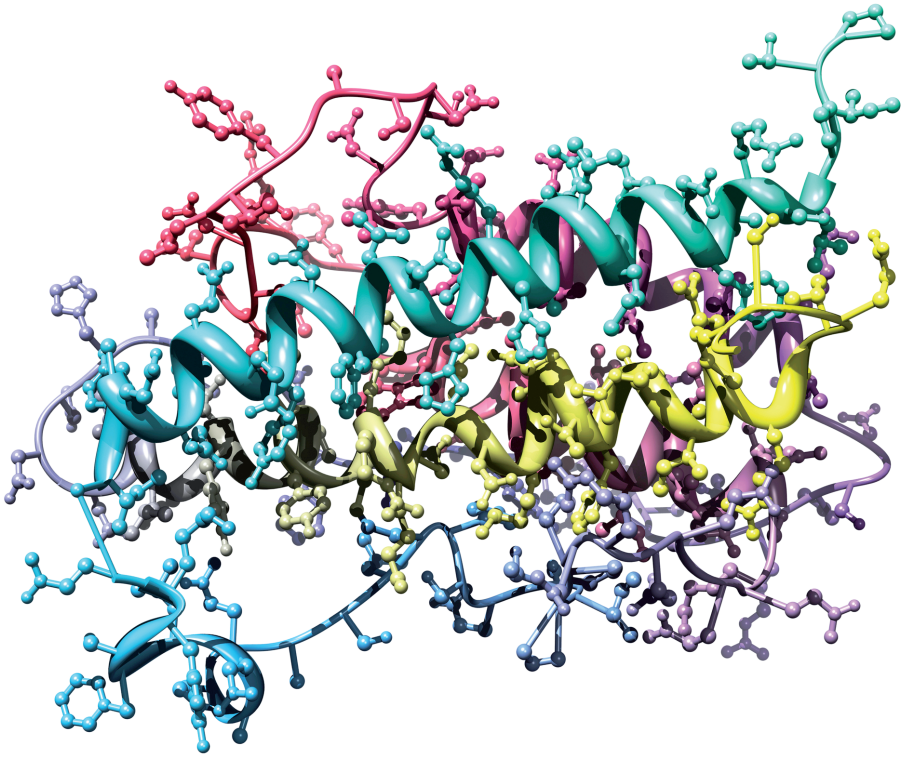
Growth Hormone Treatment in SGA

More than meets the eye



Manouk van der Steen

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Growth Hormone Treatment in SGA
More than meets the eye

Groeihormoonbehandeling bij SGA
De onzichtbare effecten

Proefschrift

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Manouk van der Steen

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	Prof.dr. S. Cianfarani

Growth is never by mere chance,
It is the result of forces working together

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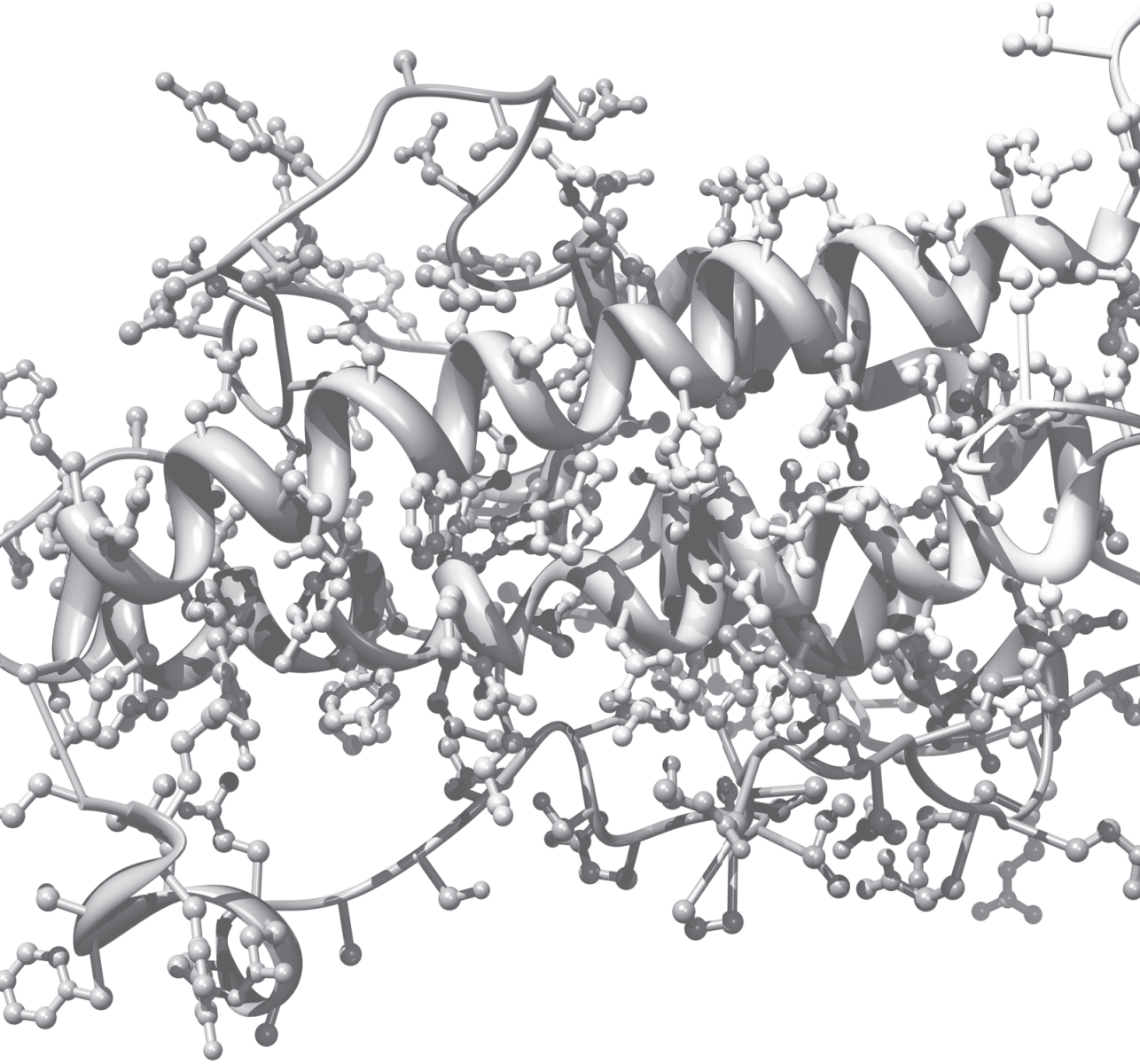
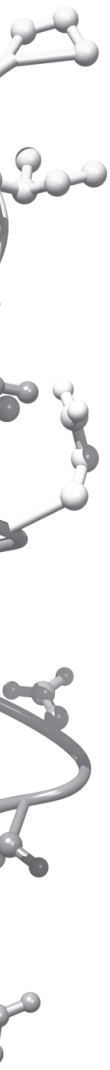


CHAPTER 1



General introduction

INTRODUCTION

Since 1991, our research group and others have been investigating children born small for gestational age (SGA) with persistent short stature, both before and during treatment with biosynthetic growth hormone (GH). Knowledge about different aspects of being born SGA and about effects of GH treatment has vastly increased over the past 25 years, but new questions emerged and needed further evaluation. This doctoral thesis describes the long-term effects of GH treatment, with or without additional gonadotropin-releasing hormone analog (GnRHa) treatment, in children born SGA, and illustrates that there is more to GH treatment than meets the eye.

SMALL FOR GESTATIONAL AGE

Small for gestational age (SGA) refers to the size of an infant at birth. Accurate information on gestational age, birth weight, and birth length is required to determine if a child is born SGA. SGA is defined as a birth length and/or weight of at least two standard deviation scores (SDS) below the median for gestational age, based on data derived from an appropriate reference population (1, 2). Children born SGA can be born full-term or premature. The term intrauterine growth retardation (IUGR) is often used synonymously with the term SGA. IUGR, however, refers to a deceleration of intrauterine fetal growth and the diagnosis is based on ultrasound measurements during pregnancy. IUGR does not always result in SGA birth and being born SGA does not necessarily mean that IUGR occurred, e.g. the fetus is small from the beginning of gestation (Figure 1).

The etiology of SGA encompasses a broad spectrum of maternal, environmental, placental, and fetal factors (Table 1) (3), but in a significant proportion of cases, the reason for being born SGA remains unclear.

SHORT STATURE

Most children born SGA experience spontaneous catch-up growth during the first years of life and reach a normal weight and height above -2 SDS. However, approximately 10-15% of children born SGA fail to show sufficient catch-up growth and remain short throughout life (height below -2 SDS) (4, 5). The reason for this insufficient catch-up growth is poorly understood but disturbances in the GH-axis have been postulated to play an important role (6-8). Short stature after SGA birth accounts for approximately 20% of all cases of short stature (9).

In 2015, 170.510 infants were live-born in the Netherlands (Central Bureau of Statistics, The Hague, the Netherlands). According to the definition that 2.3% of all live born neonates are born SGA, approximately 3922 of them fulfilled the definition of being born SGA. Since 10-15% of children born SGA show insufficient catch-up growth, 392-

588 of the live-born children in 2015 will have persistent short stature during childhood due to SGA birth.

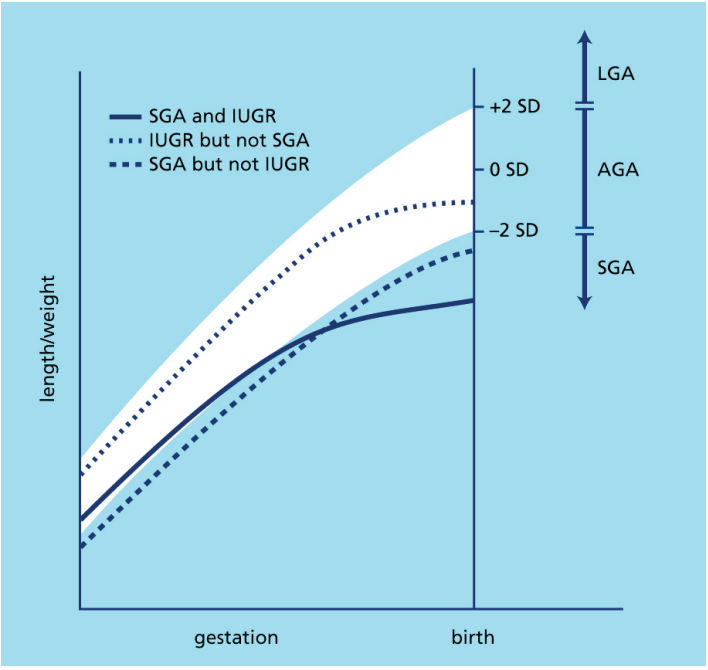


Figure 1. Fetal growth chart showing various intrauterine growth patterns.

GROWTH

Several factors influence postnatal growth including hormones, genetics, and the physical, emotional and social environment. The growth hormone axis (GH-axis) is the main hormonal axis involved in human growth and is very complex (Figure 2) (10). The anterior pituitary gland produces GH in a pulsatile pattern. Secretion of GH is under the control of the hypothalamic hormones GH-releasing hormone (GHRH) and somatostatin. GHRH binds to its receptor in the pituitary and stimulates GH secretion, whereas somatostatin inhibits GH release. Most of the effects of GH are mediated by insulin-like growth factors (IGFs). GH influences the production of IGF-I, which is synthesized in the liver and secreted into the blood under the control of GH, insulin and nutritional status. Next to growth, IGFs together with insulin and GH, regulate glucose metabolism, lipid metabolism, and body composition.

Growth assessment requires accurate measurements of height and weight over time, measurement of parental height, pubertal staging, and selection of appropriate growth references. Normal growth has periods of spurts and plateaus, and being familiar with normal patterns of growth allows practitioners to recognize and manage abnormal variations.

Table 1. Factors associated with reduced fetal growth (3)

Genetics	
Height of parents	
Chromosomal disorders of the child	Down syndrome
	Turner syndrome
Genetic disorders of the child	Silver Russell syndrome
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	Malnutrition
	Low pregnancy body mass index
	Low maternal weight gain during pregnancy
	Delivery at age <16 years or >35 years
	Low socioeconomic status
	Drug use (smoking, alcohol, illicit drugs)
Fetal factors	
Intrauterine infections	Toxoplasmosis
	Rubella
	Cytomegalovirus
	Herpes Simplex
	Syphilis
Congenital defects	
Inborn errors of metabolism	
Placental factors	
Reduced blood flow	
Reduced area for exchange of nutrients and oxygen	Infarcts
	Haematomas
	Partial abruption
Environmental factors	
High altitude	
Toxic substances	

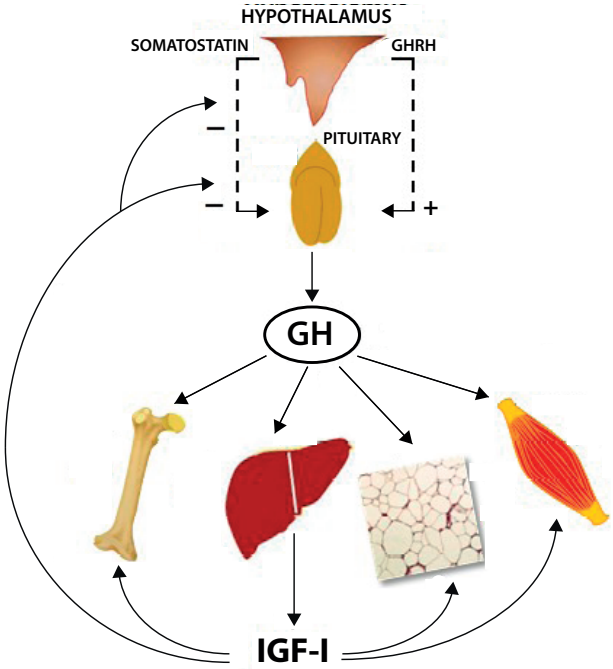


Figure 2. Physiology of the GH-IGF-I axis. Adapted from Kumar et al. (10).

