5$; $p<0.001$), tibia ($r=0.5$; $p<0.001$), armspan ($r=0.3$; $p<0.03$) and HC ($r=0.5$; $p<0.001$), but not with changes in hand SDS.

SGA_{L+W} versus SGA_L

SGA_{L+W} children had significantly lower values for gestational age, birth length SDS, birth weight SDS and birth HC SDS compared to SGA_L children (Table 3). Interestingly, at baseline, at a mean (SD) age of 5.9 (1.5) years, mean height SDS had become comparable for SGA_{L+W} and SGA_L children but the mean HC SDS was still significantly lower in SGA_{L+W} children compared to SGA_L children (-1.3 (0.8) versus –0.8 (0.9), $p=0.006$). At baseline, 14 out of 55 SGA_{L+W} children had a HC SDS ≤ -2.0 in contrast to 2 out of 32 SGA_L children (25 % versus 6 %; $p<0.001$). Also, WH SDS was significantly lower in SGA_{L+W} children (-1.8 (1.0) versus –0.8 (0.9), $p<0.001$). Three years of GH treatment induced a significant increase in height, weight and HC SDS which was similar for SGA_{L+W} and SGA_L children. HC SDS, however, remained still lower in SGA_{L+W} children compared to SGA_L children after 3 years of GH treatment, albeit the difference did not reach level of significance. The 3-year change in HC SDS correlated negatively with age at start of GH treatment in both SGA_{L+W} and SGA_L children ($r=0.4$; $p=0.01$ and $r=0.7$; $p<0.001$). Thus, the younger they started GH treatment the greater the increase in HC SDS. After 3 years of GH treatment WH SDS was still significantly lower in the SGA_{L+W} group compared to the SGA_L group.

In SGA_{L+W} and SGA_{L} controls no changes in height, weight and HC SDS were found during the 3-year follow-up period.
The SD-scores for SH, hand, tibia, foot and armspan did not differ between SGA_{L+W} and SGA_{L} children, neither at start of the study nor after 3 years of GH treatment (data not shown).

Table 1
Clinical data of the total group (n=87)

	Total group n=87	GH group n=61	Control group n=26
Boys / girls	41 / 46	25 / 36	16 / 10
Gestational age (wks)	36.1 (3.7)	36.1 (3.9)	36.0 (3.6)
Birth length SDS	-3.5 (1.4)#	-3.4 (1.5)#	-3.1 (1.3)#
Birth weight SDS	-2.4 (1.2)#	-2.3 (1.2)†	-2.7 (1.0)†
Birth HC SDS	-1.8 (1.4)*	-1.7 (1.5)*	-1.9 (1.4)*
Chronological age (yr)	5.9 (1.5)	6.0 (1.6)	5.9 (1.5)
TH SDS	-0.5 (0.8)*	-0.5 (0.8)*	-0.6 (0.7)*
Silver Russell syndrome (n)	11	6	5

All values are expressed as mean (SD) or number

* = *significantly lower than zero (p<0.001)*
\# = *significantly lower than –2.00 SDS (p<0.001)*
† = *significantly lower than –2.00 SDS (p<0.05)*

Table 2
Baseline anthropometric measurements of the total group (n=87).

	Total group n=87	GH group n=61	Control group n=26
Height SDS	-3.1 (0.6)#	-3.0 (0.6)#	-3.2 (0.5)#
SH SDS	-3.0 (0.7)#	-3.0 (0.8)#	-3.0 (0.6)#
Foot SDS	-3.0 (0.7)#	-3.0 (0.7)#	-3.1 (0.8)#
Armspan SDS	-2.5 (0.7)#	-2.4 (0.7)#	-2.6 (0.7)#
Tibia SDS	-2.3 (0.6)#	-2.2 (0.6)†	-2.3 (0.6)†
Hand SDS	-2.2 (0.7)†	-2.2 (0.7)*	-2.2 (0.7)*
HC SDS	-1.0 (0.8)*	-0.9 (0.9)*	-1.1 (0.8)*

All values are expressed as mean (SD)
* = *significantly lower than zero (p<0.001)*
\# = *significantly lower than –2.00 SDS (p<0.001)*
† = *significantly lower than –2.00 SDS (p<0.05)*

Figure 1.

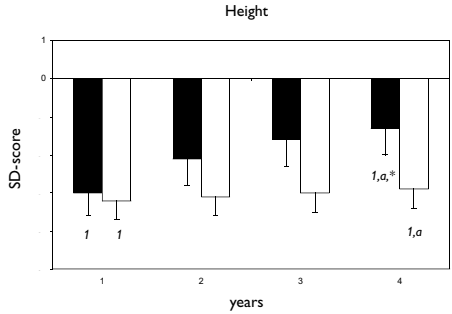
Changes in height (a), SH (b), armspan (c), hand (d), tibia (e) foot (f) and HC (g) of the GH- (black bars) and the untreated control group (open bars).

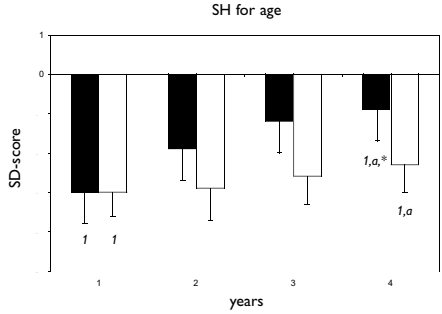
1 = significantly different from zero (p<0.001)

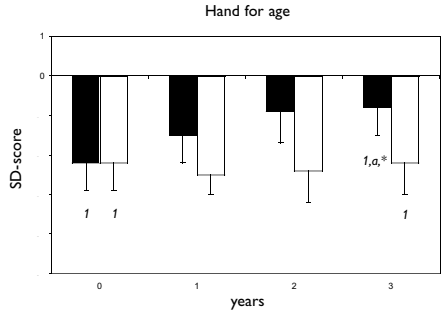
a = significantly different towards baseline value (p<0.001)

b = significantly different towards baseline value (p<0.01)

** = significantly different between the GH- and the control group (p<0.001)*






Hand for age
SD-score
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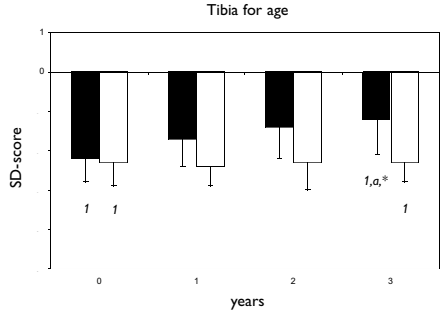
Tibia for age
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Foot for age
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Head circumference for age
SD-score
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Table 3

Gestational age and anthropometric measurements in SGA_{L+W} and SGA_L children who were either treated with GH (GH-group) or untreated (controls).

	SGA_{L+W}		SGA_L	
	GH-group	Controls	GH-group	Controls
	n = 35	n = 20	n = 26	n = 6
At birth				
Gestational age (wks)	34.7 (4.1)*	35.3 (3.5)	37.9 (2.5)	38.5 (2.6)
Birth length SDS	-4.3 (1.4)*	-3.5 (1.3)†	-2.2 (0.6)	-1.9 (0.6)
Birth weight SDS	-3.2 (0.7)*	-3.0 (0.7)*	-1.2 (0.8)	-1.4 (0.6)
Birth HC SDS	-2.4 (1.5)*	-2.2 (1.1)	-0.9 (1.0)	-0.9 (1.9)
At start of study				
Age	5.7 (1.7)	5.7 (1.6)	6.3 (1.3)	6.7 (0.9)
Height SDS	-3.0 (0.7)	-3.2 (0.6)	-3.0 (0.6)	-3.1 (0.4)
Weight SDS	-3.4 (1.0)†	-3.2 (0.9)$^{\#}$	-2.7 (0.6)	-2.5 (0.5)
HC SDS	-1.3 (0.8)$^{\#}$	-1.3 (0.9)	-0.8 (1.0)	-0.9 (0.6)
WH SDS	-2.0 (1.0)*	-1.5 (1.1)$^{\#}$	-0.9 (0.8)	-0.4 (0.8)
After 3 yrs				
Height SDS	-1.2 (0.7)^{1}	-2.9 (0.6)	-1.4 (0.8)^{1}	-2.9 (0.4)
Weight SDS	-1.5 (1.0)^{1}	-3.0 (1.1)	-1.1 (0.6)^{2}	-2.1 (0.7)
HC SDS	-0.7 (0.9)^{3}	-1.3 (0.8)	-0.3 (1.0)^{3}	-1.2 (0.5)
WH SDS	-0.9 (1.1)†	-1.1 (1.1)$^{\#}$	-0.2 (0.8)	0.2 (1.2)
3-year changes in				
Height SDS	1.8 (0.4)^{1}	0.3 (0.3)	1.6 (0.4) ^{1}	0.2 (0.3)
Weight SDS	1.9 (0.8)$^{\#1}$	0.2 (0.4)	1.6 (0.4) ^{1}	0.4 (0.7)
HC SDS	0.6 (0.5)^{1}	0.0 (0.3)	0.5 (0.5) ^{1}	-0.3 (0.2)
WH SDS	1.0 (1.0)^{3}	0.4 (0.5)	0.7 (0.5)	0.6 (0.8)

SGA_{L+W}: birth length and birth weight ≤-2.00
SGA_L: birth length ≤ -2.00 and birth weight > -2.00

Differences between SGA_{L+W} and SGA_L children (in the GH- and control group)
(* = p ≤ 0.001; † = p ≤ 0.01; $^{\#}$ = p<0.05)
Differences between the GH- and control group (in SGA_{L+W} and SGA_L children)
(^{1} = p ≤ 0.001; ^{2} = p ≤ 0.01; ^{3} = p<0.05)

Discussion

Short children born SGA show a reduced size of their sitting height (SH), armspan, tibial-, foot- and hand size, which is in proportion to their reduced height. Children who were treated with GH for 3 years had a normalisation of their height in contrast to children who remained untreated for 3 years. In the GH-treated children sitting height, armspan, tibial, foot and hand SDS increased in concordance with their increase in height SDS indicating that body proportions remained normal during 3 years of GH treatment. Conversely, height and other anthropometric measurements remained unchanged in the untreated control group. Therefore, the present study now demonstrates that the observed changes in various body parts of GH-treated children are the result of GH treatment. Our results agree with previous findings in a comparable group of short SGA children (8). In that study, however, all children received GH treatment and therefore no conclusions could be drawn concerning a causal effect of GH treatment on changes in body proportions.

Interestingly, head circumference (HC) of the total group of short SGA children was less affected. While height and all other anthropometric measurements showed a mean value which was significantly less than –2.0 SDS, the mean HC SDS was the only parameter showing a value above –2.0 SDS. Of a total of 87 short SGA children, only sixteen (18 %) had a HC SDS below the normal range, i.e. below –2.0 SDS at a mean (SD) age of 5.9 (1.5) years. Interestingly, most children (14 out of 16) with a HC SDS below –2.00 had been proportionately small at birth (SGA_{L+W}). Although the magnitude of spontaneous catch-up growth after birth in HC had been greater in SGA_{L+W} children compared to SGA_{L} children (+1.0 (1.2) vs 0.0 (1.1); $p<0.001$), HC SDS was still significantly lower in SGA_{L+W} children at a mean age of 5.9 years. This finding strongly suggests that short SGA children who have been proportionately small at birth have a greater risk for a relatively small HC during childhood.

During GH treatment HC SDS increased similarly in both SGA_{L+W} and SGA_{L} children. Since at start of the study HC SDS was significantly smaller in SGA_{L+W} children, these children still had a smaller HC after 3 years of GH treatment. Apparently, GH treatment has a similar effect on HC in both SGA_{L+W} and SGA_{L} children. In contrast, those remaining untreated for 3 years either being born as SGA_{L+W} or $SGA_{L,}$ had no gain in HC SDS during the 3-year study period. Thus, GH treatment induces a significant increase in HC SDS in both SGA_{L+W} and SGA_{L} children.

There has been some debate about the effects of being born SGA on intelligence and behaviour (15). Recently 2 large population-based studies showed lower school performances in 16- and 20 year olds who were born SGA (10,16). It has also been reported that a lower intelligence was associated with a smaller HC in SGA children at various ages (9,17). Lundgren et al found an important association between both birth length and persistent short stature and subnormal intellectual

performances (17). The first Dutch SGA study investigated psychological functioning in a group of short SGA children who had similar inclusion criteria as the present study (18). This study showed a significant reduction in intelligence and attention and an increase in behavioural and emotional problems. Both intelligence and attention were significantly associated with HC during childhood. Our present study shows that at a mean age of 5.9 years, short SGA_{L+W} children have smaller head circumferences than SGA_{L} children. These findings suggest that SGA_{L+W} children might have a higher risk for lower school achievements and psychological problems. Unfortunately, our present study did not include intelligence tests.

Since GH treatment results in an increase in HC and HC is associated with intelligence, we may speculate that GH treatment could have a positive influence on intellectual performances of short SGA children. Van der Reijden et al did perform intelligence tests in short SGA children before and after 2 years of GH treatment and did find a significant increase in Intelligence Quotient (IQ) after 2 years (18). Unfortunately this study did not include an untreated control group. Our study shows that the gain in height HC SDS during GH treatment correlated negatively with age at start. Thus, if GH treatment would stimulate intellectual and psychological development of short SGA children, it may be important to start treatment at a young age. This would especially apply for SGA children who have been proportionately small at birth since they are particularly at risk for a small HC during childhood.

Gestational age of SGA_{L+W} children was significantly shorter than that of SGA_{L} children. Despite their shorter gestational age, their birth length, birth weight and birth HC were more severely reduced suggesting that growth retardation was more severe or occurred rather early during pregnancy. Possibly, in these SGA_{L+W} children genetic causes could play a role. Recently, we investigated whether the IGF-I gene was involved in the phenotype of short children born SGA (19). In a large group of SGA_{L+W} children allele 191 of the IGF1.PC1 marker, located between exon 2 and 3 of the IGF-I gene, was transmitted more often from parent to child than one would expect based on Mendelian frequencies. Children carrying this allele had significantly lower serum IGF-I levels and had a smaller HC at the age of 1.3 years compared to children not carrying this allele. Thus, this functional IGF-I polymorphism might play a role in short SGA children who have been born proportionately small. Woods et al described severe pre- and postnatal growth failure as well as mental retardation in a child with a partial deletion of the IGF-I gene (20). These studies suggest that IGF-I plays not only an important role in somatic growth but might also be involved in the development of neuropsychological functioning. Further research is required to investigate the role of GH treatment on the increase in HC and its effect on neuropsychological functioning in SGA children.

