Short Adolescents Born Small for Gestational Age

Gonadal and thyroid function, bone mineral density, quality of life and adult height

The effects of growth hormone and additional postponement of puberty

Annemieke J. Lem

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Te kleine, SGA-geboren adolescenten

Gonadale functie, schildklierfunctie, botdichtheid,
kwaliteit van leven en eindlengte: Resultaten van groeihormoonbehandeling
en additioneel uitstel van de puberteit

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From growing to knowing
From knowing to growing

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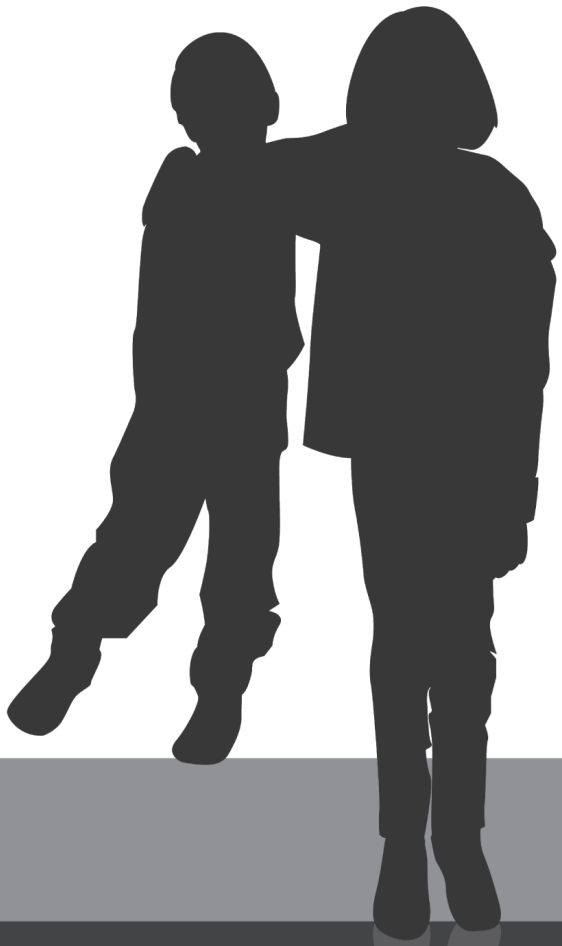

Chapter 1

General introduction

From 1991, our research group and others have been investigating children with short stature who were born small for gestational age (SGA), both before and during treatment with biosynthetic growth hormone (GH). In 2005, GH treatment was licensed for short SGA children in the Netherlands. Many questions though remained unanswered, especially about the efficacy of GH treatment when started at an older age, just before or during puberty.

This doctoral thesis describes studies evaluating short adolescents born SGA who were treated with GH, and additionally with postponement of puberty by gonadotropin-releasing hormone analogue (GnRHa).

Small for gestational age (SGA)

The term "Small for gestational age" (SGA) is used to describe newborns whose size is substantially less than expected for their gender and gestational age. SGA is defined as a birth weight and/or birth length at least two standard deviations scores (SDS) below the mean for gestational age, equivalent to the 2.3 percentile, based on data derived from an appropriate reference population(1,2). According to recent estimates, 184,397 children were live-born in the Netherlands in 2010 (Central Bureau of Statistics, The Hague, The Netherlands). By definition, approximately 4,241 of them were born SGA.

SGA children can be either born full-term or premature. To define whether or not a child is born SGA, one should have accurate gestational dating, precise measurements of weight and length at birth, as well as population based reference data. The term intrauterine growth retardation (IUGR) is often used synonymously with the term SGA. IUGR is, however, a prenatal diagnosis based on serial ultrasound measurements during pregnancy. To diagnose IUGR, at least two fetal size ultrasound measurements are necessary, whereas SGA is determined on the infant's size at birth. Being born SGA does not necessarily mean that IUGR occurred. Similarly, infants who are short after confirmed IUGR are not inevitably SGA.

Normal fetal growth is dependent on an optimal intrauterine environment, particularly in relation to the delivery of oxygen and nutrients via the placenta(3). Intrauterine factors that determine growth and growth restriction include various maternal, fetal, placental and environmental factors (Table 1). However, in about 40% of the children born SGA no underlying pathology can be identified.

Table 1. Factors associated with reduced fetal growth(4)

Maternal factors	
Medical conditions	Acute or chronic hypertension Pre-eclampsia Severe chronic disease Severe chronic infection Systemic lupus erythematosus Antiphospholipid syndrome Anemia Malignancy Abnormality of the uterus
Social conditions	Maternal nutrition Low prepregnancy body mass index (BMI) Low maternal weight gain Delivery at age <16 or >35 years Low socioeconomic status Drug use (smoking, alcohol, illicit drugs)
Fetal factors	
Multiple births	
Congenital defects	
Chromosomal anomalies	Down syndrome Turner syndrome
Inborn errors of metabolism	
Intrauterine infections	TORCHES (Toxoplasmosis, Rubella, Cytomegalovirus, Herpes Simplex, Syphilis, Others)
Placental factors	
Reduced blood flow	
Reduced area for exchange of nutrients and oxygen	Infarcts Haematomas Partial abruption
Environmental factors	
High altitude	
Toxic substances	

Persistent short stature

Most infants born SGA experience a period of accelerated linear growth during the first year of life and will achieve a normal height above the -2 SDS by the age of two years. Premature SGA infants (gestational age below 37 weeks) may need longer to catch-up to a normal length than full-term infants(5-7). Approximately 10% of children born SGA do not show spontaneous catch-up growth during the first years of life and will have persistent short stature(5,8,9). The relationship between etiology of fetal growth retardation and postnatal growth pattern is not yet delineated. Short children born SGA without signs of catch-up growth at the age of three years are not likely to catch-up to a normal height later on. These children should be referred to a pediatrician with expertise in endocrinology[1,2].

Growth hormone axis

Normal growth requires the cooperation of several hormones, such as growth hormone, thyroid hormones, sex steroids and corticosteroids. While fetal growth and early postnatal growth until the age of 3 to 6 months is mainly insulin dependent, there is progressive GH dependency after the first months of life when GH becomes the most important hormone in controlling longitudinal growth(10,11).

The GH-axis is a complex physiological axis that regulates key aspects of growth and metabolism (Figure 1). GH is secreted in a pulsatile pattern by the anterior pituitary gland, with the greatest release during sleep(12). GH has a stimulatory effect on the production of insulin-like growth factor (IGF-) I that is considered as one of the main growth factors and is involved in a large number of cellular processes, such as cell proliferation, metabolism, differentiation, motility and migration and cell survival(13). Most of the circulating IGF-I (>99%) is bound to IGF binding protein (IGFBP-) 3 in a ternary complex with acid-labile subunit (ALS).

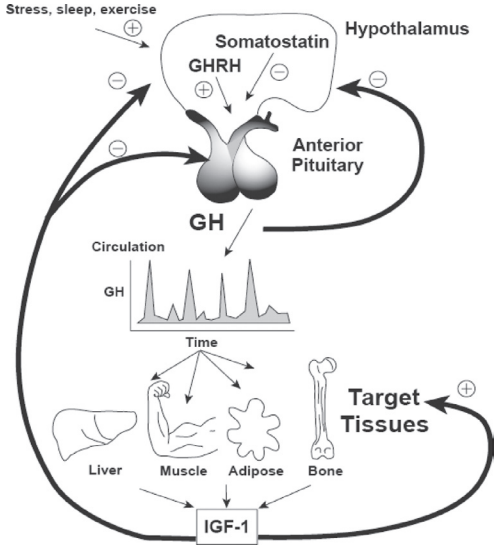

Figure 1. Regulation of GH synthesis and secretion.

The hypothalamic hormones Growth hormone releasing hormone (GHRH) and somatostatin elicit an increase and decrease in circulating growth hormone (GH) levels, respectively. Circulating GH binds to the GH receptor on peripheral tissue such as muscle, liver and bone to induce the secretion of IGF-I. Insulin-like growth factor (IGF)-I itself then has growth-promoting effects on target tissues. [Adapted from Kopchick JJ and Andry JM. Growth hormone (GH), GH receptor and signal transduction. *Mol Genet Metab.* 2000;71:293–314](14)

Puberty

During puberty, the hypothalamic-pituitary-gonadal axis is activated. The first appearance and development of secondary sexual characteristics reflects the overall physiologic development in adolescence. The continuous process of pubertal development is usually subdivided into discrete numerical stages, as proposed by Tanner(15,16). The median age of pubertal start in the Netherlands is 10.7 years in girls and 11.5 years in boys(17).

Besides the development of sexual characteristics, puberty is known to be accompanied by a pubertal growth spurt. In girls, the growth acceleration starts during the first year of breast development. In boys, the growth acceleration occurs later, during the second year of puberty when testicular size have increased to >10 milliliters. There is a large individual variation in pubertal growth pattern. Although the mechanism of these variations is essentially unknown, it is recognized that the respective levels of estradiol and testosterone on the growth plate explain the differences between girls and boys(18).

Transition through puberty is a complex and dynamic process that depends on genetic factors and numerous postnatal factors including endogenous hormones, body fat and energy consumption. Besides, the prenatal environment might play a role in the timing and progression of puberty. A Swedish population-based study showed that puberty occurred at a normal age in SGA children with spontaneous catch-up growth, but slightly earlier in SGA children with persistent short stature(19). Other studies showed that SGA boys had a normal puberty, whereas SGA girls had a more rapid progression through puberty, resulting in a slightly earlier menarche(20). Most SGA children though appear to have a pubertal onset and development within normal limits(21), also during GH treatment(22). Height and age at onset of puberty, as well as the magnitude and duration of pubertal growth are important determinants of adult height(23-25). When puberty in short SGA children starts at a normal age, this can be too early for their actual height(26). Besides, height gain during puberty in short SGA children is often reduced(21,27). Therefore, short SGA children who enter puberty at a very short stature have a poor adult height prognosis.

Although growth retardation should be evaluated as early as possible, in practice many individuals present with short stature around pubertal age. It is assumed that growth promoting treatment started during puberty has only limited effect, since by that time the epiphyseal maturation has been activated(28).

SGA and long-term risks

Epidemiological studies reported an inverse association between birth weight and risk for hypertension, cardiovascular disease and type 2 diabetes mellitus in adult life(29-31). In short SGA children, reduced insulin sensitivity and increased prevalence of cardiovascular risk factors have been described(32,33). Besides, reduced size at birth might be related to reduced bone mineral density(34,35).

Fetal life is a critical phase in the development of important organ systems. In short children born SGA, the suboptimal situation in prenatal life might induce permanent changes in function of various organ systems during childhood, adolescence and adult life.

Ovarian function in girls

The most dynamic phase of ovarian development occurs before birth. Human follicle development starts in the twelfth week of intrauterine life. Already at 20 weeks, the maximum number of primordial follicles in the ovaries is reached(36,37). During fetal life and childhood, follicles develop through primordial and primary stage, to pre-antral and small antral follicles(38,39). Fully mature oocytes are the survivors of the long selection process of folliculogenesis; they are able to undergo fertilization and embryonic development, leading eventually to offspring(40). A suboptimal intrauterine environment may have a detrimental effect on the development and preservation of primordial follicles, and may therefore impair reproductive health in later life.

The ovaries accomplish two essential functions: the synthesis and secretion of sex hormones and the development and release of the mature oocyte. An oocyte combined with surrounding granulosa and theca cells is called a follicle. Granulosa cells of primary and preantral follicles secrete anti-Müllerian hormone (AMH) that is involved in the regulation of folliculogenesis(41-43) (Figure 2). AMH reflects the number of pre-antral and small antral follicles. Since serum AMH is produced exclusively by the ovary, independently of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool(44-47).

Studies have been conducted to investigate the reproductive function of children with restricted fetal growth, but the results were controversial. Some studies showed smaller ovaries and uterus in infants and adolescent women born SGA(48,49), whereas others did not find differences in ultrasonic measurements of the uterus and ovaries in girls born SGA(50). Thus, it remains a question whether girls born SGA are at risk for impaired ovarian function. At start of this study, knowledge about using AMH levels as a marker for the ovarian follicle pool in children was very limited.

Thyroid function

Thyroid hormones are essential for fetal and postnatal development and for the regulation of neuropsychological functioning. Thyroid hormones have an important growth promoting effect, as shown by the severe growth retardation in children with thyroid deficiency. Since thyroid hormone levels change during infancy, childhood and adolescence, age-appropriate reference ranges are required to detect thyroid dysfunction during childhood.

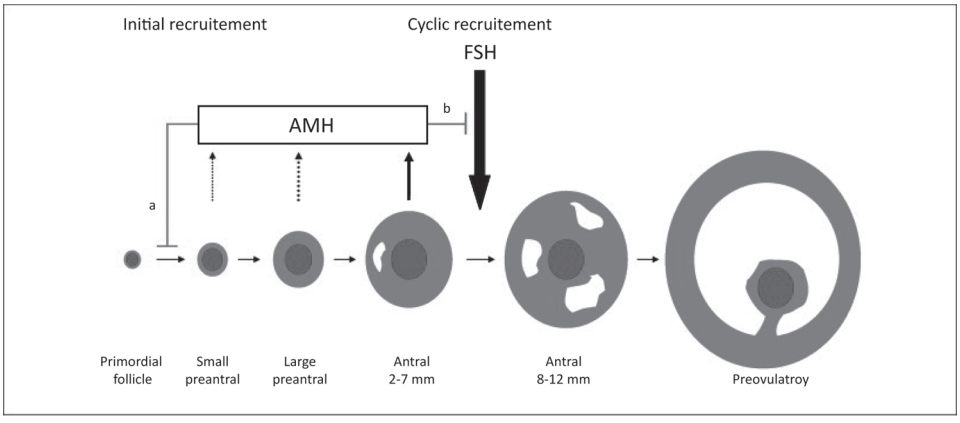

Figure 2. The role of AMH in normal ovarian follicle development.
[Adapted from Broekmans FJ et al. Anti-Müllerian hormone and ovarian dysfunction. *Trends Endocrinol Metab.* 2008; **19**(9):340-347]

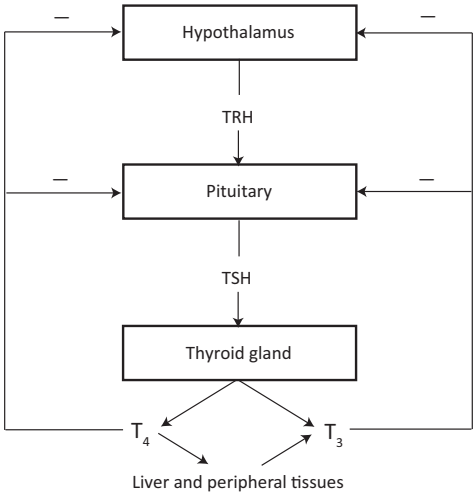

Figure 3. Hypothalamus-pituitary-thyroid gland axis

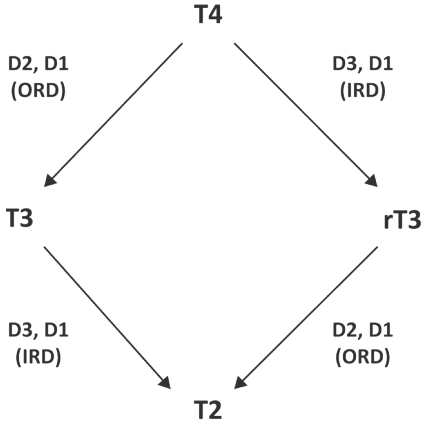

Figure 4. Peripheral thyroid deiodination pathway

TRH = thyrotropin releasing hormone, TSH = thyroid stimulating-hormone, T4 = thyroxine, T3 = triiodothyronine, ORD = outer ring deiodination, IRD = inner ring deiodination

Thyroid hormones are regulated via the hypothalamic-pituitary axis (Figure 3). Thyrotropin releasing hormone (TRH) is synthesized in the hypothalamus and mediates the pituitary release of thyroid stimulating-hormone (TSH). Subsequently, TSH stimulates the thyroid gland to synthesize and release thyroid hormones. Thyroxine (T_4), the primary secreted product of the thyroid gland, is inactive until it is converted to the active hormone triiodothyronine (T_3). Both TRH and TSH are subject to the negative feedback mechanism of T_4 and T_3. Most of the thyroid hormones T_4 and T_3 are bound to carrier proteins, mainly thyroxine-binding globulin (TBG), transthyretin and albumin, leaving less than 1 percent in the free, biologically available form.

Peripheral thyroid hormone metabolism plays an eminent role in the regulation of thyroid hormone bioactivity (Figure 4). The principal pathways of this metabolism are deiodination and conjugation, of which deiodination is the most important one. Under normal circumstances T_4 is mainly deiodinated to active T_3 by outer ring deiodination (ORD; D2 and D1). Both T_4 and T_3 may also be inactivated by inner ring deiodination (IRD; D3 and D1) forming the inactive metabolites reverse-triiodothyronine (rT_3) and 3,3'-diiodothyronine (T_2), respectively.

In short children born SGA, the suboptimal situation in prenatal life might have induced permanent changes in the regulation of thyroid function and metabolism. In fetuses with intrauterine growth retardation, a significant reduction in circulating free thyroxine (FT_4) and a modest elevation in TSH were found(52). In prepubertal short SGA children, TSH was reported to be elevated whereas FT_4 was not significantly different from controls(53,54). At start of this study, data on thyroid function in short children born SGA before and during GH treatment were not conclusive, and data on thyroid function during puberty and postponement of puberty by gonadotropin-releasing hormone analogs (GnRHa) in these children were lacking.

Growth hormone treatment

Recombinant GH has been used since 1985 and has replaced GH extracted from human pituitaries in the treatment of children with GH deficiency. The indications have gradually extended from replacement therapy in children with severe GH deficiency to an increasing number of conditions in which short stature is not due to GH deficiency.

In short children born SGA, the underlying mechanism of inadequate catch-up growth is still not fully understood. Disturbances in the GH/IGF-I axis do play a role. Sixty percent of short SGA children showed subnormal GH secretion measured over 24 hours and had low serum levels of IGF-I and IGFBP-3. About 25% showed low GH peaks during GH provocation tests(55-57).

Nowadays, GH is an approved treatment for short stature in children born SGA, in the United States of America (Food and Drugs Administration, 2001) and in Europe (European Medicines Agency, 2003). The criteria to start GH differ between these continents(2) (Table 2). The European requirement of a distance to target height (DTH) of at least 1 SDS is not used in the United States of America. Although it might be likely that short children with a small distance to their target height SDS have a lower growth response to GH because of a lower genetic growth potential, this European criterion is not evidence-based and is subject of continuous discussion.

Table 2. Criteria for GH treatment in short children born SGA(2,58).

	USA (FDA, 2001)	Europe (EMA, 2003)
Initial age in years	2	4
Initial height SDS	Not included	<-2.5SDS
Growth velocity	No catch up growth	<0SDS
Distance to target height (TH SDS – initial height SDS)	Not included	≥1SDS

The aim of GH treatment for short SGA children is achieving an AH in the normal range and/or in the target height (TH) range of the child. Although GH treatment is proven effective in children who started treatment at an early age, GH is thought to have limited effect when started during adolescence, just before or during puberty.

Although GH secretion and IGF-I levels are known to rise during puberty, the quantitative relationship between these hormones and pubertal growth is unknown. Besides, there is only limited knowledge of the effect of GH dosing on pubertal growth. At start of the Dutch SGA study, the efficacy and safety of GH treatment for short SGA adolescents, with or without additional postponement of puberty, was unknown.

Postponement of puberty

Gonadotropin-releasing hormone analogues (GnRHa) inhibit the hypothalamic-pituitary-gonadal axis (Figure 5) by suppressing the pituitary gland. Consequently, GnRHa results in an inhibition of gonadotropin secretion (luteinizing hormone (LH) and follicle stimulating hormone (FSH)), leading to decreased sex steroid production and cessation of pubertal progression. GnRHa is used to suppress the pubertal axis, mainly in children with central precocious puberty.

Starting GH treatment during puberty might have only limited effect, because the epiphyseal maturation has been activated(28). Postponement of puberty by GnRHa in addition to GH treatment might improve adult height, since GnRHa delays epiphyseal maturation. However, reduced growth velocity is an unfavorable phenomenon that might occur during GnRHa treatment(59-61).

When puberty starts relatively early in short children, the adult height (AH) prediction decreases substantially. Gaining as much height as possible from early puberty until adult height is therefore an important goal for these short children. At start of this study, it was still unknown whether postponement of puberty by GnRHa treatment in addition to GH, would be beneficial for AH improvement in early pubertal, short children born SGA. Besides, no data were available on the efficacy and safety of two different GH dosages in combination with GnRHa.

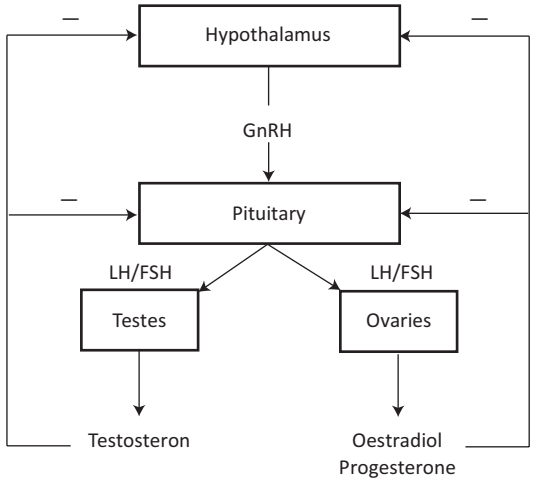

Figure 5. Hypothalamus-pituitary-gonadal axis
During puberty, gonadotropin releasing hormone analogue (GnRHa) stimulates the pituitary to secrete a pulsatile pattern of luteining hormone (LH) and follicle stimulating hormone (FSH) that stimulate the gonads (testes in boys and ovaries in girls) to produce sex steroids, either testosterone or oestradiol and progesterone.

Bone mineral density

Long-lasting negative effects of reduced birth size on bone mineral content and density have been described(35). Moreover, reduced birth size and lower growth velocity during childhood might lead to increased fracture risk in later life(62). During long-term GH treatment in short SGA children, an increase in bone mineral density (BMD) of the lumbar spine was found, independent of the height increment(34). However, after GnRHa treatment in adolescents with short stature, a substantially decreased BMD was reported(63). Other studies concluded that BMD and body composition were not impaired in patients with precocious or early puberty after GnRHa treatment(64,65).

Most of the BMD of the lumbar spine and total body is reached before the end of the second decade, with only a slight increase thereafter(66). Puberty is considered to be a crucial period for bone mass acquisition(67). It is therefore important to ascertain that children with postponed puberty will achieve sufficient peak bone mass. Reducing sex steroid levels for two years by GnHRa might have detrimental effects on bone density, particularly on the achievement of peak bone mass. At start of this study, effects of GH and GnHRa treatment on BMD in short children born SGA were not well investigated. Besides, studies on body composition and fat distribution during GnRHa treatment were lacking.

Quality of life

Being born SGA has been associated with lower intelligence and more problems in behaviour, social functioning, school competence and attention than reference children(68-74). During GH treatment, improvement of intelligence, behaviour and self-perception has been described in short SGA children(72). However, data on quality of life in short children before and longitudinally during GH treatment were very scarce(75,76).

Most psychological studies regarding short stature concentrate on limitations in general functioning, whereas it is also important to investigate specific limitations related to short stature. Besides, most studies only investigate if there are limitations due to a health problem, also called health status. It is important though to investigate the emotional impact of problems or limitations on the person's life, also called health-related quality of life (HRQoL).

HRQoL reflects the subjective perception of health and is increasingly recognized as a relevant 'patient-reported outcome'(77). A conjunction of The Netherlands Organization (TNO) for Applied Scientific Research with the Academic Hospital in Leiden (AZL, nowadays LUMC) developed an instrument (TACQOL) to assess children's HRQoL that explicitly offers respondents the possibility to differentiate between their functioning and the way they feel about it(78,79). When children or parents indicate the presence of a health problem on this questionnaire, they are asked to assess the child's emotional reaction. Besides the generic TACQOL questionnaire, a condition-specific questionnaire (TACQOL-short stature) was developed to measure the impact of short stature on HRQoL. An example of a question from the TACQOL-short stature child form is shown in Figure 6.

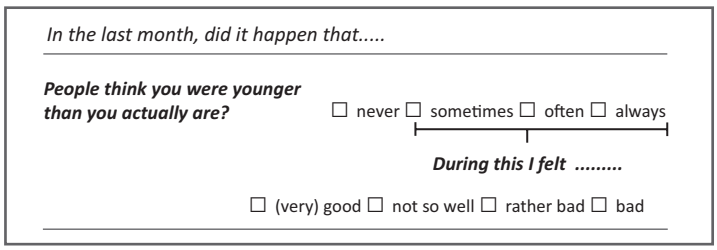

Figure 6. An example of a question from the TACQOL-short stature child form (scale: body image).

When GH treatment is combined with postponement of puberty by GnRHa to improve adult height, possible psychosocial benefits of enhancing growth must be weighed against possible adverse effects of delaying puberty(80,81). At start of this thesis no data on HRQoL in short children born SGA before and longitudinally during GH treatment were available. Especially, the HRQoL in short SGA children treated with a combination of GH and GnRHa treatment was unknown.

Aims of the study

Long-term effects of being born SGA

- To investigate whether being born SGA affects anti-Müllerian hormone (AMH), a marker for the ovarian follicle pool, in short girls born SGA. To investigate the effect of GH treatment on serum AMH levels in these girls.
- To investigate thyroid function in short children born SGA before puberty, during puberty and during postponement of puberty, in comparison to age and sex appropriate reference values. To generate of these reference values in the Dutch population (age 0-18 years). In addition, to investigate levels of thyroid hormones during GH treatment.

Criteria for starting GH treatment in Europe

- To investigate the influence of distance to target height (DTH) on the growth response during GH treatment in short children born SGA and to ascertain whether it is correct to exclude children with a DTH<1 SDS from GH treatment.

Efficacy and safety of GH and additional GnRHa treatment

- To investigate whether GH treatment in short children born SGA is effective when started during adolescence (above 8 years of age). To assess whether GH treatment 2 mg/m²·day during puberty results in significantly better adult height (AH) compared with the standard dose of 1 mg/m²·day. Also, to assess whether additional 2 years postponement of puberty by GnRHa improves AH in children with poor AH prediction at start of puberty.
- To assess bone mineral density and body composition in short children born SGA during GH treatment from start until adult height, and to assess possible adverse effects of additional postponement of puberty by 2 years of GnRHa.

Psychosocial effects of GH and additional GnRHa treatment

- To investigate health-related quality of life (HRQoL) in short adolescents born SGA during GH treatment and during combined treatment of GH and 2 years of GnRHa.

Outline of the thesis

This doctoral dissertation gives a detailed account of the various studies, not necessarily in the sequence in which these were carried out. **Chapter 1** gives an introduction in the topics described in the thesis. **Chapters 2 and 3** illustrate possible effects of impaired fetal growth on organ systems in later life. **Chapter 2** describes the effects of being born SGA on serum AMH levels, an excellent marker for the ovarian follicle pool, in prepubertal short SGA girls. **Chapter 3** describes the thyroid hormone levels in healthy controls and in short children born SGA. In addition, it shows whether the peripheral thyroid hormone metabolism changes during puberty, during postponement of puberty and during GH treatment.

The next **chapter 4** deals with the criteria for starting GH treatment in short SGA children. Although GH treatment is an approved treatment for this group of children, the criteria differ between Europe and the USA. The European requirement of a distance to target height of at least one standard deviation score, is controversial.

The following two chapters describe the longitudinal, randomized, dose-response GH trial evaluating efficacy and safety of GH treatment in older short SGA children (at or above 8 years of age). The trial was designed to investigate the effects of two GH dosages (1 versus 2 mg/m^2·day) and additional two years of GnRHa treatment on growth and adult height. **Chapter 5** presents the growth and AH results of 121 treated SGA children. **Chapter 6** gives the results on bone mineral density and body composition during and after combined GH and GnRHa treatment in short SGA children. **Chapter 7** presents the psychosocial effects of short stature in SGA children, prior to start of treatment and after two years of GH and additional GnRHa treatment.

Chapter 8 discusses the significance of the presented data and the mutual relationship in the context of literature. The effects of being born SGA and the effects of GH and additional GnRHa treatment are presented to the extent of what we know now. Our final conclusions are listed, including practical implications for treatment and follow-up of short SGA adolescents, as well as recommendations for future research. Finally, **chapter 9** summarizes the dissertation in English as well as in Dutch.

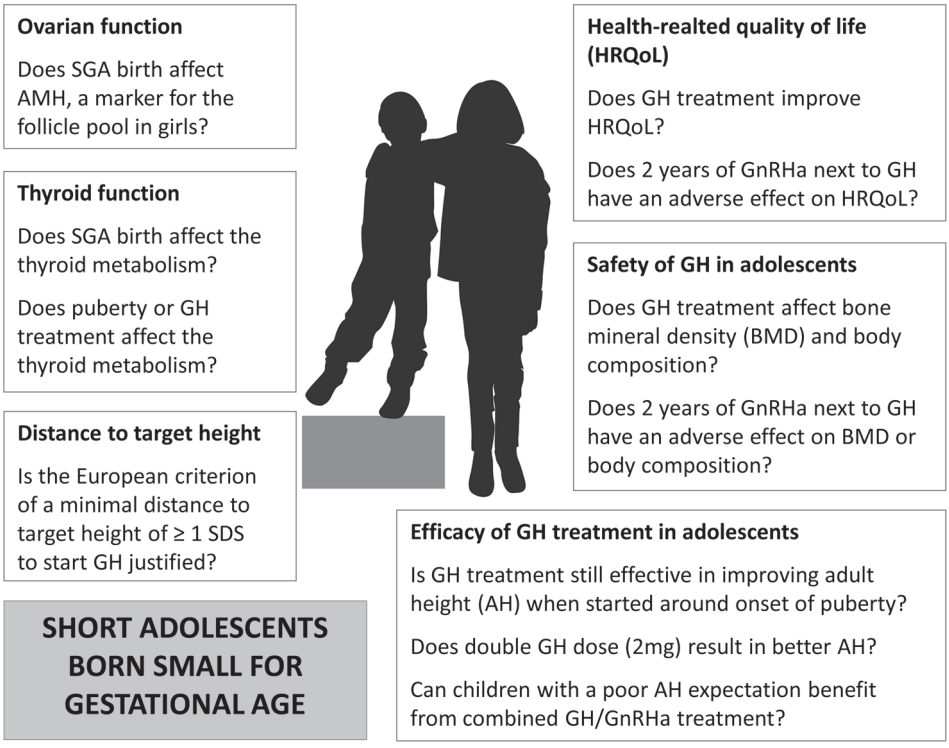

Figure 7. Research questions
SGA, small for gestational age; GH, growth hormone; GnRHa, gonadotropin releasing hormone analogue.

Appendix A

Inclusion and exclusion criteria of the Dutch SGA-study

The Dutch SGA-study included children when they met the following inclusion criteria:

1) birth length and/or birth weight SDS for gestational age (GA) <-2.0[82];
2) chronological age 8 years or older;
3) prepubertal stage (Tanner stage 1) or early pubertal stage (breast stage 2-3 in girls and testicular volume <10 ml in boys(83), with a GnRHa stimulating test indicating central puberty(84));
4) current height SDS <-2.5 SDS or a predicted adult height <-2.5 SDS (defined as height below 140 cm at start of puberty), according to Dutch references(85);
5) well documented growth data from birth to start of treatment;
6) informed consent.

Children were excluded in case of:

1) Turner syndrome in girls, known syndromes or chromosomal disorders, or serious dysmorphic symptoms suggestive for a syndrome that has not yet been described, except for Silver-Russell syndrome;
2) a complicated neonatal period with severe asphyxia (defined as Apgar score ≤3 after 5 minutes), or long-term complications of respiratory ventilation (bronchopulmonary dysplasia or other chronic lung disease);
3) celiac disease and other chronic or serious diseases of the gastrointestinal tract, heart, genito-urinary tract, liver, lugs, skeletal or central nervous system;
4) chronic or recurrent major infectious diseases or nutritional and/or vitamin deficiencies;
5) endocrine or metabolic disorders, e.g. diabetes mellitus, diabetes insipidus, hypothyroisdism, or inborn errors of metabolism;
6) medications or interventions during the previous six months that might have interfered with growth, such as coticosteroids (including high dose corticosteroid inhalation), sex steroids, growth hormone, or major surgery (particularly of the spine or extremities);
7) use of medication that might interfere with growth during GH treatment, such as cortico-steroids, sex steroids, GnRH analogue;
8) active or treated malignancy or increased risk of leukemia;
9) serious suspicion of psychosocial dwarfism (emotional deprivation);
10) expected non-compliance.

Appendix B

Design of the Dutch SGA-study

The Dutch SGA-study started in 2003. It is a longitudinal, randomized, dose-response GH trial involving short SGA children ≥8 years, evaluating GH 1 versus 2mg/m²·day from early puberty until AH. All children administered Somatropin (Genotropin®) subcutaneously daily. Every 3 months, GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1mg/m²·day (Figure 8). When these prepubertal children entered puberty or when children were in early puberty at start of treatment, they were randomly assigned to treatment with either GH 1 or 2mg/m²·day, after stratification for gender, pubertal stage and parental height (one or two parents with height <-2SDS versus both parents with height ≥-2SDS). Children who were very short at start of puberty (height <140 cm) were defined as children with a predicted AH <-2.5SDS and received GnRHa (leuprorelide acetate depots 3.75mg subcutaneously every 4 weeks) for 2 years in addition to GH treatment.

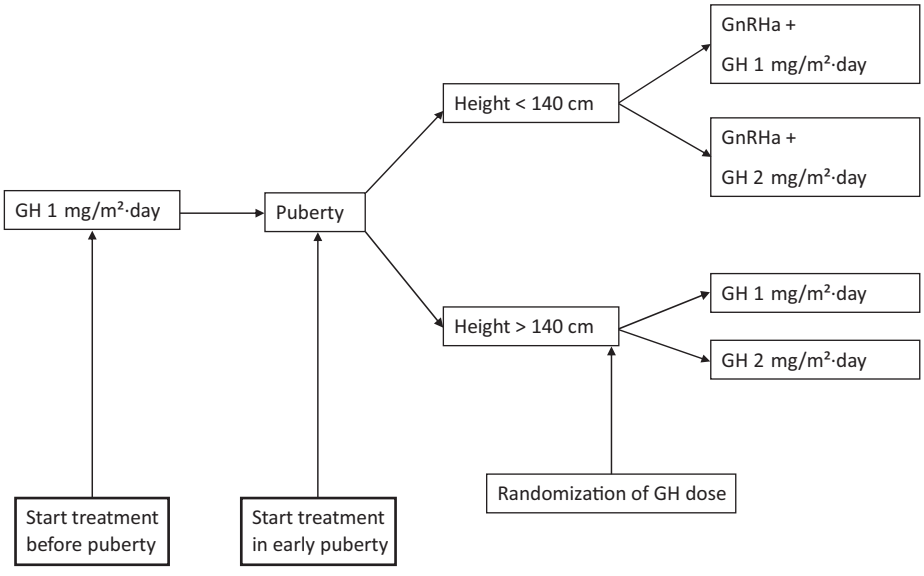

Figure 8. Flowchart treatment regimen

Appendix C

Participating centers and physicians of the Dutch SGA-study

The Dutch SGA-study is a multicenter trial coordinated by the Dutch Growth Research Foundation, Rotterdam, the Netherlands. The SGA-study team consists of one MD-researcher and a research nurse. Three-monthly, 10 hospitals throughout The Netherlands are visited by the MD-researcher and the research nurse, where children are examined, in collaboration with the local pediatrician or pediatric endocrinologist. Standardized measurements take place according schedule at the Erasmus Medical Center / Sophia Children's Hospital Rotterdam, The Netherlands.