Pubertal growth

Height and age at onset of puberty, as well as the magnitude and duration of pubertal growth, are important determinants of adult height, explaining 15-20% of adult height (11). The median age of pubertal onset in the Dutch population is 10.7 years for girls and 11.5 years for boys (12). During puberty, the hypothalamic-pituitary-gonadal axis is reactivated, which results in the development of secondary sexual characteristics, the pubertal growth spurt, and epiphyseal maturation (Figure 3). In girls, the pubertal growth spurt starts during the first year of breast development. In boys, the pubertal growth spurt occurs later, during the second year of puberty, when the testicular size has increased to >10 ml.

Although reported study results are difficult to compare due to the use of various definitions for the milestones of puberty, most authors seem to agree that short children born SGA have a normal pubertal onset and development, but relatively early for their short stature (13-20). In short children born SGA, height gain during puberty is often reduced (18, 21). They, therefore, have a poor adult height prognosis when they enter puberty with a height below -2.5 SDS.

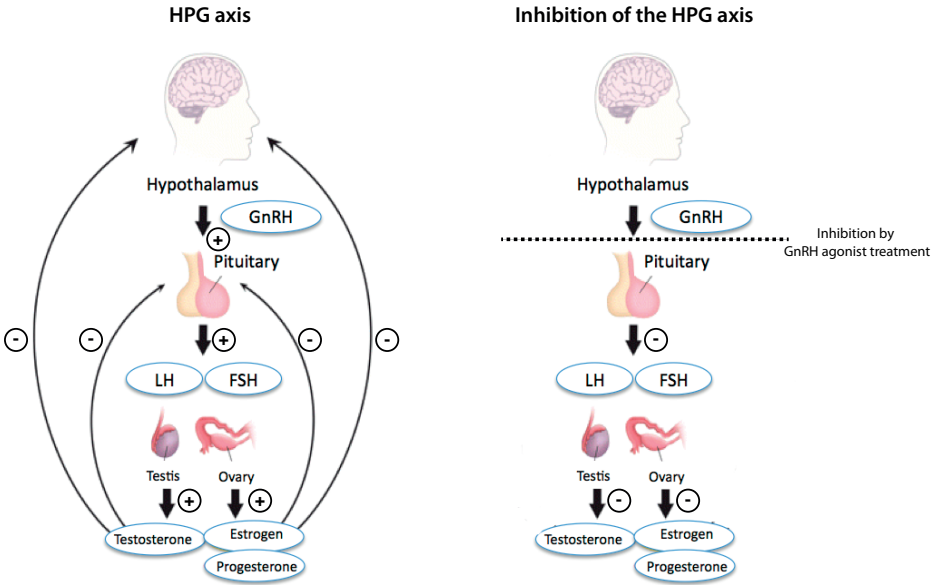


Figure 3. The hypothalamic-pituitary-gonadal (HPG) axis.
Abbreviations: GnRH, gonadotropin-releasing hormone analog; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

GROWTH HORMONE TREATMENT

Recombinant GH has been used since 1986 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 GH deficiency to an increasing number of conditions in which short stature is not due to classic GH deficiency, such as short stature after SGA birth. Since 2005, recombinant GH treatment is reimbursed for short children born SGA in the Netherlands. In Europe, the recommended dose for short children born SGA is 1 mg/m^2/day (~0.033 mg/kg/day) (The European Agency for the Evaluation of Medicinal Products, 2001).

The aim of GH treatment in short children born SGA is achieving an adult height in the normal range and/or in the target height range of the child. Studies have shown that GH treatment effectively induces catch-up growth and improves adult height in most short children born SGA (22-25). The GH-induced growth response is, however, highly variable (24). Several studies have been conducted to determine clinical predictors for growth response to GH treatment (22, 26-29). Patient characteristics found to be related with adult height SDS were: age and height SDS at start of GH treatment, target height SDS, GH dose, bone age delay at start of treatment, and baseline IGFBP-3 SDS, together explaining approximately 40% of the variability in adult height SDS.

START OF GH TREATMENT IN EARLY PUBERTY

GH treatment is most effective when started at a young age (22, 30, 31). However, some children come to medical attention at an older age, even when pubertal development has started. In 2003, the Dutch SGA study (Appendix A) was initiated to evaluate the effectiveness and safety of GH treatment in children born SGA, who start treatment at the age of 8 years or older. When GH treatment is started in early puberty, the epiphyseal maturation has been activated which might result in a limited effect of GH treatment (32). Gonadotropin-releasing hormone analogs (GnRHa) delay the epiphyseal maturation by suppressing the pubertal axis (Figure 3), and GnRHa treatment in addition to GH treatment might therefore further improve adult height in children who start GH treatment in early puberty.

At start of the Dutch SGA study, the efficacy and safety of GH treatment for short SGA children who start treatment above the age of 8 years, with or without additional postponement of puberty, was unknown. Besides, the optimal GH dose during puberty and/or postponement of puberty was unknown. Our research group recently reported adult height data of the Dutch SGA study, showing that short SGA adolescents can still have impressive catch-up growth, even when GH was started when they had already entered puberty (33). Besides, 2 years of GnRHa treatment in early puberty in addition to GH treatment further improved adult height in children born SGA who were relatively short at onset of puberty (33). In boys, a higher GH dose of 2 mg/m^2/day during puberty resulted in a better adult height, compared with the standard GH dose of 1 mg/m^2/day (33).

Since both GH and GnRHa treatment might influence body composition and insulin sensitivity, combining these treatments had raised concerns. Our research group showed short-term metabolic effects during 2 years of combined GH/GnRHa treatment in short SGA children participating in the Dutch SGA study (34). However, the long-term effects of this combined treatment, as well as the GH-dose effects on metabolic health and glucose metabolism, remained unknown.

A previous study of our group assessed the effect of GH treatment on pubertal development in short children born SGA and showed that GH had no effect on pubertal onset, progression of puberty, age at menarche, and the interval between the onset of breast development and menarche (19). Besides, no GH-dose effect was found on the onset or duration of puberty. At start of the Dutch SGA study, the effects of 2 years of GnRHa treatment, in addition to GH treatment, on pubertal development and growth, were unknown.

GENES INVOLVED IN SHORT STATURE

Adult height is one of the most heritable human traits (35), but until now only a small number of genetic mutations (<1%) explain short stature in children born SGA (36-38). Children born SGA comprise a heterogeneous group with a broad spectrum of clinical characteristics (1, 2). Uncovering the genetic basis of short stature is important for health prognosis, genetic counseling, and treatment options. Some short children born SGA have accelerated bone age maturation during childhood, resulting in early closure of the epiphyseal growth plates and cessation of growth at a young age, with a disappointing short adult height. Heterozygous mutations in the ACAN gene have been described in children with idiopathic short stature and advanced bone age (39, 40), but the presence in children born SGA with persistent short stature was unknown. Due to the variance in clinical characteristics, the identification of appropriate patients for genetic testing remained a challenge. A clinical scoring system to identify children most likely to test positive for a mutation in the ACAN gene, and for distinguishing these children from those not likely to test positive, would therefore be useful. Besides, the response to GH treatment in children with an ACAN gene mutation was unknown.

METABOLIC AND CARDIOVASCULAR RISK FACTORS IN SGA

Epidemiological studies reported an inverse association between birth weight and risk for diabetes mellitus type 2, hypertension, and cardiovascular disease in adult life (41-43). In short children born SGA, reduced insulin sensitivity and an increased prevalence of cardiovascular risk factors have been described (44, 45). Short adults born SGA have a lower lean body mass than those born appropriate for gestational age (AGA), but a similar fat mass percentage, insulin sensitivity measured by frequently sampled intravenous glucose tolerance (FSIGT) tests, and lipid profile (46-49). Systolic and diastolic blood pressure in short adults born SGA is higher compared to adults born AGA (25). In contrast, adults born SGA with spontaneous catch-up growth to a normal adult height, have a higher fat mass percentage and lower insulin sensitivity compared to adults born AGA (46, 50, 51). Thus, particularly young adults born SGA with early and spontaneous catch-up in weight, show an unfavorable body composition and lower insulin sensitivity as opposed to those who remain short (52-55). Long-term studies on metabolic and cardiovascular risk factors in these predisposed children born SGA, either with or without spontaneous catch-up growth, are scarce, but recognizing metabolic and cardiovascular diseases at an early stage is very important.

Effects of GH treatment on metabolic and cardiovascular risk factors

Besides the positive effects on linear growth, GH has well-documented lipolytic, anabolic, and insulin-antagonistic effects. Long-term GH treatment results in an increase

in lean body mass due to its anabolic effects on muscle mass, and a decline in fat mass due to the lipolytic effects on fat mass (56-60). Short children born SGA have reduced insulin sensitivity before receiving GH treatment (44). GH has well-documented insulin-antagonistic effects and treatment results in a further decline in insulin sensitivity and a compensatory increase in insulin secretion in children born SGA (57, 61-64). During long-term GH-treatment, blood pressure SDS and cholesterol levels decrease in GH-treated SGA children and become lower than in untreated SGA children (25, 56, 57, 65, 66). There are, however, only limited data on the longitudinal effects after cessation of GH treatment in adults born SGA (25, 46, 67). During 6 months after cessation of GH treatment, fat mass increases whereas lean body mass decreases (67). Studies on insulin sensitivity after cessation of GH treatment reported conflicting results, with some describing an increase in insulin sensitivity and others reporting no change in insulin sensitivity after cessation of treatment (61, 62). The number of subjects in these studies was, however, too low to draw conclusions. Our own research group showed that the GH-induced lower insulin sensitivity increased during 6 months after cessation of GH treatment (67). It remained, however, to be elucidated how body composition, insulin sensitivity, blood pressure, and lipid levels would change during several years after cessation of GH treatment.

Recent preliminary French data of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) project suggest that there might be an increased cardiovascular mortality in adults with isolated GH deficiency or adults born SGA who were treated with GH during childhood (68). These data have raised concerns about the long-term safety of GH treatment. Sävendahl et al. reported preliminary data on long-term vital status and causes of death in patients treated with GH in Belgium, the Netherlands and Sweden, and reported no deaths due to any form of cardiovascular disease (69). These data originate from the SAGhE project, in which eight European Union countries participate to evaluate long-term mortality in patients treated with GH during childhood. The main limitation of the SAGhE project is that data of ex-patient groups are compared with national reference values and not with an appropriate control group of untreated short SGA patients. To study the effect of GH treatment on the risk of diabetes mellitus type 2 and cardiovascular diseases, it is necessary to evaluate the risk for these diseases in children born SGA, and to compare data of GH-treated young adults born SGA with those of untreated young adults born SGA. We set out to study longitudinal data on metabolic and cardiovascular risk factors after cessation of GH treatment, and compared these data to age-matched untreated SGA control groups (Appendix B-C).

DETERMINANTS OF METABOLIC AND CARDIOVASCULAR DISEASES

To evaluate metabolic and cardiovascular risk factors, it is important to use appropriate measurements. The various measurements used in the studies were:

Body composition

It is well known that a higher fat mass percentage in adulthood results in an increased risk for metabolic and cardiovascular diseases. The amount of fat mass and lean body mass, components of body composition, can be measured by dual-energy X-ray absorptiometry (DXA), which is explained in appendix D.

Glucose homeostasis

Reduced insulin sensitivity plays an important role in the pathogenesis of diabetes mellitus type 2 and usually precedes the first symptoms of the disease by many years. Insulin sensitivity and insulin secretion should be balanced; reduced insulin sensitivity leads to an increased insulin secretion by the β-cells in the pancreas. When insulin secretion does not increase in relation to reduced insulin sensitivity, impaired glucose tolerance and eventually diabetes mellitus type 2 will develop. One of the best ways to accurately measure insulin sensitivity and β-cell function is by means of a frequently sampled intravenous glucose tolerance (FSIGT) test with Tolbutamide (Appendix D).

Blood pressure and lipid profile

Increased blood pressure, total cholesterol, low-density lipoprotein, and apolipoprotein B together with reduced serum levels of high-density lipoprotein and apolipoprotein A-I, are determinants of cardiovascular disease. Fasting blood samples are needed to evaluate serum lipid levels.

Carotid intima media thickness

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

AIMS OF THE THESIS

This thesis presents a detailed description of the studies that were performed to improve the knowledge about and the care for short children born SGA. The study populations consisted of GH-treated children and young adults born SGA, participating in the Dutch SGA study (Appendix A) or the IUGR studies (Appendix B), and untreated young adults born SGA or AGA (Appendix C). The aims of the studies described in this thesis are presented below.

Metabolic and cardiovascular health during GH treatment with or without GnRHa

Body composition, blood pressure, and lipid profile were investigated from start of GH treatment until adult height, to determine the long-term effects of combined GH/GnRHa treatment versus GH treatment only. Frequently sampled intravenous glucose tolerance (FSIGT) tests were used to assess the long-term effects of combined GH/GnRHa treatment versus GH treatment only on insulin sensitivity and β-cell function.

Pubertal development and growth during GH treatment with or without GnRHa

Pubertal onset, pubertal duration, and pubertal growth were assessed in GH-treated children born SGA with or without 2 years of additional GnRHa treatment, to investigate whether 2 years of additional GnRHa treatment affects pubertal development after cessation of GnRHa treatment.

Metabolic and cardiovascular risk factors after cessation of GH treatment

Body composition, insulin sensitivity, β-cell function, blood pressure, lipid profile, and carotid intima media thickness were investigated during 5 years after cessation of GH treatment to assess the long-term effects of GH treatment on metabolic and cardiovascular risk factors.

To determine the effect of GH treatment during childhood on metabolic and cardiovascular risk factors in early adulthood, the data at 5 years after cessation of GH treatment were compared to untreated young adults born SGA with or without spontaneous catch-up growth, and to young adults born AGA.

ACAN gene mutations in short children born SGA

ACAN-sequencing was performed in children born SGA with advanced bone age maturation during GH treatment, to determine the occurrence of ACAN gene mutations in children born SGA. The clinical characteristics and response to GH treatment in children with an ACAN gene mutation were investigated.