In conclusion, untreated short SGA children have generally normal body proportions. Besides height, the sizes of sitting height, armspan, tibial, foot and hand are all significantly lower than –2.0 SDS. In contrast, head circumference is less severely affected and therefore relatively large in these children (-1.1 SDS). Those who had

been born SGA_{L+W}, still had a smaller HC at the age of 5.9 years compared to those who had been born SGA_{L}. Three years of GH treatment induced a proportionate growth resulting into normalization of both height and other anthropometric measurements, including HC.

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References

1. Albertsson-Wikland K, Karlberg J 1994 Natural growth in children born small for gestational age with and without catch-up growth. Acta Paediatr Suppl 399:64-70; discussion 71.
2. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL 1995 Children born small for gestational age: do they catch up? Pediatr Res 38:267-71.
3. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
4. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
5. Boguszewski M, Albertsson-Wikland K, Aronsson S, Gustafsson J, Hagenas L, Westgren U, Westphal O, Lipsanen-Nyman M, Sipila I, Gellert P, Muller J, Madsen B 1998 Growth hormone treatment of short children born small-for-gestational- age: the Nordic Multicentre Trial. Acta Paediatr 87:257-63.
6. de Zegher F, Du Caju MV, Heinrichs C, Maes M, De Schepper J, Craen M, Vanweser K, Malvaux P, Rosenfeld RG 1999 Early, discontinuous, high dose growth hormone treatment to normalize height and weight of short children born small for gestational age: results over 6 years. J Clin Endocrinol Metab 84:1558-61.
7. Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose- response trial. J Clin Endocrinol Metab 84:3064-70.
8. Sas TC, Gerver WJ, De Bruin R, Mulder PG, Cole TJ, De Waal W, Hokken-Koelega AC 2000 Body proportions during 6 years of GH treatment in children with short stature born small for gestational age participating in a randomised, double-blind, dose-response trial. Clin Endocrinol (Oxf) 53:675-81.
9. Ounsted M, Moar VA, Scott A 1988 Head circumference and developmental ability at the age of seven years. Acta Paediatr Scand 77:374-9.
10. Strauss RS, Dietz WH 1998 Growth and development of term children born with low birth weight: effects of genetic and environmental factors. J Pediatr 133:67-72.
11. Usher R, McLean F 1969 Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J Pediatr 74:901-10.
12. Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM 2000 Continuing positive secular growth change in The Netherlands 1955-1997. Pediatr Res 47:316-23.
13. Tanner JM, Whitehouse RH 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 51:170-9.
14. Gerver WJM, De Bruin R 1996 Paediatric Morphometrics: A reference manual. Bunge, Utrecht.
15. Grantham-McGregor SM 1998 Small for gestational age, term babies, in the first six years of life. Eur J Clin Nutr 52 Suppl 1:S59-64.
16. Larroque B, Bertrais S, Czernichow P, Leger J 2001 School difficulties in 20-year-olds who were born small for gestational age at term in a regional cohort study. Pediatrics 108:111-5.
17. Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T 2001 Intellectual and psychological performance in males born small for gestational age with and without catch-up growth. Pediatr Res 50:91-6.
18. van der Reijden-Lakeman I 1996 Growing pains Thesis. Erasmus University Rotterdam, Rotterdam.
19. Arends N, Johnston L, Hokken-Koelega A, Duijn Cv C, Ridder Md M, Savage M, Clark A 2002 Polymorphism in the IGF-I Gene: Clinical Relevance for Short Children Born Small for Gestational Age (SGA). J Clin Endocrinol Metab 87:2720.
20. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ 1996 Intrauterine growth retardation and post-natal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335:1363-7.

General Discussion

General Discussion

The present thesis describes several aspects of a large cohort of short children born small for gestational age (SGA). The first part of this thesis tried to unravel some of the late consequences (insulin insensitivity) as well as etiological aspects of being born SGA. The latter consisting of genetical aspects and morphological details of the pituitary region.

The second part of this thesis contains the 3-year results of the randomised, open-labelled, controlled GH study. This study was started in October 1996 and patients were included during the following 2 years. Nine different centers participated in the study and 99 children were included. The effects of GH treatment versus no treatment on growth, bone maturation, bone mineral density, body composition and body proportions were investigated.

In this chapter our findings are discussed regarding our present knowledge in view of the current literature.

Late consequences of being born SGA

Insulin sensitivity

We found that in short SGA children insulin sensitivity (Si) was reduced to only 38 % of the levels found in the short AGA controls. As expected the acute insulin response (AIR) was nearly three times higher in short SGA children compared to the AGA controls, as compensation for this reduction in Si. In normal subjects a reciprocal relationship exists between insulin action and insulin secretion by the pancreatic β-cell (1). If insulin sensitivity decreases, β-cells will secrete larger amounts of insulin in order to maintain a normal glucose metabolism. If the β-cells fail to adapt or fail to maintain the secretion of large amounts of insulin, the risk of developing type 2 diabetes mellitus increases. Our results show that short prepubertal SGA children maintained a normal glucose tolerance due to a compensatory increase in insulin secretion. Since these abnormalities are found in young children it can be questioned how long it will take before type 2 diabetes will become overt. This important question can only be answered during long-term follow-up. Glucose effectiveness, the ability of glucose to enhance its own peripheral uptake and reduce its endogenous production in the liver independent of insulin, was not significantly different between the short SGA and AGA children. Our findings agree with results described by Hofman et al., who also found a reduced insulin sensitivity in a group of 15 short prepubertal SGA children (2). Our results are also comparable to those found in a group of young adults who were born SGA.(3). In the present study, glucose intolerance was already subclinically present in one SGA child (4 %). This finding is comparable to the results described by Sas et al, who found impaired glucose tolerance during an OGTT in 8 % of another group of 79 short prepubertal SGA children (4).

Leanness is a typical feature of short prepubertal SGA children (5). It is well known that increasing leanness is associated with a dramatic increase in insulin sensitivity (6). In contrast to what is to be expected in lean individuals, insulin sensitivity in prepubertal SGA children was significantly reduced. Johnson et al investigated longitudinal (annual) changes in fat mass, measured by Dual Energy X-ray Absorptiometry (DEXA), and insulin sensitivity in a cohort of healthy children. They found that a low insulin sensitivity at a young age was followed by a higher increase in fat mass over years, independent of sex and pubertal stage (7). This might implicate that lean and short SGA children with a low Si are at an increased risk of becoming obese in early or later adulthood. Since a high BMI is one of the features of the metabolic syndrome, the observed reduction in insulin sensitivity in SGA children may precede the changes in body composition occurring in later life. When these children would develop overweight, it would worsen their underlying reduced Si, thereby strongly increasing the risk of type 2 diabetes mellitus.

Short prepubertal children born SGA have asymptomatically a severe reduction in insulin sensitivity despite a lean appearance.

Blood pressure

Our data showed a significantly higher systolic BP in short SGA children compared to age- and height-matched children (8). In contrast, diastolic BP was not significantly different from age- and height-matched children. These data are consistent with previous data in another group of short SGA children reported by Sas et al., in which systolic blood pressure was also elevated to levels in the high-normal range (9). Hypertension in adult life has been associated with a low birth weight (10,11). Also, studies investigating blood pressure during childhood and early adulthood have shown a significant inverse relationship between systolic blood pressure and birth weight (12,13). Not much is known about the etiology of this relationship. Barker et al postulated that changes in hemodynamic load during fetal life may alter arterial structure leading to related to hypertension later in life (10). Brenner et al. hypothesized that poor nephron development could be a link between low birth weight and hypertension later in life (14). Fetal growth retardation in rats will lead to smaller kidneys and a reduced number of nephrons (15). Impaired renal development would result in a reduction of the glomerular filtration surface area. This in turn leads to systemic and glomerular hypertension later in life. As a result glomerular sclerosis could occur and even worsen the situation by further reducing glomerular filtration surface area leading to a vicious circle. Since there is evidence that blood pressure is tracking from childhood to adulthood (16), our results might indicate that short children born SGA are at increased risk of becoming hypertensive in later life. It has been reported that a high systolic BP is more important in the pathogenesis of cardiovascular disease than a high diastolic BP (17). Therefore, short children born SGA need regular evaluation of their blood pressure during childhood and adulthood.

During childhood short SGA children have a significantly increased systolic blood pressure.

Serum lipids

Fasting serum levels of total cholesterol, triglycerides, LDL and HDL-cholesterol were all within the normal range. These findings are in agreement with previous reports (9,18). Our study is the first one investigating fasting serum FFA levels in SGA children. We found that the mean fasting serum FFA level was in the high-normal range, with 6 children (21 %) having levels above the normal range. Recently, an important role was attributed to FFA in the pathogenesis of type 2 diabetes (19). Two peptides play an important role in the FFA metabolism. Acylation stimulating protein (ASP) stimulates FFA uptake by peripheral cells and esterification into TG (20). If ASP activity is low, serum FFA level will increase. Sniderman et al. found a defect in the ASP pathway leading to an ineffective FFA storage which in turn will result in insulin resistance and obesity (21). Thus, a reduction in ASP activity might play a role in the increased serum FFA levels in these children. Another possibility is an increased activity of hormone sensitive lipase (HSL). HSL is responsible for hydrolysis of TG, the rate limiting step in the breakdown of TG in adipocytes (22). A raised activity of HSL will

result in an increased breakdown of TG followed by an increase in serum FFA levels. In normal subjects HSL is deactivated by insulin when energy supply is sufficient (23). If suppression by insulin fails, HSL activity increases which in turn may result in a rise in serum FFA levels. Several studies postulated that the HSL gene is involved in type 2 diabetes (24-26). Further research is therefore required to study FFA metabolism in larger groups of SGA children.

Serum lipids, i.e. total cholesterol, triglycerides, LDL and HDL-cholesterol were all within the normal range. However, 6 children (21%) had serum FFA levels above the normal range. We hypothesize that FFA in short SGA children might play a role in the development of type 2 diabetes later in life.

Clustering

The present study investigates insulin sensitivity in relation to cardiovascular risk factors in a group of young children born SGA with short stature. Studies in healthy children have revealed that insulin sensitivity and fasting insulin levels were related to blood pressure, serum lipids and obesity (27-29). Epidemiological studies have also shown that clustering of fasting insulin and risk factors for cardiovascular disease was already present during childhood and early adulthood (30,31). Since insulin sensitivity, BMI, blood pressure and serum lipids are known to correlate with one another it is not correct to investigate correlation coefficients between only two variables. In order to investigate the underlying relationship between all variables we performed a cluster analysis. We found two important clusters. The first cluster clearly showed that insulin insensitivity (1/Si) was related to higher systolic and diastolic blood pressure and higher serum FFA levels. This is in concordance with previous epidemiological studies in children and adults reporting an association between low insulin sensitivity and cardiovascular risk factors (30,32-34). In contrast to previous reports, we found a second cluster, consisting of BMI, total cholesterol, LDL-cholesterol and triglycerides. This second cluster comprised parameters which are known to be closely related to obesity. Since our study group was far from obese it is not surprising that we did not find clustering of BMI with insulin insensitivity (1/Si). Our findings may imply that a reduction in insulin sensitivity together with a slight increase in serum FFA levels and systolic blood pressure are the first abnormalities in the development of the metabolic syndrome. Our study shows that short SGA children who are generally lean show signs of pre-diabetes mellitus type 2 which may become overt when they become overweighed. For that reason it seems particularly important to prevent overweight in children born SGA.

Although the metabolic syndrome is an adult disease, our study shows that risk factors for the development of type 2 diabetes mellitus and cardiovascular diseases are already present during childhood in short children born SGA who are far from obese.

The metabolic syndrome in SGA infants with spontaneous catch-up growth

Since a lot of data regarding the association between diabetes mellitus type 2, cardiovascular risk factors and low birth weight are based on epidemiological studies we have to be careful when extrapolating these results to our study population. A lot of these studies did not differentiate between individuals with and without postnatal catch-up growth. The present study comprises SGA children without postnatal catch-up growth during childhood. Results of studies investigating risk factors for type 2 diabetes mellitus and cardiovascular disease in SGA individuals with catch-up growth are rather conflicting. A Swedish study found an increased risk of high systolic blood pressure in men born SGA, especially if they remained short (35). Postnatal catch-up growth in height did not further increase this risk in those were born short only (SGA_L). In those who were born both light and short (SGA_{L+W}), postnatal catch-up growth in height reduced the risk of high systolic blood pressure. Also, linear catch-up growth in height reduced the risk of overweight in these men born SGA. The effects of fetal and childhood growth on type 2 diabetes and hypertension in adult life were also investigated in a Finnish population study. They found that type 2 diabetes was associated with a low birth weight, a short birth length and thinness at birth, followed by catch-up growth after birth and accelerated growth in both height and weight to above average after the age of 7 years (36). Also, they found an association between hypertension and low birth weight, short body length and thinness at birth followed by catch-up growth from birth until the age of 7 years in both height and weight up to average levels (37). The disadvantage of this study is that patients were selected by disease, i.e. by having either hypertension or type 2 diabetes mellitus. Also, no growth data were shown from birth until adulthood but only from 7 until 15 years of age, a growth period were puberty plays a major role.

Thus, it seems that spontaneous catch-up growth in height only, does not increase the risk for the development of hypertension and type 2 diabetes mellitus.

Catch-up growth in weight for height, however, may increase the risk for these adult diseases.

Further research is needed to investigate the effects of postnatal catch-up growth on the development of type 2 diabetes mellitus and cardiovascular disease in individuals born SGA.