Participating centers and pediatricians are:

Erasmus Medical Center Rotterdam / Sophia Children's Hospital, Rotterdam
A.C.S. Hokken-Koelega MD PhD
A.J. Lem MD
D.C.M. van der Kaay MD PhD
J. van Houten, research nurse
J. Bontenbal-van de Wege, research nurse

Admiraal de Ruyter Hospital, Vlissingen	E.J. Sulkers MD PhD
Canisius Hospital, Nijmegen	C. Westerlaken MD PhD
Catharina Hospital, Eindhoven	R.J. Odink MD
Leids University Medical Center, Leiden	D. Mul MD PhD, W. Oostdijk MD PhD
Rijnstate Hospital, Arnhem	J.C. Mulder MD, F. Neijens MD
University Medical Center Groningen, Groningen	W.M. Bakker-van Waarde MD PhD
University Medical Center St. Radboud, Nijmegen	C. Noordam MD PhD
Zaans Medical Center, Zaandam	J.P.C.M. van der Hulst MD
Isala Clinics Amalia, Zwolle	E.J. Schroor MD PhD

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Chapter 2

Anti-Müllerian hormone in short girls born small for gestational age and the effect of growth hormone treatment

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Abstract

Background

Fetal growth restriction is thought to negatively influence reproductive function in later life. Serum Anti-Müllerian hormone (AMH) is a marker of the primordial follicle pool. The objectives of this study were to evaluate the effect of being born small for gestational age (SGA) on serum AMH levels and to investigate the effect of growth hormone (GH) treatment on serum AMH levels in short SGA girls.

Methods

Serum AMH levels were investigated in 246 prepubertal girls aged 3 to 10 years: 119 untreated short SGA and 127 healthy controls. Associations between AMH levels and clinical characteristics were analysed using multiple regression analyses. In addition, we investigated the effect of GH treatment on serum AMH levels in short SGA girls.

Results

Serum AMH levels were similar in short SGA and healthy control girls (p=0.95). In short SGA girls, AMH levels were not significantly influenced by birth weight standard deviation score (SDS), birth length SDS and gestational age, even after adjustment for age, height SDS and body mass index (BMI) SDS at sampling, socio-economic status (SES) and maternal smoking during gestation. Serum AMH levels did not change during 4 years of GH treatment in short SGA girls (p=0.43).

Conclusion

Serum AMH levels in prepubertal short SGA girls are similar to healthy controls, indicating that the follicle pool is not compromised due to SGA birth. GH treatment has no effect on AMH levels in short SGA girls.

Introduction

Fetal life is a critical phase in the development of important organ systems, including the gonads. Already at 20 weeks of gestation, the maximum number of primordial follicles in the ovaries is reached(1,2). A suboptimal intrauterine environment may have a detrimental effect on the development and preservation of primordial follicles, and may therefore impair reproductive health in later life. Granulosa cells of primary and preantral follicles, the stages following primordial follicles, secrete anti-Müllerian hormone (AMH) that is involved in the regulation of folliculogenesis(3). Since serum AMH is produced exclusively by the ovaries, independently of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool(4-9).

Research has been conducted to investigate the reproductive function in children with restricted fetal growth, born small for gestational age (SGA), but the results were controversial. From autopsy examination of female fetuses, the developing ovary was found to increase in size with gestational age, but did not differ between growth restricted and normal fetuses(10). Although some retrospective studies found higher FSH levels and reduced uterine and ovarian size in SGA girls(11), another group of researchers could not confirm these findings(12). Recently, increased levels of AMH were found in both low and high birth weight female infants in the first three months of life, compared to normal birth weight infants(13).

Nowadays short SGA children can be treated with growth hormone (GH). Several studies showed an important role of GH, insulin-like growth factors (IGFs) and IGF binding proteins (IGFBPs) in ovarian follicular development (review(14)). However, little is known about serum AMH levels in children treated with GH.

We hypothesised that fetal growth restriction does not affect the ovarian follicle pool and therefore SGA birth would not alter serum AMH levels in girls. To test this hypothesis, we compared serum AMH levels in a large group of prepubertal short SGA girls, with those of healthy control girls. In addition, we investigated the effect of GH treatment on the serum AMH levels in short SGA girls.

Patients and methods

SGA subjects

The SGA group consisted of 119 prepubertal girls with short stature before start of GH treatment (aged 3 to 10 years). These girls were originally enrolled in Dutch multicenter GH trials(15-18). Girls were included in the present study if they met the following criteria: A) birth length and/or birth weight standard deviation score (SDS) for gestational age below -2.0(19); B) height SDS for calendar age (CA) below -2.0(20); C) height velocity SDS for CA below zero to exclude children with spontaneous catch-up growth; D) prepubertal stage. Of all short SGA girls, 7.6% had partial

growth hormone deficiency (GHD), defined as a maximum GH peak after stimulation test (arginine or clonidine) between 10 and 20 mU/l, and none of them had severe GHD with a maximum GH peak <10 mU/l. Girls were excluded if there was a complicated neonatal period, or signs of severe asphyxia (defined as Apgar score 3 or less after 5 minutes), endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, or chondrodysplasia), Turner syndrome or other syndromes (except for Silver-Russell syndrome), as well as children who were using or had used medication that could interfere with growth.

Controls

The control group consisted of 127 healthy girls, randomly recruited from the Erasmus MC in Rotterdam, The Netherlands. The girls were aged 3 to 10 years and were referred because of a minor surgical procedure. None of the girls was born preterm (gestational age <37 weeks), born SGA (birth weight <2500 gram), or had a short stature (height SDS <-1.6). Girls were excluded if they had endocrine or metabolic disorders, chromosomal defects, syndromes or serious dysmorphic symptoms suggestive of a yet unknown syndrome.

The studies were performed in accordance with the Helsinki declaration recommendation for conduct of clinical research and approved by the Medical Ethics Committees of the participating centres. Written informed consent was obtained from the parents or guardians of each child.

Methods

We analysed serum AMH levels in 119 prepubertal, short SGA girls before start of GH treatment, and 127 control girls. In addition, we investigated the effect of GH on serum AMH levels in a subgroup of short SGA children. The subgroup consisted of 44 short SGA girls who were treated with GH in a dose of either 1 mg/m²·day (~0.033 mg/kg) or 2 mg/m²·day (~0.066 mg/kg). We compared serum AMH levels before and after a median (interquartile, IQR) duration of GH treatment of 4.03 (2.02 ; 5.94) years.

Standing height, weight and Tanner stage were determined in the short SGA girls. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Height, target height (TH) and BMI were expressed in SDS, adjusting for age and gender according to Dutch reference data (Fredriks AM et al. 2000). Prepubertal stage was defined as Tanner breast stage 1(21). TH was calculated as TH = [(maternal height + paternal height - 13) / 2 + 4.5], including the secular trend of the last decades in the Dutch population(22). Information regarding socio-economic status (SES) and maternal smoking during gestation was obtained using questionnaires that were answered by the parents of the short SGA girls. Education level of the parents was used as socio-economic indicator to determine SES (categorised as lowest, low, medium, high; range 1-4) (Verweij A 2008).

Assays

All samples were kept frozen until assayed (-80°C). Serum AMH levels were determined in the same laboratory by using an in-house double antibody ELISA(24) or an ultra sensitive enzyme-linked immunosorbent assay (Immunotech-Coulter, Marseilles, France) as described elsewhere(25). The values of the Immunotech Coulter assay were adjusted (*2.147) for comparison with the in-house ELISA. The limit of detection was 0.05 microgram/litre. The intra and interassay variation coefficients were <5% and 10% in the in-house ELISA and <5% and 8% in the Immunotech Coulter assay. GH, IGF-I and IGFBP-3 were measured using specific radio immunoassays (RIAs), as previously described(26). Serum levels of total IGF-I and IGFBP-3 were expressed in SDS, adjusting for age and gender, using reference values for healthy children of normal stature determined in the same laboratory(27).

Statistical analyses

All data were expressed as median (interquartile range (IQR)). SD-scores for height, TH and BMI were calculated using Growth Analyser (version 3.5; Growth Analyser b.v., Rotterdam, The Netherlands). Due to a skewed distribution, serum AMH levels were log transformed for analyses. Power calculation was performed according to the serum AMH levels of the control group. To determine a change in mean AMH level of 1 standard deviation, the number needed to investigate is 22 (α=0.05 and β=0.9). Comparisons between the short SGA and the control group were conducted using the independent-samples *t*-test. We used the one-sample *t*-test and Chi-square test to compare variables in the SGA subgroup to the mean of the variables in the total SGA group. The paired-samples t-test was used to determine differences in two repeated measurements within the short SGA subgroup. The associations between serum AMH levels and clinical characteristics were analysed using multiple regression analyses. SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) statistical software was used for data analysis. Results were regarded statistically significant if p was <0.05.

Results

Clinical characteristics

The clinical characteristics of the total study group are presented in Table 1. The short SGA group of 119 prepubertal girls, had a median (IQR) age of 6.24 (4.70 ; 7.36) years, which was similar to that of healthy controls (p=0.72). The SD-scores of birth weight, birth length, height at sampling, TH, BMI, IGF-I and IGFBP-3 were significantly lower than zero, the expected mean value of the control girls (all p<0.01). The baseline characteristics of the subgroup of 44 short SGA girls, with AMH levels before and during GH treatment, were similar to the total SGA group, except for a significant younger age at sampling (p<0.01).

Serum AMH levels

The median (IQR) serum AMH levels were similar in the short SGA and the healthy control girls, being 5.22 (3.54 ; 8.19) and 4.79 (3.12 ; 7.82) µg/l, respectively (Table 1, p=0.95). Figure 1 shows AMH levels against age for both groups. The majority of the short SGA girls (109/119=90%) had an AMH level within the normal range (between -2 SDS and +2 SDS of controls). Serum AMH levels of short SGA and control girls were similarly distributed around the normal mean. Eight short SGA girls had an AMH level below the control -2 SDS, equivalent to 6.7% of the total SGA group, whereas 2.4% (3/127) of the control girls had by definition an AMH level below the -2 SDS. The percentages of girls with an AMH level below -2 SDS did not significantly differ between the short SGA girls and the controls (p=0.13). Since serum AMH levels did not differ between partial GH deficient and other short SGA girls (p=0.85), we analysed these two groups together.

The associations between AMH levels and clinical characteristics, were analysed using multiple regression analyses in all short SGA girls before start of GH treatment. Serum AMH levels were not significantly correlated with birth weight SDS (β=0.13 with p=0.18), birth length SDS (β=0.14 with p=0.24) and gestational age (β=-0.02 with p=0.86), also after adjustment for age, height SDS, BMI SDS at sampling, SES and maternal smoking. Also other variables, such as IGF-I SDS and IGFBP-3 SDS at time of sampling, were not significantly related to serum AMH levels.

Growth Hormone treatment

The effect of GH treatment on serum AMH levels was investigated in a subgroup of 44 short SGA girls (Table 1), who received GH for a median (IQR) duration of 4.03 (2.02 ; 5.94) years. Height SDS increased significantly with a median (IQR) gain in height SDS of 1.56 (1.23 ; 1.88) (p<0.01). Since there was no significant difference in serum AMH levels between children who received 1 mg or 2 mg GH/m^2·day, data of both dosage-groups were analysed together. Similar serum AMH levels before and after 4 years of GH treatment were found (p=0.43). The AMH levels were also similar to those of the control girls (p=0.40), also after correction for age and pubertal stage at time of sampling.

Table 1. Clinical characteristics and serum AMH levels of the total group (short SGA and control girls) and of a short SGA subgroup

	Total group			Short SGA subgroup		
	Short SGA n=119	**Control n=127**	**P-value** (a)	**At start of GH n=44**	**After 4 yrs of GH n=44**	**P-value** (b)
Age (yrs)	6.24 (4.70 ; 7.36)	6.23 (4.70 ; 7.34)	0.72	5.04 (4.33 ; 6.19)	10.12 (7.18 ; 10.90)	<0.01
Gestational age (wks)	37.7 (33.9 ; 39.0)			37.0 (32.6 ; 38.4)		
Birth weight SDS	-2.34 (-3.09 ; -1.64)	*	<0.01	-2.43 (-3.12 ; -1.63)		
Birth length SDS	-2.74 (-3.56 ; -2.11)	*	<0.01	-2.80 (-3.99 ; -2.19)		
Target height SDS	-0.25 (-0.90 ; 0.22)	*	<0.01	-0.11 (-0.42 ; 0.28)		
Height SDS	-2.91 (-3.24 ; -2.55)	*	<0.01	-2.77 (-3.17 ; -2.46)	-1.34 (-1.71 ; -0.75)	<0.01
BMI SDS	-1.43 (-2.12 ; -0.64)	*	<0.01	-1.76 (-2.19 ; -0.89)	-1.05 (-1.67 ; -0.43)	0.03
IGF-I SDS	-1.14 (-1.96 ; -0.26)	*	<0.01	-1.46 (-2.42 ; -0.26)	1.73 (0.97 ; 2.28)	<0.01
IGFBP-3 SDS	-0.86 (-1.70 ; -0.20)	*	<0.01	-1.28 (1.72 ; 0.05)	0.78 (0.16 ; 2.32)	<0.01
Maternal smoking (%)	32.5			22.9		
SES (%) 1	2.9			0		
2	60.6			56.1		
3	15.4			12.2		
4	21.2			31.7		
AMH (µg/l)	5.22 (3.54 ; 8.19)	4.79 (3.12 ; 7.82)	0.95	5.15 (3.87 ; 7.55)	4.64 (2.17 ; 7.76)	0.43

Data expressed as median (interquartile range) unless written otherwise.

SDS, Standard Deviation Score; BMI, Body Mass Index; IGF-I, Insulin-like Growth Factor-I; IGFBP-3, Insulin-like Growth Factor Binding Protein-3; SES, Socio-Economic Status; AMH, Anti-Müllerian Hormone.

* Median/mean values in the control group are expected to be zero.

a. Short SGA and control group.

b. Short SGA subgroup; at start of and after GH treatment.

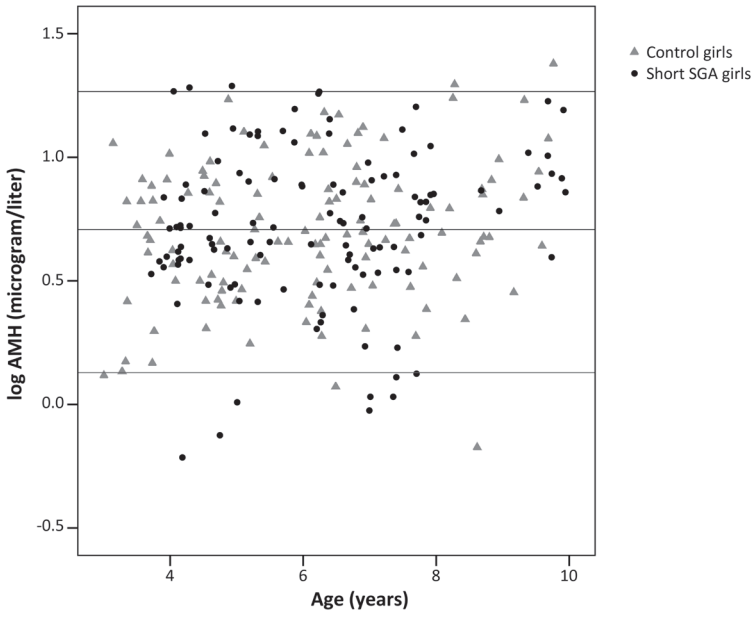

Figure 1. Serum AMH levels in untreated, short SGA and control girls
Serum AMH levels (µg/l) in 119 untreated SGA and 127 control girls (p=0.95).
Lines represent SD-scores of control group: +2 SDS, 0 SDS, -2 SDS.

Discussion

Our study shows that serum AMH levels in prepubertal short SGA children are similar to healthy control girls, indicating that the follicle pool is not compromised due to SGA birth. We found no adverse effect of birth size on serum AMH levels, even after adjustment for possible confounders as socioeconomic status (SES) and maternal smoking. Subgroup analyses revealed no effect of 4 years of GH treatment on the serum AMH levels in SGA girls.

The possible effect of birth size on the ovarian follicle pool was investigated by determination of serum AMH levels in a large group of untreated short SGA girls compared to healthy control girls. Serum AMH levels were similar in both groups. Although we excluded girls born SGA from the control group, we had no exact data on birth size of this group. Therefore, we analysed possible correlations between serum AMH levels and birth size in short SGA girls. We found no correlation between serum AMH levels and birth weight SDS, birth length SDS or gestational age, even after correction for age, height SDS, BMI SDS, IGF-I SDS, IGFBP-3 SDS, SES and maternal smoking. This demonstrates that the size of the ovarian follicle pool of prepubertal short SGA girls is not reduced because of their SGA or preterm birth. Previously, reduced prenatal growth

has been associated with FSH hypersecretion and reduced size of internal genitalia(11). However, these observations were obtained from a small and selected group. More recent studies in adolescent girls showed that fetal growth trajectories and birth size were not related to ovarian reserve(28,29), in line with our results. Our study has additional value, since we analysed a much larger group of short SGA girls, and we also investigated the effect of growth hormone treatment.

Although serum AMH levels were similar in short SGA and control girls, short SGA girls might have more often an AMH level below <-2 SDS, suggesting premature ovarian failure (6.7% in the short SGA group versus 2.4% in the control group, p=0.13). Syndromes with known gonadal dysfunction as Turner(30) and Bloom(31,32) were excluded, however, we can not rule out that a yet unknown genetic polymorphism or mutation could be present. Nonetheless, the characteristics of the short SGA girls with an AMH below -2 SDS did not appear different from the rest of the short SGA group. Our results demonstrate that in short SGA girls, small birth size does not influence the follicle pool in the majority of the cases, although short SGA girls might be at a slightly higher risk for low AMH. Underlying problems that might result in both a short stature and a reduced ovarian reserve, need further research in even larger cohorts.

In contrast to the present study, others showed increased levels of serum AMH in low birth weight infant girls. That study however, comprised infant girls who, on average, already showed evident catch-up in weight at the age of 2-3 months(13).We can speculate that SGA born children who show catch-up growth are more likely to have higher levels of AMH than controls, in contrast to short SGA girls. This is in line with a recent study showing higher AMH levels in young women with normal stature born SGA than in controls(29).

Serum AMH reflects the ovarian follicle pool, since production of this hormone is exclusively found in granulosa cells of the preantral and antral follicles of the ovary(3). Since the size and morphology of the ovaries are relatively stable during childhood(33), we investigated prepubertal children in the range of 3 to 10 years. AMH is a good marker of the ovarian follicle pool(5,7), also demonstrated in young girls(9). The relationship with spontaneous fertility is less well established(34) and is most often studied in adult patients with infertility(35). Although the range of serum AMH levels is broad and skewed, as shown in our healthy controls, several studies demonstrated that AMH offers the clinical estimate of the ovarian reserve(36-38). Nowadays the prognostic value of serum AMH on an individual basis is similar to that of the antral follicle count, another sensitive and specific marker to predict ovarian reserve(8). Hence from the current study we conclude that reduced birth size does not alter serum AMH levels and thus ovarian reserve in prepubertal short girls.

Many children born SGA who remain short after birth are nowadays treated with GH. Since the GH-IGF-I system has an important role in oocyte fertilization(39), we investigated the effect of GH treatment on serum AMH levels. Our results show that AMH levels in SGA girls, who were treated with GH for a median duration of 4 years, were similar to untreated short SGA and control girls. These results indicate that GH treatment does not change the size of the ovarian pool of growing follicles in short SGA girls. Spontaneous catch-up growth after being born SGA

has been associated with a higher risk of developing PCOS-like phenotype in sheep(40). However, our results do suggest that catch-up growth in height during GH treatment does not affect serum AMH levels and hence ovarian reserve.

In conclusion, prepubertal short SGA girls have similar serum AMH levels as healthy controls, indicating that the follicle pool is not compromised due to SGA birth. GH treatment has no effect on AMH levels in short SGA girls.

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Chapter 3

Serum thyroid hormone levels in healthy children from birth to adulthood and in short children born small for gestational age

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Abstract

Context

Age-appropriate reference ranges for thyroid hormones are required for detecting pediatric thyroid dysfunction. Data on thyroid hormones and peripheral thyroid metabolism in short children born small for gestational age (SGA) before and during growth hormone (GH) treatment are lacking.

Objectives

To obtain pediatric thyroid hormone reference ranges. To investigate thyroid hormones in short SGA children before puberty, during puberty and during postponement of puberty by gonadotropin-releasing hormone analogue (GnRHa). To evaluate thyroid hormones during GH treatment.

Patients and Design

In 512 healthy children (225 females; 0-18 years), free thyroxine (FT_4), thyroid-stimulating hormone (TSH), total thyroxine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3) and thyroxine binding globulin (TBG) were determined. Reference ranges were calculated using the linearity, median and skewness (LMS) method. In 125 short SGA children (62 females; mean age 11.3 years), thyroid hormones were analyzed before and after 2 years of GH treatment and additional GnRHa.

Results

Thyroid references showed wide ranges postnatally and age-specific patterns thereafter, similar in boys and girls. Untreated short SGA children had similar FT_4 and T_4 levels as the reference population, but significantly higher T_3, rT_3 and TBG levels. During puberty and during GH treatment, FT_4 and rT_3 significantly decreased whereas T_3 significantly increased.

Conclusion

Age-specific thyroid reference ranges are presented. Puberty and GH treatment both induce changes in peripheral thyroid metabolism, resulting in more biologically active T_3 at the expense of less inactive rT_3, possibly mediated by IGF-I. GH treatment induces altered peripheral thyroid metabolism, but does not result in thyroid dysfunction.

Introduction

Thyroid hormones are essential for fetal and postnatal development and for neuropsychological functioning. Age-appropriate reference ranges are required to detect thyroid dysfunction during childhood. Only few studies have published reference ranges of thyroid hormones in children(1-9), and comparison of results is hampered by different assays, discrepancies in methodology and type of measured thyroid analytes.

Studies have investigated thyroid function in short children born small for gestational age (SGA), but results were inconclusive. In fetuses with intrauterine growth retardation, a significant reduction in circulating free thyroxine (FT_4) and a modest elevation in thyroid stimulating hormone (TSH) were found(10) that might be explained by a reduction in the expression of thyroid receptor isoforms in the cerebral cortex and cerebellum in these fetuses(11). In prepubertal short SGA children, TSH was elevated whereas FT_4 did not differ from controls(12,13).

Growth hormone (GH) treatment is an effective and safe treatment to improve height in short SGA children(14-17). It is assumed that GH treatment started during puberty has only limited effect, since by that time the process of epiphyseal maturation has already been activated(18). Postponement of puberty in addition to GH treatment might improve adult height when puberty starts relatively early. There are no data on thyroid function during puberty and postponement of puberty in short SGA children.

Present study aimed to obtain thyroid hormone reference ranges in a large population of newborns, infants, children and adolescents. Besides, we aimed to investigate thyroid hormones in short SGA children and to evaluate levels during GH and additional GnRHa treatment. Based on previous results(10-13), we firstly hypothesized that untreated short SGA children have normal FT_4, but increased TSH. Secondly, we hypothesized that GH treatment alters peripheral thyroid metabolism, resulting in mildly decreased FT_4 and mildly increased T_3. Thirdly, we hypothesized that postponement of puberty does not influence thyroid hormones.

Patients and methods

Reference study

The reference population consisted of 512 healthy children (225 female), aged 0-18 years. The children were randomly recruited from the Erasmus MC, Rotterdam, The Netherlands. All children were referred because of a minor surgical procedure, were born term (gestational age >37 weeks), appropriate for gestational age (birth weight >-2 SDS) and had a normal stature (height >-2 SDS). Children were excluded in case of a thyroid or pituitary disorder, systemic disorder, chromosomal defect, syndrome or serious dysmorphic symptoms suggestive for a yet unknown syndrome. In case of blood sampling, one additional sample was obtained to determine FT_4, TSH, total thyroxine (T_4), total triiodothyronine (T_3), reverse triiodothyronine (rT_3) and thyroxine

binding globulin (TBG). Per age interval the number of samples was as follows: cord blood n=64; 1 to 7 days n=40; 8 days to 1 month n=31; 1 to 3 months n=54; 3 to 6 months n=63; 6 months to 1 year n=74; 1 to 2 year n=44; 2 to 5 years n=33; 5 to 8 years n=12; 8 to 12 years n=48; 12 to 15 years n=22; 15 to 18 years n=27.

SGA study

The Dutch SGA GH study (started in December 2003) aimed to investigate efficacy and safety of GH treatment in short SGA adolescents (≥8 years), in combination with 2 years of postponement of puberty by GnRHa in case puberty started before height of 140 cm was attained. Children were included when they met the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) for gestational age <-2.0(19); 2) chronological age of ≥8 years; 3) prepubertal stage (Tanner stage 1) or early pubertal stage (breast stage 2-3 in girls and testicular volume <10 ml in boys(20), with a GnRHa test result indicating central puberty(21)); 4) current height SDS <-2.5 SDS or a predicted adult height <-2.5 SDS (defined as a height at start of puberty <140 cm), according to Dutch references(22). Karyotype was normal in all girls. None of the children was GH deficient according to stimulation tests or overnight profiles. Children were excluded in case of a complicated neonatal period with signs of severe asphyxia (defined as Apgar score ≤3 after 5 minutes), long-term complications of respiratory ventilation (e.g. bronchopulmonary dysplasia), endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness or chondrodysplasia) or syndromes (except for Silver-Russell syndrome), as well as children who were using or had used medication that could interfere with growth or GH treatment.

All short SGA children administered Somatropin (Genotropin®) subcutaneously daily. Every 3 months, GH dose was adjusted to the calculated body surface area. Prepubertal children received GH 1mg/m²·day (~0.033mg/kg·day). When these prepubertal children entered puberty or when children were in early puberty at start of treatment, they were randomly assigned to treatment with either GH 1 or 2mg/m²·day, after stratification for gender, pubertal stage and parental height (one parent with height <-2 SDS or both parents with height ≥-2 SDS). Children who were very short at start of puberty (height <140 cm, with a predicted adult height <-2.5 SDS), received GnRHa (leuprorelide acetate depots 3.75mg subcutaneously every 4 weeks) for 2 years in addition to GH treatment. During GnRHa, puberty was sufficiently suppressed in all children as assessed clinically, as well as by GnRHa stimulating tests. At baseline and after 2 years of treatment, blood samples for hormonal assessments were collected.

Short SGA children who had been treated for at least 2 years were included in the thyroid analyses (n=125 children (63 boys, 62 girls: 66 prepubertal, 59 pubertal)). All children were used for baseline analyses. Subsequently, children were grouped according to pubertal stage and treatment regimen: prepubertal children who received only GH (n=31), pubertal children who received only GH (n=12) and pubertal children who received a combination treatment of GH and

GnRHa (n=47). Prepubertal children who entered puberty during GH treatment (n=35), were excluded for group analyses.

Both the reference and the SGA study were performed according to the Helsinki declaration and approved by the Medical Ethics Committees of the participating centers. Written informed consent was obtained from parents or guardians of each child and from children who were 12 years or older.

Measurements

Serum levels of FT_4, TSH, T_4 and T_3 were determined by chemiluminescence assays (Vitros Eci technology, Ortho-Clinical-Diagnostics, Amersham, UK). RT3 was measured by radioimmunoassay (RIA) as previously described(23). TBG was measured by immunometric assay (Immulite 2000, Siemens, Breda, The Netherlands) if aged ≤6 years and by Dynotest RIA if aged >6 years (Brahms, Berlin, Germany). Interassay coefficients of variation amounted to 4% for TSH, 5% for FT_4, 3.3% for T_3 and 10% for rT_3. Serum levels of insulin-like growth factor (IGF-)I and insulin-like growth factor binding protein (IGFBP-)3 were measured using specific RIA(24). IGF-I and IGFBP-3 were expressed in SDS, adjusting for age and gender, using reference values from healthy children of normal stature determined in the same laboratory(25).

At start and three-monthly during GH treatment, height, weight and Tanner stage were determined, as described elsewhere(16). Body mass index (BMI) was calculated (kg/m²). Target height (TH) was calculated as TH = [(maternal height + paternal height + 13) / 2 + 4.5] for boys and TH = [(maternal height + paternal height - 13) / 2 + 4.5] for girls(22,26). SDS for height, TH and BMI were calculated to adjust for age and gender according to Dutch references(22), using Growth Analyser (version 3.5; Growth Analyser B.V., Rotterdam, The Netherlands).

Data analysis

Reference curves of thyroid hormone levels were constructed by the LMS method of Cole and Green(27), using the LMSchartmaker Light (version 2.43, Medical Research Counsil, UK). The principle behind the LMS method is that, after transformation, data show a standard normal distribution. The method summarizes the distribution of measurement values over the age range using three smoothed age-related curves; the median (M curve), the coefficient of variation of the measurement (S curve) and a curve showing the power transformation needed at each age to convert the data to a Gaussian distribution (L curve). The L, M and S curves obtained for each thyroid analyte were combined to derive reference curves. An advantage of the LMS method is that an individual measurement can be converted into an exact SDS. Besides, the LMS method uses age as a continuous variable, instead of most methods that describe distributions within arbitrary chosen age groups. Differences in thyroid hormone levels between boys and girls were analyzed by comparing thyroid hormone SDS.

Clinical characteristics of short SGA children were presented as mean (SD) unless stated otherwise. Distribution of variables was determined by the Kolmogorov-Smirnov test and normal

Q-Q-plots. Differences in clinical characteristics between prepubertal and pubertal short SGA children were evaluated using independent Sample *t*-test for normally distributed outcomes and Mann-Whitney U-test otherwise. We used one-Sample *t*-test to compare SDS results with 0 SDS (mean value in the reference population). Changes in SDS over time were analyzed with paired sample *t*-test. Correlations between thyroid hormone levels in the reference group were analyzed using Pearson's and Spearman's correlation coefficients. The statistical package SPSS (version 17.0; SPSS Inc. Chicago, IL) for Windows was used. Results were regarded statistically significant if p was <0.05.

Results

Reference children

Age-specific reference ranges (-2, -1, 0, 1 and 2 SDS) for thyroid hormones are shown in Tables and Figures 1 A-F.

Free thyroxine

FT_4 SD-scores were similar in boys and girls (p=0.620). In umbilical cord blood, FT_4 was widely spread, distributed between 12.12 (-2 SDS) to 56.54 pmol/l (+2 SDS). At 6 months of age, the upper level of FT_4 was still 31 pmol/l. Subsequently until 2 years, the FT_4 curve narrowed. After 2 years, FT_4 gradually declined until adult age (correlation FT_4 and age: n=142, r=-0.33, p<0.001).

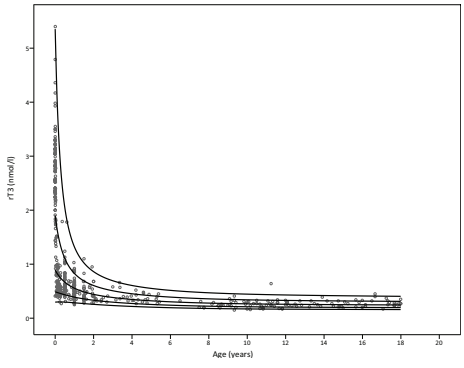
Thyroid-stimulating hormone

Serum TSH SD-scores were similar in boys and girls (p=0.686). The curve showed a wide variation directly after birth, distributed between 2.43 to 24.03 mU/l in umbilical cord blood. The values fell sharply, resulting in a much narrower range from 0.60 and 6.82 mU/l on day four. Thereafter, the width of the curve remained fairly constant. Because of the extreme variation in TSH directly after birth, we constructed two curves; one for the first days of life and one between 3 days and 18 years of age.

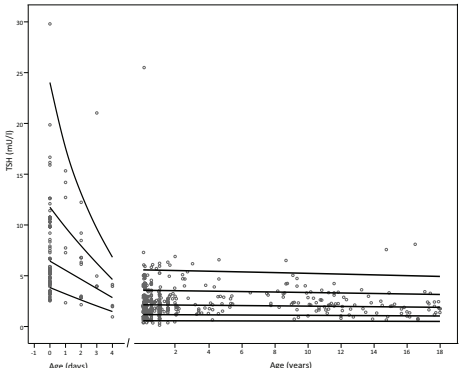
Total thyroxine

Serum T_4 SD-scores were similar in boys and girls during infancy and childhood (p=0.614). In umbilical cord blood, T_4 was distributed between 75.26 to 240.37 nmol/l. T_4 declined significantly during childhood (age 1 to 16 years, n=168, r=-0.68, p<0.001). From 16-18 years of age T_4 tended to be higher in girls compared to boys (p=0.056), so no uniform T_4 reference curve was constructed for that age-period.