OUTLINE OF THIS THESIS

Chapter 1 gives an introduction on the topics described in this thesis.
Chapter 2 describes the effects of GH treatment with or without 2 years of additional GnRHa on metabolic health in short children born SGA.
Chapter 3 describes the effects of GH treatment with or without 2 years of additional GnRHa on insulin sensitivity and β-cell function in short children born SGA.
Chapter 4 describes the effects of GH treatment with or without 2 years of additional GnRHa on puberty and pubertal growth in short children born SGA.
Chapter 5 shows longitudinal data on body composition and glucose homeostasis until 5 years after cessation of GH treatment in adults born SGA.
Chapter 6 shows longitudinal data on blood pressure, lipid levels, and carotid intima media thickness until 5 years after cessation of GH treatment in adults born SGA.
Chapter 7 reports on ACAN gene mutations in children born SGA and growth response during GH treatment.
Chapter 8 discusses our results and conclusions in relation to the current literature and comments on the clinical implications and conclusions of our study results.
Chapter 9 contains an English and Dutch summary of the results described in this thesis.
Chapter 10 summarizes the results of Dutch GH trials in patients born SGA.
Chapter 11 contains a list of abbreviations, a list of publications, and a list of co-authors affiliations. It further contains the PhD portfolio, acknowledgments, and curriculum vitae.

APPENDIX A

The Dutch SGA study

Design

The Dutch SGA study is a longitudinal, randomized, dose-response GH trial involving short SGA children of at least 8 years of age. All children received somatropin sc daily (Genotropin). Every 3 months, GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1 mg/m^2/day (Figure 4). When these children entered puberty or when children were in early puberty at the start of treatment, they were randomly assigned to treatment with either GH 1 or 2 mg/m^2/day after stratification for gender, pubertal stage, and parental height (one or two parents with a height less than −2 SDS *vs.* both parents with a height of at least −2 SDS). Because no model is known to predict AH accurately at the start of puberty, we used a pragmatic, arbitrary cutoff level. A height of less than 140 cm at the start of puberty was used to identify children with an AH expectation of less than −2.5 SDS, based on Dutch reference values (12, 70); these children received GnRHa (leuprolide acetate depots, 3.75 mg sc every 4 weeks) for 2 years in addition to GH treatment.

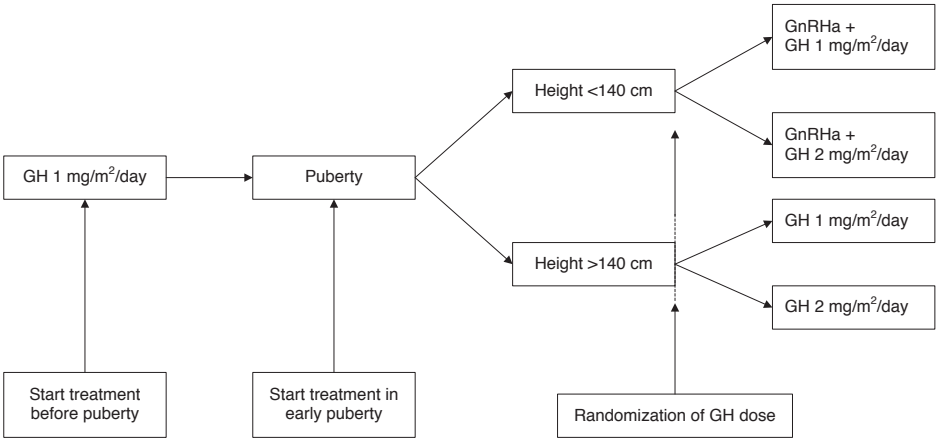


Figure 4. Flowchart of treatment regimen of the Dutch SGA study.

Inclusion and exclusion criteria

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

1) Birth length and/or birth weight SDS for gestational age less than -2.0 (71)
2) Chronological age of 8 years or older
3) Prepubertal stage (Tanner stage I) or early pubertal stage (breast stage II-III in girls and or testicular volume less than 10 ml in boys (72), with a GnRHa-stimulating test indicating central puberty (73))
4) Height SDS less than -2.5 SDS or a predicted adult height less than -2.5 SDS (defined as height at start of puberty below 140 cm), according to Dutch references (12)
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, lungs, 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, hypothyroidism, or inborn errors of metabolism
6) Medications or interventions during the previous 6 months that might have interfered with growth, such as corticosteroids (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 corticosteroids, sex steroids, GnRH analog
8) Active or treated malignancy or increased risk of leukemia
9) Serious suspicion of psychosocial dwarfism (emotional deprivation)
10) Expected non-compliance

Participating centers and physicians

The Dutch SGA study is a multicenter trial coordinated by the Dutch Growth Research Foundation, Rotterdam, the Netherlands. Every 3 months, the PhD fellow and a research nurse visit 10 hospitals throughout the Netherlands to examine children in collaboration with the local pediatrician or pediatric endocrinologist. Standardized measurements take place according to schedule at the Erasmus University Medical Center – Sophia Children's Hospital Rotterdam, the Netherlands.

Participating centers and pediatricians are:

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

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

APPENDIX B

IUGR studies

The IUGR studies were started before 2003, and included children born SGA with persistent short stature. At that time, the term intrauterine growth retardation (IUGR) was still used synonymously with the term SGA. Therefore, these initial Dutch GH studies were named 'IUGR studies'. Later on, a difference in the definitions of IUGR and SGA was made, as discussed on page 11. By that time, however, the IUGR studies were already nationwide known and their name was, therefore, not changed.

Inclusion and exclusion criteria

Children were included when they met de following inclusion criteria:

1) Birth length and/or birth weight SDS for gestational age less than -2.0 (71)
2) An uncomplicated neonatal period without signs of severe asphyxia (defined as Apgar score ≤ 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia
3) Chronological age between 3 and 8 years at start of the study
4) Height SDS less than -2.5 SDS, according to Dutch references (12)
5) Height velocity SDS below zero to exclude children with spontaneous catch-up growth (12)
6) Prepubertal stage, defined as Tanner stage I or testicular volume less than 4 ml in boys (72)
7) Normal liver, kidney and thyroid functions
8) Well-documented growth data from birth to start of treatment
9) Informed consent

Children were excluded in case of:

1) Endocrine or metabolic disorders, e.g. diabetes mellitus, diabetes insipidus, hypothyroidism, or inborn errors of metabolism
2) Celiac disease and other chronic or serious diseases of the gastrointestinal tract, heart, genito-urinary tract, liver, lungs, skeletal or central nervous system
3) Chromosomal disorders or serious dysmorphic symptoms suggestive for a syndrome that has not yet been described, except for Silver Russell syndrome
4) Chondrodysplasia
5) Active or treated malignancy or increased risk of leukemia
6) Serious suspicion of psychosocial dwarfism (emotional deprivation)
7) Use of medication that might interfere with growth during GH treatment, such as corticosteroids, sex steroids, GnRH analog
8) Expected non-compliance

APPENDIX C

PROGRAM and PREMS study cohorts

The PROgramming factors for Growth And Metabolism (PROGRAM) study consists of healthy young adults born term, whereas the Prematurity and Small for Gestational Age (PREMS) study cohort consists of healthy young adults born preterm (gestational age less than 36 weeks). In these participants, several parameters for metabolic and cardiovascular diseases were determined.

Inclusion and exclusion criteria

Inclusion criteria:

1) Chronological age at inclusion between 18 and 24 years
2) Neonatal period without signs of severe asphyxia (defined as Apgar score ≤ 3 after 5 minutes), sepsis, or long-term complications of respiratory ventilation, such as bronchopulmonary dysplasia
3) Well-documented growth data
4) Caucasian
5) Born singleton
6) Signed informed consent
7) PROGRAM study: gestational age of 36 weeks or more
8) PREMS study: gestational age of less than 36 weeks

Exclusion criteria:

1) Chromosomal disorders or serious dysmorphic symptoms suggestive for a syndrome that has not yet been described, except for Silver Russell syndrome
2) Any disease, endocrine or metabolic disorders, that could interfere with growth during childhood (e.g. diabetes, growth hormone deficiency, malignancies, severe chronic disease)
3) Treatment that could have interfered with growth (such as radiotherapy or growth hormone treatment)
4) Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood

APPENDIX D

Dual Energy X-ray Absorptiometry (DXA)

DXA is a machine used to measure body composition (fat mass and lean body mass) and bone mineral density. The person being assessed lies still for approximately 15 minutes while a scanner slides over the participant. DXA uses X-rays to assess these measurements, but the radiation dose is about 1/10th of a chest X-ray.

Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test

Several values regarding glucose homeostasis can be measured by FSIGT tests: insulin sensitivity, which quantifies the capacity of insulin to promote glucose disposal; glucose effectiveness, which reflects the capacity of glucose to mediate its own disposal; acute insulin response, which is an estimate of insulin secretory capacity; and the disposition index, which indicates the β-cell function. These indicators of glucose regulation were determined by the Bergman's minimal model, calculating paired glucose and insulin data obtained during an FSIGT test with Tolbutamide (74, 75).

When insulin sensitivity varies in healthy subjects, these changes are compensated proportionally by insulin secretion; reduced insulin sensitivity leads to increased insulin secretion by the β-cells (Figure 5, (76)). If insulin secretion does not change appropriately, impaired glucose tolerance and eventually diabetes mellitus type 2 will develop (77).

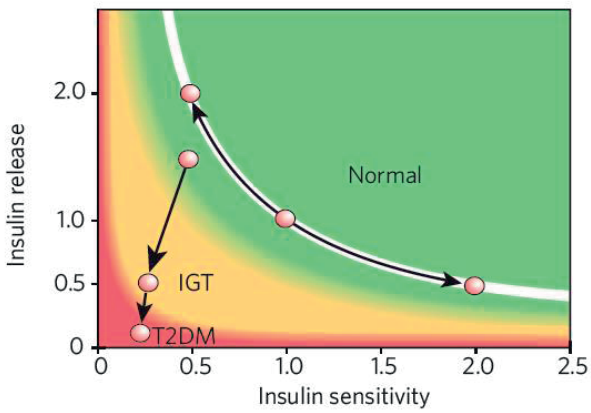


Figure 5. Hyperbolic association between insulin secretion and insulin sensitivity. Adapted from Kahn et al. (76). Abbreviations: IGT, impaired glucose tolerance; T2DM, diabetes mellitus type 2.

Carotid Intima Media Thickness

Intima media thickness (IMT) is the thickness of the two inner layers of an arterial wall. The thickness of the intima media of the carotid artery is related to atherosclerosis in later life (78, 79). Carotid IMT (cIMT) was measured in the supine position using carotid ultrasonography of both the left and right carotid artery using a 7.5 MHz linear array transducer. A longitudinal 1 cm segment of the common carotid artery, proximal to the bifurcation, was identified for measurements and the images were transferred to a computer. The B-mode of Art Lab, a special intima-media thickness assessment program, was used to measure the intima media thickness. This measurement was repeated 6 times for each common carotid artery (left and right side) and the mean was calculated.

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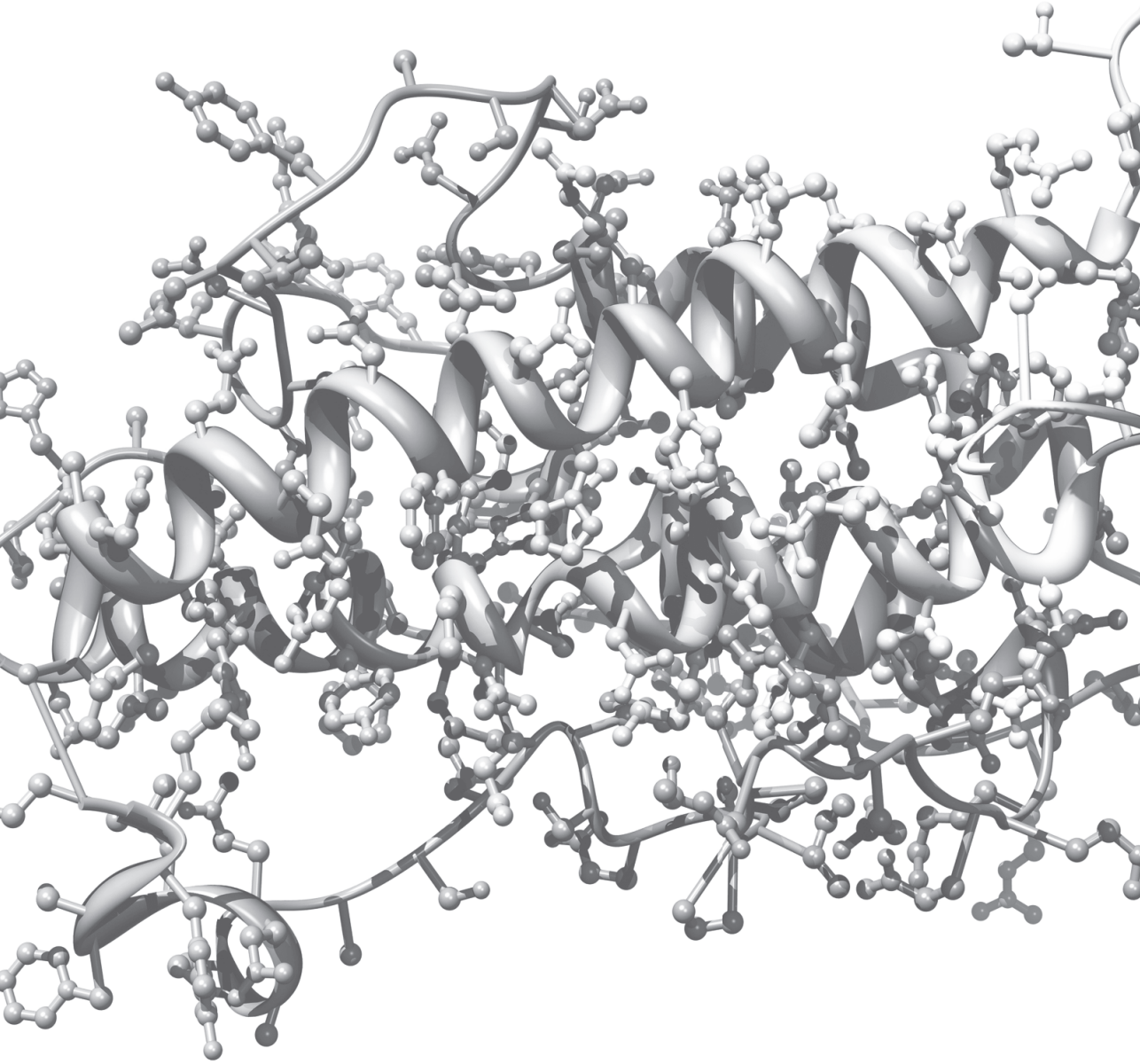
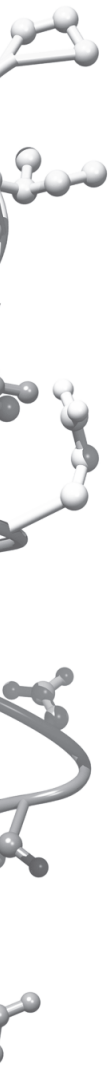


CHAPTER 2



Metabolic health in short children born small for gestational age treated with growth hormone and gonadotropin-releasing hormone analog: Results of a randomized, dose-response trial

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ABSTRACT

Context Previously we showed that pubertal children born small for gestational age (SGA) with a poor adult height (AH) expectation can benefit from treatment with growth hormone (GH) 1 mg/m^2/day (~0.033 mg/kg/day) in combination with 2 years of GnRH analog (GnRHa) and even more so with a double GH dose. GnRHa treatment is thought to have negative effects on body composition and blood pressure. Long-term effects and GH-dose effects on metabolic health in children treated with combined GH/GnRHa are unknown.