Genetic research in short SGA children

IGF-I gene in relation to growth

Several causes of being born SGA are summarized in the Introduction (Table 1, page 13). However, in most cases the cause remains unclear. GH deficiency (GHD) does not seem to play a major role as in the present study only 12 out of 99 short SGA children had GHD. In addition, classical GHD usually does not result in birth weights below –2 SDS. Since IGF-I seems to play an important role in both pre- and postnatal

growth we hypothesized that IGF-I could play an important role in these short SGA children. From studies in knock-out mice we know that the IGF-I gene plays an important role in both pre-and postnatal growth (38,39). Also in humans IGF-I is an important factor for normal prenatal and postnatal growth, development and metabolism. Several studies have shown a positive relationship between serum IGF-I levels and size at birth (40-43). It is highly remarkable that in humans only one case has been reported describing a homozygous deletion of exon 4 and 5 of the IGF-I gene in a 15-year old boy (44). This child was severely growth retarded both pre- and postnatally. We investigated whether the IGF-I gene was involved in the phenotype of short children born SGA (45). Since homogeneity is an important factor in association studies, we decided to include only those children who both had a birth length and birth weight below –2 SDS for gestational age (SGA_{L+W}). Allele 191 of the IGF1.PC1 marker, located between exon 2 and 3 of the IGF-I gene, was transmitted more often from parent to child than one would expect based on Mendelian frequencies. SGA children carrying this allele had significantly lower serum IGF-I levels compared to SGA children not carrying this allele suggesting a 'causal allele'. Our results are supported by those of Vaessen et al (46). They investigated a polymorphism in the promotor region of the IGF-I gene in a population-based study. They found that absence of the wildtype allele was associated with a reduction in birth weight of 213 gram. In contrast, Johnston et al, at first, did not find an association of the allelic variation of the IGF-I gene and birth size in a French cohort of adults who were born SGA and AGA (controls) (47). A possible explanation for their negative results can be the heterogeneity of this SGA cohort. Although the mean adult height of the SGA cohort was significantly lower compared to the AGA cohort, no distinction was made between the SGA adults who had attained normal height and those who remained short. Also, the definition of SGA differed between the two studies. However, after changing the definition of SGA into a birth weight and a birth length below –2 SD for gestational age, they could confirm our results in both this French as well as a Swedish cohort of short SGA children (48).

Another interesting finding was that children carrying the 191 allele of the IGF1.PCR marker had significantly smaller head circumference (HC) at the age of 1.3 years compared to children not carrying this allele (45). Thus, genetically determined low serum IGF-I levels may lead not only to a reduction in length, weight and head circumference at birth but also to persistent short stature and small head circumference during childhood and adulthood. IGF-I plays an important role in brain growth and development (49). Brains of IGF-I knock-out mice are significantly smaller compared to control mice (50). It has been reported that during childhood SGA children have, in general, a smaller head circumference compared to AGA children, irrespective of their height (51,52). Also, a smaller HC has been associated with lower intelligence in SGA children at various ages (51,53). A human homozygous partial deletion of the IGF-I gene resulted in severe growth retardation, sensorineural deafness and mental retardation (44). In the first Dutch SGA trial psychological tests were performed. A

significant reduction in both intelligence and attention were found, which in turn were significantly associated with a smaller HC (54). We found that SGA children who were both short and light at birth (SGA_{L+W}) have a higher risk for a smaller HC at the age of 5.9 years. Unfortunately, our present study did not include intelligence tests but we hypothesize that SGA_{L+W} children might have an increased risk for lower school achievements and psychological problems. Possibly, the IGF-I gene is not only involved in somatic growth but also plays a role in head size and neuropsychological functioning.

This study shows an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a group of short children born SGA. Genetically determined low serum IGF-I levels may lead not only to a reduction in birth length, weight and head circumference but also to persistent short stature and small head circumference during childhood and adulthood (proportionate small).

IGF-I gene in relation to the metabolic syndrome

The IGF-I gene also plays an important role in the pathogenesis of diabetes. Besides being an important contributor to pre-and postnatal growth, IGF-I has a stimulatory effect on growth and development of pancreatic beta-cells. A lifetime exposure to low-normal serum IGF-I levels has been reported as a risk factor for developing cardiovascular disease and type 2 diabetes (55-57). Until now there has been described only one human partial deletion of the IGF-I gene (58). Besides a very low birth weight, severe postnatal growth retardation and sensorineural deafness this boy had a severe insulin resistance (58). Vaessen et al investigated in a population-based study a polymorphism located in the promotor region of the IGF-I gene (57). They found that absence of the wildtype allele was significantly associated with lower serum IGF-I levels and lower body height. Also, they found an increased relative risk for the development of type 2 diabetes and myocardial infarction in non-carriers of the wildtype allele. We also found an association between a polymorphism of the IGF-I gene, located between exon 2 and 3, and low serum IGF-I levels in a specific group of short children born SGA who remained proportionate small (Chapter 3). Unfortunately we do not have data on insulin sensitivity in all children participating in the genetic part of the study. The subgroup of children who underwent an FSIGT was too small to investigate any influence of the IGF-I polymorphism.

These studies indicate the importance of the IGF-I gene in the development of adult diseases. Regarding the two hypothesis, our data support the fetal insulin hypothesis. The IGF-I gene may be one of the links between a low birth weight and the increased risk for adult diseases. Certainly, the IGF-I is not the only gene involved. The relation between birth weight and adult disease is probably multifactorial whereby several genes may play a role. In addition, environmental factors may have an additive effect especially in those individuals with a certain genetic susceptibility.

We suggest that the IGF-I gene may be one of the links between low birth weight and an increased risk of adult disease.

Pituitary region in short SGA children

This is the first study investigating the pituitary region in short children born SGA. Disturbances in the growth hormone (GH)-insulin-like growth factor-I (IGF-I)-axis might play a role in the lack of catch-up growth of these children. Studies in patients with hypopituitarism, i.e. multiple pituitary hormone deficiency (MPHD) and isolated GH deficiency (IGHD), revealed abnormalities in the pituitary region using magnetic resonance imaging (MRI) (59-61). These abnormalities included ectopia of the neurohypophysis (NH), hypoplasia or interruption of the pituitary stalk (PS) and hypoplasia of the adenohypophysis (AH). We hypothesized that disturbances in the GH/IGF-I axis, as had been reported in these children, might be related to abnormalities in the pituitary region. Therefore MRI's were performed in short SGA children without IGHD (SGA group), short SGA children with IGHD (SGA+IGHD group), non-SGA children with IGHD (IGHD group), non-SGA children with multiple pituitary hormone deficiencies (MPHD group) and in children with normal stature (control group).

However, we found no major abnormalities in the pituitary region of the SGA group. None of the SGA children had an ectopic NH or a disrupted or an absent PS. Concordantly, Nagel et al. found no abnormalities in the pituitary gland of 3 children with short stature after intrauterine growth retardation (IUGR) (62). Also no major abnormalities were found in the pituitary region of the SGA+IGHD group. Only one out of the ten showed a hypoplastic AH (10 %). As previously reported by others, 40 % of the non-SGA children with IGHD (IGHD group) showed a hypoplastic AH and an ectopic NH (38 % and 42 %, respectively) and 4 out of 24 (17 %) showed PS abnormalities (62,63). In non-SGA children with multiple pituitary hormone deficiencies (MPHD group) 87 % showed a hypoplastic AH and an ectopic NH and 67 % showed PS abnormalities. Children with MPHD showed the lowest maximum GH peaks and serum IGF-I and IGFBP-3 levels. Thus, children with the most severe form of GHD showed the most severe pituitary abnormalities. These findings agree with observations described in previous reports (62-66).

Pituitary height (PH) was measured in all patient groups. Since PH is dependent on age and sex, values are expressed in SD scores (67). Evaluating all children we found a significant positive correlation between PH SDS and the severity of GHD (maximum GH peak, IGF-I and IGFBP-3 levels). This indicates that a child with a low maximum GH peak, low serum IGF-I and low IGFBP-3 levels has a greater risk of having a small pituitary gland. These findings agree with the findings of Nagel et al who investigated 91 children with different causes of short stature (62). PH SDS in the SGA group was comparable to the PH SDS of the control group. PH SDS in the SGA+IGHD group, however, was significantly lower compared to the SGA group and the control group. So, even a moderate decrease in PH (with PH remaining within the normal range) was associated with significantly lower maximum GH peaks during provocation tests and to significantly lower serum IGF-I and IGFBP-3 levels. Therefore, assessment of PH in children with partial IGHD without MRI abnor-

malities in the pituitary region, may support the diagnosis of GHD. Since our data indicate that it is unlikely to find pituitary abnormalities in short non-IGHD children born SGA, we conclude that in these children there is no need to perform MRI's as part of a diagnostic process.

We did not find evidence in favour of our hypothesis that disturbances in the GH/IGF-I axis in short children born SGA, might be related to abnormalities in the pituitary region. Thus, subnormal GH secretion and subnormal serum IGF-I and IGFBP-3 levels in short SGA children can not be explained by anatomic abnormalities in the pituitary region. However, in the SGA+IGHD group PH was significantly lower compared to the SGA group and controls suggesting that the size of the pituitary may play a role in the GH secretion in SGA children with IGHD. It is not known whether a reduced number or a reduced volume of somatotrophic cells may lead to a smaller pituitary causing a reduction in GH secretion. Schechter et al showed that the somatotrophic cells of a patient with IGHD were morphologically identical to normal somatotrophic cells (68). The IGF system may play a role in the development and functioning of the anterior pituitary gland (69). However, further research is required to investigate this area.

Several genes are involved in the development of the pituitary gland. Abnormalities in these genes are related to both morphological and functional abnormalities (70). As short SGA do not show abnormalities in the pituitary region it is unlikely that these genes are involved in the etiology of being born SGA.

Short SGA children either with or without IGHD did not show major anatomic abnormalities in the hypothalamic-pituitary region. There is no indication to perform an MRI as part of a diagnostic process in short children born SGA without GHD.

Effects of 3 years of GH treatment: Results of the randomized, controlled GH trial.

Growth

This is the first study reporting the effects of GH treatment versus no treatment in short non-GHD children born SGA in a randomized, 3-year study design with an additional group of GHD children born SGA who were treated parallel to the randomized trial. GH treatment with a dose of 33 μg/kg/day in short SGA children either with or without GHD resulted in normalisation of their height during childhood. After two years of GH treatment, the mean H SDS of both the GH- and the GHD-group had normalised, i.e. above –2.00 (-1.6 (0.7) and -1.6 (0.6) respectively), while the H SDS of the untreated control group remained –3.0 (0.5) SDS. After 3 years of GH treatment 21.1 % of the GH-group and 20 % of the GHD-group still had a height below –2.0 SDS. All children of the untreated control group remained a height below –2.0 SDS during the 3-years study period. Our results are comparable to those

reported previously by us and others (71-73). Since GH treatment studies in short SGA children are longitudinal studies it takes on average ten years before the effects of GH treatment on adult height can be analysed. Recently, adult height data of the first Dutch randomized GH-dose-response trial treating short SGA children with either a low or a high dose of GH (33 or 66 μg/kg/day) were analysed (74). After a mean duration of 7.8 years, height SDS in children receiving the low GH dose increased from –2.9 (0.8) at start of the study to an adult height SDS of –1.1(0.8) at the end of the study. Height SDS in children receiving the high dose increased from –3.0 (0.8) at start of the study to an adult height of –0.9 (0.8). Neither adult height SDS nor gain in height during GH treatment differed significantly between the two dosage groups. GH treatment resulted in a normalisation of height, i.e. an adult height above –2.0 SDS, in 85 % of all children. Also, 98 % had an adult height within their target height range. Carel et al showed final height data of a controlled GH trial in short SGA adolescents (75). They found an increment in adult height of 0.6 SDS in those receiving GH in a dose of 66 μg/kg/day compared to an untreated control group. After a mean duration of 2.7 (0.6) years of GH treatment adult height was obtained in most children. In 50 % of the GH-treated children and in 27 % of the control group adult height appeared to be in the normal range for the general population, i.e. above –2 SDS. Two other studies reported adult height data in only small numbers of children. In one study 16 children with a mean age of 12.7 years at start of the study, were treated with a dose of 0.7 IU/kg/day (76). H SDS had increased from –2.0 SDS at start to –1.0 SDS at the end of treatment. Another study present 12 short children born SGA with a mean age of 7.6 at start of the study who were treated with a low dose of GH (77). They found an increment in H SDS from –2.9 SDS at start to –1.5 SDS at the end of treatment.

Based on combined results of randomised long-term GH trials (50 % Dutch data) it was recently decided by the US and European registration committees that short children who were born SGA form an indication for GH treatment.

> *From data of our randomized, controlled GH study, we can conclude that, in contrast to no treatment, 3 years of GH treatment results in a normalisation of height during childhood.*

Bone maturation

Bone maturation is important because it relates to the closure of the epiphyseal growth plates and therefore has an important impact on adult height. We found that before GH treatment all children had a 1-year delay in bone age, regardless of their GH-status. During the 3-year study period, bone maturation accelerated significantly in the GH-treated groups. In the GHD group this acceleration was most obvious during the first year of treatment (mean ΔBA/ΔCA 1.6 (0.6) yrs/yr) while in the GH group the maximum acceleration was observed during the second year of treatment (mean ΔBA/ΔCA 1.6 (0.7) yrs/yr). After the first two years of treatment this acceleration slowed-down and during the third year no significant difference was found

compared with the untreated control group. Bone maturation in untreated short children born SGA did neither accelerate nor decelerate. So, after the 3-year study period they still had a bone age delay of one year in contrast to the GH-treated groups who had a bone age similar to their calendar age. Data describing bone maturation during GH treatment of short SGA children are rather confusing. It has been reported that bone maturation accelerated spontaneously in untreated short SGA children (76,78,79). Our data, however, could not confirm these findings. Also, a slower bone maturation was found in both untreated and GH-treated SGA children with a relatively young age at start of the study compared to SGA children with a relatively old age at start of the study (80). In contrast, during a 3-year follow-up period, we did not observe any difference in bone maturation between younger (age 3.0 to 5.5 years) and older (age 5.5 to 8 years) SGA children, either untreated or GH-treated. Our results, however, showed that the acceleration in bone maturation is associated with a strong catch-up growth during the first 2 years of GH treatment. Sas et al showed a similar acceleration of bone maturation during the first 2 years of treatment with either a low or a high dose of GH treatment (71). Despite this acceleration of bone age during the first 2 years of GH treatment 89 % of all children attained an adult height above –2.0 SDS (74). We did neither find any differences in bone maturation between SGA children with or without SRS nor between SGA children with or without GHD.

There is no evidence to assume that the observed acceleration in bone maturation during the first 2 years of GH treatment would have a negative effect on final height.