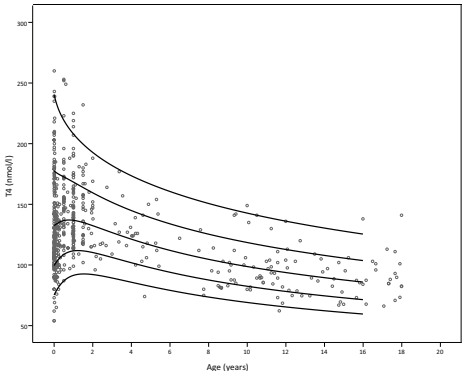
Figure 1 A-F. Reference values at selected ages



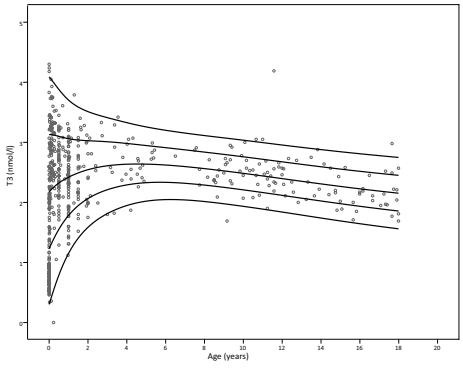
1A. Free thyroxine (FT_4)



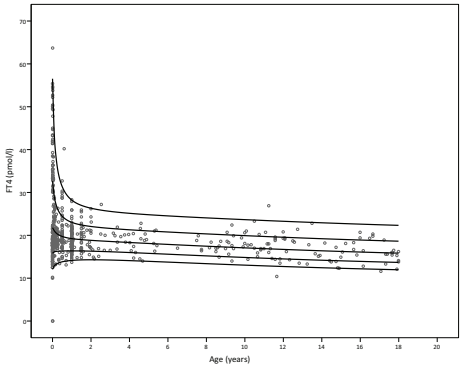
1B. Thyroid-stimulating hormone (TSH)



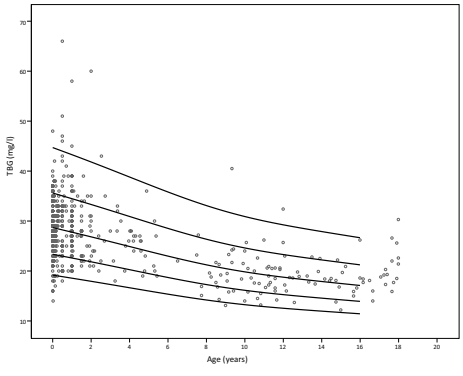
1C. Total thyroxine (T_4)



1D. Total triiodothyronine (T_3)



1E. Reverse triiodothyronine (rT_3)



1F. Thyroid binding globulin (TBG)

Table 1 A-F. Reference values at selected ages

1A

FT_4 (pmol/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	12.12	15.81	21.78	32.65	56.54
1 week	12.32	15.91	21.61	31.65	52.54
1 month	12.81	16.12	21.12	29.28	44.33
3 months	13.41	16.28	20.31	26.29	36.83
6 months	13.82	16.35	19.74	24.47	31.39
1 year	14.14	16.37	19.23	23.02	28.22
2 years	14.29	16.30	18.83	22.06	26.32
5 years	13.93	15.79	18.10	21.02	24.81
8 years	13.39	15.20	17.46	20.33	24.07
12 years	12.72	14.49	16.71	19.55	23.28
15 years	12.31	14.05	16.24	19.05	22.76
18 years	11.96	13.68	15.84	18.63	22.33

1B

TSH (mU/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	2.43	3.84	6.44	11.75	24.03
1 day	1.90	3.21	5.54	9.76	17.58
2 days	1.40	2.61	4.64	7.94	13.10
3 days	0.94	2.03	3.75	6.24	9.65
4 days	0.60	1.48	2.85	4.63	6.82
1 week	0.58	1.18	2.14	3.57	5.58
1 month	0.58	1.18	2.14	3.57	5.57
3 months	0.58	1.18	2.14	3.57	5.57
6 months	0.58	1.18	2.14	3.56	5.56
1 year	0.57	1.17	2.13	3.55	5.54
2 years	0.57	1.17	2.12	3.53	5.51
5 years	0.56	1.15	2.08	3.47	5.41
8 years	0.55	1.12	2.04	3.40	5.31
12 years	0.53	1.09	1.98	3.31	5.16
15 years	0.52	1.07	1.94	3.23	5.05
18 years	0.51	1.05	1.90	3.16	4.93

1C

T_4 (nmol/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	75.26	99.44	132.38	177.64	240.37
1 week	75.90	99.96	132.64	177.36	239.10
1 month	77.93	101.62	133.44	176.47	235.16
3 months	82.29	105.14	135.11	174.68	227.29
6 months	86.82	108.63	136.60	172.62	219.31
1 year	91.44	111.63	136.80	168.31	207.93
2 years	92.10	110.25	132.38	159.47	192.75
5 years	82.29	97.73	116.39	139.02	166.56
8 years	73.56	87.61	104.65	125.38	150.69
12 years	65.40	78.18	93.75	112.78	136.11
14 years	62.27	74.55	89.54	107.88	130.43
18 years	–	–	–	–	–

1D

T_3 (nmol/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	0.30	1.23	2.18	3.13	4.09
1 week	0.32	1.25	2.18	3.13	4.08
1 month	0.39	1.29	2.21	3.13	4.06
3 months	0.56	1.40	2.26	3.12	3.99
6 months	0.79	1.55	2.32	3.10	3.88
1 year	1.15	1.78	2.42	3.06	3.70
2 years	1.59	2.06	2.54	3.03	3.51
5 years	2.02	2.33	2.64	2.95	3.26
8 years	2.01	2.29	2.56	2.84	3.11
12 years	1.84	2.12	2.40	2.68	2.96
15 years	1.69	1.98	2.27	2.55	2.84
18 years	1.56	1.86	2.15	2.45	2.75

1E

rT_3 (nmol/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	0.30	0.48	0.87	1.88	5.51
1 week	0.30	0.48	0.86	1.82	5.20
1 month	0.30	0.47	0.83	1.67	4.36
3 months	0.30	0.46	0.75	1.38	3.04
6 months	0.30	0.43	0.66	1.11	2.11
1 year	0.28	0.39	0.55	0.83	1.32
2 years	0.26	0.33	0.44	0.60	0.86
5 years	0.20	0.25	0.32	0.42	0.55
8 years	0.18	0.22	0.28	0.36	0.47
12 years	0.17	0.21	0.26	0.33	0.43
15 years	0.16	0.20	0.25	0.32	0.42
18 years	0.16	0.20	0.25	0.31	0.40

1F

TBG (mg/l)	Standard Deviation Score				
Age	**-2**	**-1**	**0**	**1**	**2**
Day of birth	19.17	23.34	28.68	35.61	44.70
1 week	19.16	23.34	28.67	35.59	44.68
1 month	19.12	23.28	28.61	35.51	44.59
3 months	19.02	23.16	28.46	35.33	44.35
6 months	18.87	22.97	28.23	35.04	44.00
1 year	18.56	22.60	27.77	34.47	43.28
2 years	17.94	21.84	26.83	33.31	41.82
5 years	16.00	19.48	23.93	29.71	37.30
8 years	14.20	17.28	21.23	26.36	33.09
12 years	12.54	15.27	18.76	23.29	29.24
14 years	11.96	14.57	17.90	22.22	27.89
18 years	–	–	–	–	–

Triiodothyronine

Serum T_3 SD-scores were similar in boys and girls (p=0.764). The T_3 reference curve showed a wide range directly after birth, distributed between 0.30 to 4.09 nmol/l in umbilical cord blood. Until 5 years of age, the T_3 reference range narrowed while the median T_3 slightly increased. Thereafter, T_3 slowly decreased and resided within a narrower range with relatively constant

width. Between the age of 5 and 18 years, T_3 correlated negatively with age (n=108, r=-0.30, p=0.002).

Reverse triiodothyronine

Serum rT_3 SD-scores were also similar in boys and girls (p=0.721). After birth, extremely high rT_3 levels were found, distributed between 0.30 to 5.51 nmol/l in umbilical cord blood. This wide variation in rT_3 narrowed after birth, resulting in constantly low values during childhood and adolescence.

Thyroxine binding globulin

Serum TBG SD-scores were similar in boys and girls during infancy and childhood (p=0.677). In umbilical cord blood, TBG was distributed between 19.17 to 44.70 mg/l. The range became somewhat narrower for older infants and the curve declined during childhood (aged 1 to 16 years, n=168, r=-0.55, p<0.001). After the age of 16 years, TBG was significantly higher in girls than in boys (p=0.022). Since TBG differed significantly between adolescent boys and girls, no uniform TBG reference curve was constructed between 16 and 18 years.

Correlations between serum thyroid hormone levels in reference children above the age of one year

FT_4 SDS correlated positively with T_4 SDS (r=0.70, p<0.001), rT_3 SDS (r=0.52, p<0.001) and TBG SDS (r=0.26, p<0.001). TSH SDS correlated inversely with FT_4 SDS (r=-0.15, p=0.045), T_4 SDS (r=-0.23, p=0.004), T_3 SDS (r=-0.25, p=0.001), rT_3 SDS (r=-0.26, p<0.001) and tended to correlate with TBG SDS (r=-0.15, p=0.053). TBG SDS correlated strongly with T_4 SDS (r=0.67, p<0.001), and correlated also with rT_3 SDS (r=0.35, p<0.001), T_3 SDS (r=0.30, p<0.001) and FT_4 SDS (r=0.26, p<0.001).

Short SGA children

Baseline

Clinical characteristics of the short SGA children are presented in Table 2. FT_4 and T_4 SDS in short SGA children were similar to that of the reference. Short SGA children had higher TSH SDS than the reference (p=0.004). However, 96.7% had TSH levels within the normal range and only 3 children had TSH levels above the normal range. TBG, T_3 and rT_3 SDS were higher than the reference (all p<0.001). IGF-I and IGFBP-3 SDS were significantly lower than zero (p<0.001 and p=0.002, respectively).

T_4 SDS correlated positively to FT_4 SDS (r=0.62, p<0.001), T_3 SDS (r=0.34, p<0.001), rT_3 SDS (r=0.60, p<0.001) and TBG SDS (r=0.50, p<0.001). FT_4 SDS correlated with rT_3 SDS (r=0.54, p<0.001). TBG SDS correlated to T_3 SDS (r=0.34, p<0.001) and to rT3 SDS (r=0.21, p=0.024). None of the thyroid hormones was associated with gestational age, birth weight SDS, birth length SDS or height SDS at start of treatment (all p>0.3). IGF-I SDS correlated inversely to FT_4 SDS (r=-0.36, p<0.001), T_4 SDS (r=-0.23, p<0.001) and rT_3 SDS (r=-0.32, p<0.001). In contrast, IGF-I

SDS correlated positively to T_3 SDS (r=0.33, p<0.001) and T_3/rT_3 SDS (r=0.43, p<0.001). IGF-I SDS and IGFBP-3 SDS correlated with each other (r=0.473, p<0.001). IGFBP-3 SDS showed comparable correlations with FT_4, T_4, T_3 and rT_3 SDS as IGF-I SDS.

Puberty

Serum thyroid hormone levels differed significantly between pubertal and prepubertal short SGA children (Table 3A). FT_4, T_4, rT_3 and TBG SDS were significantly lower in pubertal than in prepubertal children (all p<0.001), whereas T_3 and T_3/rT_3 SDS were significantly higher (p=0.021 and p<0.001, respectively).

Postponement of puberty

After 3 months of GnRHa, all children had sufficient pubertal suppression. Thyroid hormone levels changed significantly during postponement of puberty (Table 3B); FT_4, T_4 and TBG SDS increased (all p<0.03), whereas T_3 and rT_3 SDS decreased (p=0.001). IGF-I and IGFBP-3 SDS decreased during 3 months of GnRHa, although not significantly. The thyroid hormones during postponement of puberty were in line with those of prepubertal children, except for rT_3 SDS.

GH treatment

During 2 years of GH treatment, height increased significantly with a mean (SD) of 0.7 (0.4) SDS (p<0.001). IGF-I increased with 2.3 (1.3) and IGFBP-3 increased with 2.0 (1.4) SDS (both p<0.001).

Since thyroid hormone metabolism was significantly different between prepubertal and pubertal SGA children at baseline, we analyzed children separately according to their pubertal status. During GH treatment in prepubertal children (Table 3C), FT_4 decreased with 0.5 (0.9) SDS (p=0.007) and rT_3 with 0.4 (0.9) SDS (p=0.027). T_3 increased with 0.6 (1.3) SDS (p=0.030), T_3/rT_3 with 0.5 (0.9) SDS (p=0.004) and TBG with 0.6 SDS (p<0.001). TSH and T_4 SDS remained similar. During GH treatment in puberty-postponed children (Table 3D), FT_4 decreased with 0.8 (1.2) SDS (p<0.001) and TSH with 0.8 (0.7) SDS (p<0.001). T_3 increased with 1.0 (0.9) SDS (p<0.001) and rT_3 with 0.6 (0.7) SDS (p<0.001), without a significant change in T_3/rT_3 SDS (p=0.716). In pubertal children treated with GH only (n=10 with both sample moments), no significant changes in thyroid hormone levels could be detected (all p>0.1).

Table 2. Clinical characteristics and thyroid hormone levels of untreated short SGA children

	Total group (n=125)
Boys / girls	63 / 62
Age in years	11.3 (1.6)
Gestational age in weeks	37.4 (3.4)
Birth weight SDS	-1.9 (0.9)*
Birth length SDS	-2.6 (1.2)*
Height SDS	-3.0 (0.6)*
BMI SDS	-1.1 (1.0)*
Target height SDS	-0.7 (0.7)*
FT_4 SDS	0.1 (1.2)
TSH SDS	0.2 (0.9)#
T_4 SDS	0.1 (0.9)
T_3 SDS	1.2 (1.2)*
rT_3 SDS	1.5 (0.9)*
T_3/rT_3 SDS	-0.9 (0.9)*
TBG SDS	0.5 (0.8)*
IGF-I SDS	-1.1 (1.3)*
IGFBP-3 SDS	-1.0 (1.0)*

Data expressed as mean (± SD), unless written otherwise
* Test for mean = 0, p<0.001, # Test for mean = 0, p<0.01

Table 3 A-D. Thyroid hormone levels in short SGA children

3A. Prepubertal and pubertal SGA children, untreated

	Prepuberal (n=66)	**Pubertal** (n=59)	**Difference**	**P-value**
Age (years)	10.3 (1.2)	12.4 (1.1)	2.1 (1.2)	**<0.001**
FT_4 SDS	0.5 (1.1)	-0.4 (1.3)	-0.9 (1.2)	**<0.001**
TSH SDS	0.2 (0.9)	0.3 (0.9)	0.0 (0.9)	0.840
T_4 SDS	0.4 (0.9)	-0.3 (0.8)	-0.7 (0.9)	**<0.001**
T_3 SDS	1.0 (1.2)	1.5 (1.3)	0.5 (1.2)	**0.021**
rT_3 SDS	1.8 (0.7)	1.0 (0.8)	-0.8 (0.7)	**<0.001**
T_3/rT_3 SDS	-1.3 (0.7)	-0.4 (0.9)	0.9 (0.8)	**<0.001**
TBG SDS	0.7 (0.8)	0.3 (0.8)	-0.4 (0.8)	**0.009**
IGF-I SDS	-1.6 (1.1)	-0.5 (1.2)	1.1 (1.0)	**<0.001**
IGFBP-3 SDS	-1.2 (0.8)	-0.7 (1.1)	0.6 (0.9)	**0.002**

Thyroid hormones, IGF-I and IGFBP-3 are given in mean (SD). P-value by independent samples *t*-test

3B. Pubertal SGA children before and after 3 months of GnRHa

	Before GnRHa (n=45)	During GnRHa (n=45)	Difference	P-value
FT_4 SDS	-0.3 (1.4)	0.1 (0.9)	0.4 (1.1)	**0.027**
TSH SDS	0.5 (0.8)	0.6 (0.9)	0.1 (0.6)	0.361
T_4 SDS	-0.3 (0.9)	0.0 (0.7)	0.3 (0.8)	**0.020**
T_3 SDS	1.4 (1.3)	0.7 (1.0)	-0.6 (1.2)	**0.001**
rT_3 SDS	1.0 (0.9)	0.6 (0.7)	-0.5 (0.6)	**<0.001**
T_3/rT_3 SDS	-0.4 (0.9)	-0.2 (0.7)	0.2 (0.6)	0.057
TBG SDS	0.2 (0.9)	0.5 (0.9)	0.4 (0.7)	**0.001**
IGF-I SDS	-0.7 (1.1)	-0.9 (1.1)	-0.1 (0.6)	0.168
IGFBP-3 SDS	-0.8 (1.0)	-0.9 (1.1)	-0.1 (0.5)	0.474

Thyroid hormones, IGF-I and IGFBP-3 are given in mean (SD).
P-value by paired samples *t*-test From the 47 children, 45 had measurements on both moments.

3C. Prepubertal SGA children before and after 2 years of GH

	Before GH (n=31)	During GH (n=31)	Difference	P-value
FT_4 SDS	0.4 (1.1)	-0.1 (1.2)	-0.5 (0.9)	**0.007**
TSH SDS	0.1 (1.0)	-0.1 (0.9)	-0.2 (0.9)	0.340
T_4 SDS	0.2 (0.8)	0.2 (0.9)	0.0 (0.8)	0.883
T_3 SDS	0.8 (1.2)	1.4 (1.2)	0.6 (1.3)	**0.030**
rT_3 SDS	1.7 (0.9)	1.3 (0.8)	-0.4 (0.9)	**0.027**
T_3/rT_3 SDS	-1.2 (0.8)	-0.7 (0.8)	0.5 (0.9)	**0.004**
TBG SDS	0.4 (0.7)	1.0 (0.7)	0.6 (0.7)	**<0.001**
IGF-I SDS	-1.6 (1.2)	0.9 (0.9)	2.5 (1.2)	**<0.001**
IGFBP-3 SDS	-1.2 (0.8)	0.7 (1.1)	1.9 (1.3)	**<0.001**

Thyroid hormones, IGF-I and IGFBP-3 are given in mean (SD). P-value by paired samples *t*-test

3D. Puberty-postponed SGA children before and after 2 years of GH

	Before GH (n=45)	During GH (n=45)	Difference	P-value
FT_4 SDS	0.1 (0.9)	-0.7 (1.4)	-0.8 (1.2)	**<0.001**
TSH SDS	0.6 (0.9)	-0.3 (0.8)	-0.8 (0.7)	**<0.001**
T_4 SDS	0.0 (0.7)	-0.2 (1.1)	-0.2 (0.9)	0.233
T_3 SDS	0.7 (1.0)	1.7 (0.8)	1.0 (0.9)	**<0.001**
rT_3 SDS	0.6 (0.7)	1.2 (0.9)	0.6 (0.9)	**<0.001**
T_3/rT_3 SDS	-0.2 (0.7)	-0.2 (1.0)	0.0 (0.8)	0.716
TBG SDS	0.5 (0.9)	0.6 (0.8)	0.2 (0.6)	0.123
IGF-I SDS	-0.9 (1.1)	1.5 (1.1)	2.4 (1.4)	**<0.001**
IGFBP-3 SDS	-0.9 (1.1)	1.4 (1.4)	2.3 (1.4)	**<0.001**

Thyroid hormones, IGF-I and IGFBP-3 are given in mean (SD).
P-value by paired samples *t*-test From the 47 children, 45 had measurements on both moments.

Discussion

This study presents reference ranges for serum FT_4, TSH, T_4, T_3, rT_3 and TBG. Thyroid hormones showed wide-ranged values directly after birth and subsequently a clear age-dependency. Further, we assessed thyroid hormones in 125 short SGA children. We found similar FT_4 and T_4 in untreated SGA children compared to the reference, but significantly higher TSH, T_3, rT_3 and TBG. Puberty and GH treatment resulted both in a significantly increase of biologically active T_3 and decrease of biologically inactive rT_3, suggesting an altered peripheral thyroid metabolism towards a more active setting.

Thyroid hormones are essential for normal growth and development. Our study provides age-specific reference ranges from birth to adulthood, required for interpretation of individual thyroid hormone levels. RT_3 and TSH reference values showed extremely wide-spread levels directly after birth, possibly related to delivery stress(28). Besides, monodeiodination of T_4 to rT_3 by placental deiodinase type III might explain neonatal increased rT_3 levels(29). RT_3 levels fell into a much narrower and constantly low curve from 2 years of age. Since TSH levels declined rapidly during the first days of life, we used two different LMS models. If we had used only one LMS model, the large spread in the first days would widen the normal limits for a much broader age group. Verburg et al.(8) described this difficulty of analyzing extreme TSH values after birth. Although they mentioned two separate calculations, their final TSH reference values were widely spread until 2 years of age.

The other thyroid reference values also showed wide-spread levels directly after birth, and age-specific patterns afterwards. The reference curves reveal that serum FT_4, T_4, T_3 and TBG change even during adolescence, demonstrating that adult reference values are not applicable for adolescents. TBG was significantly higher in adolescent girls compared to adolescent boys. Estrogens are known to increase serum TBG concentrations through slowing down clearance of TBG from the circulation(30) and by enhancing TBG biosynthesis(31). It is known that T_4 rises parallel to TBG, resulting in a new steady state in thyroid function with normal FT_4 and TSH levels(32). The higher serum TBG and T_4 levels in adolescent girls can be explained by a rise in estrogens, either endogenously during puberty or exogenously by administration of oral contraceptives. Unfortunately, we had no information on oral contraceptive use or estrogen levels in the reference girls. No gender specific reference curves for adolescents of 16-18 years could be constructed.

Our reference values are in line with those published by other groups(1-5, 7-9). However, comparison of exact concentrations is hampered by discrepancies in methodology and different assays. The LMS method is attractive since it provides a reference curve that changes gradually instead of abruptly at arbitrary chosen ages, complies better with the biological reality and is more reliable than discontinuous reference ranges. Besides, using this method allows correct conversion of individual measurements into exact SD-scores. Until present study, thyroid hormone reference ranges were not available for TSH, FT_4, T_4 and T_3 using Vitros ECi assay.

Throughout pregnancy, the hypothalamus-pituitary-thyroid axis matures continuously. Restriction in fetal growth may permanently influence thyroid function. Normal FT_4 levels have been described in children born SGA, but with increased serum TSH levels(12,33), more evident in preterm than in term SGA children(13). We found similar FT_4 and T_4 levels, slightly higher TSH levels, and obviously higher T_3 and rT_3 levels in short SGA than in reference children. The explanation for these higher T_3 and rT_3 levels in short SGA children is unknown. None of the thyroid hormones was correlated with gestational age, birth weight, birth length or height at start of treatment.

Peripheral thyroid hormone metabolism plays an eminent role in the regulation of thyroid hormone bioactivity. The principal pathways of thyroid hormone metabolism are deiodination and conjugation, of which deiodination is the most important one. Under normal circumstances T_4 is mainly deiodinated to active T_3 by outer ring deiodination (D1 and D2), whereas type 3 deiodinase (D3) catalyzes the inactivation of T_3 and T_4. The increase in T_3 and decrease in rT_3 levels as demonstrated during puberty and GH treatment can, at least partially, be explained by increased activation of T_4 by D1 or D2, but also by decreased inactivation of T_4 by D3.

There is a complex relationship between the GH-IGF-I axis and the hypothalamic-pituitary-thyroid axis that becomes more complex during GH treatment(34). Peripheral conversion of T_4 to T_3 occurs in liver, kidney and skeletal muscle, and the liver is considered to be important for GH-induced thyroxine conversion. A placebo-controlled crossover study of 4 months in GH deficient adults showed that GH treatment increased peripheral conversion of T_4 to T_3, without significantly affecting TSH(35). We showed that IGF-I correlates positively to T_3, and negatively to FT_4, T_4 and rT_3. Recently, an increased conversion of T_4 to T_3 during puberty was described(36). We found both in puberty as well as during GH treatment, significantly more active and less inactive thyroid hormone. These changes in thyroid metabolism might contribute to acceleration of growth during puberty and GH treatment. To summarize, our data support the relationship between the GH-IGF-I axis and the thyroid hormone metabolism.

Although GH replacement has often been reported to unmask central hypothyroidism(37, 38), defined as a reduction in FT_4 or T_4 to below the normal range, we demonstrated altered peripheral metabolism instead of altered secretion from the thyroid gland. During GH treatment in short SGA children, the decline in FT_4 was accompanied by an increase of biologically active T_3 at the expense of a decrease of inactive rT_3. We therefore conclude that GH treatment in short SGA children does not result in thyroid dysfunction.

During 3 months postponement of puberty, thyroid hormone metabolism changed in the direction of prepubertal children with less active hormones. The inactive rT_3 levels though, decreased during GnRHa and even further during GnRHa and GH combined treatment. Besides, TSH levels decreased significantly during combined treatment. Prospective studies on thyroid hormones before and during GnRHa treatment are lacking. A retrospective study in 73 girls with central precocious puberty showed no evidence of thyroid dysfunction during GnRHa

treatment(39). We assume that the changes we found in rT_3 and TSH are clinically irrelevant, since these changes did not affect active hormones.

In conclusion, we present age-specific references for interpretation of thyroid hormone levels from birth to adulthood. Short SGA children have similar FT_4 and T_4 as the reference, but increased TSH, T_3, rT_3 and TBG levels. Puberty and GH treatment resulted in more active and less inactive thyroid hormones, suggestive for an altered peripheral thyroid metabolism, possibly influenced by the GH-IGF-I system. GH treatment does not result in true hypothyroidism, since the decrease in FT_4 is accompanied by an increase in active T_3.

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Chapter 4

Should short children born small for gestational age with a distance to target height <1 SDS be excluded from growth hormone treatment?

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Abstract

Context

The criteria for starting growth hormone (GH), an approved treatment for short children born small for gestational age (SGA), differ between Europe and the USA. One European requirement for starting GH, a distance to target height (DTH) of ≥1 SDS, is controversial.

Objective

To investigate the influence of DTH on growth during GH treatment in short SGA children, and to ascertain whether it is correct to exclude children with a DTH <1 SDS from GH.

Patients

A large group of short prepubertal SGA children (baseline n=446; 4 years GH n=215).

Measurements

We analysed the prepubertal growth response during 4 years of GH. We investigated the influence of the continuous variable DTH SDS on growth response, and a possible DTH SDS cut-off level below which point the growth response is insufficient.

Results

Height gain SDS during 4 years of GH showed a wide variation at every DTH SDS level. Multiple regression analyses demonstrated that, after correction for other significant variables, an additional DTH of 1 SDS resulted in 0.13 SDS more height gain during 4 years of GH. We found no significant differences in height gain below and above certain DTH SDS cut-off levels.

Conclusions

DTH SDS had a weak positive effect on height gain during 4 years of GH, while several other determinants had much larger effects. We found no support for using any DTH cut-off level. Based on our data, excluding children with a DTH <1 SDS from GH treatment is not justified.

Introduction

Small for gestational age (SGA) is defined as a birth weight and/or birth length below -2.0 standard deviation score (SDS)(1). Approximately 10% of the children born SGA do not show spontaneous catch-up growth during the first years of life and will have persistent short stature (2-4). These short SGA children can be effectively and safely treated with growth hormone (GH) (5-7). Nowadays, GH is an approved treatment for short stature in children born SGA in the United States (Food and Drugs Administration, 2001) and Europe (European Medicines Agency, 2003). The criteria to start GH differ between these continents(5); the European requirement of a distance to target height (DTH) of at least 1 SDS is not used in the United States.

Since reduced size at birth may result from any foetal, maternal, placental or demographic influence, SGA children comprise a heterogeneous group with a broad spectrum of clinical features. Although the total group of SGA children with persistent short stature benefit from GH, there is a wide variation in the response to GH treatment. Many studies have attempted to find variables in short SGA children predicting the growth response to GH in the first year(8), until the onset of puberty(9), and up to adult height(7,9,10). Important variables appeared to be: gender, age at start of GH treatment, GH dose, height at start, bone age delay at start, target height (TH), and in a Swedish study also the difference between height SDS at start and the mid-parental height SDS(10,11). Although it might be likely that short children with a small distance to target height (DTH; TH SDS – height SDS at start) have a lower growth response to GH, the European criterion for starting GH in short SGA children with a DTH SDS>1 is not evidence-based and is subject of continuous discussion.

The aim of our study was to ascertain whether it is justified to exclude children with a DTH SDS<1 from GH treatment. We had the opportunity to investigate the growth response in a large group of prepubertal short SGA children treated with GH. We investigated the influence of the continuous variable DTH SDS on growth response, and we studied a possible DTH SDS cut-off level, below which point the growth response is insufficient.

Patients and methods

Subjects

The study cohort consisted of 446 short children born SGA, who were treated with GH in four trials (trials 1(12), 2(13), 3(14) and 4(15)), of which data have been published. Children were included in the present study if they met the following criteria: A) birth length and/or birth weight SDS for gestational age below -2.0(16); B) height SDS for calendar age (CA) at start(17) below -2.0 (trials 1 and 2) or -2.5 (trials 3 and 4); C) height velocity SDS for CA below zero to exclude children with spontaneous catch-up growth; D) CA at start of 3 yr or older; E) prepubertal stage at start of GH treatment and F) available parental height. Children were excluded if there

was a complicated neonatal period, or signs of severe asphyxia (defined as Apgar score 3 or less after 5 minutes), endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, or chondrodysplasia), or syndromes (except for Silver-Russell syndrome), as well as children who were using or had used medication that could interfere with growth. The studies were in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committees of the participating centres. Written informed consent was obtained from the parents or guardians of each participating child.

Study design

All children were treated with GH subcutaneously once daily at bedtime. Depending on the study design, the GH dose was 1 mg/m²/day (~0.033 mg/kg) or 2 mg/m²/day (~0.066 mg/kg). Every three months the GH dose was adjusted to the calculated body surface area. The growth responses of the prepubertal children after 1 and 4 years of GH treatment were analysed. We investigated data from the total group, as well as from three subgroups according to the distance to target height at start of GH treatment: DTH SDS<1, DTH SDS1-2 and DTH SDS>2.

Measurements

At the start and three monthly during GH treatment, standing height, weight and Tanner stage were determined, as described elsewhere(12). Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Height, TH, BMI and sitting height/height ratio (SH/H) were expressed in SDS, adjusting for age and gender according to Dutch reference data(17). TH was calculated as TH = [(maternal height + paternal height + 13) / 2 + 4.5] for boys and TH = [(maternal height + paternal height - 13) / 2 + 4.5] for girls(18). Target height range (THR) was defined as TH SDS +/- 1.3(17). Distance to target height SDS (DTH SDS) was determined as TH SDS minus height SDS at start. Bone age (BA) was determined according to the Greulich and Pyle method(19). Bone age delay was calculated calendar age (CA) minus BA in years. To correct for differences in GH dose over time, the mean GH dose during 1 or 4 years of GH treatment was used, instead of the GH dose at start of the study. Prepubertal stage was defined as Tanner breast stage 1 for girls and testicular volume less than 4 ml for boys(20).

Assays

At baseline and yearly during GH treatment, blood samples were taken after an overnight fast. After centrifugation, all samples were frozen (-80˚) until assayed. Insulin-like growth factor-I (IGF-I) and Insulin-like growth factor-binding protein-3 (IGFBP-3) were measured in one laboratory using specific radio immunoassays (RIAs), as previously described(21). Serum levels of total IGF-I and IGFBP-3 were expressed in SDS, adjusting for age and gender, using reference values for healthy children of normal stature determined in the same laboratory(22).

Statistics

Analyses were carried out using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. Results are expressed as the median (interquartile range), unless indicated otherwise. One-Sample *t*-test was used to compare results, expressed as SDS, with zero SDS (mean value for age-matched healthy references). We used the Pearson Chi-Square test to determine differences in categorical variables between the three subgroups. Correlations were tested with the Pearson correlation test. To evaluate the relative contribution of several determinants to the dependent variable height gain SDS, we performed multiple linear regression analyses. The potential determinants were: 1) initial characteristics: gender, birth weight SDS, birth length SDS, gestational age, TH SDS, 2) baseline characteristics at start of GH treatment: CA, height SDS, weight SDS, BMI SDS, SH/H ratio SDS, IGF-I SDS, IGFBP-3, BA delay, DTH SDS and 3) treatment characteristics: mean GH dose. A known interaction term between IGFBP-3 SDS and GH dose was added to the model(9). The best fitting models illustrating the contribution of DTH SDS are shown. Statistical significance was defined as $P<0.05$.

Results

The group characteristics and growth results are shown in Table 1. The total group comprised only those children who remained prepubertal throughout the study period. The median height SDS at start of GH was -3.02. The median TH SDS was significantly lower than that of the normal population. The median height gain SDS during the first year was 0.86, and during 4 years 1.89. After 4 years of GH treatment, 83% of all children had reached a height in the normal range (height SDS>-2); the median height SDS at that time was -1.19.

Correlations and multiple regression analyses

The continuous variable DTH SDS appeared to be negatively correlated with height SDS after 4 years of GH treatment ($R=-0.18$, $P<0.01$, Figure 1). This indicates that a larger DTH SDS was correlated with a shorter height SDS after 4 years of GH, however, the obtained height SDS showed a wide variation between the children. Comparing the three subgroups according to the DTH SDS (DTH SDS<1, DTH SDS1-2 and DTH SDS>2) showed a higher percentage of children with a height in the normal range (height SDS>-2) when the DTH SDS was smaller (not significant, Figure 1).

Since an achieved height SDS after GH treatment depends on the height SDS at start, it is more interesting to investigate height gain SDS during treatment. We therefore studied the correlation between DTH SDS and height gain SDS during GH treatment. The variable DTH SDS at start had a significant, positive correlation with height gain SDS after 1 year ($R=0.32$, $P<0.01$) and after 4 years of GH ($R= 0.35$, $P<0.01$, Figure 2). During 4 years of GH, the height gain SDS showed

a broad range with a minimum of 0.34 SDS and a maximum of 4.09 SDS. Although our data demonstrated a continuous, positive correlation between DTH SDS and height gain SDS, there was a wide variation at each level of DTH SDS, without an appropriate cut-of level for DTH SDS.

Table 1. Clinical characteristics of the total group of prepubertal short SGA children

Start of GH treatment (prepubertal)	**n**	
Boys / girls	446	249 / 197
Birth weight SDS	443	-2.23 (-3.11 | -1.60)*
Birth length SDS	325	-2.89 (-4.01 | -2.17)*
Age (years)	446	6.65 (4.90 | 8.76)
Height SDS	446	-3.02 (-3.39 | -2.61)*
TH SDS	446	-0.53 (-1.13 | 0.03)*
DTH SDS	446	2.43 (1.91 | 3.00)
Mother height SDS	446	-1.02 (-1.66 | -0.19)*
Father height SDS	446	-0.99 (-1.70 | -0.19)*
BMI SDS	446	-1.23 (-2.00 | -0.63)*
SH/H ratio SDS	437	1.20 (1.34 | 2.02)*
IGF-I SDS	419	-1.23 (-2.00 | -0.28)*
IGFBP-3 SDS	408	-1.21 (-1.82 | -0.49)*
BA delay (years)	398	1.40 (0.74 | 2.10)*
Patients before introduction of DTH >1SDS (%) #	446	402/446 (90%)
After 1 year of GH treatment (prepubertal)		
Height SDS	394	-2.18 (-2.66 | -1.74)*
Height gain SDS (0-1 yrs)	394	0.86 (0.64 | 1.05)
After 4 years of GH treatment (prepubertal)		
Height SDS	215	-1.19 (-1.75 | -0.64)*
Height gain SDS (0-4 yrs)	215	1.89 (1.53 | 2.30)
Height SDS≥-2 (%)	215	179/215 (83%)
Height in TH range (%)	215	159/215 (74%)

Data expressed as median (interquartile range) unless written otherwise.
SDS, Standard Deviation Score; TH, Target Height; BMI, Body Mass Index; BA delay, calendar age – bone age;
SH/H ratio, sitting height / height ratio.
* P<0.001 compared with zero SDS (median for age and height)
# Patients who were included before the introduction of the European requirements in 2005, including DTH ≥1 SDS, as a percentage of the total group of included patients.