Objective This study aimed to investigate body composition, blood pressure, and lipid profile during GH treatment, either with or without 2 years of additional GnRHa. To assess whether GH 2 mg/m^2/day (~0.067 mg/kg/day) results in a similar or even more favorable metabolic health at AH than GH 1 mg/m^2/day.

Methods This was a longitudinal, randomized, dose-response GH trial involving 107 short SGA children (58 girls) treated with GH until AH (GH randomized 1 or 2 mg/m^2/day during puberty). Sixty-four children received additional GnRHa. At AH, metabolic parameters were compared between children treated with combined GH/GnRHa and those with only GH. The GH-dose effect on metabolic health was evaluated in a subgroup of 47 children who started GH treatment in early puberty (randomized 1 or 2 mg/m^2/day) with 2 years of GnRHa.

Results At AH, fat mass percentage (FM%) SD score (SDS), lean body mass (LBM) SDS, blood pressure SDS, and lipid profile were similar between children treated with combined GH/GnRHa and those with only GH. In the pubertal subgroup, FM% SDS was lower during treatment with GH 2 mg/m^2/day. There was no GH dose-dependent effect on LBM SDS, blood pressure, and lipid profile.

Conclusions Combined GH/GnRHa treatment has no long-term negative effects on metabolic health compared with only GH. Started in early puberty, a GH dose of 2 mg/m^2/day results in a similar metabolic health at AH and a more favorable FM% than GH 1 mg/m^2/day.

INTRODUCTION

Being born small for gestational age (SGA) has been associated with a higher prevalence of diabetes mellitus type 2, hypertension, and hyperlipidemia at a relatively young adult age (1). Because 10% of children born SGA have persistent short stature (2-4), many of them are treated with growth hormone (GH) to increase adult height (AH) (5-10). Long-term GH treatment results in an increase in lean body mass (LBM), a decline in fat mass (FM), and a decrease in blood pressure (BP) and lipid profile (11-13).

Some short SGA children only come to medical attention around onset of puberty. We have shown that in children who start GH treatment in early puberty with an expected AH less than −2.5 SD score (SDS), additional treatment with a gonadotropin-releasing hormone analog (GnRHa) for 2 years from start of puberty, can increase AH (14). GnRHa treatment is, however, known to increase FM in children with precocious puberty (15-17). On the other hand, a GH dose of 2 mg/m^2/day (~0.067 mg/kg/day) during puberty results in a significant increase in AH compared with the standard dose of 1 mg/m^2/day (~0.033 mg/kg/day) (14). Studies investigating combined treatment of GH and GnRHa (GH/GnRHa) in children born SGA have mainly focused on AH and short-term metabolic effects (14, 18), but the long-term effects of this combined treatment as well as the GH-dose effect on metabolic health are unknown.

We present safety data of a longitudinal, randomized, dose-response GH trial involving short SGA children (≥8 years at start) who were treated with GH until AH, either with or without additional GnRHa for 2 years from start of puberty. First, metabolic parameters at AH were compared between children treated with combined GH/GnRHa and those treated with only GH. We expected that the effects of 2 years of additional GnRHa treatment on metabolic parameters would be temporary and would normalize after discontinuation of GnRHa treatment. We hypothesized that at AH, metabolic parameters would be similar between these two treatment groups. Secondly, we investigated the GH-dose effect (1 vs 2 mg/m^2/day) on body composition, BP, and lipid profile in a subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with 2 years of GnRHa treatment. We hypothesized that treatment with GH 2 mg/m^2/day from the onset of puberty until AH would result in a similar or even more favorable body composition, BP, and lipid profile than GH 1 mg/m^2/day.

METHODS

Subjects

The Dutch SGA study included children when they met the following criteria: 1) birth length and/or birth weight SDS for gestational age less than −2.0 (19); 2) chronological age at least 8 years; 3) prepubertal stage (Tanner stage I) or early pubertal stage (breast stage II-III in girls and testicular volume <10 ml in boys) with a GnRHa-stimulating test

indicating central puberty (20, 21); 4) height less than −2.5 SDS and/or expected AH less than −2.5 SDS (height at start of puberty <140 cm), based on Dutch references (22); 5) well-documented growth data from birth to start of treatment; and 6) normal karyotype in all girls (14). 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, short stature homeobox 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

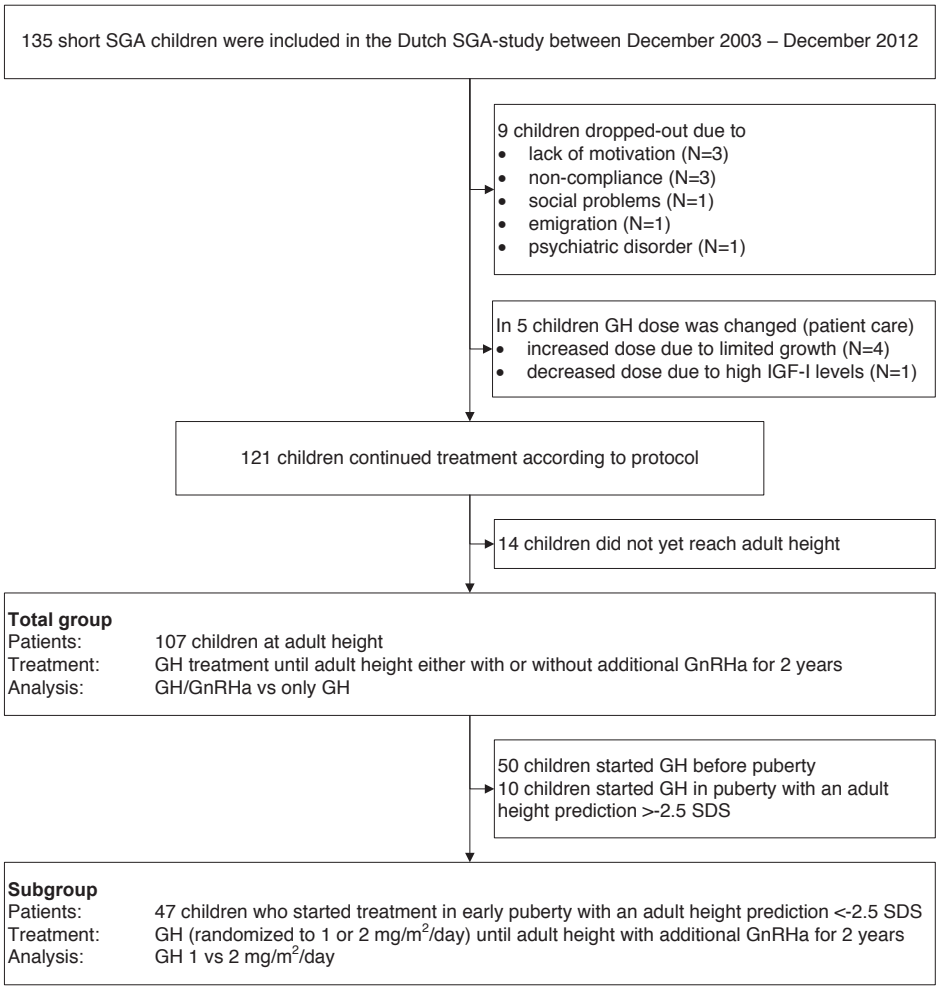

Figure 1. Flowchart of inclusion, dropout, and analyses.

the children were GH deficient according to stimulation tests (GH peak >7.7 ng/ml) or overnight GH profiles.

The SGA study included 135 short SGA children (70 girls) (Figure 1). Nine children dropped out for the following reasons: lack of motivation despite ongoing catch-up growth (n=3), noncompliance (n=3), social problems (n=1), emigration (n=1), and psychiatric disorder (n=1). In four children, the GH dose was increased due to limited catch-up growth, and in one child decreased due to high IGF-I levels. Among the 121 children who continued treatment according to protocol, 107 children reached AH (defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months, and bone age was ≥15 years for girls and ≥17 years for boys), or near AH (defined as height velocity between 0.5 and 2 cm during the last 6 months and adult pubertal stage).

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 and from children older than 12 years, and assent was obtained in children younger than 12 years of age. Due to ethical considerations, the medical ethics committees did not allow a randomized untreated short SGA group.

Design

The Dutch SGA study is a longitudinal, randomized, dose-response GH trial involving short SGA children of at least 8 years of age. All children received somatropin sc daily (Genotropin; Pfizer, Inc.). Every 3 months, GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1 mg/m^2/day (Figure 2). When these children entered puberty or when children were in early puberty at the start of treatment, they were randomly assigned to treatment with either GH 1 or 2 mg/m^2/day

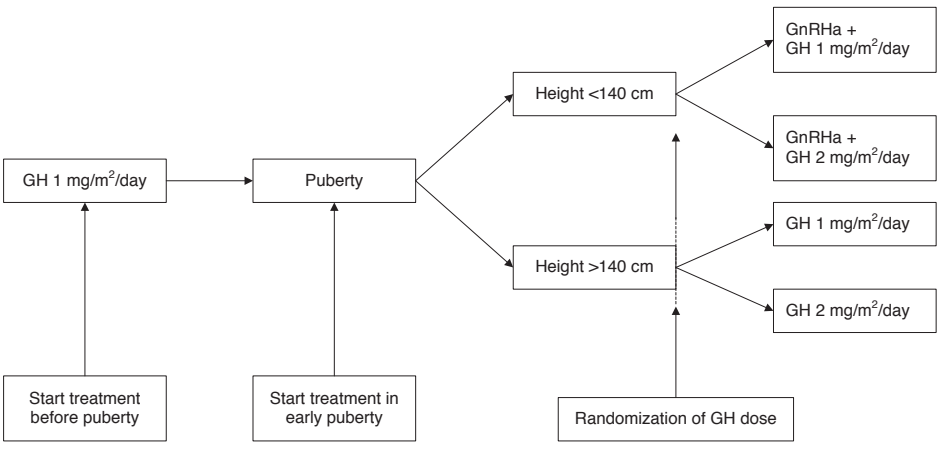

Figure 2. Flowchart of treatment regimen.

after stratification for sex, pubertal stage, and parental height (one or two parents with a height <−2 SDS vs both parents with a height ≥−2 SDS). Because no model is known to predict AH accurately at the start of puberty, we used a pragmatic, arbitrary cutoff level. A height of less than 140 cm at the start of puberty was used to identify children with an AH expectation of less than −2.5 SDS, based on Dutch reference values (22, 23); these children received GnRHa (leuprolide acetate depots, 3.75 mg sc every 4 weeks) for 2 years in addition to GH treatment. During GnRHa treatment, puberty was sufficiently suppressed in all children, both clinically and by GnRHa-stimulating tests or overnight gonadotropin profiles (24, 25).

Measurements

Height, weight, and Tanner stage were determined at start and every 3 months by the same physicians. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior). Height and weight were transformed into SDS for sex and chronological age according to Dutch references (22), using Growth Analyzer Research Calculation Tools (Growth Analyzer B.V.). AH SDS was calculated using references for Dutch adults (age 20 years) (22). As a component of metabolic syndrome, waist circumference was measured at baseline and subsequently every 6 months until AH in standing position by using a nonextensible tape at the midpoint between the iliac crest and the last rib. The mean of 2 measurements was used for analyses.

Body composition

In all children, body composition at start and at AH was measured by dual-energy X-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Healthcare). All scans were made on the same machine, and quality assurance was performed daily. For this type of DXA, the intra-assay coefficient of variation has been reported to be 0.41-0.88% for fat tissue and 1.57-4.49% for lean body mass (LBM) (26). Total fat mass (FM) was measured including head and was expressed as percentage of total body weight (FM%). FM% SDS was calculated according to age- and sex-matched Dutch reference values (27). Because LBM is strongly related to height, LBM expressed as SDS for age and sex might result in an underestimation in case of short stature. Therefore, LBM was expressed as SDS for height and sex, according to Dutch reference values (27). In the subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with 2 years of GnRHa treatment, DXA scans were performed at start and after 1, 2, 2.5, and 4 years of treatment. The scans at AH were performed at the last visit or a visit in the last year before discontinuation of GH treatment.

Blood pressure

Systolic and diastolic BP were measured at start of treatment and 3-monthly thereafter, using an appropriately sized cuff while patients were in a sitting position. The mean of two measurements was used for analysis. Because height is an important determinant of BP in childhood, BP was expressed as SDS adjusted for height and sex (28).

Assays

Fasting levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDLc), apolipoprotein A-1 (ApoA-1), and apolipoprotein B (Apo-B) were measured at start of treatment and yearly thereafter. Low-density lipoprotein (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = TC – HDLc – 0.45 x TG (29).

TC and triglyceride were measured using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit, respectively (Roche Diagnostics). HDLc was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). ApoA-1 and Apo-B were determined by rate nephelometry on the Image Immunochemistry System, according to the manufacturer's instructions (Beckman Coulter). The intra-assay variations of measurements of TC, triglyceride and HDLc were 2.9, 3.3 and 3.9%. Between-run coefficients of variation for ApoA-1 and Apo-B were 4.2 and 2.8% at levels of 0.94 and 0.53 g/l, respectively.