Differential effects of GH dose

Although the present study was not designed to study a dose dependent effect of GH treatment on height, we tried to answer the following question: Should short SGA children with a H SDS below –3.00 be treated with a higher dose of GH in order to achieve a more rapid normalisation of their height? To answer this question we compared the results of the present randomized, controlled GH trial with the results of our first randomized GH-dose-response trial, in which a part of the short SGA children who met the same in- and exclusion criteria, received GH treatment at a higher dose of 66 μg/kg/day (double dose) (71). All SGA children with a H SDS at start between –2.00 and –3.00, who received either a normal or a double dose of GH, showed a normalisation of H SDS (i.e. > -2.00) within 2 years of treatment. None of these children had a H SDS below –2.00 after 2 years of treatment indicating that all children had normalized their height. In SGA children with severe short stature at start of the study (≤ –3.00), mean H SDS normalised after 3 years of GH treatment in children receiving a normal GH dose and after 2 years in children receiving the double dose. We did, however, not find a significant effect of GH dose on the 3-year gain in H SDS in children with a H SDS at start ≤ –3.00. After three years of GH treatment 31 % of the double dose group and 41 % of the normal dose group still

had a H SDS below the normal range. This difference was not significant. Since the difference in H SDS at start between the two height groups was 1 SD, it is not surprising that children with a very low baseline H SDS need more time to catch-up in height into the normal range. Our results, however, show that treatment with a higher GH dose does not result in a more rapid catch-up growth. Thus, there is no indication to treat short SGA children with severe short stature (H SDS at start ≤ –3.00) with a double GH dose. It is however important to achieve a height within the normal range before a child enters puberty. Therefore we suggest that in children with a very low H SDS, GH treatment should start at a relatively young age. Thus, if a child born SGA is still very growth-retarded (H SDS ≤ -3.00) at the age of 3 years without showing any signs of spontaneous catch-up growth, we suggest to start treatment with a GH dose of 33 μg/kg/day (normal dose) at that young age.

We did not find an indication to treat very short SGA children (H SDS ≤ -3.00) with a higher GH dose. We rather suggest to start GH treatment at an early age in order to achieve a normal height before puberty starts.

Insulin-like growth factor–I (IGF-I) and IGF binding protein-3 (IGFBP-3)

Disturbances in the GH/IGF-I axis have been described in short SGA children (81,82). Also, it has been suggested that these disturbances might play a role in the insufficient postnatal catch-up growth in these children (81,83). At start of the present study all children underwent a GH-stimulation test in order to assess GH-deficiency (GHD). Twelve of a total of 99 children (12 %) were diagnosed as GHD. In short non-GHD children born SGA baseline serum IGF-I and IGFBP-3 levels, expressed as SDS, were significantly lower compared to normal age and sex related children. In the 12 short SGA children with GHD serum IGF-I and IGFBP-3 levels were even more severely reduced. Remarkably, during GH treatment a similar increase in both IGF-I and IGFBP-3 levels was found in children with and without GHD. The 3-year increment in IGF-I and IGFBP-3 SDS correlated well with the 3-year gain in H SDS suggesting that the good response to GH treatment was mediated through increased serum IGF-I levels. In a previous Dutch study, five-year results of GH treatment with either a normal or a double dose showed an increase in both IGF-I and IGFBP-3 levels during the first years (71). Levels did not further increase after 3 years of GH treatment. During the first 3 years of that study children receiving the double dose had significantly higher IGF-I levels compared to children receiving the normal dose. However, after 5 years no differences in IGF-I and IGFBP-3 levels were observed between the 2 dosage groups. The clinical impact of these high serum IGF-I and IGFBP-3 levels is not known. Based on the initially higher IGF-I levels in children who were treated with the double dose we prefer to treat children with the normal dose rather than the double dose. For safety reasons serum IGF-I and IGFBP-3 levels need to be checked annually until adult height is attained.

Three years of GH treatment results in a significant increase in serum IGF-I and IGFBP-3 levels which correlates well with the 3-year gain in H SDS. These data

suggest that the good response to GH treatment was mediated through increased serum IGF-I levels.

Body composition and bone mineral density (BMD)

This is the first GH-controlled study investigating body composition by Dual Energy X-ray Absorptiometry (DXA) in a group of short children born SGA before and during GH treatment. DXA is a precise method to investigate fat mass, lean body mass (LBM) and bone mineral density (BMD). It uses a low radiation dose, is very accurate and can easily be performed in children (84,85).

Bone mineral density (BMD)

In short SGA children BMD at start of the study was significantly lower compared to normal children. However, part of this reduction can be explained by the fact that these children are all short. DXA measures an areal density (g/cm^2) and therefore BMD is underestimated in short children (86). For that reason we calculated bone mineral apparent density (BMAD) which is a widely used validated method to correct for short stature (87). At baseline, mean BMAD SDS was reduced to a lesser degree than mean BMD_{TB} and mean BMD_{LS} SDS levels. However, mean BMAD SDS still differed significantly from zero, suggesting bone mineral density in these children is not only reduced because of their short stature. Other factors, besides height, might also play an important role. It is well known that muscles form a large part of the load on bone and therefore have a stimulatory effect on bone mass. Indeed, we found a severe reduction in LBM at start of the study. So, a part of the reduction in BMD in short SGA children may be explained by a reduced muscle mass. Other possible causes may comprise low serum IGF-I levels and a low nutritional intake since most parents report a poor dietary intake.

During GH treatment BMAD and BMD_{TB} normalised after 1 and 2 years, respectively, whereas BMD_{LS} normalised after 3 years of GH treatment. These results are comparable to results observed in GHD children (88,89). The observed increment in BMD could be either a result of the increase in muscle mass, taller stature or a more direct effect of GH on bone. Furthermore, a better nutritional intake and increased physical activity during GH treatment, as was reported by parents, might also have played a role in the increase of BMD.

Short children born SGA show a significant reduction in BMAD which normalises during GH treatment.

Lean body mass (LBM) and fat mass

'Leanness' is a very broad term often used in short SGA children indicating they are very thin. However, until now, this characteristic was mainly based on either a low BMI or a reduction in skinfold thickness (9). Leger et al investigated body composition of these children measuring fat and muscle mass on a cross-sectional area of the thigh by Magnetic Resonance Imaging (MRI) and found a reduction in both fat and

muscle mass in the thigh (5). However, this method does not measure total body muscle and total body fat. Measuring body composition using DXA enables us to better specify the leanness of these children.

We found that at start of the study both LBM and total body fat were significantly lower compared to children of the same age and sex. Surprisingly, we did not detect any differences in either total body fat or LBM between short SGA children with or without partial GHD. This confirms the clinical observation that short SGA children with partial GHD do not show the typical GHD appearance of truncal obesity which is a well known feature of children with partial GHD. Apparently, in short SGA children who are GHD, SGA related leanness dominates their typical GHD appearance.

Another interesting finding was the fact that at start of the study all short SGA children either with or without partial GHD, showed a more severe reduction in LBM than in total body fat. So, leanness in these short SGA children can be specified into a reduction in muscle mass and to a lesser extent a reduction in total body fat. Since our results were compared to healthy age- and sex matched children with a normal height, part of the reduction in LBM and fat mass in these short SGA children can be explained by their short stature. Other possible causes for the reduction in muscle mass include low IGF-I levels and poor nutritional intake.

The fetal program hypothesis postulates that during fetal malnutrition glucose is only available for growth of vital organs, such as the brain and the heart, at the expense of muscle mass (90). This could also be one of the explanations for the observed reduction in muscle mass in short SGA children.

GH treatment resulted in an impressive increase of LBM. In contrast, total body fat reduced only during the first year of GH treatment and remained stable thereafter. Thus GH treatment has both a lypolitic effect on fat mass and an anabolic effect on LBM. These findings are comparable to the reported effects of GH treatment in GHD children and adults (89,91,92).

In short children born SGA, leanness is the result of a reduction in LBM and to a lesser extent of a reduction in total body fat. GH treatment results in a significant increase of LBM and a slight reduction of fat mass.

Skinfold thickness and body mass index (BMI)

Both BMI and skinfold thickness are generally used tools for estimating body composition. The lean appearance of short SGA children has previously been supported by calculating BMI and by measuring skinfold thickness (9,93). Skinfold measurements assess subcutaneous fat only, in contrast to BMI, which estimates total body fat and LBM together. In our study, we compared these methods to results obtained by DXA, which is considered as the 'gold standard' for these measures. At start of the study skinfolds correlated strongly with total body fat measured by DXA whereas BMI correlated both with total body fat and LBM. During GH treatment changes in skinfolds did correlate with changes in total body fat measured by DXA but changes in

BMI neither correlated with changes in LBM nor with changes in total body fat. This implicates that measuring skinfold thickness before and during GH treatment gives a reliable estimate of total body fat in short SGA children. Furthermore, measuring skinfolds is cheap and easy to perform. Conversely, BMI can not distinguish between total body fat and LBM. For that reason BMI is not an accurate estimate of body composition, at least in short SGA children. In contrast to skinfold thickness, BMI is also not a useful tool to study changes in body composition during GH treatment.

Our study indicates that the sum of 4 skinfolds is a better tool than BMI for estimating total body fat in short SGA children both before and during GH treatment.

Body proportions

Although short children born SGA clinically appear to have normal body proportions, objective data are limited. Sas et al reported in a group of short SGA children normal body proportions which remained normal during GH treatment with either a dose of 1 or 2 mg/m^2 body surface area/day (94). However, that study did not comprise an untreated control group. The present study showed that short SGA children have a reduced size of their sitting height (SH), armspan, tibial-, foot- and hand size, which is in proportion to their reduced height. In the GH-treated children sitting height, armspan, tibial, foot and hand SDS increased in concordance with their increase in height SDS indicating that body proportions remained normal during 3 years of GH treatment. Conversely, height and other anthropometric measurements remained unchanged in the untreated control group. Therefore, we were now able to demonstrate that the observed changes in various body parts of GH-treated children are the result of GH treatment. These results agree with our previous findings in a comparable group of short SGA children (94).

Untreated short SGA children have generally normal body proportions. Three years of GH treatment results in a proportionate growth of height, sitting height, armspan, tibial, foot and hand.

Head circumference

Head circumference (HC) of the total group of short SGA children was less affected compared to other parts of the body. While height and all other anthropometric measurements showed a mean value which was significantly less than –2.0 SDS, the mean HC SDS was the only parameter showing a value above –2.0 SDS. Possibly a brain sparing effect during fetal life might have played a role. Only 18 % of all SGA children had a HC SDS below the normal range, i.e. below –2.0 SDS. Interestingly, most of the short SGA children (14 out of 16) had been proportionately small at birth (SGA_{L+W}). Although the magnitude of spontaneous catch-up growth in HC after birth had been greater in SGA_{L+W} children compared to SGA_L children, HC SDS was still significantly lower in SGA_{L+W} children at a mean age of 5.9 years. This finding strongly suggests that short SGA children who have been proportionately

small at birth have a greater risk for a relatively small HC during childhood. During GH treatment HC SDS increased similarly in both SGA_{L+W} and SGA_{L} children. Since at start of the study HC SDS was significantly smaller in SGA_{L+W} children, these children still had a smaller HC after 3 years of GH treatment.

In individuals born SGA a smaller HC has been associated with a lower intelligence (51,53). Lundgren et al found an important association between both birth length and persistent short stature and subnormal intellectual performances (53). During the first Dutch randomized GH-dose-response trial psychological functioning was investigated by a psychologist. Results showed a significant reduction in intelligence and attention and an increase in behavioural and emotional problems compared to healthy peers (54). Both intelligence and attention were significantly associated with HC during childhood. Since short SGA_{L+W} children have smaller head circumferences than SGA_{L} children at a mean age of 5.9 years, we hypothesize that SGA_{L+W} children have a higher risk for lower school achievements and psychological problems. Unfortunately, our present study did not include intelligence tests.

Compared to their height, head circumference is less severely affected and the refore relatively large in most short SGA children. Those who had been born SGA_{L+W}, still had a smaller HC at the age of 5.9 years compared to those who had been born SGA_{L} and therefore they may have an increased risk for lower school achievements and psychological problems. GH treatment induced a similar increase in HC for SGA_{L+W} and SGA_{L} children.

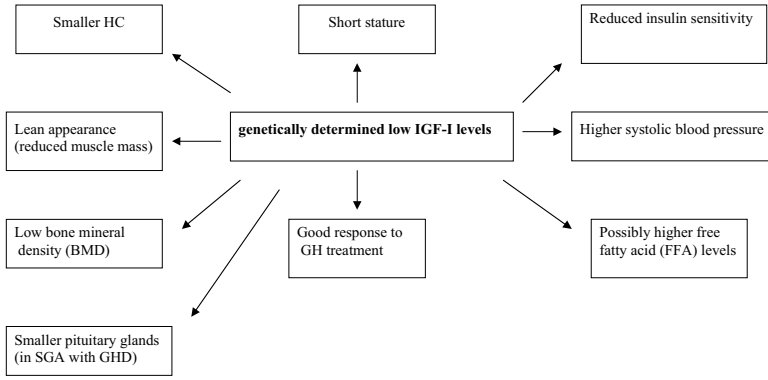
Conclusions

Our study shows that risk factors for the development of type 2 diabetes mellitus and cardiovascular disease do already exist during childhood in short and lean prepubertal children born SGA. Since low IGF-I levels are associated with type 2 diabetes and cardiovascular risk factors, we hypothesize that the IGF-I gene may be one of the genes involved in both a low birth length and weight and these diseases in later life. Our data also show that genetically determined low IGF-I levels may lead to a reduction in birth weight, length and head circumference and to persistent short stature and small head circumference in later life (proportionate small). No major abnormalities were found in the pituitary region of short children born SGA indicating that there is no need to perform MRI as part of a diagnostic process. Three years of GH treatment results in a normalisation of height during childhood and an increase in bone maturation proportionately to the gain in height. There is no indication to treat very short SGA children (H SDS ≤ -3.00) with a higher GH dose. It is however important to start GH treatment at an early age in order to achieve a normal height before puberty starts. Leanness in short SGA children proves to be the result of a significant reduction in LBM and to a lesser extent of a reduction in total body fat. In addition, BMAD is reduced in these children. Both LBM and BMAD normalized during GH treatment. Our study shows that the sum of 4 skinfolds is a better tool than BMI for estimating total body fat in short SGA children both before as well as during GH treatment. Short SGA children are proportionately short. Interestingly HC is relatively large in many of these children. However, at the age of 5.9 years children born SGAL+W still had a smaller HC compared to children born SGAL. As a smaller HC is associated with an increased risk for lower intellectual performance and psychological problems, children born SGAL+W may be at risk for lower school achievements. Three years of GH treatment induced a proportionate growth resulting in a normalization of height and other anthropometric measurements, including HC, in contrast to untreated SGA controls.

Hypothesis

The results of this thesis may be summarized in Figure 1. Although I am aware of the fact that not all effects can be attributed to the IGF-I gene, most probable other genes may have similar effects, the following hypothesis may arise. Genetically determined low IGF-I levels may play a central role in the etiology and consequences of short children born with both a small birth weight and birth length (SGA_{L+W}). Low IGF-I levels in these short SGA children may be responsible for being born SGA in the first place. Subsequently these low IGF-I levels may be responsible for the lack of catch-up growth, their typical appearance (leanness, small head circumference), their increased risk for adult diseases (type 2 diabetes mellitus, hypertension, cardiovascular disease) and their good response to GH treatment.