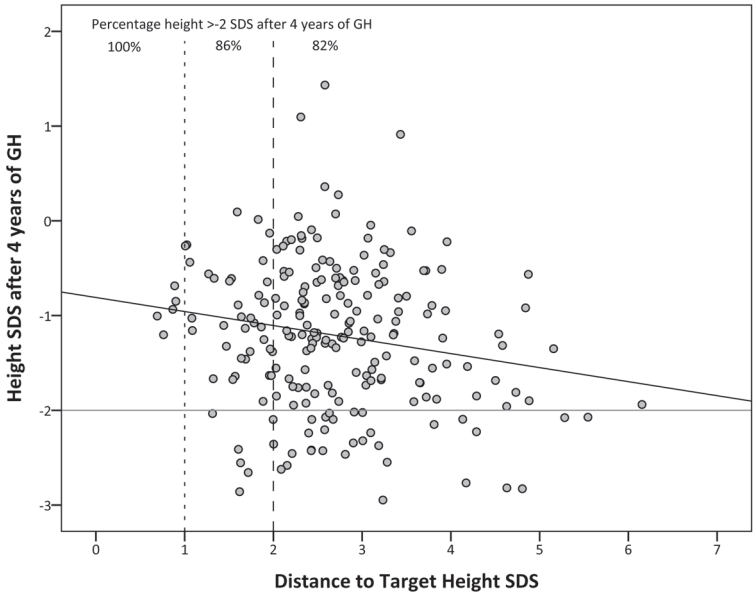

Figure 1. Height SDS in relation to DTH SDS in prepubertal children after 4 years of GH

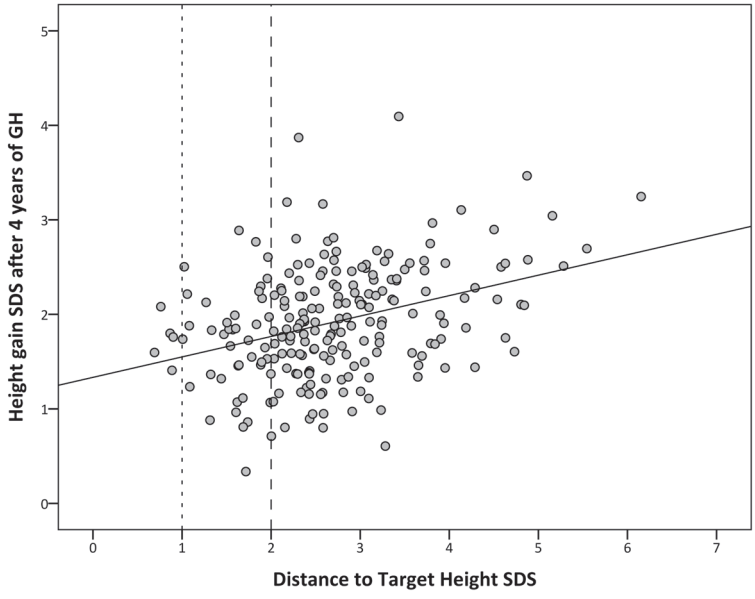

Figure 2. Height gain SDS in relation to DTH SDS in prepubertal children after 4 years of GH

To ascertain whether the correlation between DTH SDS and height gain SDS during 4 years of GH also remains after correction for differences at baseline and known influencing variables, we performed multiple regression analyses (Table 2). Model A (P<0.01) showed that gender, age at start, height SDS at start and the interaction term between GH dose and IGFBP-3 SDS at start were significant negative determinants of height gain SDS, whereas mean GH dose was a significant positive determinant. DTH SDS at start instead of height SDS showed that DTH SDS had a regression coefficient of +0.13 (P<0.01, Model B). The differences in R^2 in Model B compared to Model A demonstrated that only 3% of the explained variation in height gain SDS was due to the effect of DTH SDS.

Table 2. Multiple regression models for gain in height SDS during 4 years of GH

	Model A		**Model B**		**Model C**		**Model D**	
	Beta	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**
Constant	1.84	**<0.01**	1.70	**<0.01**	2.14	**<0.01**	2.12	**<0.01**
Gender #	-0.23	**<0.01**	-0.24	**<0.01**	-0.26	**<0.01**	-0.25	**<0.01**
Age at start in years	-0.12	**<0.01**	-0.10	**<0.01**	-0.13	**<0.01**	-0.12	**<0.01**
Height SDS at start	-0.10	0.10						
Mean GH dose mg/kg/day	0.54	**<0.01**	0.56	**<0.01**	0.61	**<0.01**	0.59	**0.03**
IGF-I SDS at start	-0.04	0.22	-0.03	0.29	-0.04	0.14	-0.04	0.19
IGFBP-3 SDS at start	0.19	0.11	0.19	0.11	0.16	0.18	0.15	0.19
GH dose*IGFBP3 SDS ^	-0.25	**0.02**	-0.25	**0.02**	-0.23	**0.03**	-0.23	**0.03**
Continuous variable								
Distance to TH SDS			0.13	**<0.01**				
Categorical variable								
DTH <1 SDS*					-0.22	0.33		
DTH <2 SDS*							-0.14	0.10
Overall		**<0.01**		**<0.01**		**<0.01**		**<0.01**
R square		0.37		0.40		0.36		0.36
R square adjusted		0.35		0.37		0.34		0.34

All variables are at start of GH treatment, except for mean GH dose. Not significant variables were BA delay, birth length SDS, birth weight SDS, gestational age and BMI SDS. Significant p-values are bold.

Gender, Boy = 0 and girl = 1

^ This interaction term indicates that the dose effect is related to the value of IGFBP-3 SDS at start. Because of the negative coefficient of this term, the relation is: for lower values of IGFBP-3, the dose effect of GH is higher(9).

* Distance to Target Height, DTH <1SDS = 1 and DTH ≥1 = 0; DTH <2SDS = 1 and DTH ≥2 = 0

In addition, we used DTH SDS as a categorical variable to investigate whether a DTH SDS below a certain cut-off level resulted in significantly less height gain during 4 years of GH (Table 2). We found that a DTH SDS<1 compared to DTH SDS≥1 did not result in a significantly different height gain (P<0.01, Model D). We also compared the growth response of all children with a DTH SDS<2 with that of the DTH SDS≥2 subgroup. The last model did not show a significantly different height gain between children with a DTH SDS<2 and children with a DTH SDS≥2 (P<0.01, Model E).

Discussion

Our study showed a weak positive correlation between the continuous variable DTH SDS and gain in height SDS during 4 years of GH, in short, prepubertal children born SGA. Each additional DTH SDS resulted in only 0.13 SDS more gain in height during 4 years of GH, while several other significant determinants had much larger effects. The growth response showed a wide variation across the entire DTH SDS range and did not support any cut-off level. Our results demonstrate that the European cut-off level of a DTH SDS≥1 is not justified; using this criterion will exclude children who can also benefit from GH treatment.

The growth response, a gain in height SDS during 4 years of GH, was significantly positively correlated with DTH SDS, in line with previous studies(10,11). After adjustment for gender, age at start, GH dose, and the interaction term between GH dose and IGFBP-3, 1 SDS smaller DTH was associated with only 0.13 SDS less height gain, equivalent to approximately 0.8 centimetre difference during 4 years of treatment. The total model explained 40% of the variance in height gain during 4 years of GH, but DTH SDS was responsible for only 3% of this gain. Thus, although DTH SDS influenced height gain, it was much less important than several other determinants. Moreover, there was a large variation in height gain SDS independent of DTH SDS, meaning that even children with a large DTH SDS responded differently to GH treatment. This variation can not be explained on the basis of current knowledge and needs further research.

We also investigated if children below and above various DTH SDS cut-off levels responded differently to GH treatment. The scatter plots of height gain SDS during 4 years of GH treatment showed no obvious cut-off level for DTH SDS. After adjusting for other influencing variables, we neither found a significant difference in height gain between children with a DTH SDS<1 and children with a DTH SDS≥1, nor between children with a DTH SDS<2 and children with a DTH SDS≥2.

Another objective to evaluate the benefit of GH treatment could have been reaching a height within the target height range (THR). But since, by definition, children with a DTH SDS<1 have a baseline height in their THR, it is in inappropriate to use this objective to investigate the influence of DTH SDS on growth response. With regard to TH SDS it is worth noting, however, that TH is a calculation including height of child's father and mother; if one parent is very short, the TH SDS will be low and therefore the DTH SDS will be lower too. This does not necessarily mean

that the child's growth potential is determined by the height of the shortest parent. Although children were excluded from GH treatment when they had major dysmorphic features, children with a smaller DTH SDS are more likely to have genetic abnormalities that restrict prenatal and/or postnatal growth and result in short stature in parents and child. On the other hand, there is increasing evidence that children with genetic variations such as IGF-1 receptor haploinsufficiency and d3-GH receptor polymorphisms do respond well to GH treatment(23-26).

We did not find a justification for any cut-off level based on DTH SDS, including the European requirement of a DTH SDS≥1. By using this requirement, children will be excluded who appeared to benefit from GH treatment like children with a larger DTH SDS. The wide individual variation in growth response suggests that treatment plans should be more individualised. Instead of using an arbitrary cut-off level for DTH SDS, it seems more appropriate to use growth prediction modelling to institute individualized GH treatment.

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Chapter 5

Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analogue: Results of a randomized, dose-response GH trial

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Abstract

Context

Growth hormone (GH) treatment is effective in improving height in short children born small for gestational age (SGA). GH is thought to have limited effect when started during adolescence.

Objective

To investigate GH treatment efficacy in short SGA children when started during adolescence. To assess whether GH 2mg/m²·day during puberty improves adult height (AH) compared with 1mg/m²·day. Also, to assess whether additional 2 years postponement of puberty by gonadotropin releasing hormone analogue (GnRHa) improves AH in children who are short at start of puberty (<140 cm), with a poor AH expectation.

Patients and design

In this longitudinal, randomized, dose-response GH trial we included 121 short SGA children (60 boys) ≥8 years of age. We performed intention-to-treat analyses on all children and uncensored cases analyses on 84 children who reached AH. Besides, we evaluated growth during 2 years of combined GH/GnRHa and subsequent GH treatment until AH, in a subgroup of 40 pubertal children with a height <140cm at start.

Outcome measures

Adult height

Results

Short SGA children started treatment at a median age of 11.2 years, when 46% had already started puberty. Median height increased from -2.9 at start to -1.7 standard deviation score (SDS) at AH ($p<0.001$). Treatment with GH 2 versus 1mg/m²·day during puberty resulted in significantly better AH ($p=0.001$), also after correction for gender, age at start, height SDS at start, treatment years before puberty and TH SDS. AH was similar in children who started puberty <140 cm and received GH/GnRHa, compared with children who started puberty >140 cm and received GH only ($p=0.795$).

Conclusion

Also when started in adolescence, GH treatment significantly improves AH in short SGA children, particularly with GH 2mg/m²·day during puberty. When SGA children are short at start of puberty, they can benefit from combined GH/GnRHa treatment.

Introduction

Approximately 10% of the children born small for gestational age (SGA) have persistent short stature(1-3). For short children born SGA, growth hormone (GH) is an effective treatment to improve growth and adult height (AH)(4-9). Some short SGA children only come to medical attention around onset of puberty. It is assumed that GH treatment started during puberty has only limited effect, since by that time the epiphyseal maturation has been activated(10). Postponement of puberty by gonadotropin releasing hormone analogue (GnRHa) can delay epiphyseal maturation by suppressing sex steroid hormones. However, reduced growth velocity is an unfavorable phenomenon that might occur during GnRHa treatment(11-13). Growth data during and after combined GH and GnRHa (GH/GnRHa) treatment in short SGA children are lacking.

The aim of GH treatment for short SGA children is achieving an AH in the normal range and/or in their target height (TH) range. When puberty starts in children with very short stature, their predicted AH decreases substantially. Therefore, any improvement in AH can be an important goal for short children who have already entered puberty. A significant GH dose-effect on short-term growth has often been found(8, 14), but an effect on the long-term is less established(9,15). The optimal GH dose for short SGA children, in particular during puberty and/or postponement of puberty, is still unknown.

We present AH results of a randomized, dose-response GH trial involving short SGA adolescents. Our primary aim was to investigate the efficacy of GH treatment when started around onset of puberty. We aimed to assess whether a double GH dose (2mg/m²·day=0.067mg/kg·day) from onset of puberty improves AH, compared with the standard GH dose (1mg/m²·day=0.033mg/kg·day). In addition, we aimed to evaluate whether combined GH/GnRHa treatment improves AH in short SGA children who are short (<140 cm) at start of puberty.

Subjects

The Dutch SGA study included children when they met the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) for gestational age (GA) <-2.0(16); 2) chronological age ≥8 years; 3) prepubertal stage (Tanner stage 1), or early pubertal stage (breast stage 2-3 in girls and testicular volume <10 ml in boys(17) with a GnRHa stimulating test indicating central puberty(18)); 4) current height <-2.5 SDS(19) and/or expected AH <-2.5 SDS (height at start of puberty <140 cm); 5) well documented growth data from birth to start of treatment, 6) normal karyotype in all girls. Children were excluded in case of a complicated neonatal period with severe asphyxia (defined as Apgar score ≤3 after 5 minutes), long-term complications of respiratory ventilation (bronchopulmonary dysplasia), endocrine or metabolic disorders, growth failure caused by other disorders (celiac disease, emotional deprivation, severe chronic illness or

chondrodysplasia), chromosomal disorders, SHOX haploinsufficiency or syndromes (except for Silver-Russell syndrome), and children who were using or had used medication interfering with growth or GH treatment. None of the children were GH deficient (GHD) according to stimulation tests or overnight profiles (GH peak >7.7 ng/ml).

From December 2003 till December 2007, we included 121 short SGA children (60 boys) (Figure 1). Eleven children dropped out for the following reasons: lack of motivation despite ongoing catch-up growth (n=5), familial problems (n=2) and increase of GH dose for patient care (limited growth due to either national-level sport activities (n=1), co morbidity (n=1), or unknown reason (n=2)). Among the 110 children who continued treatment according to protocol, a chromosome 15q26.3 microdeletion (*IGF1R* gene) was identified in one boy(20), and a ICR1 hypomethylation causing Silver-Russell syndrome was identified in another boy(21). In total, 84 children reached AH, of whom 15 stopped GH treatment prematurely because they were satisfied with their attained height.

This study was performed according to the Helsinki declaration and approved by the Medical Ethics Committees of the participating centers. Written informed consent was obtained from parents or guardians of each child and from children who were 12 years or older. Due to ethical considerations, the Medical Ethics Committees did not allow a randomized untreated short SGA group.

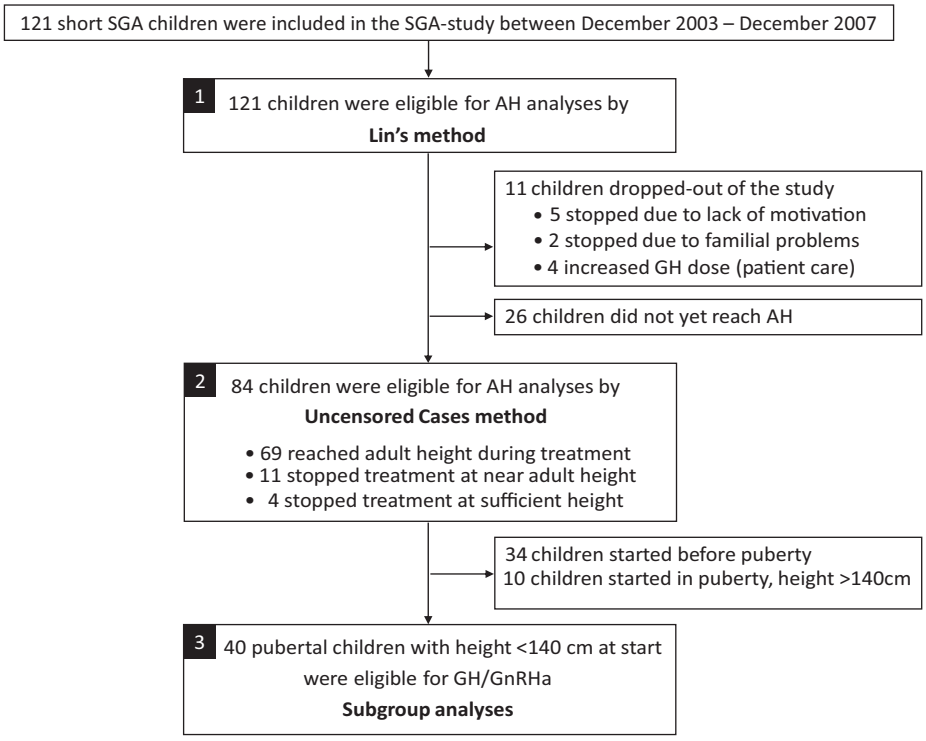

Figure 1. Flowchart of inclusion, drop-out and analysis

Design

Longitudinal, randomized, dose-response GH trial involving short SGA children ≥8 years, evaluating GH 1 versus 2mg/m²·day from early puberty until AH. All children administered Somatropin (Genotropin®) subcutaneously daily. Every 3 months, GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1mg/m²·day (Figure 2). When these prepubertal children entered puberty or when children were in early puberty at start of treatment, they were randomly assigned to treatment with either GH 1 or 2mg/m²·day, after stratification for gender, pubertal stage and parental height (one or two parents with height <-2 SDS versus both parents with height ≥-2 SDS). Because no model is known to predict AH accurately at start of puberty, we used a pragmatic, arbitrary cut-off level. A height of <140cm at start of puberty was used to identify children with a poor AH expectation <-2.5 SDS, based on Dutch reference values(19, 22); these children received GnRHa (leuprorelide acetate depots 3.75mg subcutaneously every 4 weeks) for 2 years in addition to GH treatment. During GnRHa treatment, puberty was sufficiently suppressed in all children as assessed clinically, as well as by GnRHa stimulating tests or by overnight gonadotropin profiles(23,24).

Measurements

Height, weight and Tanner stage were determined at start and three monthly by the same physicians (DvdK and AL)(8). Body mass index (BMI) was calculated (kg/m²). TH was calculated as TH = [(maternal height + paternal height + 13)/ 2 + 4.5] for boys and TH = [(maternal height + paternal height -13) / 2 + 4.5] for girls, and TH range as TH±1.3 SDS(19,25). SDS for height, TH and BMI were calculated according to Dutch references(19), using Growth Analyser (version 3.5; Growth Analyser B.V., Rotterdam, The Netherlands). Bone age (BA) was determined at start and yearly by one investigator according to Tanner and Whitehouse radius, ulna and short bones (RUS TW-2)(26). BA development was calculated as BA / calendar age (CA) in years. Height SDS at 0.5 and 1 year of age were corrected for GA in premature born children, defined as GA <36 weeks. Height SDS during childhood, before start of GH treatment, was calculated as the average of ≥4 measurements between 2 to 10 years. In case of ≤3 measurements (n=4), no average height SDS was calculated.

Outcome measures

AH was defined as the height reached when growth velocity had decreased to <1cm during the last year, and BA was ≥15 years for girls and ≥17 years for boys. At near AH, height velocity was between 0.5 - 2cm during the last 6 months. In contrast to height SDS at stop of GH treatment, AH SDS was calculated using references for Dutch adults (≥20 years)(19). TH corrected AH SDS was calculated as AH SDS minus TH SDS.

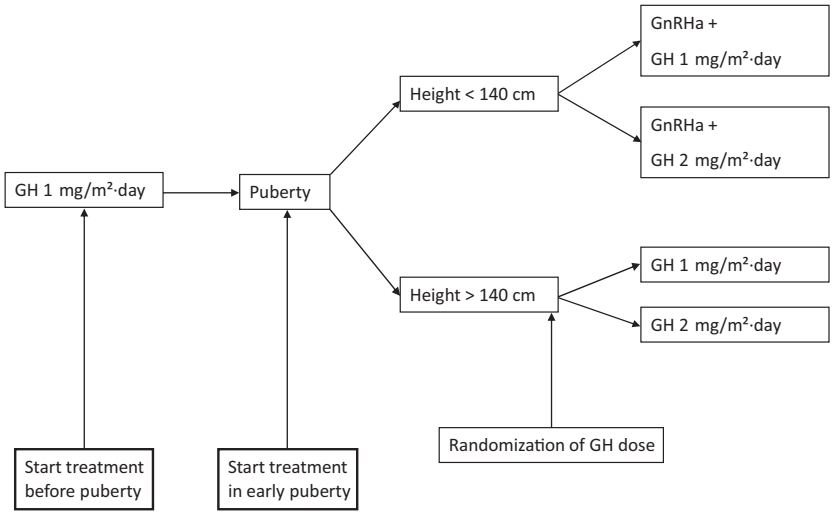

Figure 2. Flowchart treatment regimen

Assays

Blood samples were collected at baseline (prior to the administration of GnRHa or GH), yearly during treatment and at the last visit before GH was stopped. Serum levels of IGF-I and IGFBP-3 were determined in one laboratory and expressed as SDS, using reference values for healthy children with normal stature(27).

Data analysis

Most published AH results after GH treatment are of patients with complete follow-up until AH (uncensored cases). A correct method should, however, also take into account the information of patients with limited data (censored cases), to prevent biased results. The statistical method of Lin et al.(28) proved to be valid for intention-to-treat AH analyses(29), estimating a mean AH in cm for boys and girls separately by using all available growth data. Unfortunately, Lin's method does not allow adjustment for possible confounders. We therefore used both Lin's and uncensored cases method. First, we estimated mean AH using all available growth data of all 121 cases by Lin's method (Figure 1, intention-to-treat). Second, we compared the achieved mean AH in 84 uncensored cases who reached AH with the estimated mean AH by Lin's method. If achieved and estimated AH results were similar, we could use growth data of uncensored cases for detailed AH analyses, including adjustment for possible confounders. Third, we performed additional analyses in a subgroup of children who were all early pubertal with a height of <140cm at start of treatment, to evaluate growth during 2 years of combined GH/GnRHa and subsequent GH treatment until AH.

Baseline characteristics and growth results are presented as median (interquartile range, IQR) unless stated otherwise. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics between subgroups were evaluated using independent sample t-test for normal-distributed variables and Mann-Whitney-U test otherwise. One-sample *t*-test was used to compare SDS results with zero (mean value for age and sex matched references) and to compare achieved mean AH to estimated mean AH. Changes in SDS over time were analyzed with paired sample t-test. Pubertal growth reference was calculated using median age at start of puberty of Dutch boys and girls(22) and growth in centimeters from this age until 21 years of Dutch boys and girls(19), being on average 30 cm in boys and 20 cm in girls. One-sample Wilcoxon signed rank test was used to compare pubertal growth results of the subgroup with pubertal growth reference. Multiple linear regression analysis was performed to analyze the effect of GH dose and additional GnRHa on AH SDS after adjustment for influencing variables. Backward selection was used to build a model attaining variables that significantly influenced AH SDS. We used SAS 9.1 (SAS institute Inc. Cary, NC, USA) to perform Lin's analysis and PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) for other statistical analyses. Results were regarded statistically significant if p was <0.05.

Power calculation

Sample size calculation was based on the significant AH improvement in a previous Dutch randomized, double-blind, dose-response GH trial(9). The estimated sample size was based on an expected difference in AH SDS of 0.5 SDS, an SD of 0.7, testing at a significance level of 5%. Eighteen children in each GH dose group were sufficient to detect this difference with 85% power.

Results

Baseline

Birth weight, birth length and TH SDS were significantly lower than zero (Table 1). In the total group, height SDS slightly increased during the first year of life, whereas it further decreased during childhood. At start of treatment, the median (IQR) age was 11.2 (10.0 ; 12.4) years and the median height was -2.9 (-3.4 ; -2.5) SDS. Pubertal children were significantly older and taller, and had higher IGF-I and IGFBP-3 levels, compared with prepubertal children.

Table 1. Baseline characteristics

	Total group* (n=121)	Prepubertal (n=65)	Pubertal (n=56)	P-value^
Boys / girls	60 / 61	36 / 29	24 / 32	0.080
Gestational age (weeks)	38.0 (36.0 ; 40.0)	38.0 (36.1; 39.6)	39.0 (36.0 ; 40.0)	0.233
Birth weight SDS	-1.9 (-2.5 ; -1.3)	-1.8 (-2.4 ; -1.2)	-2.0 (-2.6 ; -1.4)	0.252
Birth length SDS	-2.5 (-3.2 ; -1.9)	-2.3 (-3.0 ; -1.7)	-2.7 (-3.3 ; -2.1)	0.257
Height SDS, 0.5 years	-2.3 (-2.8 ; -1.9)	-2.3 (-2.7 ; -1.8)	-2.4 (-2.8 ; -2.0)	0.189
Height SDS, 1 year	-2.4 (-2.8 ; -2.0)	-2.4 (-2.7 ; -2.0)	-2.4 (-2.9 ; -2.0)	0.516
Height SDS, childhood	-2.9 (-3.3 ; -2.6)	-3.0 (-3.4 ; -2.6)	-2.8 (-3.1 ; -2.5)	**0.046**
TH SDS	-0.6 (-1.2 ; -0.2)	-0.9 (-1.2 ; -0.3)	-0.5 (-1.3 ; -0.2)	0.395
Start of GH treatment				
Age (years)	11.2 (10.0 ; 12.4)	10.1 (9.3 ; 11.1)	12.5 (11.4 ; 13.0)	**<0.001**
Height (cm)	129.8 (124.2 ; 138.1)	124.9 (119.0 ; 128.9)	138.3 (133.2 ; 141.7)	**<0.001**
Height SDS	-2.9 (-3.4 ; -2.5)	-3.0 (-3.5 ; -2.8)	-2.6 (-3.4 ; -2.3)	**0.003**
Height – TH SDS	-2.1 (-2.8 ; -1.8)	-2.2 (-2.8 ; -1.8)	-2.0 (-2.8 ; -1.7)	0.233
BMI SDS	-1.0 (-1.7 ; -0.4)	-1.1 (-1.7 ; -0.6)	-0.8 (-1.6 ; -0.2)	**0.050**
SH/H ratio SDS	1.2 (0.8 ; 1.7)	1.3 (0.9 ; 2.1)	1.1 (0.5 ; 1.6)	0.258
Head circumference SDS	-1.3 (-1.9 ; -0.6)	-1.1 (-1.8 ; -0.5)	-1.5 (-1.9 ; -1.0)	0.056
IGF-I SDS	-1.2 (-2.0 ; -0.3)	-1.6 (-2.3 ; -1.1)	-0.4 (-1.4 ; 0.4)	**<0.001**
IGFBP-3 SDS	-1.2 (-1.6 ; -0.6)	-1.2 (-1.6 ; -0.7)	-0.9 (-1.4 ; -0.5)	**0.044**

Data are expressed as median (interquartile range), unless written otherwise. Significant p-values are bold.
* All SD scores in the total short SGA group were significantly different from zero SDS (p<0.001)
^ Prepubertal versus pubertal short SGA children
# AH analyses according to Lin's method
SDS, Standard Deviation Score; cm, centimeter; BMI, Body Mass Index; SH/H, sitting height/height; IGF-I, Insulin-like growth factor-I; IGFBP-3, Insulin-like growth factor-binding protein-3; TH, target height

1. Adult height, intention-to-treat analyses

Using all available growth data of the 121 children who started GH treatment, we estimated mean AH for boys and girls separately with Lin's method. Mean (sd) AH for 60 boys was 171.1 (7.4) cm, corresponding to -1.8 (1.1) SDS. The TH corrected AH was -1.1 (0.9) SDS. AH was significantly higher in boys randomized to double GH dose, being 173.4 (6.8) cm in the 2mg/m²·day group (n=29), compared with 168.7 (7.1) cm in the 1mg/m²·day group (p=0.009). AH was similar in boys who started GH treatment before puberty (n=36) and boys who started during puberty (p=0.259). Further, AH was similar in boys who started puberty >140 cm and received GH only (n=37), compared with boys who started puberty <140 cm and received GH/GnRHa (p=0.714). Mean AH for 61 girls was 160.0 (6.2) cm, corresponding to -1.6 (1.0) SDS. The TH

corrected AH was -0.9 (0.8) SDS. AH was significantly higher in girls randomized to double GH dose, being 161.9 (5.7) cm in the 2mg/m²·day group (n=29), compared with 158.2 (6.1) cm in the 1mg/m²·day group (p=0.015). AH did not differ between girls who started GH treatment before puberty (n=29) and girls who started during puberty (p=0.750), neither between girls with (n=40) or without additional GnRHa treatment (p=0.422). The mean estimated AH in SDS was similar in boys and girls (p=0.305).

2. Adult height, uncensored cases analyses

Eighty-four children (32 boys) reached AH during this study period, being 69% of the total group. Mean (sd) achieved AH was -1.7 (0.8) SDS, similar in boys and girls (p=0.711). Mean achieved AH was 171.0 (6.2) cm in boys and 159.5 (5.6) cm in girls, both similar to estimated AH by Lin's method (p=0.984 and p=0.650, respectively). The difference between achieved and estimated AH was less than the standard error of the estimated AH (<0.96 in boys and <0.79 in girls). Besides, baseline characteristics were similar in the uncensored and censored cases, except for significantly younger age at start of the censored cases (p<0.001). Because growth data of the uncensored cases appeared representative for all cases, we used the uncensored cases to perform detailed AH analyses, including adjustment for possible confounders.

Boys reached AH at a median (IQR) age of 18.1 (17.7;18.3) years, after 6.0 (5.1;6.5) treatment years. Girls reached AH at 16.8 (16.2;17.5) years of age, after 5.3 (4.7;6.2) treatment years. During treatment, height improved from -2.9 SDS at start of treatment to -1.7 SDS at AH (p<0.001), as shown Figures 3A and 3B. Sixty-two percent (52/84) reached an AH >-2 SDS. Median TH corrected AH was -0.5 (-1.2;-0.2) SDS. Seventy percent (57/82) reached an AH in their TH range and 10% (8/82) above their TH. Height gain ranged from -0.7 to +3.3 SDS.

The achieved AH was 0.6 SDS higher in children randomized to double GH dose (p=0.002), namely -1.5 (-2.0 ; -0.8) SDS after GH 2mg/m²·day versus -2.1 (-2.5 ; -1.5) SDS after 1mg/m²·day. TH corrected AH was -0.7 (-1.4 ; -0.3) SDS after GH 2mg/m²·day group versus -1.1 (-1.6 ; -0.9) SDS after 1mg/m²·day (p=0.010). Using multiple regression analyses, we evaluated the effect of GH dose and additional GnRHa treatment on AH SDS after adjustment for possible confounders. Non-significant variables were gestational age, birth weight SDS and birth length SDS. GH 2mg/m²·day from start of puberty resulted in significantly better AH SDS than 1mg/m²·day (B=0.49, p=0.001), after correction for gender, age at start, height SDS at start, treatment years before puberty and TH SDS. GH treatment with or without additional GnRHa resulted in similar AH SDS after correction (B=0.08, p=0.795), suggesting that children who received additional GnRHa treatment due to their poor AH expectation at start of puberty, attained comparable AH SDS as children who did not.

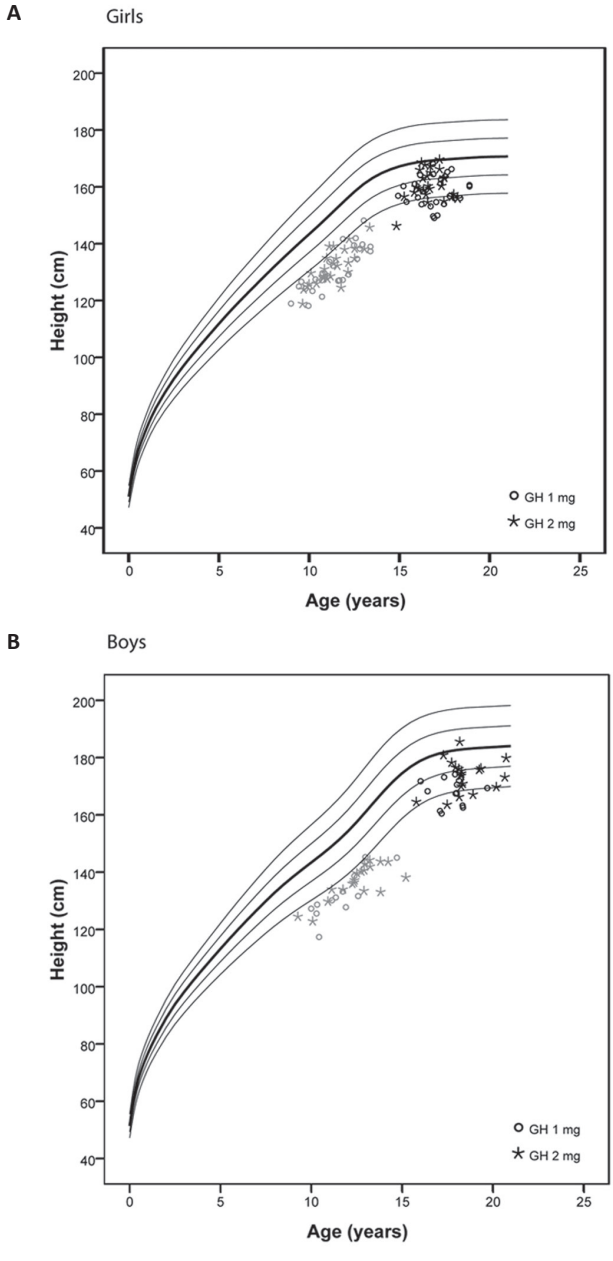

Figure 3. Individual heights at start and stop of GH treatment (A) Girls; B) Boys)

Individual heights of girls and boys at start of study (grey) and at reaching adult height (black). Reference curves for healthy Dutch girls and boys are presented in -2, -1, 0, +1 and +2 SDS lines. Circles represent children who were randomized to GH 1 mg/m²·day during puberty, and stars represent children who were randomized to GH 2 mg/m²·day during puberty.

3. Adult height, subgroup analyses

To evaluate growth during combined GH/GnRHa and subsequent GH treatment until AH, we analyzed a subgroup of 40 pubertal children with a poor AH expectation at start of treatment (height <140cm). These children received GnRHa during the first 2 years, along with GH randomized 1 or 2mg/m²·day until AH.