Metabolic syndrome

Revised criteria of the National Cholesterol Education Program (NCEP; Adult Treatment Panel III [ATP III]) were used to determine components of metabolic syndrome (30-32). Metabolic syndrome was defined as having three or more of the following risk factors: 1) abdominal obesity: waist circumference in men greater than 102 cm, and in women greater than 88 cm; 2) triglyceride above 1.7 mmol/l; 3) HDLc in men below 1.03, in women below 1.3 mmol/l; 4) BP ≥130/ ≥85 mm Hg; 5) fasting glucose above 5.6 mmol/l.

Statistics

Statistical analyses were performed using SPSS version 21 (IBM). Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics are presented as mean (SD). Differences at start between children treated with combined GH/GnRHa and those treated with only GH, were evaluated using an independent-sample t test for normally distributed variables. A one-sample t test was used to compare SDS results with zero (mean value for age- and sex-matched references).

At AH, outcome measurements between children treated with combined GH/GnRHa and those treated with only GH, were evaluated using multiple regression analysis to

correct for variables such as GH dose, GH duration, age and sex. Correlations were determined using Pearson's correlation test.

Additional analyses were performed in a subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with 2 years of GnRHa and subsequent GH until AH, to evaluate changes in body composition, BP and lipid levels, using repeated measurements analysis (linear mixed model) with an unstructured covariance matrix with GH dose as the categorical independent variable. Lipid analyses were also adjusted for age and sex. Differences between the 1mg and 2mg groups at various time-points were evaluated using an independent-sample t test. P-values < 0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Table 1 shows the clinical characteristics of the total group of 107 short SGA children (58 girls). At start of treatment, the mean (SD) age was 11.5 (1.5) years and height SDS -2.9 (0.6). Sixty-four children were treated with combined GH/GnRHa and forty-three with only GH. Height SDS at start of puberty was significantly lower in the group treated with combined GH/GnRHa (P=0.001), but AH SDS was similar between groups (P=0.540).

Total group at adult height

The total group of 107 children was treated from start to AH (64 with combined GH/GnRHa and 43 with only GH). At start, FM% SDS, systolic BP SDS and diastolic BP SDS were significantly higher and LBM SDS significantly lower than average. At AH, FM% SDS was significantly higher (0.4 SDS, P<0.001) and LBM SDS significantly lower (-0.3 SDS, P=0.03) than average for healthy children, but FM% SDS and LBM SDS were similar between children treated with combined GH/GnRHa and those treated with only GH, corrected for GH dose and GH treatment duration (Table 2). At AH, a systolic BP SDS greater than 2 SDS was found in 6.5%, in contrast to 22.4% at start. The percentage of children with a diastolic BP SDS greater than 2 SDS increased over time, resulting in 4.7% at AH vs 0.9% at start. The change in diastolic BP SDS showed a positive correlation with age (P<0.001). The percentage of children with a diastolic BP SDS greater than 2 SDS was 6.3% in those treated with combined GH/GnRHa and 2.3% in those treated with only GH. At AH, systolic and diastolic BP SDS were similar between children treated with combined GH/GnRHa and those treated with only GH, corrected for GH dose and GH treatment duration (Table 2). Lipid levels remained within the normal range and were at AH similar between children with combined GH/GnRHa and those treated with only GH, corrected for GH dose, GH treatment duration, age and sex (Table 2).

Table 1. Clinical characteristics of the total group

Characteristic	Total Group	GH/GnRHa	GH	P-value
N	107	64	43	
Boys/girls	49/58	23/41	26/17	**0.013**
Gestational age, weeks	37.5 (3.1)	37.6 (3.2)	37.3 (3.1)	0.633
Birth weight SDS	-2.0 (1.0) [b]	-2.0 (0.9) [b]	-2.0 (1.0) [b]	0.899
Birth length SDS	-2.6 (1.2) [b]	-2.7 (1.2) [b]	-2.6 (1.2) [b]	0.677
TH SDS	-0.7 (0.7) [b]	-0.7 (0.7) [b]	-0.7 (0.7) [b]	0.916
Start of study				
Age, years	11.5 (1.5)	11.8 (1.3)	11.0 (1.8)	**0.014**
Height SDS	-2.9 (0.6) [b]	-2.9 (0.6) [b]	-2.9 (0.6) [b]	0.547
Prepubertal / pubertal at start	50/57	17/47	33/10	**<0.001**
Start of puberty				
Age, years	12.3 (1.0)	11.9 (0.9)	12.8 (0.9)	**<0.001**
Height SDS	-2.6 (0.7) [b]	-2.7 (0.6) [b]	-2.3 (0.6) [b]	**0.001**
GH dose 1 vs 2 mg/m^2/day	53/54	31/33	22/21	0.782
Adult height				
Age, years	17.4 (1.2)	17.4 (1.0)	17.4 (1.3)	0.977
Height SDS [a]	-1.7 (0.8) [b]	-1.7 (0.9) [b]	-1.6 (0.8) [b]	0.540
Duration treatment, years	5.9 (1.4)	5.6 (1.2)	6.4 (1.5)	**0.003**

Abbreviations: TH, target height.
Data are expressed as mean (SDS), unless otherwise specified. Bold text indicates a significant P-value.
[a] AH SDS was calculated using Dutch references for Dutch adults (age 20 years).
[b] Variables in SDS compared with zero SDS, P<0.001.

At AH, the prevalence of metabolic syndrome was 3.7% (four of 107 adolescents), according to the revised NCEP criteria. One girl was treated with combined GH/GnRHa and three boys were treated with only GH.

Subgroup of pubertal children randomized to GH 1 or 2 mg/m^2/day and 2 years of GnRHa

The GH-dose effects on body composition, BP, and lipid profile were analyzed in detail in a subgroup of 47 pubertal children. All these children were early pubertal at the start of GH treatment with a poor AH expectation. They received GnRHa for the first 2 years in addition to GH (randomized to 1 or 2 mg/m^2/day) until AH. At start, 24 children (15 girls) were randomized to receive GH 1 mg/m^2/day (1mg group) and 23 children (15 girls) to GH 2 mg/m^2/day (2mg group). Age at discontinuation of GH treatment was 17.6 years in the 1mg group and 17.3 years in the 2mg group (P<0.001). GH treatment duration was similar between the GH-dose groups.

Table 2. Safety parameters at start and at AH in the total group

	Total group	GH/GnRHa	GH	P-value
N	107	64	43	
Boys / girls	49/58	23/41	26/17	**0.013**
Start				
Body composition				
FM% SDS	0.2 (0.7) [d]	0.2 (0.7) [d]	0.2 (0.8)	0.788
LBM SDS	-1.6 (1.1) [c]	-1.5 (1.0) [c]	-1.7 (1.2) [c]	0.204
Blood pressure				
Systolic BP SDS	1.3 (1.0) [c]	1.4 (1.0) [c]	1.2 (1.0) [c]	0.415
Diastolic BP SDS	0.2 (0.7) [e]	0.1 (0.8)	0.3 (0.7) [e]	0.288
Lipid levels [a]				
TC, mmol/l	4.42 (0.7)	4.24 (0.5)	4.69 (0.8)	**0.001**
LDLc, mmol/l	2.38 (0.6)	2.32 (0.5)	2.47 (0.7)	0.185
HDLc, mmol/l	1.48 (0.3)	1.44 (0.3)	1.54 (0.3)	0.223
TG, mmol/l	0.79 (0.3)	0.76 (0.3)	0.83 (0.3)	0.076
ApoA-I, g/l	1.46 (0.2)	1.40 (0.2)	1.55 (0.2)	**0.005**
Apo-B, g/l	0.74 (0.2)	0.71 (0.1)	0.77 (0.2)	0.075
At adult height [b]				
GH dose 1 vs 2 mg/m^2/day	53/54	31/33	22/21	0.782
Body composition				
FM% SDS	0.4 (0.8) [c]	0.4 (0.7) [c]	0.4 (0.9) [d]	0.545
LBM SDS	-0.3 (1.3) [e]	-0.4 (1.3) [e]	-0.03 (1.3)	0.423
Blood pressure				
Systolic BP SDS	0.5 (1.0) [c]	0.4 (0.9) [c]	0.6 (1.1) [c]	0.553
Diastolic BP SDS	0.7 (0.7) [c]	0.7 (0.7) [c]	0.8 (0.7) [c]	0.379
Lipid levels [a]				
TC, mmol/l	3.95 (0.7)	3.93 (0.6)	3.98 (0.7)	0.437
LDLc, mmol/l	2.23 (0.6)	2.24 (0.6)	2.22 (0.6)	0.841
HDLc, mmol/l	1.40 (0.3)	1.41 (0.3)	1.40 (0.3)	0.841
TG, mmol/l	1.01 (0.4)	0.97 (0.4)	1.07 (0.4)	0.164
ApoA-I, g/l	1.37 (0.2)	1.36 (0.2)	1.38 (0.2)	0.752
Apo-B, g/l	0.70 (0.2)	0.70 (0.2)	0.70 (0.2)	0.847

Data are expressed as mean (SDS), unless otherwise specified. Bold text indicates a significant P-value.

[a] Lipid levels at start and at adult height were additionally adjusted for age and sex.

[b] At adult height, all comparisons between children treated with combined GH/GnRHa and only GH were corrected for GH-dose and GH duration.

[c, d, e] Variables in SDS compared with zero SDS: [c] P≤0.001; [d] P<0.01; [e] P<0.04.

Body composition

There was a GH dose-dependent effect on FM% SDS during the entire treatment period with a higher FM% SDS in the 1mg group (P=0.035). At start, FM% SDS was similar between the GH-dose groups (P=0.171). During 2 years of combined GH/GnRHa treatment, FM% SDS increased significantly in the 1mg group (0.2 SDS, P=0.030) but remained similar in the 2mg group, resulting in a higher FM% SDS in the 1mg group at discontinuation of GnRHa (P=0.038) (Figure 3A). Because FM% SDS remained stable in both GH-dose groups in the years after discontinuation of GnRHa, this resulted in a higher FM% SDS at AH in the 1mg group (0.7 vs 0.2 SDS, P=0.022).

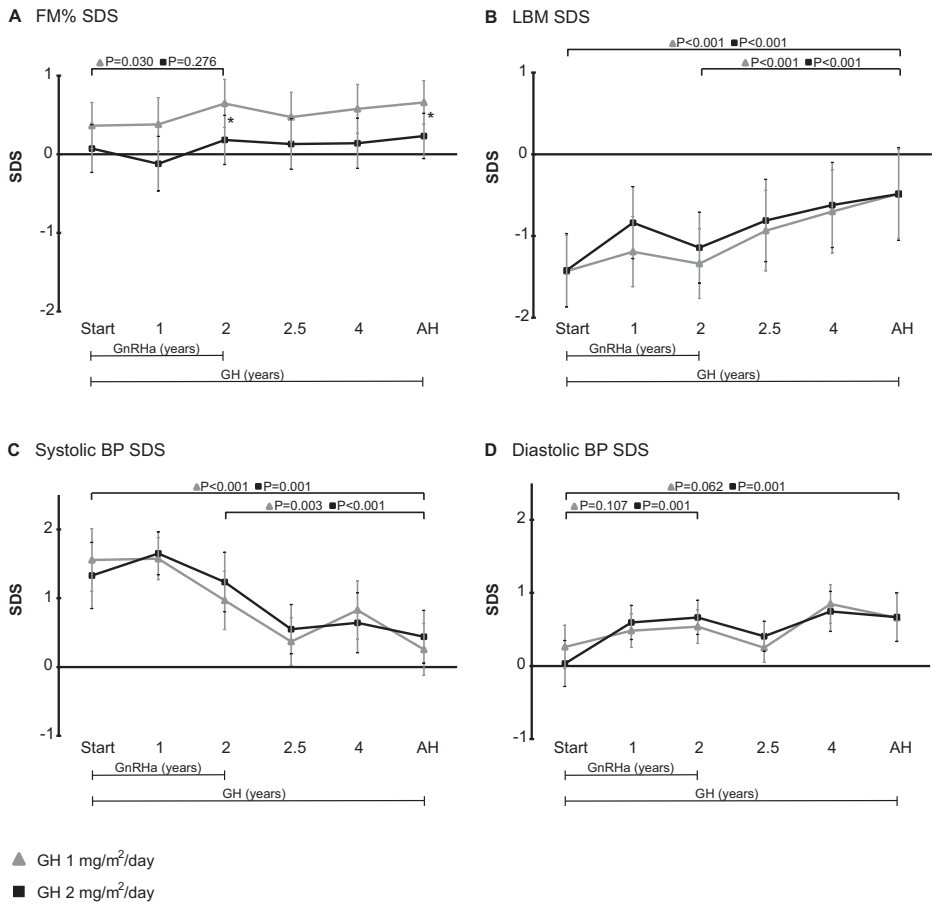

Figure 3. Body composition and BP levels in a subgroup of children who started GH treatment in early puberty with 2 years of GnRHa and subsequent GH until AH.

Data are expressed as estimated marginal means with 95% confidence intervals.

FM% SDS was calculated according to age- and sex-matched Dutch reference values. LBM and BP were expressed as SDS adjusted for height and sex (27, 28).

* P-values between GH-dose groups <0.05.

Table 3. Lipid levels in the subgroup of children who started GH treatment in early puberty with 2 years of GnRHa

	GnRHa								
		GH				P-value [a]	P-value [b]	P-value [c]	Normal range
Lipid	Start	1 year	2 years	4 years	AH	(0-2yrs)	(2yrs-AH)	(0-AH)	13-18yrs
TC, mmol/l	4.00 (3.66-4.34)	4.17 (3.89-4.44)	4.24 (4.06-4.42)	4.18 (3.88-4.49)	4.08 (3.67-4.49)	0.121	0.432	0.819	3.0-5.5
LDLc, mmol/l	2.19 (1.88-2.50)	2.37 (2.11-2.64)	2.35 (2.19-2.51)	2.35 (2.08-2.61)	2.33 (1.97-2.70)	0.252	0.933	0.642	1.7-3.8
HDLc, mmol/l	1.34 (1.17-1.50)	1.49 (1.36-1.63)	1.52 (1.42-1.62)	1.52 (1.38-1.66)	1.50 (1.31-1.69)	**0.015**	0.827	0.296	0.9-1.9
TG, mmol/l	0.74 (0.52-0.96)	0.89 (0.71-1.08)	0.96 (0.76-1.15)	1.01 (0.79-1.22)	1.02 (0.76-1.28)	0.080	0.680	0.220	0.4-1.6
ApoA-1, g/l	1.40 (1.28-1.51)	1.42 (1.33-1.50)	1.42 (1.36-1.48)	1.38 (1.28-1.48)	1.34 (1.20-1.47)	0.656	0.240	0.601	0.94-1.99
Apo-B, g/l	0.70 (0.61-0.78)	0.71 (0.65-0.78)	0.72 (0.68-0.76)	0.75 (0.66-0.83)	0.71 (0.60-0.83)	0.588	0.901	0.871	0.60-1.33

There was no GH dose-dependent effect on any lipid level during the entire treatment period, results are therefore shown for the GH-dose groups together. Data are expressed as estimated means (SEM) (95% confidence interval) after adjustment for age and sex. Bold text indicates a significant P-value.