Figure 1.
Consequences of genetically determined low IGF-I levels in short children born SGAL+W



Future research and recommendations

Future research

Epidemiological studies demonstrated that spontaneous postnatal catch-up growth in weight and height during childhood has been associated with an increased for developing risk type 2 diabetes in adult life (36). Our results show a reduction in insulin sensitivity in short children born SGA. It might be relevant, however, to investigate risk factors for type 2 diabetes during childhood in a group of prepubertal SGA children who did show catch-up growth during the first years of life. In the first Dutch study an oral glucose tolerance test (OGTT) was used to investigate glucose metabolism. The disadvantage of an OGTT is that glucose needs to be absorbed in the gastrointestinal tract. Therefore the exact amount of intravascular glucose is not known. During an FSIGT test glucose is administered intravenously, which makes the test very accurate for research of insulin metabolism. Body composition seems to play an important role in the development of type 2 diabetes mellitus. However, in short SGA children BMI is not a valuable estimate of body composition neither before nor during GH treatment (95). In order to investigate the relation between body composition (i.e. body fat and muscle mass) and glucose and insulin metabolism we recommend to perform FSIGT tests and DXA's (at the same day).

Our results showed that serum lipids in short prepubertal SGA children were in the normal range. However, we did find an elevation of mean serum FFA levels in the high-normal range with 21 % of the children having serum FFA levels above the normal range. These findings have not been previously described. In order to confirm these findings it is necessary to measure fasting FFA levels in larger cohort of short

SGA children. Moreover, it might be very interesting to evaluate these findings in SGA children with spontaneous catch-up growth after birth. As both acylation stimulating protein (ASP) and hormone sensitive lipase (HSL) play a role in FFA metabolism further research is needed to investigate their role in SGA children.

Also, hypertension has been associated with being born SGA and catch-up growth in height and weight during childhood (37). Therefore, it would also be interesting to investigate blood pressure and serum lipids in a large cohort of short SGA children and investigate these parameters in relation to FSIGT tests to see whether clustering occurs. In order to evaluate the presence of clustering in a cohort of SGA children with spontaneous catch-up growth the results of the FSIGT tests in combination with the DXA results should be analysed together with the results of blood pressure and serum lipids.

Our results suggest the existence of a functional variant of the IGF-I gene located between the promoter region and exon 3 which results in significantly lower serum IGF-I levels. We do not know whether this polymorphism itself is involved in regulating serum IGF-I levels or that this polymorphism influences other polymorphisms regulating IGF-I production. In order to unravel the role of this IGF-I polymorphism sequencing of the coding regions of the IGF-I gene is needed. Since IGF-I is not the only gene involved in fetal and postnatal growth it is important to further investigate other genes. Possible candidate genes may include the insulin and the IGF-II gene and their receptor genes.

Recommendations during GH treatment

Long-term continuous GH treatment in short SGA children results in a normalization of height and proportionate growth of other body parts. GH treatment seems a safe treatment since no adverse events occurred during either the present or the randomised dose-response trial. However, long-term follow-up is required to reassure that GH treatment during childhood does not induce adverse events in adulthood.

We evaluated the influence of the severity of growth retardation at start and the GH dose on the gain in height. Our results showed that treatment with a double GH dose did not result in a more rapid catch-up growth. Since it is important to achieve a height within the normal range before a child enters puberty, we suggest to start GH treatment with a normal dose at a relatively young age (e.g. 3 years of age). Obviously, other causes for short stature (e.g. Turner syndrome, coeliac disease) should be excluded before starting GH treatment.

Since short stature after being born SGA increases the risk of overweight in adult life it is interesting to further evaluate how GH treatment interferes with this weight gain. GH treatment also results in an increase in weight. However, this increase in

weight is not due to an increase in body fat but due to an increase in muscle mass. It is not yet known if body composition further changes after discontinuation of GH treatment and if the risk on overweight is changed after GH treatment. Therefore long term follow-up of these children/adults is necessary. Also, it is important to educate these children about the risks of obesity and measures to prevent obesity.

GH induces relative insulin resistance with an increase in fasting insulin levels. Although a recent report showed that these changes were reversible after discontinuation of GH treatment, it is important to regularly evaluate glucose metabolism during and after discontinuation of GH treatment (96). This can be performed by measuring serum HbA1c levels and fasting glucose and insulin levels. If these values are abnormal an FSIGT test can be performed. It is however not necessary to perform FSIGT tests on a regular base in all short SGA children receiving GH treatment.

Blood pressure and serum lipids need to be monitored during and after GH treatment. Since epidemiological studies show an increased risk of systolic blood pressure which do not further increase in those SGA children showing postnatal catch-up growth it is relevant to evaluate the effect of GH treatment during and after discontinuation of GH treatment. Although it has been reported that serum lipids, i.e. total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides remained within the normal range during 5 years of GH treatment it still is important to monitor these lipids during and after discontinuation of GH treatment (9). Also, it might be interesting to investigate FFA levels during GH treatment.

Lower intellectual performance and problems with psychological functioning have been described in those born SGA. Both were associated with a smaller HC. GH treatment appears to improve the growth of the head. The effect of GH treatment on intellect and beviour has only been evaluated in one study. Although a beneficial effect on the long term was reported, these findings need confirmation. Unfortunately, no psychologist was involved in the present study to evaluate these parameters in comparison to an untreated control group. It is however important to study in the coming years the psychological effects before, during and after GH treatment.

References

1. Bergman RN 1989 Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38:1512-27.
2. Hofman PL, Cutfield WS, Robinson EM, Bergman RN, Menon RK, Sperling MA, Gluckman PD 1997 Insulin resistance in short children with intrauterine growth retardation. J Clin Endocrinol Metab 82:402-6.
3. Jaquet D, Gaboriau A, Czernichow P, Levy-Marchal C 2000 Insulin resistance early in adulthood in subjects born with intrauterine growth retardation. J Clin Endocrinol Metab 85:1401-6.
4. Sas T, Mulder P, Aanstoot HJ, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. Clin Endocrinol (Oxf) 54:243-51.
5. Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. J Clin Endocrinol Metab 78:904-9.
6. Cutfield WS, Bergman RN, Menon RK, Sperling MA 1990 The modified minimal model: application to measurement of insulin sensitivity in children. J Clin Endocrinol Metab 70:1644-50.
7. Johnson MS, Figueroa-Colon R, Huang TT, Dwyer JH, Goran MI 2001 Longitudinal changes in body fat in African American and Caucasian children: influence of fasting insulin and insulin sensitivity. J Clin Endocrinol Metab 86:3182-7.
8. 1987 Task force on blood pressure control in children. 1987 Report of the second task force on blood pressure control in children. Pediatrics 79:1-25.
9. Sas T, Mulder P, Hokken-Koelega A 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-92.
10. Barker DJ, Bull AR, Osmond C, Simmonds SJ 1990 Fetal and placental size and risk of hypertension in adult life. Bmj 301:259-62.
11. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia 36:62-7.
12. Law CM, Barker DJ, Bull AR, Osmond C 1991 Maternal and fetal influences on blood pressure. Arch Dis Child 66:1291-5.
13. Lurbe E, Torro I, Rodriguez C, Alvarez V, Redon J 2001 Birth weight influences blood pressure values and variability in children and adolescents. Hypertension 38:389-93.
14. Brenner BM, Chertow GM 1994 Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. Am J Kidney Dis 23:171-5.
15. Merlet-Benichou C, Gilbert T, Muffat-Joly M, Lelievre-Pegorier M, Leroy B 1994 Intrauterine growth retardation leads to a permanent nephron deficit in the rat. Pediatr Nephrol 8:175-80.
16. Fuentes RM, Notkola IL, Shemeikka S, Tuomilehto J, Nissinen A 2002 Tracking of systolic blood pressure during childhood: a 15-year follow- up population-based family study in eastern Finland. J Hypertens 20:195-202.
17. Kannel WB 2000 Elevated systolic blood pressure as a cardiovascular risk factor. Am J Cardiol 85:251-5.
18. Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R 2000 Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. Pediatr Res 48:623-8.
19. McGarry JD 2002 Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 51:7-18.
20. Baldo A, Sniderman AD, St-Luce S, Avramoglu RK, Maslowska M, Hoang B, Monge JC, Bell A, Mulay S, Cianflone K 1993 The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. J Clin Invest 92:1543-7.
21. Sniderman AD, Cianflone K, Arner P, Summers LK, Frayn KN 1998 The adipocyte, fatty acid trapping, and atherogenesis. Arterioscler Thromb Vasc Biol 18:147-51.
22. Langin D, Holm C, Lafontan M 1996 Adipocyte hormone-sensitive lipase: a major regulator of lipid metabolism. Proc Nutr Soc 55:93-109.
23. Holm C, Osterlund T, Laurell H, Contreras JA 2000 Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. Annu Rev Nutr 20:365-93.
24. Klannemark M, Orho M, Langin D, Laurell H, Holm C, Reynisdottir S, Arner P, Groop L 1998 The putative role of the hormone-sensitive lipase gene in the pathogenesis of Type II diabetes mellitus and abdominal obesity. Diabetologia 41:1516-22.

25. Magre J, Laurell H, Fizames C, Antoine PJ, Dib C, Vigouroux C, Bourut C, Capeau J, Weissenbach J, Langin D 1998 Human hormone-sensitive lipase: genetic mapping, identification of a new dinucleotide repeat, and association with obesity and NIDDM. Diabetes 47:284-6.
26. Talmud PJ, Palmen J, Luan J, Flavell D, Byrne CD, Waterworth DM, Wareham NJ 2001 Variation in the promoter of the human hormone sensitive lipase gene shows gender specific effects on insulin and lipid levels: results from the Ely study. Biochim Biophys Acta 1537:239-44.
27. Jiang X, Srinivasan SR, Webber LS, Wattigney WA, Berenson GS 1995 Association of fasting insulin level with serum lipid and lipoprotein levels in children, adolescents, and young adults: the Bogalusa Heart Study. Arch Intern Med 155:190-6.
28. Steinberger J, Moorehead C, Katch V, Rocchini AP 1995 Relationship between insulin resistance and abnormal lipid profile in obese adolescents. J Pediatr 126:690-5.
29. Sinaiko AR, Gomez-Marin O, Prineas RJ 1997 Relation of fasting insulin to blood pressure and lipids in adolescents and parents. Hypertension 30:1554-9.
30. Raitakari OT, Porkka KV, Ronnemaa T, Knip M, Uhari M, Akerblom HK, Viikari JS 1995 The role of insulin in clustering of serum lipids and blood pressure in children and adolescents. The Cardiovascular Risk in Young Finns Study. Diabetologia 38:1042-50.
31. Chen W, Srinivasan SR, Elkasabany A, Berenson GS 1999 Cardiovascular risk factors clustering features of insulin resistance syndrome (Syndrome X) in a biracial (Black-White) population of children, adolescents, and young adults: the Bogalusa Heart Study. Am J Epidemiol 150:667-74.
32. Ferrannini E, Haffner SM, Mitchell BD, Stern MP 1991 Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 34:416-22.
33. Mykkanen L, Haffner SM, Ronnemaa T, Bergman RN, Laakso M 1997 Low insulin sensitivity is associated with clustering of cardiovascular disease risk factors. Am J Epidemiol 146:315-21.
34. Srinivasan SR, Myers L, Berenson GS 2002 Predictability of childhood adiposity and insulin for developing insulin resistance syndrome (syndrome X) in young adulthood: the Bogalusa Heart Study. Diabetes 51:204-9.
35. Lundgren EM, Cnattingius HM, Jonsson GB, Tuvemo TH 2001 Linear catch-up growth does not increase the risk of elevated blood pressure and reduces the risk of overweight in males. J Hypertens 19:1533-8.
36. Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D 2000 The fetal and childhood growth of persons who develop type 2 diabetes. Ann Intern Med 133:176-82.
37. Eriksson J, Forsen T, Tuomilehto J, Osmond C, Barker D 2000 Fetal and childhood growth and hypertension in adult life. Hypertension 36:790-4.
38. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell 75:59-72.
39. Baker J, Liu JP, Robertson EJ, Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75:73-82.
40. Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M 1991 Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. Pediatr Res 29:219-25.
41. Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, Hintz RL, Rosenfeld RG 1995 Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 80:1548-55.
42. Spencer JA, Chang TC, Jones J, Robson SC, Preece MA 1995 Third trimester fetal growth and umbilical venous blood concentrations of IGF-1, IGFBP-1, and growth hormone at term. Arch Dis Child Fetal Neonatal Ed 73:F87-90.
43. Leger J, Noel M, Limal JM, Czernichow P 1996 Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF) I, and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. Pediatr Res 40:101-7.
44. Woods KA, Camacho-Hubner C, Savage MO, Clark AJ 1996 Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335:1363-7.
45. Arends N, Johnston L, Hokken-Koelega A, Duijn Cv C, Ridder Md M, Savage M, Clark A 2002 Polymorphism in the IGF-I Gene: Clinical Relevance for Short Children Born Small for Gestational Age (SGA). J Clin Endocrinol Metab 87:2720.
46. Vaessen N, Janssen JA, Heutink P, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM 2002 Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight. Lancet 359:1036-7.
47. Johnston LB, Leger J, Savage MO, Clark AJ, Czernichow P 1999 The insulin-like growth factor-I (IGF-I) gene in individuals born small for gestational age (SGA). Clin Endocrinol (Oxf) 51:423-7.