Characteristics of both GH dose groups from start to AH are shown in Table 2. Boys grew 13.8 (12.8 ; 15.5) cm during 2 years of combined GH/GnRHa treatment, significantly more when treated with GH 2mg/m²·day (p=0.015). They grew 19.8 (16.4 ; 23.0) cm during subsequent GH treatment until AH. Girls grew 10.9 (8.6 ; 14.1) cm during 2 years of combined GH/GnRHa treatment, and 13.4 (11.4 ; 16.7) cm during subsequent GH treatment until AH. From start to AH, boys grew 34.5 (29.1 ; 36.5) cm and girls 24.2 (21.4 ; 29.5) cm. Although all children had proven central puberty at start of treatment, most boys grew more than 30 cm and most girls grew more than 20 cm, thus more than the pubertal growth reference of boys and girls. One girl had menarche before start, but she grew 22 cm, changing height from -3.4 to -2.2 SDS. The boy with an *IGF1R* gene microdeletion was pubertal at start, but he grew 37 cm, changing height from -4.2 to -1.9 SDS.

At start of treatment, median BA/CA was 1.0 (1.0 ; 1.0). During 2 years of GH/GnRHa treatment, BA progression was delayed as indicated by median ΔBA/ΔCA of 0.6 (0.3 ; 0.8) years (p<0.001 compared to one). After stop of GnRHa, during two years of GH treatment, ΔBA/ΔCA was 1.2 (1.0 ; 1.3) years (p=0.002 compared to one). BA before and yearly during treatment were comparable between the two GH groups (all p>0.3). Boys treated with GH 2mg/m²·day gained more height SDS and reached a better AH SDS than boys treated with 1mg/m²·day (p=0.028 and p=0.042, respectively). However, TH corrected AH SDS was similar (p=0.161). Girls treated with GH 2mg/m²·day tended to have better height gain, AH and TH corrected AH SDS, but these differences were not significant.

IGF-I and IGFBP-3

In the total group, median IGF-I increased from -1.2 (-2.0 ; -0.3) before, to 1.0 (0.4 ; 1.5) SDS after one year of GH treatment (p<0.001), while IGFBP-3 increased from -1.2 (-1.6 ; -0.6) to -0.1 (-0.5 ; 0.3) SDS (p<0.001). In pubertal children, yearly sampling during GH treatment showed median IGF-I levels of 1.5 (1.0 ; 2.0) SDS and median IGFBP-3 levels of 0.1 (-0.3 ; 0.5) SDS. Pubertal children treated with GH 2mg/m²·day had significantly higher IGF-I levels compared with pubertal children treated with 1mg/m²·day (1.6 (0.7 ; 2.3) versus 1.1 (0.1 ; 1.5) SDS, respectively (p<0.001)). Consequently, 33% of the IGF-I levels from the GH 2mg/m²·day group were >2 SDS, versus 6% from 1mg/m²·day group (p<0.001). IGF-I levels during GH/GnRHa treatment were significantly lower than during GH treatment only (p<0.001)

Table 2. Subgroup of pubertal children treated with combined GH/GnRHa

		GH treatment and additional GnRHa		
		GH 1 mg/m²·day (n=19)	**GH 2mg/m²·day (n=21)**	**P-value ***
Start				
Boys / girls		5 / 14	7 / 14	0.629
Age in years		12.4 (11.6 ; 12.8)	12.2 (11.2 ; 12.9)	0.839
Height cm		134.7 (127.8 ; 139.5)	134.9 (133.1 ; 139.2)	0.579
Height SDS		-3.0 (-3.5 ; -2.4)	-2.6 (-3.4 ; -2.2)	0.336
BA/CA		1.0 (1.0;1.0)	1.0 (1.0 ; 1.1)	0.394
IGF-I SDS		-0.9 (-1.8 ; -0.2)	-0.6 (-1.6 ; -0.3)	0.895
Adult height				
Age in years		18.0 (17.5 ; 18.6)	18.2 (17.0 ; 18.7)	0.490
Height SDS at stop GH		-2.0 (-2.7 ; -1.1)	-1.4 (-2.2 ; -0.5)	0.101
AH SDS		-2.1 (-2.8 ; -1.3)	-1.7 (-2.2 ; -0.6)	0.107
Boys		-2.8 (-3.0 ; -1.8)	-1.2 (-2.3 ; -1.0)	**0.042**
Girls		-1.8 (-2.4 ; -1.2)	-1.7 (-2.3 ; -0.6)	0.491
TH corrected AH SDS		-1.3 (-1.9 ; -0.9)	-0.9 (-1.7 ; -0.3)	0.161
Boys		-0.9 (-1.9 ; -0.8)	-0.9 (-1.6 ; -0.3)	0.450
Girls		-1.5 (-1.9 ; -0.9)	-1.0 (-1.8 ; -0.3)	0.264
IGF-I SDS		0.8 (0.4 ; 1.2)	1.8 (0.7 ; 2.1)	**0.005**
Start – stop GnRHa (2 years)				
Δ Height gain cm	Boys	12.7 (11.2 ; 13.6)	15.2 (13.6 ; 17.5)	**0.015**
	Girls	13.2 (11.2 ; 14.7)	15.0 (11.7 ; 18.0)	0.250
Δ Height gain SDS	Boys	0.0 (-0.3 ; 0.1)	0.7 (0.0 ; 0.5)	0.223
	Girls	0.6 (0.3 ; 0.8)	0.7 (0.2 ; 1.1)	0.550
ΔBA / ΔCA		0.6 (0.4;0.8)	0.6 (0.3 ; 0.9)	0.888
Δ IGF-I SDS		1.9 (1.4 ; 2.3)	2.5 (2.0 ; 3.0)	0.202
Stop GnRHa – AH				
Δ Height gain cm	Boys	18.6 (13.9 ; 22.6)	20.0 (18.7 ; 23.3)	0.416
	Girls	10.7 (9.4 ; 12.0)	11.4 (7.2 ; 14.5)	0.982
Δ Height gain SDS	Boys	0.6 (-0.1 ; 09)	1.3 (0.4 ; 2.1)	0.088
	Girls	0.7 (0.5 ; 1.0)	0.8 (0.3 ; 1.1)	0.963
Start – AH				
Duration treatment	Boys	5.3 (5.0 ; 5.9)	5.7 (5.0 ; 6.1)	0.808
	Girls	4.9 (4.6 ; 5.4)	5.1 (4.5 ; 5.2)	0.491
Δ Height gain cm	Boys	31.8 (25.4 ; 35.6)	34.7 (33.6 ; 42.6)#	0.223
	Girls	23.5 (21.3 ; 28.5)^	26.3 (21.1 ; 32.0)^	0.435
Δ Height gain SDS	Boys	0.7 (-0.2 ; 1.0)	1.6 (1.3 ; 2.5)	**0.028**
	Girls	1.1 (1.0 ; 1.7)	1.3 (0.6 ; 2.2)	0.905

Clinical characteristics of the subgroup of 40 pubertal children with a poor AH expectation at start of treatment (height <140cm), who received GnRHa during the first 2 years, along with GH until AH (1 or 2mg/m²·day). Data are expressed as median (interquartile range), unless written otherwise. Cm, centimeter; SDS, Standard Deviation Score; BMI, Body Mass Index; BA delay, calendar age minus bone age; Δ, change in variable. * Difference between GH dosage groups. ^ Compared with 20 cm p-value <0.02. # Compared with 30 cm p-value = 0.051

Adverse events

GH treatment was well tolerated and no adverse events considered to be drug-related were observed. One girl, who had been treated with GH 2mg/m²·day for 27 months, had a headache during 10 days. No abnormalities were found, especially no papilledema, hypertension or signs of increased intracranial pressure on the CT-scan. The headache did not change after GH discontinuation or after restarting, but disappeared spontaneously. In retrospect, a causal relationship with GH treatment was unlikely. During GH treatment, 5 boys and 3 girls started thyroxine supplement since free thyroxine (FT4) had decreased below the normal range. In retrospect, the decrease of FT4 during GH treatment appeared the result of altered peripheral thyroid metabolism, and not of hypothyroidism(30). All yearly evaluated HbA1c and fasting glucose levels remained in the normal range.

Discussion

We present AH results of 121 short SGA children who were treated in a randomized, dose-response GH trial evaluating the effects of GH alone or in combination with GnRHa treatment. Children started GH treatment at a median age of 11.2 years, when 46% had already started puberty. Median height increased from -2.9 at start to -1.7 SDS at AH. Sixty-two percent reached an AH >-2SDS and 70% reached an AH in their TH range. Double GH dose of 2mg/m²·day during puberty, resulted in significantly better height gain and AH SDS.

High-quality trials on AH in GH-treated short SGA children are scarce, according to a systematic review in 2009(31). Three randomized-controlled GH trials showed the following AH results: 2 SDS height gain during 7.8 years in 54 children(9), 1.3 SDS height gain during 5.5-8.5 years in 77 children(6) and 1.1 SDS height gain during 2.7 years in 91 adolescents(4). These studies demonstrate significant AH improvement during GH treatment, compared with spontaneous catch-up growth of 0.2-0.5 SDS in untreated controls(4,6,9). However, a limitation of these reports is the use of uncensored cases only. It was demonstrated that uncensored case analysis of AH data can be biased(29), whereas the intention-to-treat analysis by Lin's method(28) results in much higher accuracy(29). We therefore used both methods and showed that our group of uncensored cases was a good representation of the total group.

GH treatment in our group of older short SGA children resulted in 1.2 SDS height gain, bringing 62% of the adolescents in the normal AH range. Previously, Carel et al. showed that 2mg/m²·day GH in older short SGA children resulted in 0.6 SDS increase of AH, bringing 47% of the adolescents in the normal AH range(4). In that study though, hardly any child completed treatment as planned and the treatment duration ranged from 6 months to 3.2 years. We found that adolescents can still have impressive catch-up growth, even when they already entered puberty at start of treatment. Our results should not lead to delaying GH treatment until puberty, since a normal height during childhood and adolescence has important advantages. In clinical

practice, some parents and/or healthcare workers wait for spontaneous catch-up growth, but realize at start of puberty that time is pressing. When children present with short stature around pubertal age, they should not be excluded from GH treatment.

Although GH secretion and IGF-I levels are known to rise during puberty, the quantitative relationship between these hormones and pubertal growth is unknown. Besides, there is only limited knowledge of GH dosing effect on pubertal growth. In GHD children, raising GH dose during pubertal years has been reported to result in a significant height gain of 4.6 cm(32), as well as an insignificant gain only(33). In short SGA children, no benefit of doubling GH dose on AH SDS has been found(6,9). In our total group of 121 short SGA children we demonstrated that doubling GH dose during puberty results in 0.5-0.6 SDS better AH, using intention-to-treat analyses as well as uncensored cases analyses, also after adjustment for influencing variables. Our group of pubertal children had only a few years left to attain a normal height, so doubling GH dose appears most effective during a relatively short treatment period. Besides, children might need a higher GH dose during GH/GnRHa. Previously, we found decreased GH levels during 3 months of GnRHa treatment(34). We found lower IGF-I levels during GH/GnRHa treatment than during GH alone, probably due to suppression of sex steroids. In the GH/GnRHa subgroup, growth tended to be better when treated with the double GH dose, but not significantly. This can be explained by smaller numbers in the subgroup, large individual growth variation, and differences in TH SDS. In the total group though, GH 2mg/m^2·day resulted in significantly better AH SDS.

Comparable to a study on IGF-I levels in prepubertal short SGA children(35), we found significantly higher IGF-I levels in pubertal children treated with GH 2mg/m^2·day, even resulting in 33% of the measurements above the normal range. Concerns have been expressed regarding possible detrimental effects of persistently high serum GH and IGF-I levels(36). For that reason, we exclusively recommend high GH dosing during puberty when only few years of growth are left, or in combination with GnRHa. Yearly monitoring of IGF-I levels during GH treatment is advised.

Using subgroup analyses we showed detailed growth results during combined GH/GnRHa treatment and subsequent GH treatment until AH. We demonstrated that Dutch short SGA adolescents treated with GH/GnRHa treatment grew on average 34.5 cm (boys) and 24.2 cm (girls) until AH, more than the reference population during puberty(19,22). This is remarkable since these short SGA children always had a height below the reference range, with an even worse AH expectation at start of treatment. Van Gool et al. showed in a randomized-controlled trial that 3 years of GH/GnRHa treatment had a positive but modest effect on AH in short adolescents born SGA (n=6 treated) or with normal birth size (n=11 treated)(37). These adolescents received 3 years of GH/GnRHa, but no GH treatment until AH. From discontinuation of GH/GnRHa until AH, boys grew on average 5.9 cm and girls 7.7 cm, compared to 19.8 cm (boys) and 11 cm (girls) in our group. We therefore conclude that continuation of GH treatment until AH is of crucial importance to attain maximum height gain.

There is a clear discrepancy between large-scale use of GH/GnRHa treatment in clinical practice as observed from large registries or databases, and the small amount of evidence supporting the use of this combined treatment(38). In our study, treatment with GnRHa in addition to GH was not randomized but depended on absolute height at start of puberty below 140 cm. No model is known to predict AH accurately at start of puberty. We used a pragmatic cut-off level; Dutch children with a height <140 cm in early puberty were expected not to reach a normal AH, even with an average pubertal growth spurt. Despite this limitation, our study suggests that short SGA children with a poor AH expectation at start of puberty can benefit from combined GH/GnRHa treatment to improve their AH. Importantly, we showed that health-related quality of life improves during 2 years of GH/GnRHa treatment, comparable to prepubertal and pubertal children treated with GH only(39).

In conclusion, GH significantly improves AH SDS in short SGA children who start treatment around puberty. GH 2mg/m²·day during puberty results in significantly better AH compared with the standard dose of 1mg/m²·day. Since GH 2mg/m²·day results in significantly higher IGF-I levels, these need to be closely monitored. Pubertal children with a poor AH expectation can benefit from combined GH/GnRHa treatment.

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Chapter 6

Bone mineral density and body composition in short children born SGA during growth hormone and gonadotropin releasing hormone analogue treatment

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Abstract

Context

Postponement of puberty by gonadotropin releasing hormone analogue (GnRHa) in addition to growth hormone (GH) treatment might increase adult height (AH) in short adolescents born small for gestational age (SGA). GnRHa treatment is thought to have negative effects on bone mineral density (BMD) and body composition.

Objective

To assess BMD of total body (BMD_{TB}), lumbar spine (BMD_{LS}), bone mineral apparent density lumbar spine ($BMAD_{LS}$), lean body mass (LBM), fat mass (FM) and fat distribution during GH treatment, with or without additional two years of GnRHa.

Patients and design

A prospective GH trial involving short SGA adolescents (≥8 years). Eighty-eight children (50 girls) were treated until AH (GH randomized 1 or 2mg/m²·day during puberty); 52 of them received additional GnRHa. BMD and body composition were longitudinally assessed by dual-energy X-ray absorptiometry (DXA).

Results

Baseline BMD_{TB} SDS and BMD_{LS} SDS were significantly reduced (both $p<0.001$), but $BMAD_{LS}$ SDS was comparable to zero ($p=0.129$). BMD_{TB} SDS and BMD_{LS} SDS improved (both $p<0.001$) from start until AH, whereas $BMAD_{LS}$ SDS remained similar ($p=0.168$). At AH, 93% of patients had a normal BMD_{TB}, 99% a normal BMD_{LS} and 98% a normal $BMAD_{LS}$ (>-2 and <+2 SDS). From start until AH, LBM SDS_{height} and FM SDS increased significantly towards zero (both $p<0.001$). Multiple regression analyses showed that additional GnRHa treatment had no adverse effect on the changes in BMD and body composition during GH treatment, also after correction for influencing variables.

Conclusion

Untreated short SGA adolescents had reduced BMD_{TB} and BMD_{LS}, but normal bone-size-corrected $BMAD_{LS}$. During GH treatment, BMD_{TB} and BMD_{LS} increased significantly, leading to a normal adult BMD in almost all patients. Two years of GnRHa in addition to GH treatment had no adverse effect on BMD or body composition.

Introduction

Children born small for gestational age (SGA) are likely to have suffered from restricted fetal growth. Long-lasting negative effects of reduced birth size on bone mineral content and density have been described(1). Moreover, reduced birth size and lower growth velocity during childhood might lead to increased fracture risk in later life(2). Two recent studies showed associations between BMD and postnatal growth, and reported that weight gain during childhood and adolescence is a major determinant for BMD(3,4).

For children born SGA with persistent short stature, growth hormone (GH) is an effective treatment to improve height(5-8). Since it is assumed that GH treatment has limited effect when started during puberty, postponement of puberty in addition to GH might improve adult height (AH) of short SGA adolescents. Although the combination treatment of GH and GnRHa (GH/GnRHa) is widely used in clinical practice, there is very limited evidence supporting the efficacy and safety of this combination treatment(9). Moreover, the GnRHa consensus statement of 2009 did not suggest routine use of GnRHa in children for conditions other than central precocious puberty, because of lack of prospective studies(10).

BMD in later life depends largely on the achieved peak bone mass in early adulthood and the bone loss in the years thereafter(11,12). Puberty is considered to be a crucial period for bone mass acquisition(13,14). During long-term GH treatment in short SGA children, an increase in bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) was found, independent of the height increment(15). However, after GnRHa treatment in adolescents with short stature, a substantially decreased BMD was reported(16). Other studies concluded that BMD or body composition was not impaired in patients with precocious or early puberty after GnRHa treatment(17-19). Since reducing sex steroid levels by GnRHa might have detrimental effects on the achievement of peak bone mass, it is important to ascertain that children with postponed puberty will achieve sufficient bone mass. Nowadays, longitudinal data on BMD in short SGA adolescents treated with combined GH/GnRHa are not available. Besides, studies on body composition and fat distribution during GnRHa treatment are needed(10).

In this article we present data of a longitudinal, randomized, dose-response GH trial involving short SGA adolescents (8 years or older at start), who were treated with GH alone or with combined GH/GnRHa treatment. BMD and body composition were investigated longitudinally by dual-energy X-ray absorptiometry (DXA). Based on previous literature, we expected that BMD and lean body mass (LBM) in short adolescents born SGA increase during GH treatment. We hypothesized that GnRHa would inhibit the acquisition of BMD during treatment, but that BMD would be restored after cessation. We expected unfavorable effects of GnRHa treatment on fat mass (FM) and trunk fat percentage (TF%) during treatment.

Subjects

The inclusion and exclusion criteria of the Dutch SGA study have been described before(20). Briefly, children were included when they met the following criteria: 1) birth length and/or birth weight standard deviation score (SDS) for gestational age (GA) <-2.0(21); 2) chronological age of ≥8 years; 3) prepubertal stage (Tanner stage 1) or early pubertal stage (breast stage 2-3 in girls and testicular volume <10 ml in boys(22), with a GnRHa stimulating test indicating central puberty(23)); 4) current height SDS <-2.5 SDS and/or AH expectation <-2.5 SDS (defined as a height at start of puberty <140 cm), based on Dutch references(24); 5) well documented growth data from birth to start of treatment, 6) normal karyotype in all girls.

From December 2003 till April 2008, we included 121 short SGA children (60 boys). Eleven children dropped out for the following reasons: lack of motivation despite ongoing catch-up growth (n=5), familial problems (n=2) and increase of GH dose for patient care (limited growth due to either national-level sport activities (n=1), co morbidity (n=1), or unknown reason (n=2)). Among the 110 children who continued treatment according to protocol, 88 children reached AH (defined as the height reached when growth velocity had decreased <1cm during the last year, and bone age was ≥15 years for girls and ≥17 years for boys), or near AH (defined as height velocity between 0.5-2 cm during the last 6 months, and adult pubertal stage). Four girls used oral contraceptives to regulate their menstrual cycle for more than 3 months at reaching AH (10-14 months (n=2), 6 months (n=2). Since the changes in BMD SDS, LBM SDS and FM SDS were similar in girls with or without oral contraceptive use, these girls were included in BMD and body composition analyses.

This study was performed according to the Helsinki declaration and approved by the Medical Ethics Committees of the participating centers. Written informed consent was obtained from parents or guardians of each child and from children who were 12 years or older. Due to ethical considerations, the Medical Ethics Committees did not allow a randomized untreated short SGA group.

Design

A prospective GH trial involving short SGA adolescents, evaluating the effects of GH treatment (randomized 1 or 2mg/m²·day during puberty) with or without additional GnRHa for two years. All children daily administered Somatropin (Genotropin®) subcutaneously. Every 3 months, the GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1mg/m²·day. When these prepubertal children entered puberty or when children were in early puberty at start of treatment, they were randomly assigned to treatment with either GH 1 or 2mg/m²·day, after stratification for gender, pubertal stage and parental height (one or two parents with height <-2 SDS versus both parents with height ≥-2 SDS). Children with a height <140 cm at start of puberty were defined as children with an expected AH <-2.5 SDS and received GnRHa (leuprorelide acetate depots 3.75mg subcutaneously every 4 weeks) for 2 years in addition to GH

treatment. During GnRHa treatment, puberty was clinically fully suppressed in all children, which was confirmed by GnRHa stimulation tests or overnight gonadotropin profiles(25,26).

Measurements

Height, weight and Tanner stage were determined at start and three monthly by the same physicians (DvdK and AL)(27). Body mass index (BMI) was calculated (kg/m²). TH was calculated as TH = [(maternal height + paternal height + 13) / 2 + 4.5] for boys and TH = [(maternal height + paternal height - 13) / 2 + 4.5] for girls(24). Height and BMI were transformed into SDS for sex and chronological age according to Dutch references(24), using Growth Analyser (version 3.5; Growth Analyser B.V., Rotterdam, The Netherlands).

Dual-Energy X-ray Absorptiometry

Bone mineral density (g/cm²) and body composition were measured by a dual-energy X-ray absorptiometry scan (DXA, type Lunar-Prodigy, GE Health-care, Chalfont St Giles, UK). All scans were made on the same machine, and quality assurance was performed daily. The coefficient of variation was 0.5% for total body BMD (BMD_{TB}) and 1.0% for lumbar spine BMD (BMD_{LS})(28-30). The coefficients of variation were 0.7% for LBM and 1.2% for FM (28,29). Scans were performed at start of treatment and 2-yearly thereafter. In the subgroup of pubertal children who were treated with combined GH/GnRHa from start, DXA-scans were performed according to the following schedule: at start and after 1, 2, 2.5 and 4 years of treatment. The scans at AH were performed at the last visit or at a visit in the last year before discontinuation of GH treatment.

To adjust for differences in bone size, we also used the bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) (g/cm³). $BMAD_{LS}$ was calculated by the model $BMD_{LS} \cdot (4/(\pi \cdot width))$(31), with the width as the mean width of the second to fourth lumbar vertebral body. All BMD and body composition values were transformed into SDS for sex and chronological age, according to Dutch reference values(32,33). Since LBM is strongly correlated with height, LBM was also expressed as SDS for sex and height (SDS_{height})(32). Trunk fat percentage (TF%) was calculated as $fat\ mass_{trunk}$ / ($fat\ mass_{trunk}$ + $lean\ mass_{trunk}$).

Data analysis

Baseline characteristics and growth results are presented as mean (standard deviation, sd) for the total group and as median (interquartile range, IQR) for the subgroup. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics between subgroups were evaluated using independent Sample *t*-test for normally distributed variables and Mann-Whitney-U test otherwise. One-Sample *t*-test was used to compare SDS results with zero (mean value for age and sex matched references). Changes in SDS over time were analyzed with paired sample *t*-test for normally distributed variables and with Wilcoxon signed-ranks test otherwise. Univariate correlations were determined using Pearson's correlation test. Multiple regression analyses were performed to determine possible associations between

treatment regimens (GH dose and additional GnRHa treatment) and the change in BMD or body composition, after correction for influencing variables. BMD_{LS} SDS, BMD_{TB} SDS, LBM SDS (for age), FM SDS and TF% were used as independent variables. We did not use $BMAD_{LS}$ SDS as an independent variable, since we corrected for the change in height SDS in the regression models. Analyses were carried out using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) for Windows. Statistical significance was defined as $p<0.05$.

Results

Baseline

Table 1 shows the clinical characteristics of the total group of 88 short SGA children (50 girls). The mean (sd) age at start of treatment was 11.5 (1.5) years. Mean height at start of treatment was -3.0 (0.6) SDS ($p<0.001$ compared to zero SDS). BMI SDS, LBM SDS_{height} and FM SDS were also significantly lower than zero (all $p<0.001$). BMD_{TB} and BMD_{LS} SDS were significantly lower than zero (both $p<0.001$), but $BMAD_{LS}$ SDS was comparable to zero ($p=0.129$). In addition, we divided the total group of children in those treated with only GH and those who were treated with combined GH/GnRHa. At baseline, $BMAD_{LS}$ SDS was lower in the group who received only GH treatment ($p=0.038$).

In the total group at baseline, BMD_{TB} SDS correlated significantly with BMD_{LS} SDS ($r=0.69$, $p<0.001$), $BMAD_{LS}$ SDS ($r=0.49$, $p<0.001$), height SDS ($r=0.35$, $p=0.001$), BMI SDS ($r=0.34$, $p=0.001$) and LBM SDS_{height} ($r=0.28$, $p=0.008$). BMD_{LS} SDS correlated with height SDS ($r=0.43$, $p<0.001$), BMI SDS ($r=0.41$, $p<0.001$), FM SDS ($r=0.37$, $p<0.001$) and LBM SDS_{height} ($r=0.33$, $p=0.002$). LMB SDS_{height} correlated with BMI SDS ($r=0.70$, $p<0.001$) and IGF-I SDS ($r=0.264$, $p=0.014$), and FM SDS correlated with BMI SDS ($r=0.60$, $p<0.001$). Birth characteristics as gestational age, birth weight SDS and birth length SDS were not significantly correlated with BMD_{TB}, BMD_{LS} or $BMAD_{LS}$ SDS.

Changes during treatment

The total group of 88 children was treated from start to AH, 36 of them with GH only and 52 children with combined GH/GnRHa (Table 1). During treatment, height increased from -3.0 at start of treatment to -1.4 SDS at AH ($p<0.001$). BMI SDS increased with 0.8 SDS ($p<0.001$), LBM SDS_{height} with 1.1 ($p<0.001$) and FM SDS with 0.5 ($p<0.001$).

Changes in BMD during treatment are shown in Table 1 and, compared to reference boys and girls, in Figures 1 and 2. BMD_{TB} improved with 0.3 SDS ($p<0.001$) and BMD_{LS} improved with 0.6 SDS ($p<0.001$). $BMAD_{LS}$ SDS remained similar ($p=0.168$). BMD at start and stop of treatment showed a wide variation. At AH, 93% of patients had a normal BMD_{TB}, 99% a normal BMD_{LS} and 98% a normal $BMAD_{LS}$ (>-2 and <+2 SDS), with similar percentages for boys and girls (all $p>0.5$). Characteristics at AH did not differ between children treated with only GH and those treated with combined GH/GnRHa (Table 1, all >0.1). Children treated with only GH were treated longer

(p=0.018). From start to AH, children treated with only GH gained more BMI SDS and BMD_{LS} SDS than those treated with combined GH/GnRHa (p=0.002 and p=0.032, respectively).

Table 1. Clinical characteristics at start and stop of treatment

	Total group (n=88)	GH (n=36)	GH/GnRHa (n=52)	P-value[1]	P-value[2]
Boys / girls	38 / 50	21 / 15	17 / 35	**0.017**	-
Gestational age (weeks)	37.4 (3.2)	36.3 (3.8)	38.0 (2.6)	**0.024**	-
Birth weight SDS	-1.9 (0.9)*	-1.8 (1.1)*	-1.8 (0.8)*	0.804	-
Birth length SDS	-2.5 (1.1)*	-2.3 (1.0)*	-2.6 (1.2)*	0.271	-
TH SDS	-0.7 (0.8)*	-0.6 (0.7)*	-0.7 (0.8)*	0.668	-
Start of treatment					
Age (years)	11.5 (1.5)	11.1 (1.7)	11.7 (1.2)	0.083	-
Prepubertal / pubertal	41 / 47	28 / 8	13 / 39	**<0.001**	-
Height SDS	-3.0 (0.6)*	-2.9 (0.6)*	-2.9 (0.7)*	0.863	-
BMI SDS	-0.9 (0.9)*	-1.1 (0.9)*	-0.8 (0.9)*	0.104	-
BMD TB SDS	-1.0 (0.8)*	-1.1 (0.9)*	-0.9 (0.7)*	0.190	-
BMD LS SDS	-1.2 (0.7)*	-1.3 (0.6)*	-1.0 (0.8)*	0.084	-
BMAD LS SDS	-0.2 (1.0)	-0.4 (0.7)*	0.0 (1.1)	**0.038**	-
$LBM\ SDS_{height}$	-1.5 (1.1)*	-1.7 (1.2)*	-1.3 (1.0)*	0.109	-
FM SDS	-0.4 (0.7)*	-0.4 (0.8)*	-0.4 (0.7)*	0.976	-
Trunk fat %	0.2 (0.1)*	0.1 (0.1)*	0.2 (0.1)*	0.136	-
Stop of treatment					
GH dose 1 / 2 mg	43 / 45	16 / 20	27 / 25	0.490	-
Age (years)	17.3 (1.2)	17.2 (1.4)	17.3 (1.1)	0.676	**0.018**
Height SDS	-1.4 (0.8)*	-1.3 (0.8)*	-1.6 (0.8)*	0.126	0.069
BMI SDS	-0.1 (1.0)	0.0 (1.1)	-0.2 (1.0)	0.537	**0.002**
BMD TB SDS	-0.7 (0.9)*	-0.8 (0.9)*	-0.7 (0.9)*	0.562	0.311
BMD LS SDS	-0.6 (0.8)*	-0.5 (0.7)*	-0.6 (0.9)*	0.954	0.032
BMAD LS SDS	-0.3 (1.0)*	-0.4 (0.8)*	-0.2 (1.1)	0.462	0.113
$LBM\ SDS_{height}$	-0.4 (1.2)*	-0.2 (1.2)	-0.4 (1.3)*	0.496	0.357
FM SDS	0.1 (0.7)	0.2 (0.7)	0.0 (0.6)	0.449	0.476
Trunk fat %	0.2 (0.1)*	0.2 (0.1)*	0.2 (0.1)*	0.177	0.688

Data are expressed as mean (standard deviation), unless written otherwise.

* Variables in SDS compared with zero SDS, p-value <0.05.

P-value[1] Comparison of variables at start and at stop of treatment between GH and GH/GnRHa group.

P-value[2] Comparison of change in variables from start to stop of treatment between GH and GH/GnRHa group.

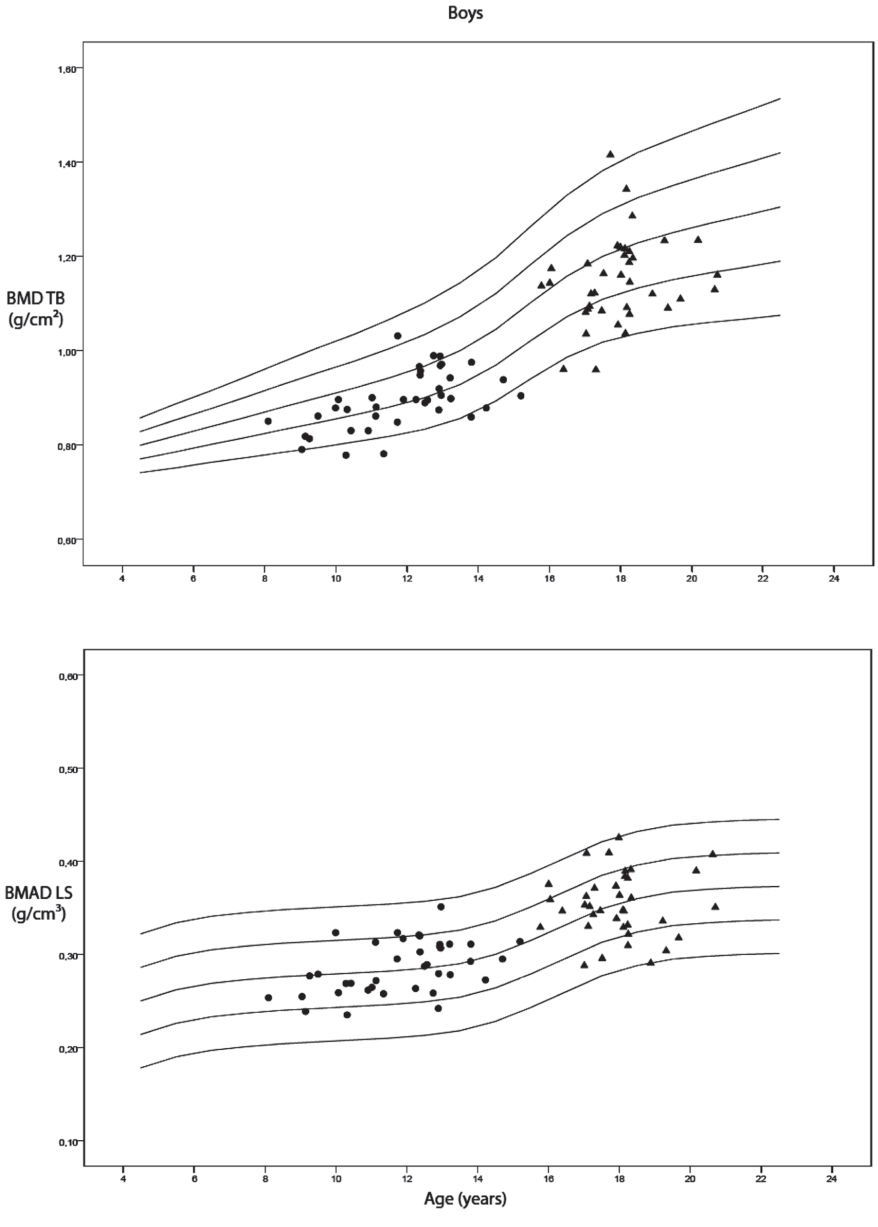

Figure 1. Change in bone mineral density, boys

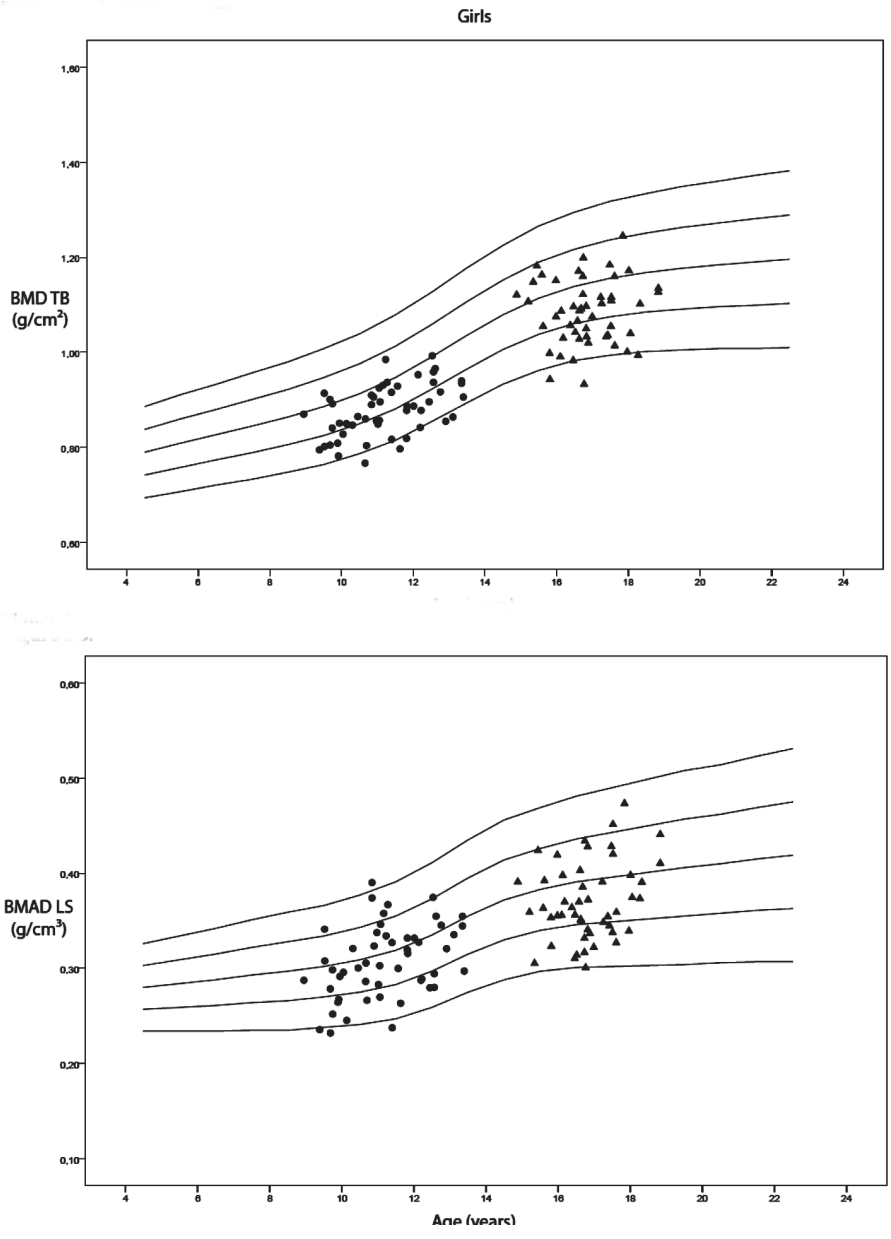

Figure 2. Change in bone mineral density, girls

Table 2. Correlations between changes in BMD and body composition during treatment

	ΔBMD_{TB} SDS	**ΔBMD_{LS} SDS**	**Δheight SDS**	**ΔBMI SDS**	**ΔIGF-I SDS**	**ΔTrunk fat %**	**ΔFM SDS**
ΔBMD_{TB} SDS	-						
ΔBMD_{LS} SDS	**0.73***	-					
Δheight SDS	0.18	**0.40***	-				
ΔBMI SDS	0.42*	**0.30***	0.02	-			
ΔIGF-I SDS	0.10	**0.24**	**0.61***	0.22	-		
ΔTrunk fat %	0.14	0.10	-0.01	**0.38***	-0.09	-	
ΔFM SDS	**0.22**	0.15	0.06	**0.54***	0.00	**0.89***	-
ΔLBM SDS	**0.26**	**0.41***	**0.76***	**0.28***	**0.46***	-0.03	0.02

Correlations between the changes (Δ) from start of treatment to adult height. Pearson correlation coefficients are shown. Correlations are bolded when p<0.05 and also marked (*) when p<0.01.