[a] At discontinuation of GnRHa treatment vs start of treatment.

[b] At adult height vs discontinuation of GnRHa treatment.

[c] At adult height vs start of treatment.

LBM SDS showed no GH dose-dependent effect during the entire treatment period (P=0.691). At start, LBM SDS was -1.4 SDS in both GH-dose groups (P=0.955). During 2 years of combined GH/GnRHa treatment, GH 2 mg/m^2/day tended to induce an increase in LBM SDS compared with start, but this did not reach statistical significance (P=0.074) (Figure 3B). At discontinuation of GnRHa, LBM SDS was similar between the GH-dose groups (P=0.518). From discontinuation of GnRHa to AH, LBM SDS increased significantly in both GH-dose groups (P<0.001), leading to a significantly higher LBM SDS at AH compared with start. At AH, LBM SDS was similar between GH-dose groups (P=0.669).

Blood pressure

There was no GH dose-dependent effect on BP SDS during the entire treatment period. At start, systolic and diastolic BP SDS were similar between the GH-dose groups. During 2 years of combined GH/GnRHa treatment, systolic BP SDS started to decline in both groups, which continued after discontinuation of GnRHa, resulting in a significantly lower systolic BP SDS at AH compared with start (P≤0.001), which was similar between groups (Figure 3C).

Diastolic BP SDS tended to increase in both GH-dose groups during 2 years of combined GH/GnRHa treatment, resulting in a diastolic BP SDS of 0.5 in the 1mg group and 0.7 in the 2mg group at discontinuation of GnRHa treatment (between dose groups P=0.439) (Figure 3D). After discontinuation of GnRHa treatment, diastolic BP SDS remained stable in both GH-dose groups resulting in a similar diastolic BP SDS between the GH-dose groups at AH (P=0.974).

Lipid profiles

There was no GH dose-dependent effect on any lipid level during the entire treatment period after adjustment for age and sex. Results are therefore shown for the GH-dose groups together (Table 3). At start, all lipid levels were similar between the GH-dose groups. During 2 years of combined GH/GnRHa treatment, HDLc and TG tended to increase but this was only significant for HDLc (P=0.015). At discontinuation of GnRHa, all lipid levels were similar between the GH-dose groups. At AH, all lipid levels were similar to start and only one patient had elevated TC and LDLc and three patients had elevated TG, but there was no difference in lipid levels between the 1mg and 2mg groups.

DISCUSSION

Our study shows long-term data on metabolic health in short children born SGA treated with GH until AH, and randomized to GH 1 or 2 mg/m^2/day from start of puberty, either with or without additional GnRHa for 2 years. At AH, after 5.9 years of GH treatment,

body composition, BP, and lipid levels were similar between children treated with combined GH/GnRHa and those treated with only GH. In the subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with 2 years of GnRHa treatment, the 1mg group showed an increase in FM% during 2 years of GH/GnRHa treatment, which remained stable after discontinuation of GnRHa. This resulted in a significantly higher FM% in the 1mg group than the 2mg group at AH. LBM, BP, and lipid levels were similar between the GH-dose groups during the entire treatment period until AH.

Our findings show that 2 years of GnRHa treatment in addition to GH treatment compared with only GH treatment has no adverse effect on metabolic health at AH. In particular, there was no difference in FM% at AH between children treated with combined GH/GnRHa and those treated with only GH, also after correction for GH dose and duration. Studies in children with precocious puberty showed that GnRHa treatment leads to an increase in FM% (15-17). Our study shows that at AH, when GnRHa treatment has been discontinued for many years, there is no indication that 2 years of GnRHa treatment had a long-term enhancing effect on FM%. From start to AH, we found a significant decline in systolic BP in both treatment groups. This resulted in much fewer children with a systolic BP greater than 2 SDS at AH (6.5% vs 21.5% at start). GH treatment is known to cause a decline in systolic BP in children born SGA (12, 13, 33, 34). Our data showed that systolic BP declined similarly in children treated with GH/GnRHa and only GH, leading to a similar systolic BP at AH. It indicates that the addition of 2 years of GnRHa treatment in early puberty does not influence systolic BP in the long-term. Diastolic BP increased from start to AH, which is in contrast with previous studies (13, 34). The increase in our study was, however, small with only five out of 107 subjects having a diastolic BP greater than 2 SDS at AH. Because diastolic BP SDS increased in both the GH/GnRHa-treated and GH-only-treated children, this increase was not caused by the additional GnRHa treatment. The increase in diastolic BP might be explained by the fact that BP was adjusted for height and sex and not for age, while diastolic BP tends to increase during puberty. Indeed, a positive correlation was found between diastolic BP SDS and age in our study population. From start to AH, we found favorable changes in lipid levels, which is consistent with previous studies in which a beneficial effect of GH treatment on lipid metabolism in children born SGA was shown (12, 13, 34). At AH, lipid levels were not higher in children treated with GH/GnRHa, indicating no long-term adverse effect of 2 years of additional GnRHa treatment on lipid levels. Metabolic syndrome was defined according to the revised NCEP criteria. At AH, it was present in 3.7% of all participants, which was lower compared with untreated, short SGA adolescents in whom a prevalence of 8.0% was found (34).

In addition, we analyzed body composition, BP, and lipid profile in detail in a subgroup of 47 children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with 2 years of GnRHa and subsequent GH until AH.

During 2 years of combined GH/GnRHa treatment, we found an increase in FM% in the 1mg group, whereas FM% remained similar in the 2mg group. This supports our hypothesis that a higher GH dose of 2 mg/m^2/day results in a more favorable body composition by counteracting the FM-enhancing effect of GnRHa treatment, whereas treatment with GH 1 mg/m^2/day might be insufficient to prevent children from gaining FM during GnRHa treatment. Because FM% in children treated with GH 1 mg/m^2/day remained less than 1SDS, the effect might not be clinically relevant in the long-term. The clinical relevance of a higher FM% also depends on the distribution of visceral and subcutaneous fat given that it is the former that is likely more related to a worse metabolic health. DXA scan is the gold standard to measure body fat and lean body mass, but it cannot separate visceral fat from subcutaneous fat. An abdominal ultrasonography would be needed to distinguish between visceral and subcutaneous fat, but was not available in our study.

We expected a temporary increase in systolic and diastolic BP during 2 years of additional GnRHa treatment as found in a previous study (18), but systolic BP remained stable during combined GH/GnRHa treatment in both GH-dose groups. In contrast, diastolic BP tended to increase in both GH-dose groups during 2 years of combined GH/GnRHa treatment. However, the increase was very small and diastolic BP remained well within the normal range. At AH, there was no difference in systolic and diastolic BP between the GH-dose groups, which is in line with another study (12).

During the entire treatment period, all lipid levels were similar in both GH-dose groups, which is consistent with a previous report (12). HDLc and triglycerides tended to temporarily increase during 2 years of additional GnRHa treatment, which is consistent with a study in children with precocious puberty and GnRHa treatment (35), but the actual increase was very small and values returned to baseline after discontinuation of GnRHa.

Overall, there seem to be no long-term adverse effects on metabolic health of 2 additional years of GnRHa treatment and a higher GH dose from pubertal onset until AH. Possible psychosocial effects of suppressing on-time puberty have been studied in this cohort showing that additional GnRHa treatment had no adverse effect on the gain in quality of life (QOL) during 2 years (36). Long-term data on QOL are, however, needed before definite conclusions can be drawn. In addition, a cost-benefit analysis should be carried out when the long-term data on QOL come available in the next years, to determine the cost/benefit ratio of combined GH/GnRHa treatment and a higher GH dose.

Although short stature should be evaluated as early as possible, in clinical practice some SGA children present with short stature around pubertal age. Previously we showed

that pubertal SGA children with a poor AH expectation can benefit from treatment with GH 1 mg/m^2/day in combination with 2 years of GnRHa and benefit even more so with a double GH dose (14). Our current data show that combined GH/GnRHa has no long-term negative effects on metabolic health compared with only GH. Started in early puberty, a GH dose of 2 mg/m^2/day results in a similar metabolic health at AH and a more favorable FM% than the standard 1 mg/m^2/day and can therefore be considered in children who start GH treatment in early puberty with a poor AH expectation.

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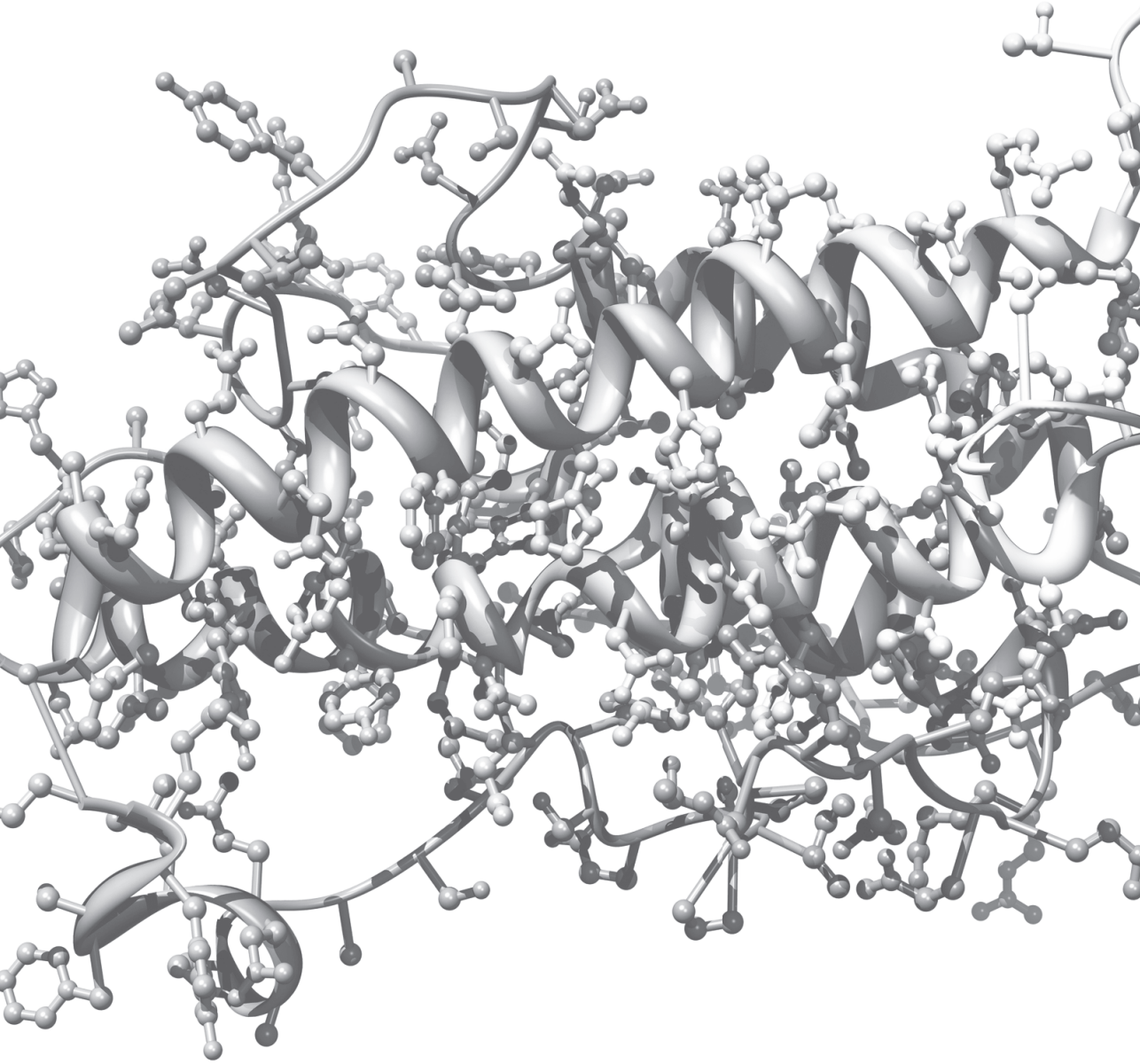
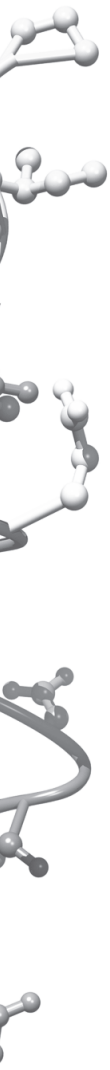


CHAPTER 3



Insulin sensitivity and β-cell function in SGA children treated with GH and GnRHa: Results of a long-term trial

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ABSTRACT

Context Pubertal children born small for gestational age with a poor adult height (AH) expectation can benefit from treatment with growth hormone (GH) 1 mg/m^2/day (~0.033 mg/kg/day) in combination with 2 years of GnRH analog (GnRHa) and even more so with GH 2 mg/m^2/day. Because both GH and GnRHa can negatively influence insulin sensitivity, combining these treatments has raised concerns. The long-term GH-dose effects on insulin sensitivity in children treated with combined GH/GnRHa are unknown.

Objective The purpose of this study was to investigate insulin sensitivity and β-cell function by a very precise method during long-term GH treatment, either with or without 2 years of additional GnRHa and to study differences in insulin sensitivity during treatment until AH between GH at 1 or 2 mg/m^2/day.