48. Johnston LB, Arends N, Dahlgren J, Leger J, Czernichow P, Albertsson-Wikland K, van Duijn C, Hokken-Koelega A, Savage MO, Clark AJ 2002 Gene association studies in small for gestational age infants. J Pediatr Endocrinol Metab 15:1459.
49. Werther GA, Russo V, Baker N, Butler G 1998 The role of the insulin-like growth factor system in the developing brain. Horm Res 49:37-40.
50. Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F 1995 Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron 14:717-30.
51. Ounsted M, Moar VA, Scott A 1988 Head circumference and developmental ability at the age of seven years. Acta Paediatr Scand 77:374-9.
52. Strauss RS, Dietz WH 1998 Growth and development of term children born with low birth weight: effects of genetic and environmental factors. J Pediatr 133:67-72.
53. Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T 2001 Intellectual and psychological performance in males born small for gestational age with and without catch-up growth. Pediatr Res 50:91-6.
54. van der Reijden-Lakeman I 1996 Growing pains Thesis. Erasmus University Rotterdam, Rotterdam.
55. Spallarossa P, Brunelli C, Minuto F, Caruso D, Battistini M, Caponnetto S, Cordera R 1996 Insulin-like growth factor-I and angiographically documented coronary artery disease. Am J Cardiol 77:200-2.
56. Janssen JA, Jacobs ML, Derkx FH, Weber RF, van der Lely AJ, Lamberts SW 1997 Free and total insulin-like growth factor I (IGF-I), IGF-binding protein-1 (IGFBP-1), and IGFBP-3 and their relationships to the presence of diabetic retinopathy and glomerular hyperfiltration in insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 82:2809-15.
57. Vaessen N, Heutink P, Janssen JA, Witteman JC, Testers L, Hofman A, Lamberts SW, Oostra BA, Pols HA, van Duijn CM 2001 A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 50:637-42.
58. Woods KA, Camacho-Hubner C, Bergman RN, Barter D, Clark AJ, Savage MO 2000 Effects of insulin-like growth factor I (IGF-I) therapy on body composition and insulin resistance in IGF-I gene deletion. J Clin Endocrinol Metab 85:1407-11.
59. Fujisawa I, Kikuchi K, Nishimura K, Togashi K, Itoh K, Noma S, Minami S, Sagoh T, Hiraoka T, Momoi T, et al. 1987 Transection of the pituitary stalk: development of an ectopic posterior lobe assessed with MR imaging. Radiology 165:487-9.
60. Kikuchi K, Fujisawa I, Momoi T, Yamanaka C, Kaji M, Nakano Y, Konishi J, Mikawa H, Sudo M 1988 Hypothalamic-pituitary function in growth hormone-deficient patients with pituitary stalk transection. J Clin Endocrinol Metab 67:817-23.
61. Pellini C, di Natale B, De Angelis R, Bressani N, Scotti G, Triulzi F, Chiumello G 1990 Growth hormone deficiency in children: role of magnetic resonance imaging in assessing aetiopathogenesis and prognosis in idiopathic hypopituitarism. Eur J Pediatr 149:536-41.
62. Nagel BH, Palmbach M, Petersen D, Ranke MB 1997 Magnetic resonance images of 91 children with different causes of short stature: pituitary size reflects growth hormone secretion. Eur J Pediatr 156:758-63.
63. Ochi M, Morikawa M, Yoshimoto M, Kinoshita E, Hayashi K 1992 Growth retardation due to idiopathic growth hormone deficiencies: MR findings in 24 patients. Pediatr Radiol 22:477-80.
64. Maghnie M, Triulzi F, Larizza D, Preti P, Priora C, Scotti G, Severi F 1991 Hypothalamic-pituitary dysfunction in growth hormone-deficient patients with pituitary abnormalities. J Clin Endocrinol Metab 73:79-83.
65. Argyropoulou M, Perignon F, Brauner R, Brunelle F 1992 Magnetic resonance imaging in the diagnosis of growth hormone deficiency. J Pediatr 120:886-91.
66. Vannelli S, Avataneo T, Benso L, Potenzoni F, Cirillo S, Mostert M, Bona G 1993 Magnetic resonance and the diagnosis of short stature of hypothalamic- hypophyseal origin. Acta Paediatr 82:155-61.
67. Argyropoulou M, Perignon F, Brunelle F, Brauner R, Rappaport R 1991 Height of normal pituitary gland as a function of age evaluated by magnetic resonance imaging in children. Pediatr Radiol 21:247-9.
68. Schechter J, Kovacs K, Rimoin D 1984 Isolated growth hormone deficiency: immunocytochemistry. J Clin Endocrinol Metab 59:798-800.
69. Gonzalez-Parra S, Argente J, Chowen JA, van Kleffens M, van Neck JW, Lindenbeigh-Kortleve DJ, Drop SL 2001 Gene expression of the insulin-like growth factor system during postnatal development of the rat pituitary gland. J Neuroendocrinol 13:86-93.
70. Mullis PE 2000 Transcription factors in pituitary gland development and their clinical impact on phenotype. Horm Res 54:107-19.
71. Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose- response trial. J Clin Endocrinol Metab 84:3064-70.

72. Boguszewski M, Albertsson-Wikland K, Aronsson S, Gustafsson J, Hagenas L, Westgren U, Westphal O, Lipsanen-Nyman M, Sipila I, Gellert P, Muller J, Madsen B 1998 Growth hormone treatment of short children born small-for-gestational- age: the Nordic Multicentre Trial. Acta Paediatr 87:257-63.
73. de Zegher F, Du Caju MV, Heinrichs C, Maes M, De Schepper J, Craen M, Vanweser K, Malvaux P, Rosenfeld RG 1999 Early, discontinuous, high dose growth hormone treatment to normalize height and weight of short children born small for gestational age: results over 6 years. J Clin Endocrinol Metab 84:1558-61.
74. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2003 Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. J Clin Endocrinol Metab 88:3584-90.
75. Carel JC, Chatelain P, Rochiccioli P, Chaussain JL 2003 Improvement in adult height after growth hormone treatment in adolescents with short stature born small for gestational age: results of a randomized controlled study. J Clin Endocrinol Metab 88:1587-93.
76. Ranke MB, Lindberg A 1996 Growth hormone treatment of short children born small for gestational age or with Silver-Russell syndrome: results from KIGS (Kabi International Growth Study), including the first report on final height. Acta Paediatr Suppl 417:18-26.
77. Albanese A, Azcona C, Stanhope R 1998 Final height in children with IUGR receiving GH treatment. Horm Res [Suppl 3] 50:46.
78. Job JC, Rolland A 1986 [Natural history of intrauterine growth retardation: pubertal growth and adult height]. Arch Fr Pediatr 43:301-6.
79. Davies PS, Valley R, Preece MA 1988 Adolescent growth and pubertal progression in the Silver-Russell syndrome. Arch Dis Child 63:130-5.
80. de Zegher F, Butenandt O, Chatelain P, Albertsson-Wikland K, Jonsson B, Lofstrom A, Chaussain JL 1997 Growth hormone treatment of short children born small for gestational age: reappraisal of the rate of bone maturation over 2 years and metanalysis of height gain over 4 years. Acta Paediatr Suppl 423:207-12.
81. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. Clin Endocrinol (Oxf) 41:621-30.
82. Boguszewski M, Rosberg S, Albertsson-Wikland K 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. J Clin Endocrinol Metab 80:2599-606.
83. Boguszewski M, Jansson C, Rosberg S, Albertsson-Wikland K 1996 Changes in serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 levels during growth hormone treatment in prepubertal short children born small for gestational age. J Clin Endocrinol Metab 81:3902-8.
84. Svendsen OL, Haarbo J, Hassager C, Christiansen C 1993 Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. Am J Clin Nutr 57:605-8.
85. Pintauro SJ, Nagy TR, Duthie CM, Goran MI 1996 Cross-calibration of fat and lean measurements by dual-energy X-ray absorptiometry to pig carcass analysis in the pediatric body weight range. Am J Clin Nutr 63:293-8.
86. Mazess RB, Barden H, Mautalen C, Vega E 1994 Normalization of spine densitometry. J Bone Miner Res 9:541-8.
87. Kroger H, Vainio P, Nieminen J, Kotaniemi A 1995 Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. Bone 17:157-9.
88. Saggese G, Baroncelli GI, Bertelloni S, Barsanti S 1996 The effect of long-term growth hormone (GH) treatment on bone mineral density in children with GH deficiency. Role of GH in the attainment of peak bone mass. J Clin Endocrinol Metab 81:3077-83.
89. Boot AM, Engels MA, Boerma GJ, Krenning EP, De Muinck Keizer-Schrama SM 1997 Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. J Clin Endocrinol Metab 82:2423-8.
90. Barker DJ 1997 The fetal origins of coronary heart disease. Acta Paediatr Suppl 422:78-82.
91. Baum HB, Biller BM, Finkelstein JS, Cannistraro KB, Oppenhein DS, Schoenfeld DA, Michel TH, Wittink H, Klibanski A 1996 Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. A randomized, placebo-controlled trial. Ann Intern Med 125:883-90.
92. Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3- year treatment period and after 1 year's withdrawal. J Clin Endocrinol Metab 83:3512-6.
93. Boguszewski M, Dahlgren J, Bjarnason R, Rosberg S, Carlsson LM, Carlsson B, Albertsson-Wikland K 1997 Serum leptin in short children born small for gestational age: relationship with the growth response to growth hormone treatment. The Swedish Study Group for Growth Hormone Treatment. Eur J Endocrinol 137:387-95.

94. Sas TC, Gerver WJ, De Bruin R, Mulder PG, Cole TJ, De Waal W, Hokken-Koelega AC 2000 Body proportions during 6 years of GH treatment in children with short stature born small for gestational age participating in a randomised, double-blind, dose-response trial. Clin Endocrinol (Oxf) 53:675-81.
95. Arends NJT, Blum WF, Stijnen T, Hokken-Koelega ACS 2003 Body composition by Dual Energy X-ray Absorptiometry (DXA) and serum leptin levels in short children born small for gestational age (SGA) before and during growth hormone (GH) treatment: Results of a randomised GH-controlled study. submitted.
96. van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A 2003 Effect of discontinuation of growth hormone treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. J Clin Endocrinol Metab 88:347-53.

Summary

Summary

The present thesis describes several aspects of short children who were born small for gestational age (SGA). In the first part (Chapter 2, 3 and 4) we studied possible consequences of being born SGA (i.e. insulin sensitivity, serum lipids and blood pressure) as well as etiological factors that might have played a role in the development of SGA (i.e. genetical causes and anatomical abnormalities in the pituitary region). In the second part of the thesis (Chapter 5, 6 and 7) results are presented of the second Dutch randomised, controlled GH trial. This study investigates the effects of GH treatment versus no treatment on growth, bone maturation, bone mineral density (BMD), body composition, i.e. fat mass, lean body mass (LBM), BMI and skinfold thickness, and body proportions.

Chapter 1

An overview is given of the literature regarding the definition of SGA, the prevalence and etiology of SGA, Silver-Russell syndrome, growth factors playing a role in fetal growth, postnatal effects during childhood and late consequences of being born SGA. Also, an overview of the literature until 1997 (the start of the present study) is given of the most important trials investigating the effects of GH treatment on linear growth in short children born SGA. At the end of this chapter the aims of the present study and the study design of the second Dutch GH trial are described.

Chapter 2

Epidemiological studies have shown that the metabolic syndrome, a combination of type 2 diabetes mellitus, hypertension, dyslipidemia and a high body mass index (BMI), occurs more frequently among adults who were born with a low birth weight. Since insulin is thought to play a key role in the pathogenesis of this syndrome we investigated insulin sensitivity and possible risk factors for cardiovascular disease in a group of short prepubertal children born small for gestational age (SGA).

Frequently sampled intravenous glucose tolerance tests (FSIGT) were performed in order to investigate insulin sensitivity (Si) in 28 short prepubertal children born SGA. Twelve short children born appropriate for gestational age (AGA) were used as controls for the FSIGT's results. In short SGA children, blood pressure (BP) and fasting levels of serum free fatty acids (FFA), triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured. In order to study the interrelationship between insulin insensitivity (1/Si), BMI, blood pressure and serum lipids a cluster analysis was performed in short SGA children.

Insulin sensitivity (Si) in short SGA children was significantly reduced to 38 % of the mean Si level measured in short AGA controls. As a result the mean acute insulin response (AIR) was significantly higher in SGA children compared to short AGA controls. Differences in Si and AIR between the 2 groups remained significant

after adjusting for age and BMI. The mean systolic BP SDS was 1.3 (1.1), being significantly higher than zero. Mean fasting serum levels of FFA, TC, TG, HDL and LDL were all within the normal range. However, 6 of the 28 SGA children (21 %) had serum FFA levels above the normal range. Two important clusters emerged from cluster analysis. The first cluster clearly showed that insulin insensitivity (1/Si) was related to higher systolic and diastolic blood pressure and higher serum FFA levels. A second cluster showed a relation between BMI, total cholesterol, LDL-cholesterol and triglycerides.

In conclusion, although the metabolic syndrome has been described as an adult disease, our study showed that risk factors for the development of type 2 diabetes mellitus and cardiovascular disease are already present during childhood in short prepubertal children born SGA, suggesting a pre-type 2 diabetes mellitus phenotype.

Chapter 3

Low birth weight is associated with an increased risk in adult life of type 2 diabetes, hypertension and cardiovascular disease. The fetal insulin hypothesis postulates that genes involving insulin resistance could effect birth weight and disease in later life. Besides insulin, there is extensive evidence that insulin-like growth factor-I (IGF-I) plays an important role in fetal growth. We hypothesized that minor genetic variations in the IGF-I gene could influence pre- and postnatal growth.

Three microsatellite markers located in the IGF-I gene in 124 short children (height <-1.88 SDS) who were born small for gestational age (SGA) and their parents were studied. SGA was defined as both a birth weight and birth length below –1.88 SDS for gestational age.

Two polymorphic markers showed transmission disequilibrium. Allele 191 of the IGF1.PCR1 marker was transmitted more frequently from parent to child and allele 198 of the 737/738 marker was transmitted less frequently from parent to child. Children carrying the 191-allele had significantly lower IGF-I levels than children not carrying this allele (-1.1 SDS vs. –0.50 SDS; p=0.03). Also, head circumference SDS remained smaller in children with allele 191 compared to children without allele 191 (-2.1 SDS vs. –0.9 SDS; p=0.003).

Our results show that genetically determined low IGF-I levels may lead to a reduction in birth weight, length and head circumference and to persistent short stature and small head circumference in later life. Since low IGF-I levels are associated with type 2 diabetes and cardiovascular disease, we propose that the IGF-I gene may also provide a link between low birth weight and such diseases in later life.

Chapter 4

Disturbances in the GH / IGF-I axis are reported in 25 – 60 % of short children born small for gestational age (SGA). Pituitary abnormalities, i.e. ectopia of the neurohypophysis (NH), hypoplasia or interruption of the pituitary stalk (PS) and hypoplasia of the adenohypophysis (AH) have been described in patients with either isolated GH deficiency (IGHD) or multiple pituitary hormone deficiencies (MPHD). We hypo-

thesized that in short SGA children abnormalities in the IGF-I / GH axis might be related to abnormalities in the pituitary region. MRI was performed in four groups of short children: SGA children without GH deficiency (SGA group; n=17), SGA children with isolated GH deficiency (SGA + IGHD group; n=10), non-SGA children with isolated GH deficiency (IGHD group; n=24) and non-SGA children with multiple pituitary hormone deficiencies (MPHD group; n=15). MRI was also performed in children with normal stature (control group; n=13). Pituitary height (PH) and thickness of the pituitary stalk (PS) were measured and the relationship with the maximum GH peak during a GH stimulation test, serum IGF-I and IGFBP-3 levels was evaluated.