The changes in BMD during treatment showed correlations with changes in body composition and IGF-I SDS (Table 2). We performed multiple regression analyses to investigate the influence of treatment regimens on the changes in BMD and body composition from start of treatment to AH, after correction for influencing variables (Table 3, best explaining models). For gain in BMD_{TB} SDS, the significant determinants were Δheight SDS (β=0.15, p=0.029) and ΔBMI SDS (β=0.29, p<0.001), whereas GH dose and addition of GnRHa had no influence (Model BMD_{TB} SDS). For gain in BMD_{LS} SDS, the significant determinants were Δheight SDS (β=0.34, p<0.001) and ΔBMI SDS (β=0.23, p=0.011), whereas GH dose and addition of GnRHa had no influence (Model BMD_{LS} SDS). In girls, duration from menarche until AH appeared to have an extra influence on the change in BMD_{LS} SDS (β=0.35, p=0.007), after correction for Δheight SDS, ΔBMI SDS, GH dose and GnRHa treatment (Model not shown). For gain in LBM SDS, the main determinant was Δheight SDS (β=0.89, p<0.001), while GH dose or addition of GnRHa had no influence (Model LBM SDS). For change in FM SDS, the model showed that females gained more FM (β=0.62, p<0.001) and that a higher GH dose and addition of GnRHa resulted in less gain in FM (β=-0.38, p=0.005 and β=-0.29, p=0.036, respectively) (Model FM SDS). For change in TF%, the model showed that girls gained more TF (β=0.66, p<0.001), whereas a higher GH dose resulted in less gain in TF (β=-0.29, p=0.005 and β=-0.21, p=0.036, respectively). Addition of GnRHa tended to result in less gain in TF (β=-0.16, p=0.070) (Model TF%).

Table 3. Multiple regression models for the changes in BMD and body composition during treatment

	Δ BMD_{TB} SDS		Δ BMD_{LS} SDS		Δ LBM SDS		Δ Fat mass SDS		Δ Trunk fat %	
	Beta	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**	**Beta**	**p-value**
Gender					0.04	0.644	0.46	**<0.001**	0.66	**<0.001**
Δ height SDS	0.23	**0.029**	0.42	**<0.001**	0.73	**<0.001**	0.02	0.840	-0.11	0.239
Δ BMI SDS	0.42	**<0.001**	0.26	**0.011**						
GH dose	-0.15	0.144	-0.10	0.319	0.07	0.396	-0.29	**0.005**	-0.22	**0.016**
GnRHa	0.06	0.578	-0.07	0.486	0.04	0.609	-0.21	**0.036**	-0.16	0.070
Overall		**<0.001**		**<0.001**		**<0.001**		**<0.001**		**<0.001**
R square		0.23		0.26		0.58		0.30		0.46
R square adjusted		0.19		0.23		0.56		0.26		0.44

Independent variables are the changes (Δ) in BMD_{TB} SDS, BMD_{LS} SDS, LBM SDS_{age}, fat mass SDS_{age} and trunk fat percentage from start of treatment to adult height. There was no significant interaction between GH dose and Δ height SDS in these models. Beta, unstandardized regression coefficients; randomized GH dose 1 or 2 mg/m²·day; addition of GnRHa yes (1) or no (0). Significant p-values are bold.

Subgroup of pubertal children treated with combined GH/GnRHa

The BMD of a subgroup of 40 pubertal children was analyzed in detail. All these children were pubertal at start of treatment with a poor AH expectation, so they received GnRHa for the fist two years in addition to GH (randomized 1 or 2mg/m²·day), and subsequent GH until AH.

During treatment, absolute values of BMD_{TB} and BMD_{LS} increased gradually from start of treatment to AH: median (IQR) BMD_{TB} increased from 0.90 (0.87 ; 0.94) to 1.11 (1.06 ; 1.18) g/cm² and BMD_{LS} from 0.77 (0.70 ; 0.83) to 1.16 (1.04 ; 1.25) g/cm² (both $p<0.001$). Compared to the reference population, BMD_{TB} SDS and BMD_{LS} SDS decreased significantly during the two years of combined GH/GnRHa treatment (Figure 3A+B, both $p<0.001$). During subsequent GH treatment, BMD_{TB} SDS and BMD_{LS} SDS increased significantly, resulting in a significantly higher BMD_{TB} SDS and BMD_{LS} SDS at AH than at baseline ($p=0.021$ and $p<0.001$, respectively).

Baseline characteristics as well as gender distribution were similar in the GH 1 and 2mg group (all $p>0.4$). Median (IQR) BMD_{TB} SDS was similar in both GH groups at start of treatment, namely -1.1 (-1.3 ; -0.2) in the 1mg group and -0.9 (-1.5 ; -0.4) in the 2mg group ($p=0.681$). BMD_{TB} SDS decreased significantly during combined GH/GnRHa treatment in both GH dose groups ($p<0.001$), to -1.4 (-1.7 ; -0.7) in the 1mg group and to -1.4 (-2.1 ; -0.7) in the 2mg group (between dose groups, $p=0.602$). During two years of subsequent GH treatment, after stop of GnRHa, BMD_{TB} SDS increased in both treatment groups, to -0.6 (-1.4 ; 0.0) in the 1mg group and to -1.1 (-2.0 ; -0.1) in the 2mg group (between dose groups, $p=0.162$). Also, adult BMD_{TB} SDS did not differ between the two GH dose groups ($p=0.129$).

Median (IQR) BMD_{LS} SDS was similar in both GH dose groups at start of treatment, namely -0.9 (-1.5;-0.2) in the 1mg group and -1.0 (-1.9;-0.2) in the 2mg group ($p=0.329$). BMD_{LS} SDS decreased significantly during combined GH/GnRHa treatment in the GH 1 and 2mg group ($p=0.015$ and $p=0.013$, respectively), to -1.1 (-1.7;-0.6) in the 1mg group and to -1.0 (-2.3;-0.5) in the 2mg group (between dose groups, $p=0.570$). During two years of subsequent GH treatment, after stop of GnRHa, BMD_{LS} SDS increased in both treatment groups to -0.6 (-1.2;0.0) in the 1mg group and to -0.8 (-1.7;-0.2) in the 2mg group (between dose groups, $p=0.155$). Also adult BMD_{LS} SDS was similar in both GH groups ($p=0.098$).

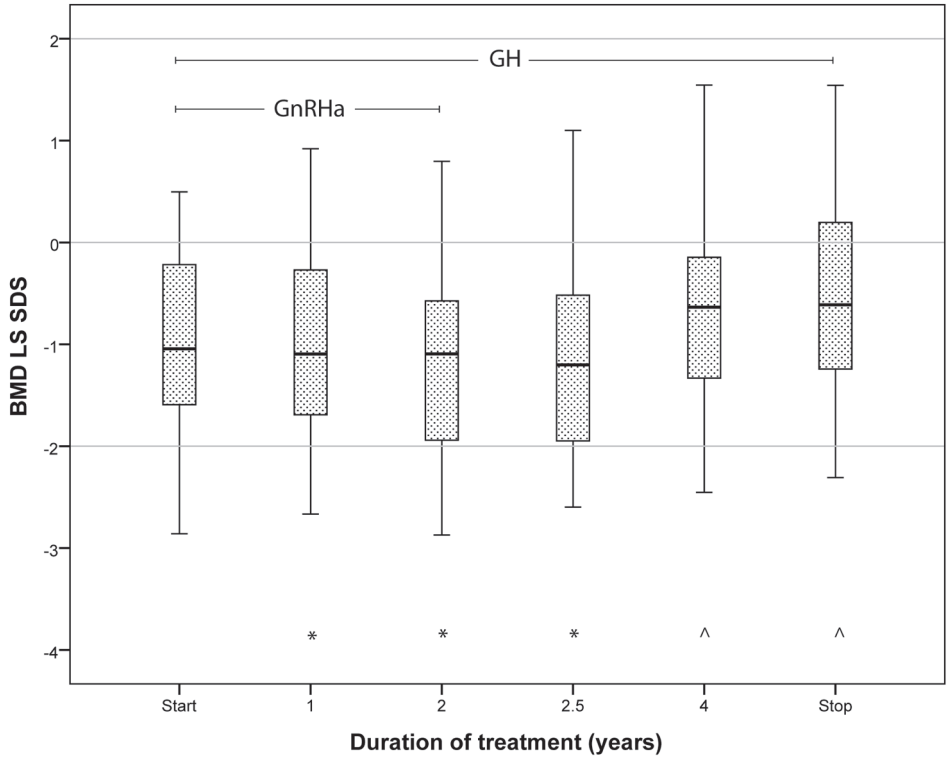

Figure 3. Change in bone mineral density, subgroup

Discussion

This paper reports longitudinal data on BMD and body composition in short SGA adolescents during GH and combined GH/GnRHa treatment. Untreated short SGA children had lower BMD_{TB} SDS and BMD_{LS} SDS, but similar bone-size-corrected $BMAD_{LS}$ SDS compared with the reference population. During GH treatment, BMD_{TB} SDS and BMD_{LS} SDS increased significantly, resulting in an adult BMD in the normal range in almost all patients. Two years of GnRHa in addition to GH treatment had no adverse effect on BMD or body composition. A higher GH dose resulted in less Fm SDS and a more favorable fat distribution.

Our study shows that untreated short SGA adolescents have lower BMD_{TB} SDS and BMD_{LS} SDS than the reference, but similar $BMAD_{LS}$ SDS. Both BMD_{TB} SDS and BMD_{LS} SDS correlated significantly with baseline height SDS and BMI SDS, but not with birth weight and/or birth length SDS. Several cohort studies suggested that poor growth during fetal life and infancy is associated with decreased bone mass in adulthood(34-37), and increased risk of hip fracture in later life(2).

However, Leunissen et al. could not confirm any association between birth size and BMD(3). Instead, age, gender, LBM and weight gain during childhood were significant determinants of BMD of the lumbar spine in early adulthood. These latter findings in combination with our results reveal that BMD in short SGA children is not impaired due to their restricted fetal growth, but is lower due to their explicitly short and lean stature.

During GH treatment in our population of short SGA adolescents, BMD_{TB} SDS and BMD_{LS} SDS increased. Improvement in BMD_{TB} SDS or BMD_{LS} SDS was significantly determined by the changes in height SDS and BMI SDS. Previous GH studies in prepubertal, short SGA children (mean age at start of GH 6 years) also reported improvement of BMD during GH treatment(15,38). Those studies reported a significantly reduced baseline $BMAD_{LS}$ SDS that increased during GH treatment. Baseline $BMAD_{LS}$ SDS in our group of adolescents was not reduced and remained similar during treatment. Although mean adult BMAD SDS was significantly lower than zero SDS, 98% of the children had a normal adult BMAD SDS. Our results imply that the BMD improvement during GH treatment in short SGA adolescents is strongly associated with enlargement of body size, besides possible effects of GH treatment.

Puberty is accompanied by rapid changes in bone length, mass and structure. According to an estimation, a quarter of adult bone mass accumulates during two years around the period of fastest bone mass accrual in adolescence(39). It is important to know whether children with postponement of puberty achieve an adequate bone mass. Several studies reported reduced BMD after GnRHa treatment in girls with central precocious puberty(40) and in adolescents with short stature(16,41). Studies investigating BMD longitudinally during and after GnRHa treatment though, did not find impaired BMD(18,19,42). Van Gool et al. reported lower BMD after three years of combined GH/GnRHa treatment in short adolescent boys, with either SGA or idiopathic short stature(41). However, this conclusion was based on non-significant findings in only 6 treated and 2 control boys. Our study is unique since it longitudinally evaluated BMD in 88 short SGA adolescents during GH or GH/GnRHa treatment, showing no adverse effect of GnHRa on BMD when given during two years in addition to GH treatment. Besides, we performed detailed BMD analyses in 40 pubertal children during combined GH/GnRHa treatment. Although absolute values of BMD_{TB} and BMD_{LS} increased, SD scores of BMD_{TB} and BMD_{LS} decreased during 2 years of GnRHa treatment. After cessation of GnRHa, during GH treatment until AH, both absolute and SD scores of BMD_{TB} and BMD_{LS} increased to significantly higher values than at start of treatment. The children in our study did not have precocious puberty, but received GnRHa because they entered puberty too early for their short stature. We conclude that the relative decrease in BMD SDS is explained by the comparison of BMD in children with postponement of puberty with the BMD of age-matched, but pubertal children. Additional research is warranted to investigate whether GnRHa impairs BMD when used for a much longer period than two years, or when it is not used in combination with GH treatment. Nevertheless, our data reveal that two years of combined GH/GnRHa treatment and subsequent GH treatment until AH does not persistently impair BMD in short SGA adolescents.

Concerns have been raised that GnRHa may affect BMI and increases the risk for obesity. BMI during GnRHa treatment have been mainly investigated in children treated for central precocious puberty, while this group is known to have an above-average BMI at diagnosis(10). Data on body composition and fat distribution during GnRHa treatment in short SGA children are lacking. We found significantly reduced baseline BMI SDS, LBM SDS and FM SDS in short SGA adolescents. During GH treatment, these body composition parameters tended to normalize. The double GH dose resulted in lower FM SDS and trunk fat percentage than the regular dose of 1mg/m²·day. Additional GnRHa treatment resulted in lower FM SDS and tended to result in lower trunk fat percentage, after correction for gender, change in height SDS and GH dose. We can not draw conclusions for GnRHa treatment alone, since any effect of GnRHa treatment on fat mass might be counterbalanced by an opposite effect of GH, but we can conclude that combined GH/GnRHa treatment has no adverse effect on body composition in short SGA adolescents.

Although growth retardation should be evaluated as early as possible, in clinical practice some SGA children present with short stature around pubertal age, who can still benefit from GH treatment(43). In case of a poor AH prognosis at start of puberty, 2 years of GnRHa in addition to GH treatment can improve AH, without an adverse effect on health-related quality of life(20,43) and, as shown here, without adverse effects on BMD or body composition.

In conclusion, BMD_{TB} SDS and BMD_{LS} SDS improve during GH treatment in short SGA adolescents, which is largely explained by the increase in height and BMI SDS. Although BMD levels show a wide variation, almost all children have an adult BMD in the normal range after treatment. Our longitudinal data on combined GH/GnHRa treatment in short SGA adolescents show no adverse effect of 2 years postponement of puberty byGnRHa on BMD or body composition when GH treatment is continued until AH.

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Chapter 7

Health-related quality of life in short children born small for gestational age: Effects of growth hormone treatment and postponement of puberty

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Abstract

Aims

To investigate health-related quality of life (HRQoL) in short children born small for gestational age (SGA) during growth hormone (GH) treatment, and additional postponement of puberty by Gonadotropin Releasing Hormone analogue (GnRHa).

Methods

HRQoL was studied longitudinally during 2 years of treatment in 97 short SGA children, mean age 11.6 years at start. They were divided in 3 groups: prepubertal GH-treated (prep-GH), pubertal GH-treated (pub-GH), and pubertal GH-treated with additional GnRHa (pub-GH/GnRHa). HRQoL was measured by generic (TACQOL) and short stature specific (TACQOL-S) questionnaires.

Results

TACQOL-S showed that prep-GH children experienced significant HRQoL improvement on 'contact with adults', 'body image' and 'vitality', and pub-GH/GnRHa children on 'contact with adults', 'contact with peers' and 'physical abilities'. Parents of prep-GH and pub-GH/GnRHa children reported significant HRQoL improvement on most TACQOL-S scales, whereas HRQoL improvement in pub-GH children reached significance on 'future prospects' only. HRQoL gain was similar in the 3 groups, also after correction for confounders. The generic questionnaire TACQOL did not reveal changes.

Conclusions

HRQoL improved in prepubertal and pubertal short SGA children during GH treatment. Additional GnRHa had no adverse effect on the HRQoL gain. Disorder-specific questionnaires were particularly appropriate to evaluate HRQoL in children treated for short stature.

Introduction

For children born Small for Gestational Age (SGA) with persistent short stature, growth hormone (GH) treatment is an effective and safe treatment to improve height during childhood, adolescence and adulthood(1-4). It is assumed that GH treatment started during puberty has only limited effect, since by that time the process of epiphyseal maturation has already been activated(5). Postponement of puberty in addition to GH treatment might improve adult height of short SGA children who entered puberty.

Health-related quality of life (HRQoL) reflects the subjective perception of health and is increasingly recognized as a relevant 'patient-reported outcome'(6). HRQoL has been studied in children and adolescents with short stature with contradictory results as reviewed in 2005(7). More recently, a British population study demonstrated that short adult stature is associated with reduced HRQoL(8). Long-term GH treatment was reported to improve HRQoL in short SGA children(9,10), but data on HRQoL during GH treatment is still limited(11).

Some short SGA children only come under medical attention at onset of puberty. When GH treatment in these children is combined with gonadotropin-releasing hormone agonist (GnRHa) to improve adult height, possible psychosocial benefits of enhancing growth must be weighed against possible adverse effects of delaying puberty(12,13). For short children, physical changes in puberty may be as important as additional growth. However, HRQoL in short SGA children treated with GH and additional GnRHa is unknown.

The aim of our study was to investigate HRQoL in short SGA children longitudinally during GH treatment, with or without additional postponement of puberty. We hypothesized that GH treatment in short SGA children results in an improvement of HRQoL, most obviously on scales about social functioning and physical abilities. Secondly, we hypothesized that additional postponement of puberty has an adverse effect, especially on scales about contact with parents and peers, and body image.

Subjects and Methods

Subjects

The Dutch SGA GH trial evaluates the efficacy of GH and additional GnRHa treatment in short SGA children at or above the age of 8 years. The study started in December 2003 and included children when they met the following criteria: A) birth length and/or birth weight standard deviation score (SDS) for gestational age <-2.0(14); B) chronological age of ≥8 years; C) prepubertal stage (Tanner stage 1) or early pubertal stage (Tanner breast stage 2-3 in girls or testicular volume 4-10 ml in boys(15), together with a GnRHa test indicating central puberty(16)); D) current height SDS for calendar age <-2.5 SDS or a predicted adult height <-2.5 SDS (predicted adult height calculated as height at start of puberty plus 20 cm for girls and plus 30 cm for boys), according

to Dutch references(17). Children were excluded in case of a complicated neonatal period, with signs of severe asphyxia (defined as Apgar score ≤3 after 5 minutes), long-term complications of respiratory ventilation such as bronchopulmonary dysplasia, endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, or chondrodysplasia) or syndromes (except for Silver-Russell syndrome), as well as children who were using or had used medication that could interfere with growth or GH treatment. The study was performed according to the Helsinki declaration and approved by the Medical Ethics Committees of the participating centres. Written informed consent was obtained from parents or guardians of each child and from children who were 12 years or older. Due to ethical considerations, the Medical Ethics Committees did not allow a randomised control group. From this trial, no long-term growth outcomes are available yet.

Study design

Prepubertal children started Genotropin® (Somatropin) treatment in a dose of 1 mg/m²·day (~0.033 mg/kg/day). Early pubertal children at start of GH treatment were randomised into an open, dose-response study evaluating the effects of 2 dosages of Genotropin® (1 mg/m²·day or 2 mg/m²·day) on pubertal growth, adult height and safety. Children were stratified for gender, pubertal stage (Tanner stage 2 or 3) and parental height (one parent with height SDS below -2 SDS or both parents with height SDS within the normal range). Children who were very short at start of puberty (height <140 cm, resulting in a predicted adult height <-2.5 SDS) received GnRHa treatment (leuprorelide acetate depots 3.75 mg subcutaneously every 4 weeks) for 2 years in addition to GH treatment. Pubertal arrest was evaluated clinically and by GnRHa tests. GH was administered subcutaneously once daily at bedtime. Every 3 months, the GH dose was adjusted to the calculated body surface area.

The study included 122 children (60 girls) and their parents/guardians. They filled out questionnaires at start and after 2 years of GH treatment. Children were divided in three groups: prepubertal children, who remained prepubertal during the analysed period and received only GH (prep-GH, n=35), pubertal children who received only GH (pub-GH, n=14) and pubertal children who received a combination treatment of GH and GnRHa (pub-GH/GnRHa, n=48), leaving 97 children eligible for analysis (Figure 1). The main reason for missing questionnaires was logistic such as loss of questionnaires. Four children were treated shorter than 2 years and therefore filled out questionnaires at start only.

HRQoL measurements

HRQoL is a combination of health problems plus emotional responses towards such problems. A conjunction of The Netherlands Organization (TNO) for Applied Scientific Research with the Academic Hospital in Leiden (AZL, nowadays LUMC) developed a reliable and valid Dutch instrument to assess children's HRQoL, which explicitly offers respondents the possibility to differentiate between their functioning and the way they feel about it(18,19). We used 3 TNO-

AZL Children's Quality of Life (TACQOL) questionnaires: the TACQOL-S CF, a short stature specific child form (CF)(10), the TACQOL-S PF, a short stature specific parent form (PF), and the generic TACQOL PF, a non disease-specific PF(18,19). When children or parents indicated the presence of a health status problem, they were asked to assess the child's emotional reaction. HRQoL was longitudinally evaluated in the participants at start and after 2 years of GH treatment.

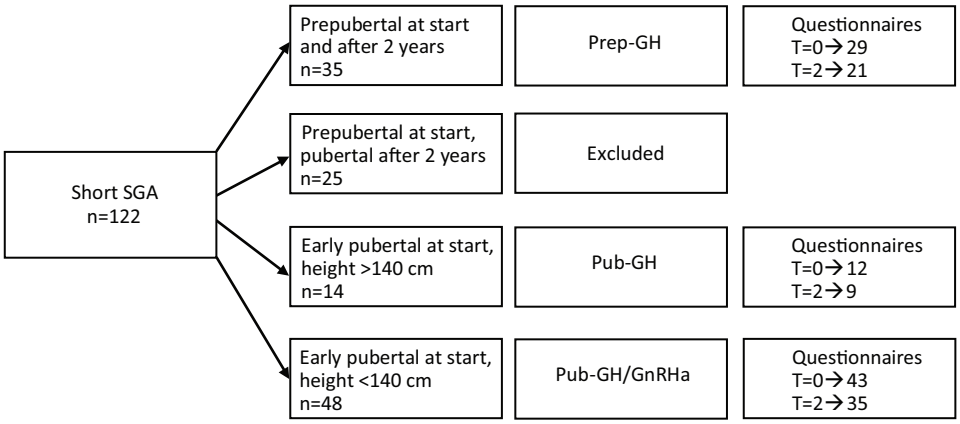

Figure 1. Flowchart of groups and questionnaires

TACQOL-S

The TACQOL-S is a condition-specific questionnaire measuring the impact of short stature on HRQoL in children aged 5-15 years(10). The TACQOL-S CF is filled out by the child, whereas the TACQOL-S PF is filled out by parents from the perspective of their child. The TACQOL-S comprises 6 scales: contact with adults (e.g. 'Were adults surprised when they heard your age?'), contact with peers (e.g. 'Have other children been bullying you?'), body image (e.g. 'Would you like to look different?'), physical abilities (e.g. 'Did you experience the tables at school as being too high?'), future prospects ('Do you think the future will be enjoyable?'), and vitality (e.g. 'Have you been getting tired quickly?'). In case of a normal stature it is possible to give the response: not applicable or never.

TACQOL

The TACQOL is a generic instrument to assess HRQoL in children aged 6-15 years(18,19). The generic TACQOL PF contains 7 scales: social functioning, autonomous functioning, physical complaints, motoric functioning, cognitive functioning, positive emotions and negative emotions. The last 2 scales do not have a separate HRQoL part, since the questions already include an emotional dimension. According to the Dutch population study(19), the Cronbach's α(20) ranges from 0.65 to 0.84, indicating that comparisons on group level are justified(21).

Other measurements

At start and three monthly during GH treatment, height, weight and Tanner stage were determined, as described elsewhere(1)]. Body mass index (BMI) was calculated as weight divided by height squared (kg/m²). Target height (TH) was calculated as TH = [(maternal height + paternal height + 13) / 2 + 4.5] for boys and TH = [(maternal height + paternal height - 13) / 2 + 4.5] for girls, with addition of 4.5 cm for secular trend in the Netherlands(17,22). SD scores for height, TH and BMI were calculated to adjust for age and gender according to Dutch references(17), using Growth Analyser (version 3.5; Growth Analyser b.v., Rotterdam, The Netherlands). Information regarding socio-economic status (SES) and serious life events (e.g. death of family member and divorce of parents) was obtained using questionnaires filled out by the parents. The highest of 2 education levels (father and mother) was used as socio-economic indicator to determine SES (categorized as lowest (1), low (2), medium (3), high (4))(23).

Data analysis

Clinical characteristics are presented as mean (SDS) unless stated otherwise. Differences in characteristics between the groups were tested by the ANOVA for continuous variables and by the Pearson chi-square test for categorical variables, using the statistical package SPSS (version 16.0; SPSS Inc. Chicago, IL) for Windows. We combined the randomised 1 and 2mg/m^2/day GH groups, since there were no significant differences in height SDS and HRQoL scores at start, nor in height gain SDS during 2 years.

Scoring of items and calculation of scale scores were assessed as previously described for the TACQOL questionnaire(19). Since HRQoL is seen as a multidimensional construct, no total score could be calculated. The TACQOL-S scale results were normally distributed, whereas the generic TACQOL scale results were skewed. As presented in the TACQOL manual, we used t-tests to compare the generic TACQOL results of the prep-GH and the pub-GH/GnRHa group to a reference sample of Dutch children(19), using mean scale scores of 1318 Dutch children without chronic conditions or diseases and none from ethnic minorities. Since the pubertal GH group was too small (n<30), *t*-tests to compare results to the reference sample were not performed in this group. Scale scores of the SGA children and mean scale scores of the reference sample were linearly transformed to a 0 to 100 scale, with higher scores indicating better HRQoL.

To handle missing data, we analysed differences in HRQoL between the groups and changes in HRQoL over time with repeated measurement analysis, using SAS 9.1 (SAS institute Inc. Cary, /NC, USA). Model estimates are presented as mean (standard error, SE). The analysed changes over time were also adjusted for factors influencing HRQoL outcome such as gender, age, height SDS or height SDS corrected for TH SDS (height SDS minus TH SDS), height gain SDS during 2 years, GH dosage, ethnicity and SES (range 1-4) of the child, type and age of respondent, and serious life events. Per subscale the significantly contributing variables were determined and used for adjustment. Overall, results of statistic analyses were reported as significant with a p-value of <0.05. A power calculation was performed for analysis in 3 groups with a total number

of 100: to determine a largest difference in HRQoL change of 15 with an SE equal to 15, the power is 0.96 (alpha = 0.05).

Results

Baseline characteristics of the three groups (prep-GH, pub-GH and pub-GH/GnRHa) are shown in Table 1. Age and height at start differed significantly in line with the definition of the groups: prepubertal children were younger and shorter, whereas pubertal children were older and longer. Moreover, pubertal children receiving GH and additional GnRHa treatment had a height <140 cm at start of treatment. Height SDS at start though, was comparable between the three groups.

Table 1. Baseline characteristics

	Prep-GH (n=35)	**Pub-GH (n=14)**	**Pub-GH/GnRHa (n=48)**	**p-value***
Child				
Boys / girls	22 / 13	10 / 4	17 / 31	**<0.001**
Age in years	9.8 (1.1) [a,b]	13.5 (1.1) [a,c]	12.3 (1.1) [b,c]	**<0.001**
Gestational age in weeks	37.1 (3.7)	38.1 (2.5)	38.1 (2.9)	0.110
Birth weight SDS	-2.2 (1.0)	-2.6 (1.1)	-2.2 (0.8)	0.797
Birth length SDS	-2.1 (1.0)	-2.7 (1.0)	-2.2 (0.9)	0.878
Height in centimetres	122.5 (6.1) [a,b]	144.2 (2.4) [a,c]	136.8 (5.3) [b,c]	**<0.001**
Height SDS	-3.1 (0.5)	-2.6 (1.0)	-2.8 (0.8)	0.356
BMI SDS	-1.2 (0.7)	-0.6 (1.1)	-0.8 (1.2)	0.205
Target height SDS	-0.6 (0.9)	-0.6 (0.8)	-0.6 (0.8)	0.662
Dutch Caucasian ethnicity (%)	28 (82)	13 (93)	40 (83)	0.635
Socio-economic Status	[b]	[c]	[b,c]	**0.010**
Lowest (%)	0	0	1 (2)	
Low (%)	21 (66)	7 (54)	20 (43)	
Medium (%)	5 (15)	4 (31)	2 (4)	
High (%)	6 (19)	2 (14)	24 (51)	
Respondent of parent form				
Father / mother / other	2 / 24 / 9	0 / 11 / 3	5 / 38 / 5	0.464
Age in years	38.5 (4.5) [a,b]	42.4 (4.2) [a]	42.4 (4.3) [b]	**0.001**

Baseline characteristics of the children and parents/guardians who filled the questionnaires.
Data expressed as mean (± SD), unless written otherwise.
p-value* Overall difference between the groups: significant p-values are bold
[a] Prep-GH versus pub-GH, p<0.01; [b] Prep-GH versus pub-GH/GnRHa, p<0.01; [c] Pub-GH versus pub-GH/GnRHa, p<0.01.

Baseline HRQoL

Table 2 shows the baseline HRQoL results. According to the TACQOL-S CF, pub-GH children reported significantly higher 'physical abilities' than pub-GH/GnRHa children (p=0.016). For 'contact with adults', 'contact with peers' and 'vitality', pub-GH children also reported higher HRQoL, but these did not reach significance. Analysing the TACQOL-S PF and the generic TACQOL PF, all HRQoL subscales were similar in the 3 groups at baseline.

Table 2. Baseline HRQoL

	Prep-GH (n=35)	Pub-GH (n=14)	Pub-GH/GnRHa (n=48)	Reference[#]	p-value[*]
TACQOL-S					
Child form					
Contact with adults	67.8 (2.9)	78.0 (4.6) [c]	65.6 (2.4) [c]	-	0.062
Contact with peers	65.1 (3.5) [a]	79.6 (5.5) [ac]	64.8 (2.9) [c]	-	0.052
Body image	74.3 (3.1)	75.7 (4.9)	71.8 (2.6)	-	0.715
Physical abilities	78.7 (2.6)	84.8 (4.0) [c]	72.4 (2.1) [c]	-	**0.016**
Future prospects	74.4 (3.0)	70.9 (4.4)	73.5 (2.3)	-	0.810
Vitality	77.4 (2.2)	82.2 (3.4)	78.8 (1.8)	-	0.493
TACQOL-S					
Parent form					
Contact with adults	77.4 (5.0)	68.8 (3.5)	66.4 (2.7)	-	0.160
Contact with peers	81.1 (5.6)	71.2 (3.7)	68.5 (3.1)	-	0.147
Body image	63.2 (5.9)	68.3 (3.8)	63.8 (3.2)	-	0.620
Physical abilities	82.0 (3.7)	76.3 (2.5)	76.4 (2.0)	-	0.386
Future prospects	75.9 (3.0)	77.0 (2.1)	75.0 (1.6)	-	0.758
Vitality	89.7 (3.5)	87.2 (2.5)	84.5 (1.9)	-	0.365
TACQOL, generic					
Parent form					
Social functioning	88.5 (3.9) [d]	86.0 (2.4)	85.6 (2.0) [d]	93.7	0.800
Autonomous functioning	99.8 (2.1)	96.5 (1.3)	96.6 (1.1)	98.0	0.367
Physical complaints	84.6 (3.8)	82.6 (2.3)	81.8 (2.0) [d]	86.3	0.802
Motoric functioning	99.3 (2.7)	94.2 (1.6)	94.1 (1.4)	96.9	0.208
Cognitive functioning	84.2 (6.7) [d]	74.5 (4.0)	78.1 (3.5) [d]	91.1	0.463
Positive emotions	88.1 (4.0) [d]	87.2 (2.4)	89.9 (2.1)	93.6	0.689
Negative emotions	71.9 (5.4)	67.5 (3.2)	72.7 (2.8)	73.0	0.467

Data expressed as unadjusted mean (± SE) HRQoL.

p-value[*] Overall differences between the groups: significant p-value are bold

[#] Means of reference sample, children without chronic diseases (TACQOL manual), shown on a 0-100 scale.