Methods This was a randomized, dose-response GH trial involving 110 short small for gestational age children (59 girls) treated with GH until AH (GH randomized to 1 or 2 mg/m^2/day). Sixty-seven children received additional GnRHa treatment. Frequently sampled intravenous glucose tolerance tests were performed and insulin sensitivity (Si), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD. The GH-dose effect was evaluated in a subgroup of 48 children who started GH treatment in early puberty (random to 1 or 2 mg/m^2/day) combined with 2 years of GnRHa.

Results At AH, after 5.9 years of GH treatment, Si, AIR, and DI were similar between children treated with combined GH/GnRHa and those treated with GH only. In the subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) together with 2 years of GnRHa treatment, there were no significant differences in Si, AIR, or DI between the GH-dose groups during the treatment.

Conclusions Combined GH/GnRHa treatment has no long-term negative effects on insulin sensitivity and β-cell function compared with GH only. Started in early puberty, a GH dose of 2 mg/m^2/day results in a similar insulin sensitivity at AH as a GH dose of 1 mg/m^2/day.

INTRODUCTION

Being born small for gestational age (SGA) has been associated with a higher prevalence of diabetes mellitus type 2 at a relative young adult age (1). We have shown that additional treatment with a gonadotropin-releasing hormone analog (GnRHa) for 2 years at start of puberty can improve adult height (AH) in children who start growth hormone (GH) treatment in early puberty with an expected AH of < −2.5 standard deviation score (SDS) (2). Additional GnRHa treatment for 2 years has no long-term negative effects on body composition, blood pressure, and lipids (3). The long-term effects of combined GH/GnRHa treatment on insulin sensitivity and β-cell function in SGA-born children are unknown (4). Some studies in children with central precocious puberty (CPP) showed a deterioration of insulin sensitivity during GnRHa treatment (5, 6). Because GH treatment is also known to reduce insulin sensitivity (7-9), the combination of GH with GnRHa treatment has raised concern. The changes in insulin sensitivity during GH treatment seem to be independent of the GH dose, at least within the dose range studied (8, 10, 11).

We present metabolic data for a longitudinal, randomized, dose-response GH trial involving short SGA children (≥8 years at start) who were treated with GH until AH, either with or without additional GnRHa for 2 years from start of puberty. First, insulin sensitivity and β-cell function at AH were compared between children treated with combined GH/GnRHa and those treated with GH only. We expected that the effects of 2 years of additional GnRHa treatment would be temporary and would normalize after discontinuation of GnRHa treatment, resulting in similar insulin sensitivity and β-cell function at AH between these 2 treatment groups. Second, we investigated the GH dose effect (1 vs 2 mg/m^2/day) on insulin sensitivity and β-cell function in a subgroup of children who started GH treatment (randomized to 1 or 2 mg/m^2/day) and 2 years of GnRHa treatment in early puberty. We hypothesized that treatment with GH 2 mg/m^2/day (~0.067 mg/kg/day) from the onset of puberty until AH would result in similar insulin sensitivity and β-cell function during treatment until AH compared with GH 1 mg/m^2/day (~0.033 mg/kg/day).

METHODS

Subjects

The Dutch SGA study included children when they met the following criteria: 1) birth length and/or birth weight SDS for gestational age less than −2.0 (12); 2) chronological age of ≥8 years; 3) prepubertal stage (Tanner stage I) or early pubertal stage (breast stage II-III in girls and testicular volume of <10 ml in boys) with a GnRHa-stimulating test indicating central puberty (13, 14); 4) height at inclusion of less than −2.5 SDS in prepubertal children or when included in early puberty a height at the start of puberty

of <140 cm, which would result in an expected AH less than −2.5 SDS, based on Dutch references (15); 5) well-documented growth data from birth to the start of treatment; and 6) normal karyotype in all girls (2). Children were excluded in cases 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, short stature homeobox haploinsufficiency or syndromes (except for Silver-Russell syndrome), and use of medication interfering with growth or GH treatment. None of the children were GH deficient according to stimulation tests (GH peak >7.7 ng/ml) (16) or overnight GH profiles (17, 18).

The SGA study included 135 short SGA children (70 girls) (Figure 1). Nine children dropped out for the following reasons: lack of motivation despite ongoing catch-up growth (n=3), non-compliance (n=3), social problems (n=1), emigration (n=1), and psychiatric disorder (n=1). In 4 children, the GH dose was increased because of limited catch-up growth and in 1 child was decreased because of high serum IGF-I levels. Among the 121 children who continued treatment according to protocol, 110 children reached AH (defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months and bone age was ≥15 years for girls and ≥17 years for boys) or near AH (defined as height velocity between 0.5 and 2 cm during the last 6 months and adult pubertal stage). In the 110 children who were eligible for analyses at AH, a frequently sampled intravenous glucose tolerance (FSIGT) test at AH was performed in 76 children. Reasons for no FSIGT test at AH were patient fear for the intravenous catheters or stopping of GH treatment >24 hours before the FSIGT test.

This study was performed according to the Declaration of Helsinki and approved by the medical ethics committees of the participating centers. Written informed consent was obtained from the 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

The Dutch SGA study is a longitudinal, randomized, dose-response GH trial involving short SGA children of at least 8 years of age. All children received somatropin sc daily (Genotropin; Pfizer Inc). Every 3 months, the GH dose was adjusted to calculated body surface area. Prepubertal children received GH 1 mg/m^2/day (Figure 2). When these 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 2 mg/m^2/day after stratification for sex, pubertal stage, and parental height (1 or 2 parents with a height less than −2 SDS vs both parents with a height of at least −2 SDS). Because no model is known

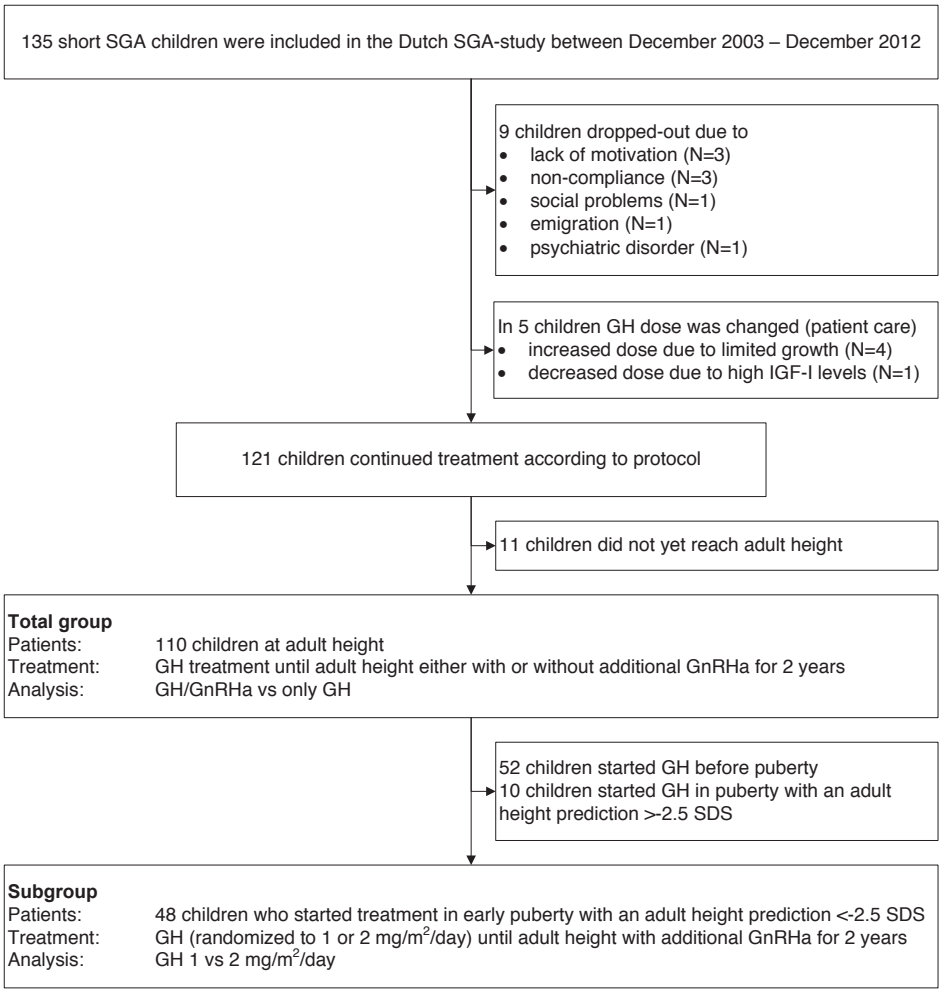

Figure 1. Flowchart of inclusion, dropout, and analyses.

to predict AH accurately at the start of puberty, we used a pragmatic, arbitrary cutoff level. A height of <140 cm at the start of puberty was used to identify children with an AH expectation of less than −2.5 SDS, based on Dutch reference values (15, 19); these children received GnRHa (leuprolide acetate depots, 3.75 mg sc every 4 weeks) for 2 years in addition to GH treatment. During GnRHa treatment, puberty was sufficiently suppressed in all children, both clinically and by GnRHa-stimulating tests or overnight gonadotropin profiles (18, 20).

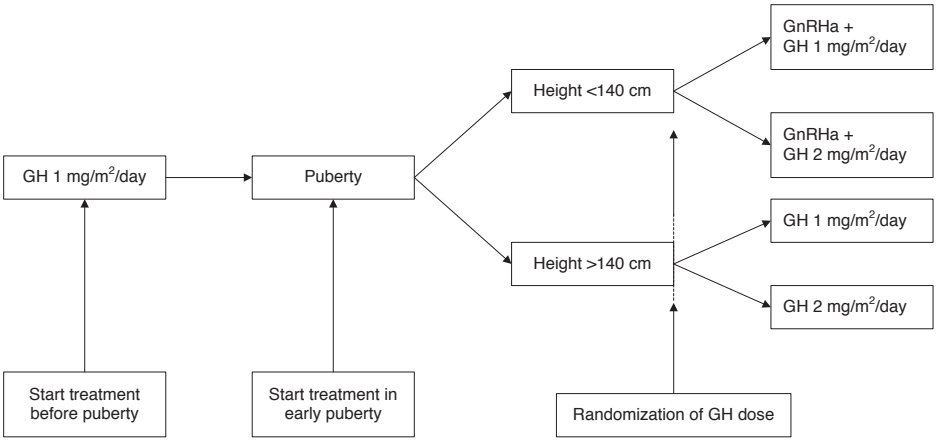

Figure 2. Flowchart of treatment regimen.

Measurements

Height, weight, and Tanner stage were determined at start and every 3 months. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior). Height and weight were transformed into SDS for sex and chronological age according to Dutch references (15), using Growth Analyzer Research Calculation Tools (https://growthanalyser.org).

Assays

At the start of treatment and yearly until AH, blood samples were taken after an overnight fast. After centrifugation, all samples were kept frozen until assayed (-80°C). Serum fasting glucose levels were determined on an Architect ci8200 system (Abbott), and serum fasting insulin levels were measured by IRMA (Medgenix, Biosource Europe). The intra-assay coefficient of variation was 2.1% to 1.5% (6.6-53.3 milligram equivalents [mE]/l) and the interassay coefficient of variation was 6.5% to 6.1% (14.4-100.4 mE/l). Homeostasis model assessment of insulin resistance (HOMA-IR) was performed using the model HOMA-IR = (fasting glucose [millimoles per liter] x fasting insulin [milliunits per liter])/22.5 (21).

Insulin sensitivity and β-cell function

To assess glucose homeostasis, a modified FSIGT test with Tolbutamide was performed at AH after an overnight fast (22). Insulin sensitivity (Si), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD Millennium software (23). Si quantifies the capacity of insulin to stimulate glucose disposal, and AIR is an estimate of insulin secretory capacity, measured as the area under the curve from 0 to

10 minutes, corrected for baseline insulin levels. The DI equals AIR * Si and indicates the β-cell function.

In the subgroup of children who started GH treatment (randomized to 1 or 2 mg/m^2/day) in early puberty with additional 2 years of GnRHa treatment, an FSIGT was performed at baseline, after 1 year of combined GH/GnRHa treatment, and at AH attainment. After approval of a protocol amendment, the last 15 children included in the SGA study underwent an additional FSIGT test 3 months after GnRHa treatment only, to determine the independent effects of GnRHa treatment on insulin sensitivity.

Body composition

In all children, body composition at the start and at AH was measured by dual-energy X-ray absorptiometry (DXA) scan (Lunar Prodigy; GE Healthcare, Chalfont St. Giles, UK). All scans of the study group and reference population were performed on the same machine, and tests for quality assurance were performed daily. For measuring total body fat and lean body mass, this type of DXA machine has an intra-assay coefficient of 0.41% to 0.88% for fat tissue and 1.57% to 4.49% for lean body mass (LBM) (24). Total fat mass was measured and fat mass SDS was calculated according to age- and sex-matched Dutch reference values (25).

In the subgroup of children who started GH treatment (randomized to 1 or 2 mg/m^2/day) in early puberty combined with 2 years of GnRHa treatment, 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.

Statistics

Statistical analyses were performed using SPSS version 21. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q plots. Clinical characteristics are presented as mean (SD). A one-sample t test was used to compare SDS results with 0 (mean value for age- and sex-matched references). Because of a skewed distribution, fasting insulin and HOMA-IR were square root-transformed, whereas Si, AIR, and DI were log-transformed for analyses. Sample size calculation was based on FSIGT test results in a previous Dutch study in short SGA children (26). The estimated sample size was based on an expected difference in mean Si between 2 groups of $10x10^{-4}/min^{-1}$ (μU/ml), SD of 10, testing at a significance level of 5%. Seventeen children in each group were sufficient to detect this difference with 80% power.

Total group analyses: differences at AH between children treated with combined GH/GnRHa and those treated with GH only, were evaluated using an independent-sample t test.