Short SGA children either with or without IGHD did not show major anatomic abnormalities in the hypothalamic-pituitary region in contrast to 58 % of the non-SGA IGHD children and 87 % of the MPHD children who had anatomic abnormalities. PH in SGA children without GHD was normal whereas it was significantly lower in SGA children with IGHD. The lowest PHs were measured in non-SGA children with MPHD. Evaluating all children we found a significant positive correlation between pituitary height SDS (PH SDS) and the severity of GHD (maximum GH peak, IGF-I and IGFBP-3 levels). Even a moderate decrease in PH was associated with significantly lower maximum serum GH peaks and lower serum IGF-I and IGFBP-3 levels.

We concluded that PH measurements in children with less severe GHD, who underwent MRI as part of the diagnostic process, might support the diagnosis of GHD even in the absence of anatomical abnormalities. Our study demonstrates that there is no indication to perform MRI of the pituitary region in short children born SGA without GHD.

Chapter 5

The effects of 3 years of GH treatment versus no treatment on height, bone maturation and bone mineral density (BMD) in a large cohort of short children born SGA were presented. Also, the influence of the severity of growth retardation at start and the GH dose on the gain in height was evaluated.

The study design was an open-labelled, controlled multicenter GH study for 3 years. Non-growth hormone deficient (GHD) children (n=87) were randomized to either a GH-group (n=61) or an untreated control group (n=26). In addition, 12 SGA children had GHD (GHD-group) and were treated in parallel. Both the GH- and the GHD-group were treated with a GH dose of 33 μg/kg/day (normal dose). Height, bone age (BA) and bone mineral density (BMD) were evaluated. BMD was investigated using Dual Energy X-ray Absorptiometry (DXA). Since height is an important determinator of BMD, bone mineral apparent density (BMAD) was calculated in order to adjust for short stature. Data of our first GH trial in which short SGA children were treated with a GH dose of 66 μg/kg/day (double dose) were used to study the effect of a double GH dose versus a normal dose on the gain in height.

Both the GH-group and the parallel treated GHD-group showed a significant increase in height, in contrast to the untreated control group ($p<0.001$). Bone matu-

ration (Δ bone age (BA)/Δ calendar age(CA)) increased significantly during the first two years of GH treatment but slowed-down thereafter. The 3-year ΔBA/ΔCA ratio correlated significantly with the gain in height ($r=0.6$, $p<0.001$). At baseline, mean BMD SDS and BMAD SDS were significantly lower than zero. Both BMD and BMAD SDS increased significantly during GH treatment ($p<0.001$). An analysis was performed investigating the effect of GH dose (33 μg/kg/day versus 66 μg/kg/day) on height gain in children with either a H SDS at start between –2.00 and –3.00 or a H SDS at start ≤ -3.00. The gain in height of children with severe short stature at start (≤ -3.00 SDS), did not differ between those receiving either a normal or double dose.

In conclusion, 3 years of GH treatment in short children born SGA results in a normalisation of height during childhood. During GH treatment, bone maturation increased proportionately to the gain in height. BMAD in short SGA children is significantly reduced compared to normal children but normalises during GH treatment. Since the height gain of children with severe short stature at start of the study (≤-3.00 SDS) does not differ between those receiving 33 μg GH /kg/day versus those receiving 66 μg GH /kg/day, we conclude that there is no indication to treat very short SGA children with the higher GH dose. We want to emphasize, however, that severely growth-retarded SGA children should start GH treatment at a young age to enable sufficient catch-up growth before onset of puberty.

Chapter 6

Short children born SGA have a lean appearance. Therefore, body composition was investigated before and during GH treatment by Dual Energy X-ray Absorptiometry (DXA) measuring fat mass, lean body mass (LBM) and bone mineral density (BMD). Results were compared to estimates of body composition, i.e. measurement of skinfold thickness and calculation of BMI.

All children participated in a 3-year randomized, controlled GH treatment study. Non-GHD SGA children (n=87) were randomized to receive GH (GH-group, n=61) or remain untreated (control group, n=26). Twelve SGA children with GH-deficiency served as a comparison cohort (GHD-group, n=12). All GH treated children were treated with 33 μg GH/kg/day. In a subgroup BMD, LBM and fat mass were measured using DXA. The sum of 4 skinfolds (skinfolds), BMI, and serum IGF-I levels were measured in all children.

At start of the study, SGA children showed a significant reduction of LBM, fat mass and BMD measured by DXA. Also, skinfolds and BMI were significantly reduced. Skinfolds correlated strongly with fat mass measured by DXA, whereas BMI correlated with both fat mass and LBM. During 3 years of GH treatment, both LBM and BMD increased significantly in contrast to the control group. Fat mass decreased significantly during the first year of GH treatment followed by an increase to levels comparable to baseline. Changes in fat mass correlated with changes in skinfolds whereas changes in BMI did neither correlate with changes in fat mass nor LBM.

Thus, leanness in short children born SGA can now be specified as a reduction

in LBM and to a lesser extent in a reduction of total body fat. Our study also indicates that, in contrast to BMI, measuring skinfold thickness gives a reliable estimate of total body fat in short SGA children both before and during GH treatment.

Chapter 7

Short SGA children appear to have normal body proportions, however, objective data before and during GH treatment were very sparse. The effects of GH treatment versus no treatment on height, weight, head circumference (HC), sitting height, armspan and hand, tibial and foot size were evaluated. Furthermore, differences in linear growth and head circumference between SGA children born with a low birth length and birth weight (SGA_{L+W}) and SGA children born with a low birth length only (SGA_L) were investigated.

The study was an open-labelled, GH-controlled, multicenter study for 3 years. Non-growth hormone deficient short SGA children (n=87) were randomised to either a GH-group (n=61), receiving GH in a dose of 33 μg/kg/day, or an untreated control group (n=26). Height, weight, HC, sitting height, armspan and hand, tibial and foot size were measured 3-monthly before and during GH treatment.

At onset of the study all anthropometric measurements, except HC SDS, were significantly lower compared to –2 SDS. During GH treatment all anthropometric measurements normalized in accordance to the normalisation of height SDS. At start of the study, mean HC SDS was significantly lower in SGA_{L+W} children compared to SGA_L children. Interestingly, most children (14 out of 16) with a HC SDS below –2.00 had been born SGA_{L+W}. During GH treatment, the 3-year increase in height, HC and other anthropometric measurements was comparable between SGA_{L+W} and SGA_L children. In untreated SGA_{L+W} and SGA_L children, however, no changes in SD-scores of height, HC and other anthropometric measurements were found during the 3-year follow-up period.

Thus, short SGA children have normal body proportions with the exception of HC which is relatively large in many of these children. SGA_{L+W} children still had a smaller HC at the start of GH treatment (mean age of 5.9 years) compared to SGA_L children. Three years of GH treatment induced a proportionate growth resulting in a normalization of height and other anthropometric measurements, including HC, in contrast to untreated SGA controls.

Chapter 8

In the general discussion our findings are discussed in relation to the current literature. Also suggestions for future research are given.

Chapter 10 Samenvatting

Samenvatting

Samenvatting

Dit proefschrift beschrijft verschillende aspecten van kinderen met een te kleine lengte die bij de geboorte reeds te klein waren voor de zwangerschapsduur (small for gestational age, SGA). In Deel 1 (Hoofdstuk 2, 3 en 4) worden mogelijke late gevolgen beschreven van SGA zoals veranderingen in insuline gevoeligheid, serum lipiden en bloeddruk. Tevens worden etiologische aspecten beschreven die een rol zouden kunnen spelen bij het ontstaan van SGA zoals genetische oorzaken en anatomische afwijkingen in het hypofyse gebied. In Deel 2 van dit proefschrift (Hoofdstuk 5, 6 en 7) worden de resultaten beschreven van de tweede Nederlandse gerandomiseerde, gecontroleerde groeihormoon (GH) studie. Deze studie onderzocht de effecten van GH behandeling versus geen behandeling op groei, botrijping, botdichtheid, lichaamssamenstelling (vetmassa, spiermassa) en lichaamsverhoudingen.

Hoofdstuk 1

Hier wordt een overzicht gegeven van de literatuur betreffende de definitie van SGA, de prevalentie en etiologie van SGA, Silver-Russell syndroom (SRS), groeifactoren die een rol spelen bij de foetale groei, postnatale effecten van SGA op de kinderleeftijd en late gevolgen van SGA op de volwassen leeftijd. Tevens wordt een literatuur overzicht tot 1997 (start van de huidige studie) gegeven van de meest belangrijke GH studies bij SGA kinderen met een te kleine lengte. Tenslotte worden de doelen van de huidige studie en de opzet van de gerandomiseerde, gecontroleerde GH trial beschreven.

Hoofdstuk 2

Epidemiologische studies laten zien dat type 2 diabetes mellitus (DM), hypertensie en cardiovasculaire ziekten vaker optreden bij personen die geboren zijn met een laag geboortegewicht. De combinatie van type 2 DM, hypertensie, dyslipidemie en een hoge body mass index (BMI) wordt ook wel "metabolic syndrome" genoemd en is eveneens geassocieerd met een laag geboortegewicht. De onderliggende mechanismen zijn echter niet goed bekend maar aangenomen wordt dat insuline resistentie en hyperinsulinisme een belangrijke rol spelen in de pathogenese van zowel diabetes als cardiovasculaire ziekten. Om te bestuderen of deze afwijkingen reeds op de kinderleeftijd aanwezig zijn, werden insuline gevoeligheid en risicofactoren voor cardio vasculaire ziekten onderzocht bij een groep prepubertaire SGA kinderen met een te kleine lengte.

Bij 28 te kleine SGA kinderen werd een Frequently Sampled Intravenous Glucose Tolerance test (FSIGT test) verricht om de insuline gevoeligheid (Si) te bepalen. De resultaten werden vergeleken met de resultaten van FSIGT tests bij 12 te kleine kinderen die geboren waren met een normaal geboortegewicht. Bloeddruk en serum lipiden (free fatty acids (FFA), triglycerides (TG), totaal cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol)

werden gemeten bij de SGA kinderen en vergeleken met normaal waarden. Om de relatie te bestuderen tussen insuline ongevoeligheid, BMI, bloeddruk en serum lipiden werd een cluster analyse verricht.

Insuline gevoeligheid (SI) was significant lager in de SGA groep vergeleken met de controle groep (p=0.004). Gemiddelde Si waardes in SGA kinderen bedroeg 38 % van de gemiddelde Si waardes gemeten in de controle groep. Een verminderde insuline gevoeligheid kan worden gecompenseerd door een verhoogde uitscheiding van insuline, gemeten als de acute insuline respons (AIR). Zoals verwacht was de AIR bij de SGA kinderen significant hoger dan bij de kinderen uit de controle groep (p< 0.001). Deze verschillen bleven significant na correctie voor leeftijd en body mass index (BMI). Bloeddruk en serum lipiden werden vergeleken met referentie waarden die gelden voor 'normale' kinderen. De gemiddelde systolische bloeddruk was significant hoger dan de gemiddelde bloeddruk voor kinderen met dezelfde lengte, leeftijd en geslacht. Nuchtere gemiddelde FFA, TC, TG, HDL en LDL waardes lagen allen binnen het normale gebied. Echter, 6 van de 21 kinderen (21%) hadden verhoogde nuchtere FFA waardes die buiten het normale gebied lagen. Cluster analyse werd verricht om het onderlinge verband tussen insuline ongevoeligheid, bloeddruk, BMI en serum lipiden te bestuderen. Uit deze analyse kwamen 2 belangrijke 'clusters ' naar voren. De eerste cluster laat zien dat insuline ongevoeligheid gerelateerd was aan een hogere diastolische en systolische bloeddruk en hogere nuchtere FFA's. De tweede cluster omvatte een relatie tussen TC, LDL, TG and BMI.

Concluderend, kinderen met een te kleine lengte die bij de geboorte reeds te klein waren, hebben op de kinderleeftijd al een verminderde insuline gevoeligheid die asymptomatisch aanwezig is. Tevens hebben ze reeds een verhoogde systolische bloeddruk en heeft 21 % verhoogde FFA spiegels. Dus, alhoewel het "metabolic syndrome" een volwassen ziekte is, laat deze studie zien dat risico factoren voor het ontwikkelen van type 2 diabetes mellitus en cardiovasculaire ziekten reeds aanwezig zijn op de kinderleeftijd in deze (nu nog) tengere en te kleine SGA kinderen.

Hoofdstuk 3

Een laag geboortegewicht is geassocieerd met een verhoogd risico op het ontstaan van type 2 diabetes mellitus, hypertensie en cardiovasculaire ziekten tijdens de volwassen leeftijd. De "fetal insulin hypothesis" veronderstelt dat erfelijke factoren (genen) die betrokken bij het ontstaan van insuline resistentie, mogelijk een effect kunnen hebben op zowel het geboortegewicht als op volwassen ziekten. Behalve insuline, is er overtuigend bewijs dat insulin-like growth factor-I (IGF-I) een belangrijke rol speelt bij foetale groei. Wij veronderstelden dat geringe genetische variatie in het IGF-I gen van invloed zou kunnen zijn op zowel pre- als postnatale groei.

Drie microsateliet markers gelokaliseerd op het IGF-I gen werden onderzocht in een groep van 124 te kleine SGA kinderen en hun ouders. SGA werd gedefinieerd als zowel een geboortelengte als een geboortegewicht kleiner dan –2.00 SDS voor de zwangerschapsduur. De resultaten werden geanalyseerd middels een Transmission

disequilibrium test (TDT test). De test onderzoekt het transmissie disequilibrium oftewel de overerving van een allel. Twee markers lieten “”transmission disequilibrium” zien. Allel 191 van de IGF1.PCR1 marker werd vaker doorgegeven van ouder naar kind en allel 198 van de 737/738 marker werd minder vaak doorgegeven van ouder naar kind. Kinderen die drager zijn van het 191-allel hadden significant lagere serum IGF-I waarden dan kinderen die dit allel niet hadden (-1.1 SDS vs. –0.50 SDS; p=0.03). Tevens bleken dragers van het 191-allel op de leeftijd van 1,5 jaar een kleinere hoofdomtrek te hebben in vergelijking met niet-dragers van dit allel (-2.1 SDS vs. –0.9 SDS; p=0.003).