[a] Prep-GH versus pub-GH, p<0.05; [b] Prep-GH versus pub-GH/GnRHa, p<0.05; [c] Pub-GH versus pub-GH / GnRHa, p<0.05;

[d] Short SGA group lower than reference, p<0.05.

Comparing the TACQOL-S PF and CF, parents reported significantly higher HRQoL than their children on 'contact with peers' (difference 4.63, p=0.046) and 'vitality' (difference 6.92, p<0.001). On 'body image' though, parents reported significantly lower (difference -7.37, p<0.001). These differences between parental and children reports were comparable between the 3 groups.

The generic TACQOL PF showed lower 'social functioning' in the short SGA children compared to the reference population (prep-GH p=0.006 and pub-GH/GnRHa group p<0.001, respectively). Also 'cognitive functioning' was reported significantly lower (prep-GH p=0.016 and pub-GH/GnRHa group p<0.001, respectively). Parents reported significantly lower 'physical complaints' in the pub-GH/GnRHa group (p=0.028), and less 'positive emotions' in the prep-GH group (p=0.027), compared to the reference population.

All three groups showed a significant increase in height SDS during 2 years of GH treatment (Table 3). Height gain SDS was lower in the pub-GH/GnRHa group than in the prep-GH group (p=0.001).

Table 3. Change in HRQOL-S during 2 years of treatment

TACQOL-S	Prep-GH (n=35)	p-value*	Pub-GH (n=14)	p-value*	Pub-GH/GnRHa (n=48)	p-value*	p-value^
Child form							
Contact with adults	7.1 (3.4)	**0.040**	6.8 (5.1)	0.184	7.9 (2.6)	**0.003**	0.879
Contact with peers	5.0 (4.3)	0.254	-0.2 (6.5)	0.973	7.2 (3.3)	**0.032**	0.532
Body image	8.1 (4.0)	**0.049**	7.6 (5.9)	0.202	-0.5 (3.2)	0.885	0.279
Physical abilities	4.5 (2.7)	0.100	3.3 (4.2)	0.429	8.1 (2.1)	**<0.001**	0.384
Future prospects	5.6 (3.5)	0.115	7.7 (5.2)	0.142	5.2 (2.7)	0.060	0.903
Vitality	5.3 (2.5)	**0.038**	0.1 (3.8)	0.988	0.6 (2.0)	0.751	0.266
Parent form							
Contact with adults	11.7 (5.0)	**0.022**	0.8 (5.3)	0.885	6.4 (3.3)	0.059	0.359
Contact with peers	9.4 (7.2)	0.194	3.6 (4.9)	0.468	11.5 (3.9)	**0.004**	0.512
Body image	20.6 (5.6)	**<0.001**	6.5 (3.7)	0.083	9.5 (3.0)	**0.002**	0.111
Physical abilities	9.5 (4.8)	**0.049**	4.1 (3.0)	0.184	7.3 (2.3)	**0.002**	0.678
Future prospects	8.6 (2.3)	**<0.001**	10.5 (2.0)	**<0.001**	3.7 (1.7)	**0.033**	0.117
Vitality	1.9 (5.0)	0.708	2.0 (4.4)	0.653	4.0 (3.2)	0.220	0.910
Height gain SDS	0.9 (0.4) [b]	**<0.001**	0.7 (0.5)	**<0.001**	0.5 (0.5) [b]	**<0.001**	**0.001**

Change in HRQoL during 2 years of treatment (HRQoL after 2 years minus HRQoL at start), according to the TACQOL-S. Data are expressed as unadjusted mean (± SE).

p-value* Change in HRQoL compared to zero: significant p-values are bold.

p-value^ Overall difference in HRQoL change between the three groups: significant p-values are bold.

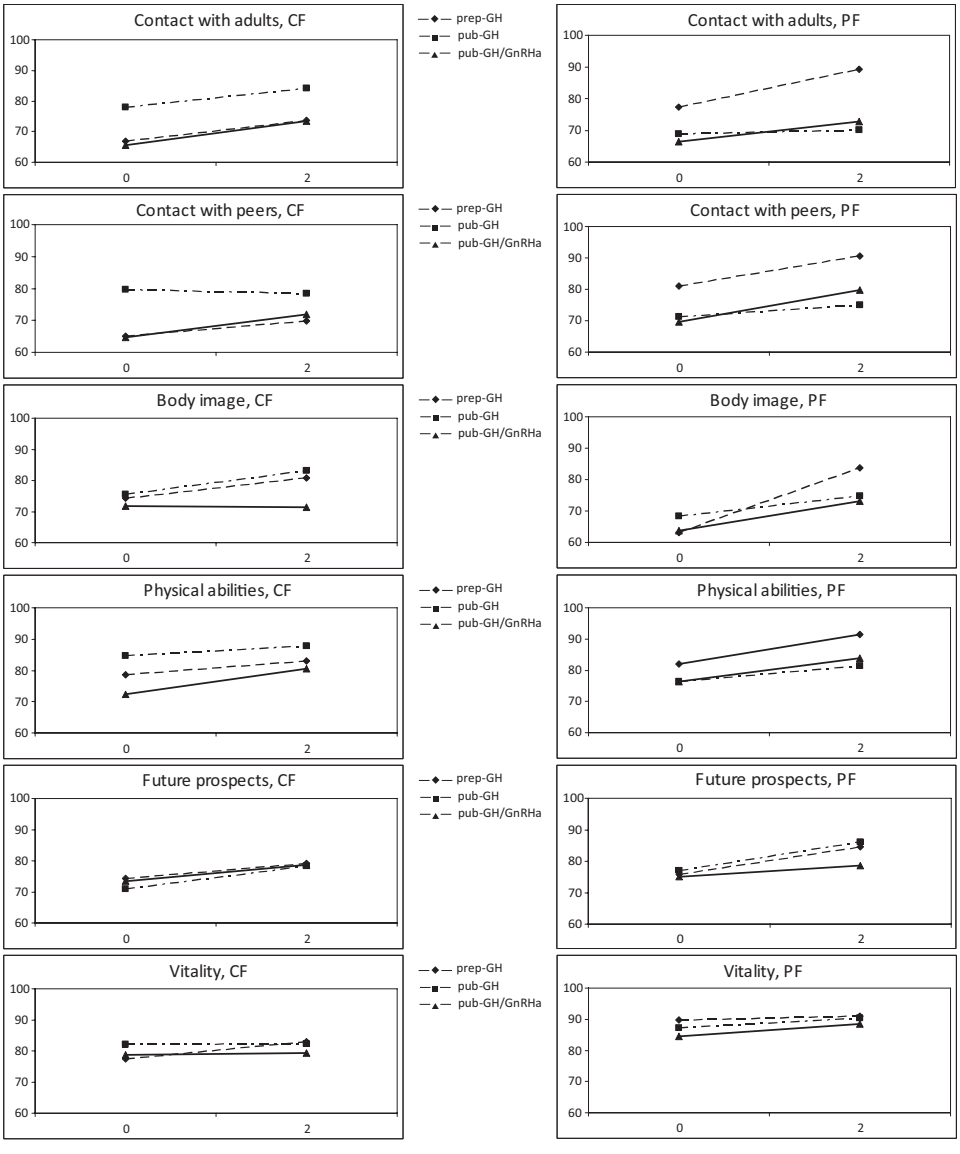

Figure 2. Change in HRQoL-S during 2 years of treatment

Change in HRQoL during 2 years of treatment (HRQoL after 2 years minus HRQoL at start), according to the TACQOL-S
X-axis: at start (0) and after 2 years of treatment (2)
Y-axis: unadjusted mean HRQoL

Change in HRQoL during 2 years of treatment

Prep-GH children reported HRQoL improvement on all TACQoL-S CF subscales, significantly on 'contact with adults' (p=0.040), 'body image' (p=0.049), and 'vitality' (p=0.038) (Table 3 and Figure 2). The pub-GH children reported improvements on 'contact with adults', 'body image', 'physical abilities' and 'future prospects'. These changes, however, did not reach significance, probably due to the smaller group size. The pub-GH/GnRHa children reported significant HRQoL improvement on 'contact with adults' (p=0.003), 'contact with peers' (p=0.032), 'physical abilities' (p<0.001), and nearly significant improvement on 'future prospects' (p=0.060). The changes on HRQoL scales reported by pub-GH/GnRHa children were similar to the other groups, also after adjustment for possible confounders as gender, age at start, height SDS at start, height gain SDS during 2 years, GH dosage, SES and life events. The gain in HRQoL was positively associated with height gain SDS for 'contact with adults' (p=0.046) and almost significantly for 'future prospects' (0.082).

Parents of prep-GH children reported improvement of HRQoL on all TACQOL-S PF scales (Table 3 and Figure 2). The improvement was significant for 'contact with adults' (p=0.022), 'body image' (p<0.001), 'physical abilities' (p=0.049) and 'future prospects' (p<0.001). Parents of pub-GH children reported HRQoL improvement on various scales, only significant on 'future prospects' (p<0.001). Parents of pub-GH/GnRHa children reported significantly better 'contact with peers' (p=0.004), 'body image' (p=0.002) and 'future prospects' (p=0.033), and almost significantly better 'contact with adults' (p=0.059). The improvements on HRQoL scales reported by parents were similar in the 3 groups, also after adjustment for possible confounders as gender, age at start, height SDS at start, height gain SDS during 2 years, GH dosage, characteristics of the respondent, SES and life events. The gain in HRQoL was positively associated with height gain SDS for 'physical abilities' (p=0.039).

According to the generic TACQOL PF subscales, no change in HRQoL was reported during 2 years of treatment with and without adjustment for possible confounding characteristics of child and parents. We found no differences of parental reports between the 3 groups during 2 years of treatment (data not shown).

Discussion

Our study demonstrates an improvement in HRQoL in short SGA children treated with GnRHa in addition to GH, similar to that of prepubertal and pubertal children who were treated with GH only. This indicates that postponement of puberty for 2 years has no adverse effect on HRQoL in GH-treated short SGA children.

We found significantly lower scores on cognitive and social functioning in short SGA children, compared to the reference population. This is in line with previous studies, showing on average lower intelligence and more behaviour, social functioning, school competence and attention problems in short SGA children(24-28). During GH treatment, improvement of intelligence, behaviour and self-perception has been described in short SGA children(28), as well as improvement of behaviour and self-esteem in short children with GH deficiency and idiopathic short stature(29,30). According to a recent systematic review however, data on QoL before and during GH treatment in short children is lacking(11). Some studies showed better HRQoL in GH-treated children, compared to untreated children with short stature(10,31), but these data were collected cross-sectional. We present longitudinal data on HRQoL in prepubertal and pubertal short SGA children during 2 years of GH treatment.

On the TACQOL-S, parents reported significantly better 'contact with peers' and 'vitality', but lower 'body image' than their children. Differences in proxy agreement at individual child-parent level have been described for the generic TACQOL(32,33). It has been indicated that children report less consistent and are more influenced by single experiences, while parents give information based on a more structured and general observation(18). On the other hand, parents may over- or underestimate the prevalence and emotional impact of health status problems in their child. Peer-related issues might be more important to a child than parents can imagine, resulting in lower 'contact with peers' reports by children in our study. Further, parents might be more capable to look ahead, resulting in better 'future prospects' during 2 years of treatment. In conclusion, both perspectives are important in the evaluation of HRQoL in children.

Measuring HRQoL with the TACQOL questionnaires combines physical, emotional and social well-being in one outcome measure. A review on HRQoL assessment in clinical trials advised to use a combination of questionnaire types, since generic questionnaires are generally less sensitive to the impact of specific diseases than disease-specific instruments(34). Since short stature is not a disease, but rather a statistical definition and clinical condition, generic questionnaires can miss problems related to short stature. A study in children with idiopathic short stature found similar HRQoL to the norm population, except for social functioning, by using the generic TACQoL(35), but might have found different results with a short stature specific questionnaire. In our study, the generic TACQoL did not show the reduced HRQoL shown by the TACQOL-S. In addition, the generic questionnaire did not reveal HRQoL improvement during 2 years of treatment, whereas the TACQOL-S demonstrated significant improvements on various scales. In line with previous

results(10,34), we therefore advise to use additional disease-specific questionnaires in children with short stature.

Due to ethical reasons we could not include a randomised control group. Although this would have been of additional value, we were able to use TACQOL data from a large reference sample at baseline. Further, our main question was answered by the change in HRQoL, from baseline to 2 years of treatment. Our study is unique in evaluating HRQoL in children before puberty, during puberty, and during postponement of puberty. We aimed to investigate three homogenous groups for pubertal status, so we excluded children who entered puberty and started postponement of puberty at a random moment during the 2 years of treatment. A limitation of the study might be the smaller number of children in the pub-GH group. Since pubertal children experience increased intensity of emotions, we can speculate that prepubertal children are a more stringent reference. To investigate the possible adverse effect of postponement of puberty on HRQoL in SGA children treated with GH, we compared the change in HRQoL in pub-GH/GnRHa children to pub-GH children as well as to prep-GH children. The gain in HRQoL in pub-GH/GnRHa children was similar to pubertal and prepubertal children treated with GH only. Therefore, we can conclude that postponement of puberty during to 2 years in addition to GH treatment has no adverse effect on HRQoL.

Early pubertal development has been associated with more mental health problems in boys and girls(36,37), and late maturation with a higher risk of psychopathology in boys [36]. Data regarding psychosocial and behaviour problems in children with precocious puberty, both untreated and treated with GnRHa, are inconclusive(38). Short children born SGA generally start their puberty at a normal age, but relatively early for their short stature(39). Since it is assumed that starting GH treatment during puberty has only limited effect, we hypothesized that postponement of puberty in addition to GH treatment improves adult height in older short SGA children, but might have some adverse psychological consequences. This was reported in a small group of ISS and SGA children treated with GH and GnRHa for 3 years(13). This combination treatment, however, did not show psychosocial consequences on the long-term(12). In our study, short SGA children treated with GH and GnRHa and their parents reported HRQoL improvement on important scales as ‘contact with peers’ and ‘future prospects’. However, these pub-GH/GnRHa children reported no HRQoL improvement on ‘body image’, whereas the other groups and parents did. Although the change in ‘body image’ during 2 treatment years did not differ significantly between the groups, the absence of improvement might be related to pubertal postponement. The pub-GH/GnRHa group experienced less gain in height during 2 years of treatment. This could be expected since pubertal hormones were fully suppressed and height was compared to children with a normal pubertal growth spurt. Despite the smaller gain in height SDS, interestingly, pub-GH/GnRHa children and their parents reported similar HRQoL improvement as the other groups did. Long-term data on height gain and HRQoL are needed before definite conclusions can be drawn, but from our results we conclude that GH treatment improves HRQoL and that additional postponement of puberty has no adverse effect on the HRQoL gain.

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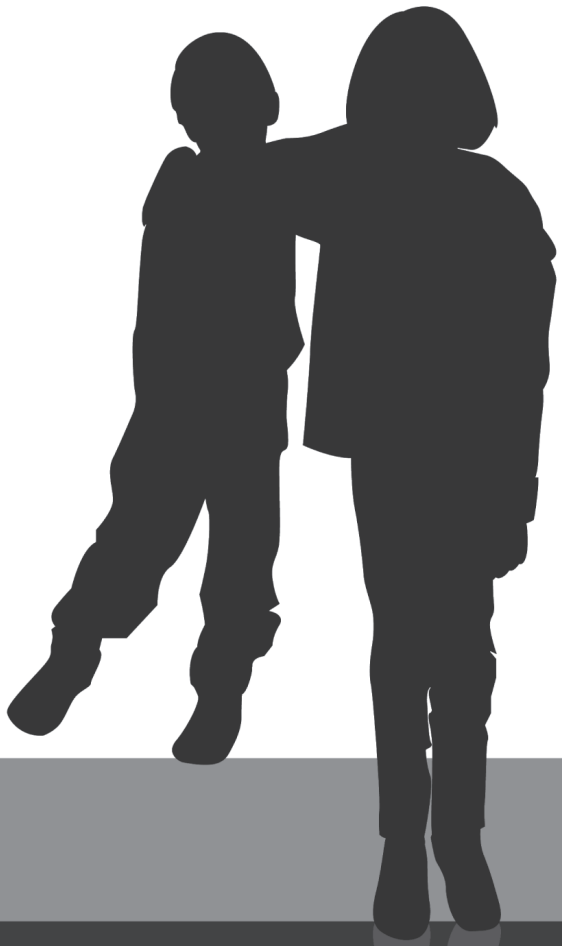

Chapter 8

General discussion and conclusions

In the studies presented in this thesis, we investigated long-term effects of being born small for gestational age (SGA) as well as the effects of growth hormone (GH) treatment and additional postponement of puberty by gonadotropin releasing hormone analogue (GnRHa) in short adolescents born SGA. In this chapter, the main findings of the studies described in this thesis are discussed, also in the context of current literature. We will emphasize clinical implications, and will also give directions for future research.

Long-term effects of being born SGA: Ovarian follicle pool

Fetal life is a critical phase in the development of important organ systems, including the gonads. Since the primordial follicle pool, the basis of female fertility, is formed at and after midgestation, restricted fetal growth might impair reproductive function in later life. Some studies investigated reproductive function in children born SGA, but the results were inconclusive.

Serum AMH levels in short girls born SGA

Anti-Müllerian hormone (AMH) is a marker of the ovarian follicle pool. Our study (Chapter 2) showed that serum AHM levels in prepubertal short children born SGA were similar to levels of healthy girls. Ninety percent of the short girls born SGA had an AMH level in the normal range. Serum AMH levels were not associated with birth weight, birth length or gestational age, also after correction for possible confounders.

We conclude that SGA birth does not compromise the follicle pool. Previously, SGA birth have been associated with FSH hypersecretion and reduced size of internal genitalia(1,2). However, these observations were based on small sample sizes and highly selected subjects. More recent studies in large numbers of adolescent and young-adult women, showed that fetal growth trajectories and birth size were not related to gonadal function(3,4). According to a questionnaire in adults, fertility (time to pregnancy and monthly pregnancy probability) was not reduced in those born SGA(5). These results are in line with ours, showing that gonadal function is not reduced by SGA birth.

Short children born SGA are at higher risk for syndromes, genetic polymorphisms or mutations. We excluded children with syndromes like Turner and Bloom, both known to be associated with gonadal dysfunction(6,7). Nonetheless, the percentage of girls with a serum AMH level below the normal range tended to be higher in those born SGA than in controls. Our data demonstrate that impaired gonadal function in short girls born SGA is rare. However, some underlying genetic problems might result in SGA birth, short stature and impaired gonadal function.

Serum AMH levels during GH treatment

There is increasing evidence, that GH and members of the insulin-like growth factor (IGF) system play a key role in the development of preantral to preovulatory follicles and in the process of

follicular atresia(8,9). The ovary can respond to GH, since GH-receptors and IGF-I receptors are present in the ovary(10). Under in vitro conditions in the rat, GH was reported to stimulate the preantral follicle development and to improve the morphology of cultured preantral follicles(11).

Our study was not designed to investigate possible effects of GH treatment on the follicle pool in detail, but we assessed whether AMH levels altered during GH treatment. Our results show that AMH levels in short SGA girls remain unchanged during GH treatment.

Conclusions, clinical implications and directions for future research

Being born SGA does not impair the follicle pool in the majority of the short SGA girls. In a few cases though, an underlying genetic problem might affect gonadal function. We advise clinicians to be aware of uncommon underlying genetic problems resulting in SGA birth, short stature and impaired gonadal function. Further research is warranted to identify characteristics of girls who are at risk of an impaired gonadal function, in even larger cohorts of children born SGA. As our study was not designed to investigate GH and/or IGF-I effects on follicle development, future clinical research with a long-term follow-up is reasonable.

Long-term effects of being born SGA: Thyroid function

Thyroid hormone reference values

Using blood samples of 512 healthy newborns, infants, children and adolescents, we determined thyroid hormones reference values (Chapter 3). Thyroid hormones showed postnatally a wide spread and an age-specific-pattern thereafter, even during adolescence. Our reference values are in line with those published by other groups(12-19). An important advantage of our study is that we determined reference curves of almost all thyroid hormones, using the LMS method. This statistical method provides gradually changing reference curves that comply better with the biological reality and are more reliable than discontinuous reference ranges. Using our reference values, it is possible to detect pediatric thyroid dysfunction by conversing individual measurements into exact SD-scores.

Thyroid hormone values in short children born SGA

Since restriction in fetal growth may permanently influence thyroid function, we investigated whether thyroid function was impaired in short children born SGA. Our data reveal that untreated short SGA children have similar free T_4 (FT_4) and T_4 levels as the reference population, but have significantly higher T_3, reverse T_3 (rT_3) and TBG levels. Normal FT_4 levels, but mildly increased serum TSH levels have been described in short children born SGA(20,21), more evident in preterm than in term SGA children(22). Recently, normal thyroid function in young adults who were born preterm and SGA was described(23). The explanation for the higher T_3 and rT_3 levels in short children born SGA is unknown. None of the thyroid hormones was correlated with birth

weight, birth length, gestational age or height at start of treatment. Based on our and others' results, we expect that thyroid hormones in short children born SGA have only mild alterations, not clinically relevant.

Thyroid hormone values during puberty and growth hormone treatment

We also investigated the effect of puberty and GH treatment on thyroid hormone values. Our findings demonstrate that puberty and GH treatment both result in a significantly increase of biologically active T_3 and a simultaneous decrease of biologically inactive rT_3, suggesting an altered peripheral thyroid metabolism towards a more active setting. There is a complex relationship between the GH-IGF-I axis and the hypothalamic-pituitary-thyroid axis(24), which becomes more complex during GH treatment(25). Although GH replacement has often been reported to unmask central hypothyroidism(26,27), defined as a reduction in FT_4 or T_4 to below the normal range, we demonstrate altered peripheral metabolism instead of altered secretion from the thyroid gland. In line with our results, a recent study also indicated an increased conversion of T_4 to active T_3 during puberty(28). The changes we found in thyroid metabolism are likely to contribute to acceleration of growth during puberty as well as during GH treatment.

Conclusions, clinical implications and directions for future research

Based on our findings, we conclude that age-specific reference values are crucial for interpretation of pediatric thyroid hormone levels, even during adolescence. Thyroid function does not seem to be impaired due to SGA birth. Puberty and GH treatment both result in more active and less inactive thyroid hormones, supporting the relationship between the GH-IGF-I axis and the thyroid hormone metabolism. When monitoring thyroid function during GH treatment, one needs to distinguish true hypothyroidism from altered peripheral thyroid metabolism. When the decrease in FT_4 or T_4 is accompanied by an increase in active T3, dysfunction of the thyroid gland is unlikely.

Criteria for starting GH treatment

One European requirement for starting GH in short children born SGA, a distance to target height (DTH) of ≥1 SDS, is controversial. In a group of 215 prepubertal short SGA children, we investigated the influence of DTH on growth during 4 years of GH treatment, to ascertain whether it is correct to exclude children with a DTH <1 SDS from GH treatment (Chapter 4).

DTH criterion for starting GH treatment in short children born SGA

Our study in short prepubertal children born SGA, showed that the gain in height SDS during 4 years of GH was positively correlated with DTH SDS, in line with previous results(29,30). However, after adjustment for gender, age at start, GH dose and the interaction term between GH dose and IGFBP-3, 1 SDS smaller DTH was associated with only 0.13 SDS less height gain, equivalent to

approximately 0.8 centimeter difference during 4 years of treatment. The total model explained 40% of the variance in height gain during 4 years of GH, whereas DTH SDS was responsible for only 3% of this variance.

Although the total group of short children born SGA benefits from GH, there is a wide variation in the response to GH treatment. Important variables influencing growth response appeared to be gender, age at start of GH treatment, GH dose, height at start, bone age delay at start and TH(29,30). The growth response in our study showed a wide variation across the entire DTH SDS range and did not support any cut-off level.

In the past two decades, an enhanced understanding of genetics has identified several potential causes for SGA, such as mutations that affect the GH/IGF-I axis, as recently reviewed(31). Children with d3-GH receptor polymorphisms showed increased responsiveness to GH treatment, and also children with IGF-1 receptor haploinsufficiency have been reported to benefit from GH treatment(32-35).

Conclusions, clinical implications and directions for future research

We conclude that the European requirement for starting GH in short children born SGA, a DTH of ≥1 SDS, is not justified. Since this criterion excludes children who can also benefit from GH treatment, it should not be used, in line with the USA and Latin America(36-38). Further research is necessary to explain the wide individual variation in growth response and to identify the good and poor responders to GH treatment. Future prediction models can hopefully serve to optimize and individualize treatment in terms of height outcomes and costs.

Efficacy of GH and additional GnRHa treatment

Management of a short, pubertal child is a controversial topic in pediatric endocrinology. It is assumed that GH treatment started during puberty has only limited effect. Postponement of puberty by GnRHa might improve adult height (AH) by slowing down the fusion of the growth plate. At start of our study, growth data during combined GH and GnRHa (GH/GnRHa) treatment in short children born SGA were limited. Besides, the optimal GH dose for short SGA children, in particular during puberty and/or postponement of puberty, was unknown.

GH treatment in short children born SGA when started around puberty

In our long-term randomized, dose-response GH trial, we evaluated AH results of 121 children born SGA after GH treatment alone or in combination with 2 years of GnRHa treatment (Chapter 5). Children started GH treatment at a median age of 11.2 years, when 46% had already started puberty. Median height increased from -2.9 at start to -1.7 SDS at AH, bringing 62% of the adolescents in the normal AH range. Previously, Carel et al. showed that GH treatment in older short SGA children resulted in only 0.6 SDS increase of AH, bringing 47% of the adolescents in the

normal AH range(39). In that study though, hardly any child completed treatment as planned and the treatment duration ranged from 6 months to 3.2 years. Based on our results, we conclude that short SGA adolescents can still have impressive catch-up growth, even when they already entered puberty at start of treatment.

GH dose during puberty and postponement of puberty

Double GH dose from onset of puberty was shown to result in 0.5-0.6 SDS better AH SDS than the standard dose of 1mg/m²·day. Previously, no benefit of doubling GH dose on AH SDS has been found in short children born SGA who started GH before puberty(29,40). Our group of adolescents had only a few years left to attain a normal height, so we can speculate that doubling GH dose is most effective during a relatively short treatment period. Besides, children might need a higher GH dose during combined GH/GnRHa. Previously, we showed that 3 months of GnRHa treatment resulted in a decrease in mean and maximum GH levels in short SGA girls(41), but not significantly in boys. During combined GH/GnRHa treatment, mean and maximum GH levels were lower in our study group(42) compared with levels in a group of prepubertal SGA children treated with GH only(43). Also the results presented in this thesis indicated lower IGF-I levels during GH/GnRHa treatment than during GH alone, possibly due to suppression of sex steroids. The double GH dose was not associated with a more rapid progression of puberty or bone maturation, neither with a greater frequency of adverse events. However, the higher GH dose was associated with significantly higher IGF-I levels, more often above the normal range.

Combined GH/GnRHa treatment in short children born SGA with poor AH expectation

When children are short at start of puberty, their AH is expected to be poor. Any improvement in AH can be an important goal for these children. Increasing height potential during puberty is complicated since the epiphyseal fusion caused by the pubertal sex steroids greatly limits the time available for linear growth. In a subgroup of pubertal children with a poor AH expectation (height at start of puberty <140 cm), we analyzed the growth during 2 years of GH/GnRHa and subsequent GH treatment until AH. Median height increased from -3.0 at start to -2.1 SDS at AH in the GH 1 mg/m²·day group, and from -2.6 at start to -1.7 SDS at AH in the GH 2 mg/m²·day group. An average growth of 34.5 cm in boys and 24.2 cm in girls was still possible, more than the pubertal growth of the Dutch reference population(44,45). This is remarkable since these children always had a height below the reference range, with an even worse AH expectation at start of treatment.

Conclusions, clinical implications and directions for future research

We conclude that GH treatment is beneficial and safe for increasing AH of adolescents who start treatment around onset of puberty. Our results should not lead to delaying GH treatment until puberty, since a normal height during childhood and adolescence has important advantages. But

in clinical practice, when children present with short stature around pubertal age, they must not be excluded from GH treatment.

A double GH dose of 2 mg/m²·day from onset of puberty results in significantly better AH. Since concerns have been expressed regarding possible detrimental effects of persistently high serum GH and IGF-I levels(46), we exclusively recommend high GH dosing during puberty when only few years of growth are left, or in combination with GnRHa. Yearly monitoring of IGF-I levels during GH treatment is advised and the GH dose should be reduced in children with repeated serum IGF-I levels above the normal range. In children with repeatedly high IGF-I levels during standard dose GH treatment (1 mg/m²·day), an *IGF1R* gene mutation should be considered. Future studies are warranted to evaluate individualized GH treatment. Determining the most advantageous individual GH dose, can help in optimizing AH as well as in controlling high levels of IGF-I or adverse events.

When SGA children are short at start of puberty, they can benefit from combined GH/GnRHa treatment. Since no model is known to predict AH precisely at start of puberty, future studies are needed. Accurate identification of short pubertal children who benefit most from combined GH/GnRHa treatment and/or from higher GH dose is essential.

Safety of GH and additional GnRHa treatment

Bone mineral density in short children born SGA

Several cohort studies suggested that poor growth during fetal life and infancy is associated with decreased bone mass in adulthood(47-50), but others could not confirm any association between birth size and bone mineral density (BMD)(51). We therefore investigated BMD and body composition before and during GH treatment, with or without additional GnRHa (Chapter 6). Our study shows that BMD in untreated short children born SGA is not impaired due to their restricted fetal growth, but is lower due to their short and lean stature.

Bone mineral density during GH with or without additional GnRHa treatment

We longitudinally evaluated BMD in 88 adolescents during GH treatment from start to AH. We demonstrated a significant improvement in BMD during GH treatment, in line with previous reports(52,53). This improvement was strongly associated with the enlargement of body size and pubertal development. Besides, we revealed that combined GH/GnRHa treatment did not persistently impair BMD. Several studies reported reduced BMD after GnRHa treatment in girls with central precocious puberty(54) and in adolescents with short stature(55,56). Studies investigating BMD longitudinally during and after GnRHa treatment though, did not find impaired BMD(57-59). Our results demonstrated no adverse effect of combined GH/GnRHa treatment on adult BMD.

Body composition during GH with or without additional GnRHa treatment

Our study demonstrated that baseline body mass index (BMI), lean body mass (LBM), and fat mass (FM) SDS were significantly reduced in short adolescents born SGA. During GH treatment, these body composition parameters tended to normalize. The double GH dose was shown to result in 0.3 SDS less fat mass and 0.2 SDS less percentage of trunk fat compared with the standard GH dose of 1 mg/m²·day, after adjustment for gender, change in height SDS and additional GnRHa treatment. These GH dose effects on fat mass can be explained by the known lipolytic effects of GH(60). Since data on body composition and fat distribution during GnRHa treatment in short SGA children are scarce, we determined LBM, FM and fat distribution longitudinally during combined GH/GnRHa treatment. Our results showed that combined GH/GnRHa treatment had no adverse effect on body composition in short adolescents born SGA.

Conclusions, clinical implications and directions for future research

Our study is unique since it longitudinally evaluated BMD and body composition in a large group of short adolescents born SGA during GH or combined GH/GnRHa treatment. Based on our findings we conclude that BMD in short adolescents born SGA is not impaired by SGA birth, but matches their short and lean stature. Adult BMD after GH treatment is normal in almost all cases. In addition, GnRHa treatment has no persisting adverse effect on BMD, when given for two years next to GH treatment. It is reassuring that GH treatment alone or combined with GnRHa treatment does not have adverse effects on BMD or body composition. Follow-up into adulthood is required to investigate the very long-term effects of changes in BMD, body composition and metabolic profile in short SGA children treated with GH and GnRHa.

Psychosocial effects of GH and additional GnRHa treatment

Health-related quality of life (HRQoL) reflects the subjective perception of health and is increasingly recognized as a relevant ‘patient-reported outcome’(61). We investigated HRQoL in short children born SGA at baseline and after two years of GH treatment, with or without additional postponement of puberty by GnRHa. HRQoL was measured by generic (TACQOL) and short stature-specific questionnaires (TACQOL-S) in children and parents.

Health-related quality of life before and during treatment

At baseline, short children born SGA had lower scores on cognitive and social functioning according to the TACQOL, compared with the reference population. This is in line with previous studies, showing on average lower intelligence and more problems in behaviour, social functioning, school competence and attention in short SGA children(62-66).

During two years of GH treatment, children and parents reported significant HRQoL improvement on various scales of the short-stature-specific TACQOL-S. Our results demonstrated that prepubertal children experienced better 'contact with adults', 'body image' and 'vitality' during GH treatment. Children with combined GH/GnRHa treatment experienced better 'contact with adults', 'contact with peers' and 'physical abilities'. According to parents' reports, children experienced better HRQoL on most short-stature-specific scales. Previously, some studies showed better HRQoL in GH-treated children, compared to untreated children with short stature(67,68), but these data were cross-sectional. We present longitudinal data on HRQoL, showing improvement in prepubertal, pubertal and puberty-postponed children during two years of GH treatment. Moreover, the HRQoL improvement was similar in prepubertal, pubertal and puberty-postponed children. Previously, impaired HRQoL was reported in a small group of ISS and SGA children treated with combined GH/GnRHa for 3 years, but the authors did not show psychosocial consequences on the long-term(69). Our results demonstrate that postponement of puberty during to 2 years in addition to GH treatment has no adverse effect on HRQoL.

The generic questionnaire did not reveal HRQoL improvement during 2 years of treatment, whereas the short stature-specific questionnaire demonstrated significant improvements on various scales. Generic questionnaires therefore can miss problems related to short stature. A study in children with idiopathic short stature found similar HRQoL to the norm population, except for social functioning, by using the generic TACQoL(70), but might have found different results with a short stature specific questionnaire. In line with previous results(67,71), we therefore advise to use an additional disease-specific questionnaire when evaluating HRQoL of children with short stature.

Conclusions, clinical implications and directions for future research

From our results we conclude that GH treatment improves HRQoL and that additional postponement of puberty has no adverse effect on HRQoL, although long-term data on HRQoL are needed before definite conclusions can be drawn. In children treated for short stature, the TACQOL-S is an appropriate tool to evaluate HRQoL. We advise the use of disorder-specific questionnaires.