Deze resultaten laten zien dat genetische bepaalde lage IGF-I spiegels kunnen niet alleen leiden tot een lager geboortegewicht, een kleinere geboortelengte en een kleinere hoofdomtrek bij de geboorte maar ook tot een persisterende kleine lengte en kleine hoofdomtrek op latere leeftijd. Aangezien lage serum IGF-I spiegels geassocieerd zijn met type 2 diabetes mellitus en cardiovasculaire ziekten veronderstellen wij dat het IGF-I gen mogelijk een link kan zijn tussen een laag geboortegewicht en ziekten op de volwassen leeftijd.

Hoofdstuk 4

Afwijkingen in de groeihormoon (GH) / insulin-like growth factor-I (IGF-I) as zijn beschreven in 25 – 60 % van de kinderen met een te kleine lengte die bij de geboorte reeds te klein waren. Hypofysaire afwijkingen zoals een ectopische neurohypofyse (NH), hypoplasie of een onderbroken hypofysesteel en hypoplasie van de adenohypofyse (AH) zijn beschreven bij patiënten met zowel een geïsoleerde GH-deficiëntie (IGHD) als met multipele hypofysaire hormoon deficiënties (MPHD). Wij onderzochten of de afwijkingen in de GH/IGF-I as bij te kleine SGA kinderen gerelateerd zouden kunnen zijn aan eventuele afwijkingen in het hypofyse gebied.

MRI scans werden verricht bij 4 groepen kinderen met een te kleine lengte: SGA kinderen zonder IGHD (SGA groep; n=17), SGA kinderen met een IGHD (SGA + IGHD groep; n=10), niet-SGA kinderen met IGHD (IGHD groep; n=24) en niet-SGA kinderen met multipele hypofysaire hormoon deficiënties (MPHD groep; n=15). MRI scans werden eveneens verricht bij kinderen met een normale lengte (controle groep; n=13). Hypofyse lengte (PH) en dikte van de hypofysesteel (PS) werden gemeten en de relatie met de GH piek tijdens een GH stimulatie test, serum IGF-I en IGFBP-3 spiegels werden bestudeerd.

Te kleine SGA kinderen met of zonder IGHD lieten geen belangrijke anatomische afwijkingen zien in het hypothalamus-hypofyse gebied in tegenstelling tot 58 % van de kinderen uit de niet-SGA IGHD groep en 87 % van de kinderen uit de niet-SGA MPHD groep die wel anatomische afwijkingen lieten zien. De hypofyse lengte van SGA kinderen zonder IGHD was normaal maar bij SGA kinderen met IGHD was deze significant kleiner. De kleinste hypofyse lengte werd gemeten bij niet-SGA kinderen met MPHD. Wanneer de hypofyse lengtes van alle kinderen werden geanalyseerd, werd een significant positieve correlatie gevonden tussen de hypofyse lengte en

de ernst van de GHD (maximum GH piek, IGF-I en IGFBP-3 waarden). Zelfs een geringe afname van de hypofyse lengte was geassocieerd met significant lagere GH piek en lagere serum IGF-I en IGFBP-3 waarden.

Wij concludeerden dat het meten van de hypofyse lengte bij kinderen met een minder ernstige GHD, de diagnose GHD kan steunen zelfs bij de afwezigheid van anatomische afwijkingen. Bij SGA kinderen met een te kleine lengte zonder GHD is er geen indicatie om een MRI van de hypofyse te verrichten als onderdeel van een diagnostisch proces.

Hoofdstuk 5

Hier worden de effecten beschreven van 3 jaar GH behandeling versus geen behandeling op lengtegroei, botrijping en botdichtheid (bone mineral density = BMD) bij een grote groep SGA kinderen met een te kleine lengte. Tevens werd de invloed van zowel de ernst van de groeiretardatie bij start van de studie, als de GH dosis op de toename in lengte tijdens de behandeling bestudeerd.

De studie opzet van deze multicenter studie was een open-labelled, gecontroleerde GH studie voor een periode van 3 jaar. Voor de start van de studie werd een GH-stimulatie test verricht om een eventuele GH-deficiëntie (GHD) te diagnostiseren Niet-GHD kinderen (n=87) werden gerandomiseerd tot een GH-groep (n=61) of een controle groep (n=26). De GH-groep startte direct met GH behandeling terwijl de controlegroep de eerste 3 jaar van het onderzoek onbehandeld bleef. Kinderen die GHD bleken te zijn werden niet gerandomiseerd maar startten direct met GH-behandeling (GHD-groep; n=12). Zowel de GH- als de GHD groep werden behandeld met een GH dosis van 33 μg/kg/dag (normale dosis). Lengte, botleeftijd en botdichtheid (BMD) werden gemeten. BMD werd bepaald met behulp van de Dual Energy X-ray Absorptiometry (DXA). Omdat BMD mede bepaald wordt door de lengte werd de zogenaamde bone mineral apparent density (BMAD) berekend om zo te corrigeren voor een te kleine lichaamslengte. Data van de eerste Nederlandse GH trial, waarbij te kleine SGA kinderen werden behandeld met een GH dosis van 66 μg/kg/dag (dubbele dosis), werden gebruikt om het effect te bestuderen van een normale versus een dubbele dosis op de toename in lengte.

Zowel de GH- als de GHD-groep lieten een significante toename zien in lengte, in tegenstelling tot de onbehandelde controle groep. Tijdens de eerste 2 jaar met GH behandeling nam de botrijping (Δ botleeftijd (BA)/Δ kalender leeftijd(CA)) significant toe Tijdens het derde jaar vertraagde de botrijping. De 3-jaars ΔBA/ΔCA ratio correleerde positief met de lengte toename. Bij start van de studie waren BMD en BMAD bij de SGA kinderen significant lager in vergelijking met normale kinderen. Beide lieten tijdens GH behandeling een duidelijke toename zien. Tenslotte werd het effect van GH dosis (33 μg/kg/dag versus 66 μg/kg/dag) op de lengte toename bestudeerd bij kinderen die bij start van de studie of een lengte SDS hadden tussen –2.00 en –3.00 of een lengte hadden ≤ -3.00. De lengte toename van kinderen met een lengte SDS ≤ -3.00 verschilde niet tussen kinderen die behandeld werden met

een normale of een dubbele dosis.

Concluderend, 3 jaar behandeling met GH resulteert bij SGA kinderen met een te kleine lengte in een normalisatie van de lengte tijdens de kinderjaren. Tijdens GH behandeling nam de botrijping toe overeenkomstig met de toename in lengte. BMAD was aanvankelijk verlaagd in vergelijking met normale kinderen maar normaliseerde tijdens GH behandeling. Aangezien er geen effect van een dubbele dosis GH waarneembaar was op de lengte toename van kinderen met een ernstige groeiretardatie (lengte SDS ≤ -3.00) concluderen wij dat er geen indicatie is om SGA kinderen met een ernstige groei retardatie te behandelen met een dubbele dosis GH. We willen echter wel benadrukken dat het van belang is om deze ernstig groei-geretardeerde SGA kinderen reeds op jonge leeftijd te behandelen om zo voldoende inhaalgroei te bewerkstelligen alvorens de puberteit begint.

Hoofdstuk 6

SGA kinderen met een te kleine lengte zien er in het algemeen zeer tenger uit. Om de lichaamsamenstelling van deze kinderen te bestuderen voor en tijdens GH behandeling werden vetmassa, vetvrije massa (lean body mass = LBM) en botdichtheid (BMD) bepaald middels Dual Energy X-ray Absorptiometry (DXA). De resultaten werden vergeleken met frekwent gebruikte afgeleiden van de lichaamsamenstelling: het meten van de huidplooidikte en het berekenen van de body mass index (BMI).

Alle kinderen namen deel aan de 3-jaars open-labelled, gerandomiseerde, gecontroleerde GH trial. Niet-GHD kinderen (n=87) werden gerandomiseerd tot of de GH-groep (n=61) of de onbehandelde controle groep (n=26). Twaalf SGA kinderen met GHD dienden als een vergelijkend cohort (GHD-groep, n=12). Kinderen uit GH- en GHD-groep werden beide behandeld met een dosis van 33 μg GH/kg/dag. Bij een subgroep van de kinderen werden DXA's verricht om vetmassa, LBM en BMD te bepalen. Huidplooidiktes en BMI werden gemeten bij alle kinderen.
Bij start van de studie hadden te kleine SGA kinderen een verminderde vetmassa, LBM en BMD. Tevens waren de huiplooidiktes en BMI lager in vergelijking met normale kinderen. Huidplooidikte was goed gecorreleerd met vetmassa gemeten met behulp van DXA, terwijl BMI correleerde met zowel vetmassa als LBM. Tijdens 3 jaar GH behandeling nam zowel LBM als BMD significant toe in tegenstelling tot de controle groep. Vetmassa nam tijdens het eerste jaar af om vervolgens weer toe te nemen tot waardes vergelijkbaar met waardes bij start. Veranderingen in vetmassa correleerden goed met veranderingen in huidplooidiktes. Echter veranderingen in BMI correleerden noch met veranderingen in vetmassa noch met veranderingen in LBM. Dus, het begrip 'tenger' bij kleine SGA kinderen kan nu gespecificeerd worden als een reductie in LBM en in mindere mate een reductie in de totale vetmassa. Deze studie laat eveneens zien dat het meten van huidplooi diktes, in tegenstelling tot het berekenen van BMI, een betrouwbare schatting geeft van de totale vetmassa bij kleine SGA kinderen zowel voor als tijdens GH behandeling.

Hoofdstuk 7

Te kleine SGA kinderen lijken normaal geproportioneerd. Echter objectieve data betreffende lichaamsproporties voor en tijdens GH behandeling zijn zeer spaarzaam. Daarom werden de effecten van GH behandeling versus geen behandeling op lengte, gewicht, hoofdomtrek (HC), zithoogte, spanwijdte en hand, voet en tibia lengte bepaald. Tevens werden verschillen in lengte groei en toename van HC bestudeerd tussen SGA kinderen geboren met zowel een te kleine geboortelengte als een te laag geboortegewicht (SGA_{L+W}) en SGA kinderen met alleen een te kleine geboortelengte (SGA_L).

De studie betreft een open-labelled, gecontroleerde GH trial gedurende 3 jaar. Niet-GHD SGA kinderen (n=87) werden gerandomiseerd tot een GH-groep (n=61) of een onbehandelde controle groep (n=26). De GH-groep werd behandeld met een GH dosis van 33 μg/kg/dag. Lengte, gewicht, hoofdomtrek (HC), zithoogte, spanwijdte en hand, voet en tibia lengte werden voor en tijdens GH behandeling bepaald.
Bij start van de studie waren alle antropometrische metingen, behalve de HC SDS, significant lager dan –2 SDS. Tijdens GH behandeling normaliseerde alle metingen overeenkomstig het normaliseren van de lengte. Bij start van de studie bleken SGA_{L+W} een significant kleinere HC SDS te hebben in vergelijking met SGA_L kinderen. Opmerkelijk, de meeste kinderen (14 van de 16) met een HC SDS kleiner dan –2.00 SDS, waren geboren met zowel een te kleine lengte als een te laag gewicht (SGA_{L+W}). Tijdens GH behandeling was er geen verschil in toename van lengte, HC en de overige antropometrische metingen tussen de SGA_{L+W} kinderen en de SGA_L kinderen. Kinderen uit de onbehandelde controle groep lieten geen toename zien van lengte, HC en overige antropometrische metingen tijdens de 3-jarige follow-up periode.

Kortom, SGA kinderen met een te kleine lengte zijn normaal geproportioneerd met uitzondering van de hoofdomtrek, welke relatief groot is bij de meeste kinderen. SGA_{L+W} kinderen hadden bij start van de studie (op een gemiddelde leeftijd van 5.9 jaar) nog steeds een kleinere hoofdomtrek in vergelijking met SGA_L kinderen. Drie jaar GH behandeling induceert een geproportioneerde groei resulterend in een normalisatie van lengte en andere antropometrische metingen, inclusief hoofdomtrek, in tegenstelling tot onbehandelde kleine SGA kinderen.

Hoofdstuk 8

In de algemene discussie worden de bevindingen besproken tegen de achtergrond van de huidige kennis en literatuur. Tevens worden suggesties gedaan voor eventueel toekomstig onderzoek.

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SGA

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Curriculum Vitae

Nicolette Johanna Theodora Arends was born on April 23, 1969 in Zevenaar, The Netherlands. She finished secondary school in 1987 (VWO-B; Antonius College in Gouda) and started medical school at the Erasmus University in Rotterdam. As a medical student she worked as a nurse in Sophia Children's Hospital in Rotterdam. At the end of her internships she performed research on hypokalemia in meningococcal disease at the Pediatric Department of St. Mary's Hospital in London (Prof.dr. M. Levin). After obtaining her medical degree in 1996 she started working as an intern at the department of Pediatric Surgery in Wilhelmina Children's Hospital in Utrecht and performed research on the results of the laparoscopic Thal procedure in children with gastro-esophageal reflux (Prof.dr. N.M.A. Bax). From 1996 until 2002 she worked as a research-fellow at the Department of Pediatrics, Subdivision of Endocrinology in Sophia Children's Hospital in Rotterdam (Prof.dr. S.L.S. Drop) which has led to the present thesis. In 2000 she received a grant from the Ter Meulen Fonds (Royal Netherlands Academy of Arts and Sciences) to perform genetic research in the Dutch study population at the Department of Molecular Endocrinology of St. Bartholomew's Hospital in London (Prof.dr. M.O. Savage and Prof.dr. A.J.L. Clark). In 2002 she started her pediatric trainingship in Sophia Children's Hospital in Rotterdam (Prof.dr. H.A. Büller and Prof.dr. A.J. van der Heijden).
Nicolette is married to Vincent Ollefers and they have two sons: Stijn (2001) and Job (2003).

List of Abbreviations

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BA = bone age
BMAD = Bone mineral apparent density
BMC = Bone mineral content
BMD = Bone mineral density
BMD_{LS} = Bone mineral density of the lumbar spine
BMD_{TB} = Bone mineral density of the total body
BMI = Body mass index
CA = Calendar age
GH = Growth hormone
GHD = GH-deficiency
H = height
HC = Head circumference
IGF-I = Insulin-like growth factor-I
IGFBP-3 = IGF-binding protein-3
LBM = Lean body mass
SDS = Standard deviation score
SGA = Small for gestational age
SGA_{L+W} = SGA children born with a low birth length and birth weight
SGA_{L} = SGA children born with a low birth length only
SH = Sitting height
TH = Target height
WH = Weight for height