General conclusions

Nowadays, GH is an approved treatment for short stature in children born SGA, in the United States (Food and Drugs Administration, 2001) and in Europe (European Medicines Agency, 2003). Although GH treatment is proven effective in children who started treatment at an early age, GH was thought to have limited effect when started during adolescence, just before or during puberty. In the present thesis we investigated long-term effects of being born SGA as well as the

effects of GH treatment and additional postponement of puberty by GnRHa in short adolescents born SGA, who started treatment around onset of puberty.

We showed that being born SGA does not impair the follicle pool in the majority of the short SGA girls, although rarely a syndrome or genetic problem might be underlying causing short stature and impaired gonadal function. Thyroid function does not seem to be disturbed in short children born SGA. Puberty and GH treatment both result in more active and less inactive thyroid hormones, indicating altered peripheral metabolism. Further, we showed that the European requirement for starting GH in short children born SGA, a DTH of ≥1 SDS, is not justified. This criterion should not be used, which would be in line with the USA and Latin America(36-38).

The studies in this thesis demonstrate that GH treatment is beneficial and safe for increasing AH in adolescents who start treatment around onset of puberty. A double GH dose of 2 mg/m²·day during puberty results in significantly better adult height, but monitoring of IGF-I levels during GH treatment is recommended. When SGA adolescents are short at start of puberty, they can benefit from combined GH/GnRHa treatment. GH treatment resulted in a significant improvement of health-related quality of life, also when combined with postponement of puberty. Finally, our results revealed no adverse effects of GH and/or GnRHa treatment on bone mineral density and body composition.

Directions for future research

More research is required to explain the wide individual variation in growth response and to identify the good and poor responders to GH treatment. Future prediction models, particularly for short children in early puberty, might serve to individualize and optimize treatment. A major challenge is to determine the most advantageous individual GH dose, since this will help in optimizing growth results as well as in controlling high levels of IGF-I or adverse events. Besides, accurate identification of short pubertal children who benefit most from combined GH/GnRHa treatment is essential.

Follow-up into adulthood is required to investigate the very long-term effects of GH or combined GH/GnRHa treatment on bone mineral density, body composition and metabolic profile in short SGA children after cessation of treatment. The Dutch Nationwide Growth Register is an unique and important database that collects a large amount of prospective data for safety issues, and needs to be maintained. Finally, long-term data on health-related quality of life, preferable determined by disorder-specific questionnaires, are needed.

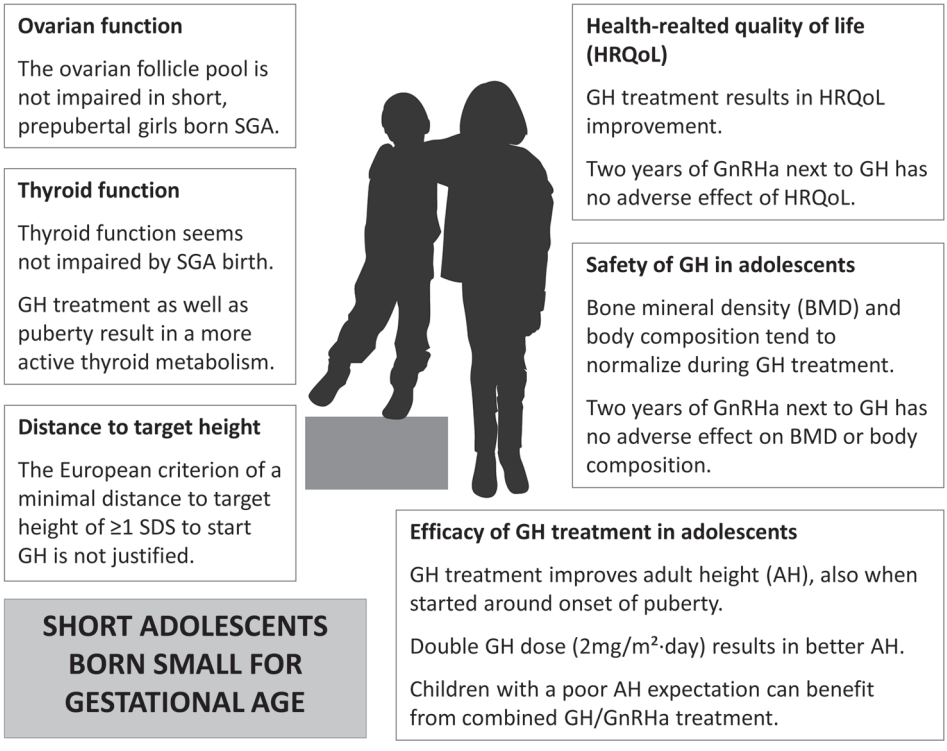

Figure 1. General conclusions
SGA, small for gestational age; GH, growth hormone; GnRHa, gonadotropin releasing hormone analogue.

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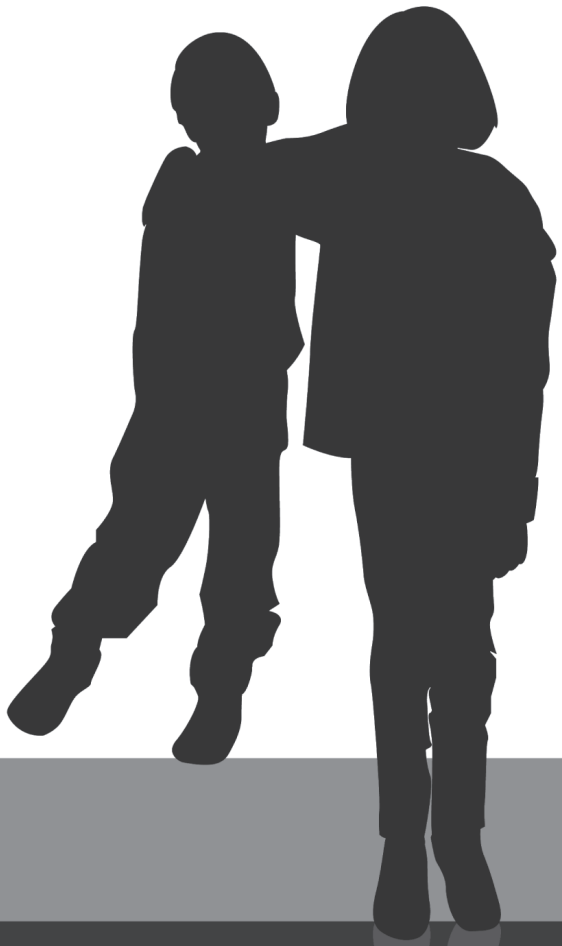

Chapter 9

Summary / Samenvatting

Summary

Chapter 1

This chapter gives an overview of definitions, prevalence and possible causes of small for gestational age (SGA) birth. It provides a general introduction on growth, puberty and the pituitary hormone axes. Treatment options for short children born SGA who present around puberty are discussed. Finally, we describe our study population, provide the aims of the studies performed and present the outline of this thesis.

Chapter 2

Fetal growth restriction is thought to negatively influence gonadal function in later life. However, studies on gonadal function in children born SGA have been inconclusive. We aimed to evaluate the effect of being born SGA on serum anti-Müllerian Hormone (AMH), a good marker of the primordial follicle pool in girls. Besides, we aimed to investigate the effect of growth hormone (GH) treatment on serum AMH levels in short girls born SGA.

Serum AMH levels in 119 untreated short SGA girls were similar to these in 127 healthy control girls (all prepubertal, aged 3-10). In short SGA girls, serum AMH levels were not significantly influenced by birth weight standard deviation score (SDS), birth length SDS and gestational age, even after adjustment for age, height SDS and body mass index SDS at sampling, socio-economic status and maternal smoking during gestation. Serum AMH levels did not change during 4 years of GH treatment in short SGA girls.

In conclusion, serum AMH levels in prepubertal short SGA girls are similar to healthy controls, indicating that the follicle pool is not compromised due to SGA birth. AMH levels in short SGA girls remain similar during GH treatment.

Chapter 3

Age-appropriate reference values for thyroid hormones are required for detecting pediatric thyroid dysfunction. Data on thyroid hormones and peripheral thyroid metabolism in short children born SGA before and during GH treatment were lacking. In chapter 3, we obtained pediatric reference ranges for free thyroxine (FT_4), thyroid-stimulating hormone (TSH), total thyroxine (T_4), triiodothyronine (T_3), reverse triiodothyronine (rT_3) and thyroxine binding globulin (TBG), using blood samples of 512 healthy children (225 females; 0-18 years). Besides, we investigated these thyroid hormones in 125 short SGA children (62 females; mean age 11.3 years) before puberty, during puberty and during postponement of puberty by gonadotropin-releasing hormone analogue (GnRHa), and we investigated these hormones during 2 years of GH treatment.

Thyroid references showed wide ranges postnatally and age-specific patterns thereafter, even during adolescence. Untreated short SGA children had similar FT_4 and T_4 levels as the reference

population, but higher T_3, rT_3 and TBG levels. During puberty and GH treatment, FT_4 and rT_3 significantly decreased whereas T_3 significantly increased.

In conclusion, short children born SGA have mild alterations in thyroid hormone levels, according to age-specific thyroid references values, not expected to be clinically relevant. Puberty and GH treatment both induce changes in peripheral thyroid metabolism, resulting in more biologically active T_3 at the expense of less inactive rT_3. Our findings indicate that GH treatment induces altered peripheral thyroid metabolism, but does not result in thyroid dysfunction.

Chapter 4

The criteria for starting GH, an approved treatment for short children born SGA, differ between Europe and the USA. Controversy exists on one European requirement for starting GH, a distance to target height (DTH) of ≥1 SDS. We therefore aimed to investigate the influence of DTH on growth during GH treatment in a large group of short prepubertal children born SGA (baseline n=446; 4 years GH n=215).

Height gain SDS during 4 years of GH treatment showed a wide variation at every DTH SDS level. Multiple regression analyses demonstrated that, after correction for other significant variables, an additional DTH of 1 SDS resulted in 0.13 SDS more height gain during 4 years of GH, comparable to only 0.8 cm. We found no support for the use of any DTH cut-off level.

In conclusion, DTH SDS has a weak positive effect on height gain during GH treatment, but several other determinants have much larger effects. Based on our data, excluding children with a DTH <1 SDS from GH treatment is not justified. We recommend that the European criterion based on DTH should not be used, in line with the USA criteria for starting GH treatment in short children born SGA.

Chapter 5

Although GH treatment is effective in improving height in short children born SGA, it is thought to have limited effect when started during adolescence. Therefore, we aimed to investigate the efficacy of GH treatment when started around onset of puberty in a longitudinal, randomized, dose-response GH trial that included 121 short SGA children (60 boys) above the age of 8 years. We aimed to assess whether GH 2 mg/m²·day during puberty improves adult height (AH) compared with the standard dose of 1 mg/m²·day. Also, we aimed to assess whether 2 years of additional postponement of puberty by GnRHa improves AH in children who are short at start of puberty (<140 cm), and have a poor AH expectation.

In our study, short SGA children started treatment at an average age of 11.2 years, when 46% had already started puberty. Height increased significantly from -2.9 at start to -1.7 SDS at AH. Treatment with GH 2 versus 1 mg/m²·day during puberty resulted in significantly better AH, also after correction for gender, age at start, height SDS at start, treatment years before puberty and TH SDS. AH was similar in children who started puberty <140 cm and received GH/GnRHa, compared with children who started puberty >140 cm and received GH only.

In conclusion, also when started around onset of puberty, GH treatment significantly improves AH in short SGA children, particularly when GH 2 mg/m²·day is administered during puberty. When SGA children are short at start of puberty, they can benefit from combined GH/GnRHa treatment.

Chapter 6

GH treatment in short children born SGA was thought to improve bone mineral density (BMD) and to have a favorable effect on body composition. In contrast, postponement of puberty by GnRHa was thought to have negative effects on BMD and body composition. We therefore aimed to evaluate BMD of total body (BMD_{TB}), lumbar spine (BMD_{LS}), bone mineral apparent density lumbar spine ($BMAD_{LS}$), lean body mass (LBM), fat mass (FM) and fat distribution during GH treatment, with or without additional two years of GnRHa. We performed dual-energy X-ray absorptiometry (DXA) scans longitudinally, in 88 children (50 girls) who were treated with GH until AH; 52 of them received additional GnRHa.

Baseline BMD_{TB} and BMD_{LS} SDS were significantly reduced, but $BMAD_{LS}$ was normal. BMD_{TB} and BMD_{LS} improved significantly from start until AH, whereas $BMAD_{LS}$ SDS remained similar. At AH, 93% of patients had a normal BMD_{TB}, 99% a normal BMD_{LS} and 98% a normal $BMAD_{LS}$ (>-2 and <+2 SDS). From start until AH, LBM SDS and FM SDS tended to normalize. Additional GnRHa treatment had no adverse effect on the changes in BMD and body composition during GH treatment, also after correction for influencing variables.

In conclusion, untreated short SGA adolescents have reduced BMD_{TB} and BMD_{LS}, but normal bone-size-corrected $BMAD_{LS}$. During GH treatment, BMD_{TB} and BMD_{LS} increase significantly, leading to a normal adult BMD in almost all patients. Two years of GnRHa in addition to GH treatment have no adverse effect on BMD or body composition.

Chapter 7

Besides height improvement, health-related quality of life (HRQoL) is an important outcome of GH treatment in short children born SGA. Based on previous literature, we hypothesized that GH treatment improves HRQoL in short SGA children, but that postponement of puberty attenuates this effect. The study described in chapter 7 investigated HRQoL longitudinally during 2 years of treatment in 97 short SGA children (mean age 11.6 years at start). The children were divided in 3 groups: prepubertal GH-treated (prep-GH), pubertal GH-treated (pub-GH), and pubertal GH-treated with additional GnRHa (pub-GH/GnRHa). HRQoL was measured by short stature specific questionnaires (TACQOL-S) in children and parents, and by a generic questionnaire (TACQOL) in parents.

Based on the TACQOL-S, prep-GH children experienced significant HRQoL improvement on ‘contact with adults’, ‘body image’ and ‘vitality’, and pub-GH/GnRHa children on ‘contact with adults’, ‘contact with peers’ and ‘physical abilities’. Parents of prep-GH and pub-GH/GnRHa children reported significant HRQoL improvement on most TACQOL-S scales, whereas the

improvement in pub-GH children reached significance on 'future prospects' only, probably due to the smaller number of children in this group. The short stature specific HRQoL gain during 2 years of treatment, was similar in the 3 groups, also after correction for potential confounders. The generic questionnaire TACQOL did not reveal any changes.

In conclusion, HRQoL can improve in prepubertal and pubertal short SGA children during 2 years of GH treatment. Two years of additional postponement of puberty has no adverse effect on the HRQoL gain. We advise to use the disorder-specific questionnaires, since these are particularly appropriate to evaluate HRQoL in children treated for short stature.

Chapter 8

In the general discussion and conclusions, we discuss the main findings of the studies described in this thesis, also in relation to current literature. We emphasize on clinical implications and give directions for future research.

Samenvatting

Hoofdstuk 1

Dit hoofdstuk beschrijft de definities, de prevalentie en de mogelijke oorzaken van een kleine lengte en/of laag gewicht bij de geboorte (small for gestational age, SGA). Het geeft achtergrondinformatie over groei, puberteitsontwikkeling en de werking van diverse hormonale assen. Het hoofdstuk zet behandelingsmogelijkheden uiteen voor te kleine, SGA-geboren kinderen die zich rond de start van de puberteit presenteren. Daarnaast worden de doelstellingen en de opzet van de SGA-studie beschreven.

Hoofdstuk 2

Suboptimale groei in de baarmoeder heeft mogelijk een negatief effect op de ontwikkeling van de voortplantingsorganen en daarmee de gonadale functie op latere leeftijd. Studies naar gonadale functie in SGA-geboren kinderen lieten echter uiteenlopende resultaten zien. Daarom onderzochten wij serum anti-Müllerian hormoon (AMH) waarden in SGA-geboren meisjes, een goede maat voor de follikel voorraad in de ovariae. Daarnaast onderzochten wij het effect van GH behandeling op serum AMH waarden.

Serum AMH waarden van 119 te kleine, SGA-geboren meisjes bleken vergelijkbaar met de waarden van 127 gezonde controle meisjes (allen prepubertair, leeftijd 3-10 jaar). In SGA-geboren meisjes bleek AMH niet gecorreleerd met geboortegewicht, geboortelengte of zwangerschapsduur, ook na correctie voor variabelen zoals leeftijd, lengte SDS, body mass index SDS, sociaal-economische status en maternaal roken tijdens de zwangerschap. Serum AMH waarden bleven gelijk tijdens 4 jaar GH behandeling.

Concluderend, serum AMH waarden in prepubertaire te kleine, SGA-geboren meisjes zijn vergelijkbaar met die van gezonde controle meisjes, implicerend dat de follikelvoorraad niet beperkt is in SGA-geboren meisjes. Tijdens 4 jaar GH behandeling, veranderen serum AMH waarden veranderen niet.

Hoofdstuk 3

Een normale schildklierfunctie is essentieel voor groei en ontwikkeling van kinderen. Om stoornissen in schildklierfunctie bij kinderen te kunnen detecteren, zijn leeftijdsafhankelijke referentiewaarden nodig. Studies naar schildklierfunctie in te kleine, SGA-geboren kinderen voor en tijdens GH behandeling gaven tegenstrijdige resultaten. In hoofdstuk 3 bepaalden wij referentiewaarden voor thyroidstimulerend hormoon (TSH), vrij thyroxine (FT_4), thyroxine (T_4), trijoodthyronine (T_3), reverse trijoodthyronine (rT_3) en thyroxine-bindend globuline (TBG), door middel van serum samples van 512 gezonde pasgeborenen, kleuters, kinderen en adolescenten (287 jongens). Vervolgens bepaalden wij deze schildklierhormonen in 125 te kleine, SGA-geboren kinderen (63 jongens, gemiddelde leeftijd 11,3 jaar) vóór de puberteit, tijdens de puberteit en

tijdens het onderdrukken van de puberteit middels gonadotrofine-stimulerend hormoon analoog (GnRHa), en bepaalden wij het verloop tijdens 2 jaar GH behandeling.

Schildklierhormoonwaarden toonden direct na de geboorte een zeer grote spreiding. Vervolgens toonden de referentiewaarden een leeftijdsspecifiek patroon, zelfs tijdens adolescentie. Onbehandelde SGA-geboren kinderen hadden vergelijkbare FT_4 en T_4 waarden als de referentiepopulatie, maar significant hogere T_3, rT_3 en TBG waarden. Zowel puberteit als GH behandeling resulteerden in een significante verlaging van FT_4 en rT_3, en een gelijktijdige, significante verhoging van T_3.

Concluderend, schildklierhormoon referentiewaarden zijn essentieel tot en met adolescentie. Schildklierhormonen in te kleine, SGA-geboren kinderen tonen milde veranderingen ten opzichte van de referentiepopulatie. Puberteit en GH behandeling induceren beide een verandering van het perifere schildkliermetabolisme, resulterend in een verhoging van het actieve T_3 tezamen met een verlaging van het inactieve rT_3. Onze bevindingen impliceren dat GH behandeling het perifere schildkliermetabolisme beïnvloedt, maar dat het geen verstoring van de schildklierfunctie veroorzaakt.

Hoofdstuk 4

De criteria om GH behandeling te starten bij te kleine, SGA-geboren kinderen verschillen tussen Europa en Amerika. Eén van de Europese criteria is een minimaal verschil tussen lengte SDS en doellengte SDS (distance to target height, DTH) van 1 SDA. Dit is al jaren onderwerp van discussie. Om te onderzoeken of het gebruik van dit DTH criterium gerechtvaardigd is, bepaalden wij de invloed van DTH op de lengte en lengtewinst in een grote groep prepubertaire te kleine, SGA-geboren kinderen voor (n=446) en na 4 jaar GH behandeling (n=215).

De lengtewinst tijdens 4 jaar GH behandeling toonde een grote variatie op elk niveau van DTH. Multipele regressie analyse toonde dat, na correctie voor significante variabelen, één extra SDS DTH resulteerde in 0.13 SDS meer lengtewinst tijdens 4 jaar GH, overeenkomend met slechts 0,8 cm. Wij vonden geen significant verschil in lengtewinst tussen kinderen boven en onder een bepaalde DTH waarde, implicerend dat het gebruik van een DTH cut-off grens niet gerechtvaardigd is.

Concluderend, de afstand tot doellengte heeft een zwak positieve correlatie met de lengtewinst tijdens 4 jaar GH behandeling, terwijl andere variabelen beduidend meer invloed hebben. Wij vonden geen ondersteunend bewijs voor het gebruik van cut-off level gebaseerd op DTH. Omdat het gebruiken van het Europese criterium DTH≥1 SDS kinderen ten onrechte uitsluit van GH behandeling, adviseren wij dit criterium niet meer te gebruiken.

Hoofdstuk 5

SGA-geboren kinderen met een blijvend te kleine lengte hebben baat bij GH behandeling. Algemeen wordt aangenomen dat starten van GH op oudere leeftijd, rondom het begin van de puberteit, onvoldoende lengtewinst oplevert. Daarom evalueerden wij de effectiviteit van GH

behandeling wanneer gestart wordt rondom het begin van de puberteit, in onze longitudinale, gerandomiseerde dosis-respons GH studie in 121 te kleine SGA-geboren kinderen, ouder dan 8 jaar. Wij vergeleken 2 doseringen GH tijdens de puberteit (middels randomisatie 1 mg of 2 mg/m²·dag). Daarnaast onderzochten wij het effect van 2 jaar GnHRa naast GH behandeling in kinderen met een slechte lengteprognose aan het begin van de puberteit.

In onze studie was de leeftijd bij start van de behandeling gemiddeld 11,2 jaar en was 46% van de kinderen al in de puberteit. De lengte verbeterde significant van -2,9 bij start naar -1,7 SDS bij volwassen lengte. GH 2 mg/m²·dag tijdens de puberteit resulteerde in 0,6 SDS betere volwassen lengte vergeleken met de standaard dosering van 1 mg/m²·dag. Kinderen die in de puberteit kwamen bij een lengte <140 cm en gecombineerde GH/GnRHa behandeling kregen, behaalden een vergelijkbare eindlengte als kinderen die in de puberteit kwamen bij een lengte >140 cm en alleen GH behandeling kregen.

Concluderend, ook wanneer gestart wordt rondom het begin van de puberteit, kan GH een normale volwassen lengte realiseren in te kleine, SGA-geboren kinderen, vooral met een dubbele dosis GH. Als de lengteprognose bij start van de puberteit slecht is, kunnen kinderen baat hebben bij gecombineerde GH/GnRHa behandeling.

Hoofdstuk 6

Gebaseerd op voorgaand onderzoek, zou GH behandeling een verbetering van de botdichtheid en de lichaamssamenstelling geven, terwijl GnRHa juist een negatief effect op deze parameters zou hebben. Wij onderzochten de botdichtheid van het gehele lichaam (BMD_{TB}), de botdichtheid van de wervelkolom (BMD_{LS}) en de botdichtheid van de wervelkolom gecorrigeerd voor de lengte ($BMAD_{LS}$), de spiermassa, de vetmassa en de vetverdeling tijdens GH behandeling met of zonder 2 jaar GnRHa. De botdichtheid en de lichaamssamenstelling werden gemeten met Dual-Energy X-Ray Absorptiometry (DXA) scans in 88 kinderen (50 meisjes) die met GH werden behandeld tot eindlengte; 52 kinderen werden tevens behandeld met 2 jaar GnRHa.

Bij aanvang van de behandeling was de BMD_{TB} en BMD_{LS} significant verlaagd, terwijl de $BMAD_{LS}$ vergelijkbaar was met de referentiepopulatie. Tijdens behandeling verbeterden de BMD_{TB} en BMD_{LS} significant, terwijl de $BMAD_{LS}$ gelijk bleef. Na het bereiken van de eindlengte had 93% van de kinderen een normale BMD_{TB}, 99% een normale BMD_{LS} en 98% een normale $BMAD_{LS}$ (>-2 and <+2 SDS). Tijdens behandeling normaliseerden de spiermassa en de vetmassa. Twee jaar GnRHa naast GH behandeling had geen negatief effect heeft op de veranderingen in botdichtheid of lichaamssamenstelling, ook na correctie voor belangrijke variabelen.

Concluderend, te kleine, SGA-geboren adolescenten hebben een verlaagde BMD_{TB} en BMD_{LS}, maar een normale $BMAD_{LS}$. Tijdens GH behandeling verbetert de botdichtheid, resulterend in een normale volwassen botdichtheid voor vrijwel iedereen. Twee jaar GnRHa naast GH behandeling heeft geen negatief effect op de botdichtheid of de lichaamssamenstelling.

Hoofdstuk 7

Te kleine, SGA-geboren kinderen hebben baat bij GH behandeling, en op oudere leeftijd mogelijk ook baat bij het uitstellen van de puberteit middels GnRHa. Naast het bereiken van een normale lengte is verbetering van de kwaliteit van leven health-related quality of life, HRQoL een belangrijk doel van de behandeling. In hoofdstuk 7 hebben wij daarom de HRQoL in 97 te kleine, SGA-geboren kinderen longitudinaal in kaart gebracht, gedurende behandeling met GH en additioneel 2 jaar GnRHa (gemiddelde leeftijd bij start 11,6 jaar). De kinderen werden verdeeld in 3 groepen: prepubertair met alleen GH (prep-GH), pubertair met alleen GH (pub-GH) en pubertair met GH en GnRHa (pub-GH/GnRHa). Wij gebruikten een algemene HRQoL vragenlijst (TACQOL) voor ouders en een HRQoL vragenlijst specifiek voor kleine lengte (TACQOL-S) voor ouders en kinderen.

Volgens de kleine-lengte-specifieke TACQOL-S ervoeren prep-GH kinderen tijdens behandeling significante verbetering van 'contact met volwassenen', 'lichaamsbeeld' en 'vitaliteit', en ervoeren pub-GH/GnRHa kinderen significante verbetering van 'contact met volwassenen', 'contact met leef-tijdsgenoten' en 'lichamelijke ongemakken'. Ouders van prep-GH en pub-GH/GnRHa kinderen rapporteerden tijdens behandeling significante verbetering van vrijwel alle schalen van de TACQOL-S. Ouders van pub-GH kinderen rapporteerden ook verbetering, maar dit werd alleen significant voor 'toekomstbeeld', waarschijnlijk verklaard door het kleine aantal kinderen in deze groep. De HRQoL verbetering gedurende 2 jaar behandeling was vergelijkbaar tussen de 3 groepen, ook na correctie voor geslacht, leeftijd, lengte en sociaal economische status. De algemene KvL vragenlijst toonde geen verschillen tijdens behandeling.

Concluderend, de HRQoL van prepubertaire en pubertaire te kleine, SGA-geboren kinderen verbetert tijdens 2 jaar GH behandeling. Het additioneel uitstellen van de puberteit middels GnRHa heeft geen negatief effect op deze verbetering in HRQoL. Wij adviseren om een HRQoL vragenlijst specifiek voor kleine lengte te gebruiken voor kinderen die behandeld worden vanwege kleine lengte.

Hoofdstuk 8

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

Chapter 10

List of abbreviations

List of co-authors and affiliations

Dankwoord

List of publications

PhD portfolio

List of abbreviations

AH	Adult Height
AMH	Anti-Müllerian Hormone
BMD	Bone Mineral Density
BMI	Body Mass Index
DTH	Distance to Target Height
DXA	Dual-Energy X-ray Absorptiometry
FM	Fat Mass
FSH	Follicle Stimulating Hormone
FT4	Free Thyroxine
GH	Growth Hormone
GnRHa	Gonadotropin Releasing Hormone analogue
HRQoL	Health-Related Quality of Life
IGF-I	Insulin-like Growth Factor-I
IGFBP-3	Insulin-like Growth Factor Binding Protein-3
IQR	Inter-Quartile Range
LBM	Lean Body Mass
LH	Luteinizing Hormone
SDS	Standard Deviation Score
SES	Socio-Economic Status
SGA	Small for Gestational Age
TH	Target Height
T4	Thyroxine
T3	Triiodothyronine
TSH	Thyroid Stimulating Hormone
rT3	Reverse Triiodothyronine
TBG	Thyroxine-Binding Globulin

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List of publications

Publications (this thesis)

Lem AJ, de Kort SWK, de Ridder MAJ, Hokken-Koelega ACS. Should short children born small for gestational age with a distance to target height <1 SDS be excluded from growth hormone treatment? *Clinical Endocrinology* (2010) 73:355-360

Lem AJ, Boonstra VH, Renes JS, Breukhoven PE, de Jong FH, Laven JSE, Hokken-Koelega ACS. Anti-müllerian hormone in short girls born small for gestational age and the effect of growth hormone treatment. *Human Reproduction* (2011) 26:898-903

Lem AJ, Jobse I, van der Kaay DCM, de Ridder M, Raat H, Hokken-Koelega ACS. Health-related quality of life in SGA children: effects of growth hormone and postponement of puberty. *Hormone Research in Paediatrics* (2012) 77:170-179

Lem AJ, de Rijke YB, van Toor H, de Ridder MAJ, Hokken-Koelega ACS. Serum thyroid hormone levels in healthy children from birth to adulthood and in short children born small for gestational age. *J Clin Endocrinol Metab* (2012) 97(9):3170-8

Lem AJ, van der Kaay DCM , de Ridder MAJ, Bakker-van Waarde WM, van der Hulst JPCM, Mulder JC, Noordam C, Odink RJ, Oostdijk W, Schroor EJ, Sulkers EJ, Westerlaken C, Hokken-Koelega ACS. Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analogue: Results of a randomized, dose-response GH trial. *J Clin Endocrinol Metab* (2012), *Epub ahead of print*

Lem AJ, van der Kaay DCM , Hokken-Koelega ACS. Bone mineral density and body composition in short children born SGA during growth hormone and gonadotropin releasing hormone analogue treatment. *J Clin Endocrinol Metab, In press*

Publications (other)

Thio GKKE, Muiño Mosquera L, **Lem AJ**, van Kempen AAMW, Valerio PG, Franssen EJF. Lokale anesthesie met prilocaïne tijdens de behandeling; effect op de pasgeborene. *Wetenschappelijk platform* (2010) 4(6):94-98

Lem AJ. Verbetering van lengte en kwaliteit van leven van SGA-geboren kinderen zonder spontane inhaalgroei. *Kinderarts en Wetenschap* (2012) 2:27-29

Renes JS, de Ridder MAJ, Breukhoven PE, **Lem AJ**, Hokken-Koelega ACS. Methylphenidate and the response to growth hormone treatment in short children born small for gestational age. *Submitted*

Muiño Mosquera L, **Lem AJ**, Thio GKKE, Aleman J, Franssen EJF, van Kempen AAMW, Valerio PG. Pudendal perineal block with prilocaine during labor: Is it safe for the newborn? *Submitted*

Thio GKKE, Muiño Mosquera L, **Lem AJ**, Valerio PG, van Kempen AAMW, Franssen EJF. Sensitive HPLC-MS assay for the determination of placental exposure of prilocaine in mother and child. *Submitted*

Renes JS, van Doorn J, Breukhoven PE, **Lem AJ**, de Ridder MAJ, Hokken-Koelega ACS. Acid-labile subunit: serum levels, polymorphisms and association with response to growth hormone treatment in short children born small for gestational age. *Submitted*

Renes JS, van Doorn J, Breukhoven PE, Gorbenko del Blanco D, **Lem AJ**, Hokken-Koelega ACS. IGFALS Gene Mutations in Short Children born Small for Gestation Age. *Submitted*

PhD portfolio

Summary of PhD training and teaching

Department:	Erasmus MC, Department of Pediatrics, Subdivision of Endocrinology
Research School:	Molecular Medicine Postgraduates School (MolMed)
PhD period:	June 2008 – June 2012
Promotor:	Prof.dr. A.C.S. Hokken-Koelega

1. PhD training	**Year**	**Workload (ECTS)**
General courses		
Classical methods for data-analysis, NIHES, Erasmus MC	2008	5.7
Biomedical English writing and communication, MolMed, Erasmus MC	2009	4
Good clinical practice, Erasmus MC	2009	1
Integrity in medical research, Medische Ethiek en Filosofie, Erasmus MC	2009	1
Missing values in clinical research, NIHES, Erasmus MC		0.7
Specific courses		
SNP's and human diseases, MolMed, Erasmus MC	2008	2
PubMed and Endnote, Medical Library, Erasmus MC	2008	0.3
InDesign CS5, MolMed, Erasmus MC	2011	0.3
Photoshop and Illustrator CS5, MolMed, Erasmus MC	2011	0.3
Seminars and workshops		
Annual pediatric research day, Erasmus MC Sophia	2008 – 09	0.6
Young investigators day, TULIPS / NVK	2008 – 10	0.9
Weekly research meeting, department of Pediatric Endocrinology	2008 – 12	4
Weekly patient presentation, department of Pediatrics	2008 – 12	4
Annual MolMed Day	2009 – 10	0.6
Annual PhD Day	2009 – 11	0.9
TULIPS Workshop	2010	0.3
International and national conferences		
LWPES/ESPE 8th Joint Meeting, New York, USA (poster presentation)	2009	1
Dutch society for clinical pharmacology en biopharmacology (NVKFB), Utrecht (oral presentation)	2010	1
Meeting North European region Pediatric Endocrinology, Horsens, Denmark (oral presentation)	2010	1
49th Annual Meeting of the ESPE, Prague, Czech Republic (2 poster presentations)	2010	1

Summary of PhD training and teaching of Annemieke J. Lem (continued)

5th International congress of the IGF/GRS society, New York, USA (poster presentation)	2010	1
50th Annual Meeting of the ESPE, Glasgow, Scotland (poster presentation)	2011	1
Congress Dutch society for pediatrics (NVK), Veldhoven (oral and poster presentation)	2011	1
51st Annual Meeting of the ESPE, Leipzig, Germany (poster presentation)	2012	1
Congress Dutch society for pediatrics (NVK), Veldhoven (oral presentation)	2012	1

2. Teaching	**Year**	**Workload (ECTS)**
Lecturing		
Symposium "5 years of research in short SGA adolescents", Rotterdam (oral presentation)	2008	0.7
Dutch Advisory Board Growth Hormone / Pediatric Endocrinology, Utrecht (2 oral presentations)	2010 – 11	1
Annual SGA Day (SGA Platform) (2 oral presentations and 2 workshops)	2010 – 11	1
Annual IMC Weekendschool day "Growth and Development", Rotterdam	2010 – 12	3
Educational lecture pediatricians Dordrecht/Gorinchem, Sliedrecht (oral presentation)	2011	1
Educational lecture minor students, Pediatric Endocrinology, Rotterdam (oral presentation)	2012	1
Advising		
Medical advisor SGA Platform	2010 – 12	2
Supervising		
Supervising research internship of medical student M. van der Grinten	2010	4
Supervising research internship of medical student S. Aammari	2011	4
3. Other activities		
Board of the PhD students, Sophia Onderzoekers Vertegenwoordiging	2009 – 11	4
Peer review of articles for international scientific journals	2012	0.5