Subgroup analyses: to evaluate GH dose-dependent effects and changes in insulin sensitivity and β-cell function from start to AH, additional analyses were performed in a subgroup of children who started GH treatment (randomized to 1 or 2 mg/m^2/day) in early puberty combined with 2 years of GnRHa and subsequently continued GH treatment until AH. We used repeated-measurements analysis (linear mixed model) with an unstructured covariance matrix with GH dose as the categorical independent variable and fat mass percentage (FM%) SDS as a covariate. Differences between the 1mg and 2mg group at various time points were evaluated using an independent-sample t test. A paired-sample t test was used to compare insulin sensitivity and β-cell function at start of treatment and after 3 months of GnRHa treatment only. We assessed linear correlations using Pearson correlation coefficient. P-values < 0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Table 1 shows the baseline clinical characteristics of the total group of 110 short SGA children (59 girls) who were treated until AH. At start of treatment, mean (SD) age was 11.4 (1.5) years and height SDS was -2.9 (0.6). Sixty-seven children were treated with combined GH/GnRHa and 43 with GH only. The combined GH/GnRHa group consisted of more girls (P=0.018) and more children that started treatment in early puberty with a more progressed Tanner stage (P<0.001).

Insulin sensitivity and β-cell function measured by FSIGT tests

Total group at adult height

Table 2 shows the insulin sensitivity and β-cell function at AH measured by FSIGT tests with Tolbutamide. The average GH treatment duration was 5.9 years of GH treatment. At AH, FM% SDS was similar between children treated with GH/GnRHa and those treated with GH only (P=0.832) (Table 2).

FSIGT tests at AH were performed in 76 patients (48 after combined GH/GnRHa and 28 after GH treatment only) at a mean age of 17.4 (1.2) years. Fasting glucose, fasting insulin, Si, and AIR were similar in children treated with combined GH/GnRHa and those treated with GH only (Table 2). DI, a measure of β-cell function, tended to be slightly higher in the combined GH/GnRHa group, but this did not reach statistical significance (P=0.066). All children were randomized to GH 1 or 2 mg/m^2/day from pubertal onset until AH. In both the combined GH/GnRHa and GH groups, Si, AIR, and DI at AH were similar between children treated with GH 1 mg/m^2/day and those treated with GH 2 mg/m^2/day. During the 5.9 (1.4) years of study, none of the patients developed diabetes mellitus type 2.

Table 1. Baseline clinical characteristics

	Total group	GH/GnRHa	GH	P-value
N	110	67	43	
Boys/girls	51/59	25/42	26/17	**0.018**
Gestational age, weeks	37.4 (3.3)	37.4 (3.6)	37.5 (3.0)	0.886
Birth weight SDS	-2.0 (1.0) [a]	-2.0 (0.9) [a]	-2.0 (1.0) [a]	0.888
Birth length SDS	-2.7 (1.3) [a]	-2.8 (1.3) [a]	-2.6 (1.2) [a]	0.513
Target height SDS	-0.7 (0.7) [a]	-0.7 (0.7) [a]	-0.7 (0.7) [a]	0.849
Age, years	11.4 (1.5)	11.6 (1.2)	11.0 (1.8)	0.065
Height SDS	-2.9 (0.6) [a]	-2.9 (0.7) [a]	-2.9 (0.6) [a]	0.591
Body Mass Index SDS	-1.0 (1.0)	-0.9 (1.0)	-1.2 (1.0)	0.202
IGF-I SDS	-1.17 (1.2)	-1.08 (1.1)	-1.30 (1.5)	0.374
Prepubertal / pubertal	52/58	19/48	33/10	**<0.001**
Pubertal stage [14]				
Tanner I	52	19	33	
Tanner II	41	36	5	
Tanner III	17	12	5	
Fasting glucose, mmol/l	5.0 (0.5)	5.0 (0.5)	4.9 (0.5)	0.605
Fasting insulin, mU/l	5.3 (4.8)	6.3 (5.5)	3.8 (3.0)	0.406
HOMA-IR	1.2 (1.2)	1.5 (1.4)	0.9 (0.8)	0.471

Data are expressed as mean (SDS), unless written otherwise. Fasting insulin and HOMA-IR were square-root transformed before analyses and adjusted for Tanner stage at baseline. Bold text indicates a significant P-value (P<0.05).

[a] Variables in SDS compared with zero SDS, P<0.01.

Subgroup of early pubertal children randomized to GH 1 or 2 mg/m^2/day and 2 years of GnRHa

In a subgroup of 48 children who started combined GH/GnRHa treatment in early puberty, the GH-dose effects on insulin sensitivity and β-cell function were analyzed in detail. All children received GnRHa for the first 2 years in addition to GH (randomized to 1 or 2 mg/m^2/day). Twenty-five children (16 girls) were randomized to treatment with GH 1 mg/m^2/day (1mg group) and 23 (15 girls) to GH 2 mg/m^2/day (2mg group). The boy/girl ratio and age at start were similar between the 1mg and 2mg groups. Because FM% SDS was significantly lower in the 2mg group during the entire treatment period (P=0.030), all analyses were adjusted for FM% SDS.

In this subgroup, FSIGT tests were performed at start of treatment, after 1 year of combined GH/GnRHa treatment and at AH. At all these time points, Si, AIR, and DI were similar in children treated with GH 1 mg/m^2/day and those treated with GH 2 mg/m^2/day. In both GH-dose groups, Si decreased during the first year of combined GH/GnRHa treatment after which Si remained similar until AH, resulting in significantly lower Si

Table 2. Insulin sensitivity and β-cell function by FSIGT

	Total group	GH/GnRHa	GH	P-value
N	110	67	43	
GH dose 1 vs 2 mg/m^2/day	55/55	33/34	22/21	0.845
At onset of puberty				
Height SDS	-2.6 (0.7)	-2.7 (0.6)	-2.3 (0.7)	**0.002**
At adult height				
Age, years	17.4 (1.2)	17.4 (1.1)	17.4 (1.3)	0.853
Adult height SDS [a]	-1.8 (0.8)	-1.8 (0.9)	-1.7 (0.8)	0.526
FM% SDS	0.4 (0.8)	0.4 (0.7)	0.4 (0.9)	0.832
Body Mass Index SDS	-0.2 (1.1)	-0.3 (1.0)	-0.05 (1.1)	0.297
FSIGT (N=76)		48	28	
Si x 10^{-4}/min, mU/l	6.1 (5.2)	6.8 (5.8)	5.0 (3.9)	0.176
AIR, mU/l	706.9 (564.4)	726.3 (616.7)	673.8 (470.2)	0.881
DI (Si x AIR)	2929.3 (1762.9)	3159.4 (1871.4)	2534.7(1510.2)	0.066
Fasting glucose, mmol/l	5.0 (0.5)	5.0 (0.5)	5.1 (0.6)	0.506
Fasting insulin, mU/l	13.6 (6.6)	13.6 (7.3)	13.7 (5.2)	0.752

Data are expressed as mean (SDS), unless written otherwise. Fasting insulin and HOMA-IR were square-root transformed and Si, AIR, and DI were log-transformed before analyses. Bold text indicates a significant P-value (P<0.05).

[a] Adult height SDS was calculated using references for Dutch adults (21 years).

values at AH than at start (Figure 3A). A compensatory increase in AIR was found during the first year of combined GH/GnRHa treatment in both GH-dose groups after which it decreased until AH. At AH, AIR was similar to start in both GH-dose groups (Figure 3B). During the first year of combined GH/GnRHa treatment, DI remained similar in both GH-dose groups. In both GH-dose groups, DI was lower at AH than at start, but this did not reach statistical significance (P=0.12 and P=0.05) (Figure 3C). The 2mg group had a higher IGF-I SDS at AH than the 1mg group (1.3 vs 0.8 SDS, P=0.042), but Si, AIR, and DI were not significantly different between the GH-dose groups.

Insulin sensitivity during 3 months of only GnRHa treatment

To investigate whether the changes during the first year of combined GH/GnRHa treatment were due to the effect of GH or could also be caused by a negative effect of GnRHa on insulin sensitivity, we performed an additional FSIGT test after 3 months of GnRHa treatment without GH in the last 15 children (9 girls) included in this study. After 3 months of GnRHa treatment, there were no significant changes in Si, AIR, or DI compared with baseline values (P=0.377, P=0.615, and P=0.181, respectively).

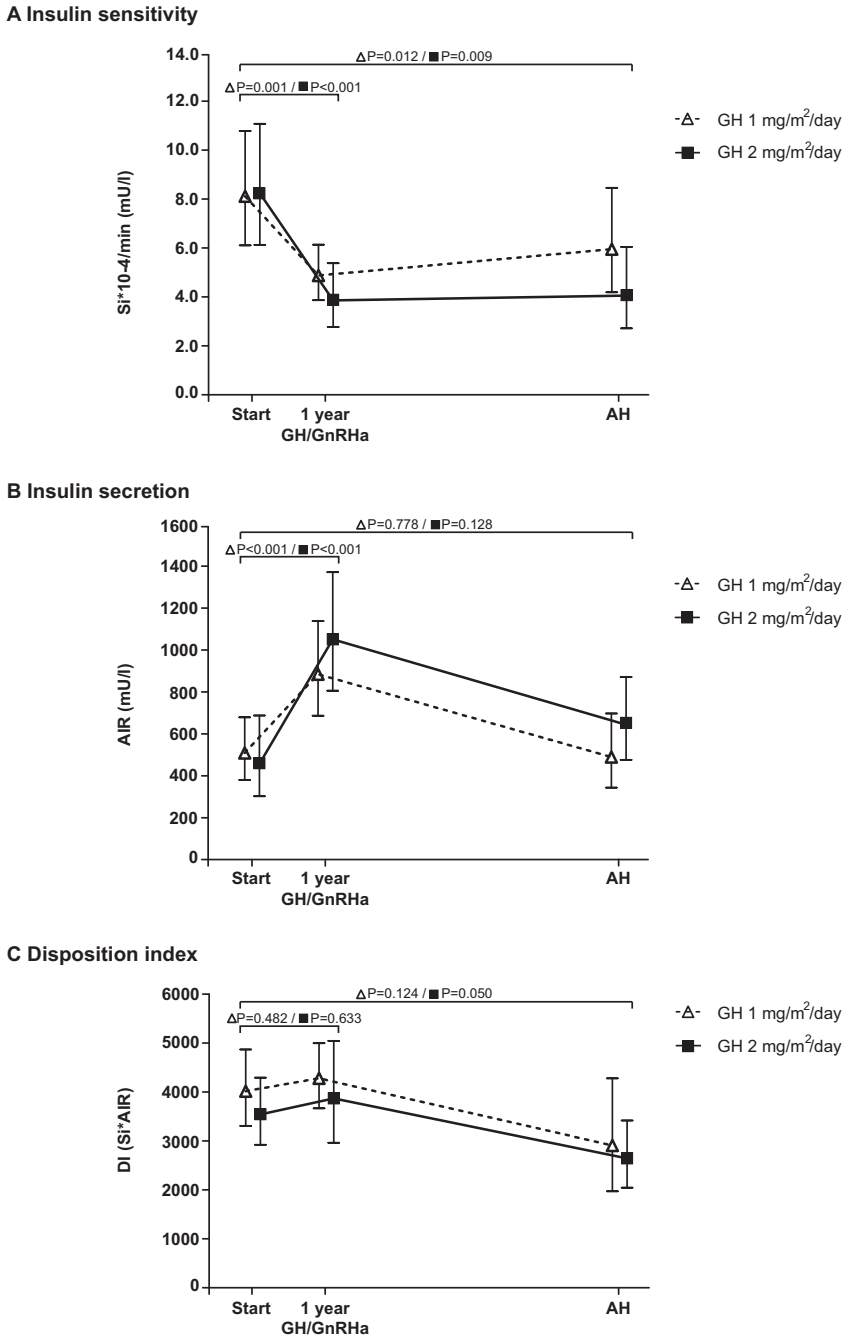

Figure 3. FSIGT results in a subgroup of children who started GH treatment in early puberty with 2 years of GnRHa and subsequent GH until adult height (AH).
Data are expressed as estimated marginal means with 95% confidence intervals.
There were no significant differences between the 2 GH-dose groups at any time point during the study.

Insulin sensitivity and β-cell function at adult height by HOMA-IR

The FSIGT test is not easily applied in large populations because of its complexity, costs, and invasiveness; thus, we tested in the total group how the FSIGT test results correlated with data obtained by HOMA-IR, which is a more practical, cheaper, and less invasive measure. At start, fasting insulin levels and HOMA-IR were higher in the combined GH/GnRHa group (P=0.004 and P=0.005, respectively). However, after adjustment for the difference in Tanner stage at baseline, fasting insulin levels and HOMA-IR were similar between the combined GH/GnRHa group and GH group (Table 1). In all 110 children who reached AH, fasting glucose, fasting insulin, and HOMA-IR at AH were similar between children treated with GH/GnRHa and those treated with GH only. The correlation between HOMA-IR and Si measured by the FSIGT test was significant but not strong enough for HOMA-IR to serve as a proxy for Si measured by the FSIGT test in individual patients (r=-0.396, P<0.001).

DISCUSSION

This is the first study describing the long-term changes in insulin sensitivity and β-cell function determined by the FSIGT test with Tolbutamide in short children born SGA who started GH treatment at ages older than 8 years. They were randomized to GH 1 or 2 mg/m^2/day from start of puberty, either with or without additional GnRHa for 2 years. At AH, after an average of 5.9 years of GH treatment, Si, AIR, DI, fasting glucose, and fasting insulin were similar between children treated with combined GH/GnRHa and those treated with GH only. In the subgroup of children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) together with 2 years of GnRHa treatment, there were no significant differences in Si, AIR, or DI between the GH-dose groups during the entire treatment.

Our study shows that 2 years of GnRHa in addition to GH treatment results in a similar insulin sensitivity at AH compared with GH treatment only. Short-term studies in girls with CPP compared combined GH/GnRHa treatment with GH only or placebo and showed no negative metabolic side effects (27, 28). A long-term study until AH about the effects of GnRHa treatment in girls with CPP showed a lower insulin sensitivity at AH in treated girls than in untreated girls (5). In that study, however, insulin sensitivity was determined by HOMA-IR, which is not comparable with the gold standard FSIGT test with Tolbutamide that we used. Furthermore, the difference in insulin sensitivity, could be explained by the higher body mass index in the girls with CPP treated with GnRHa as there is a strong negative correlation between total body fat mass and insulin sensitivity (29). At AH, we found neither a higher fat mass in the SGA children treated with combined GH/GnRHa nor a lower insulin sensitivity. Combined GH/GnRHa treatment tended

to cause a slightly higher DI, a measure of β-cell function, than GH treatment only, but the difference was not significant.

In the subgroup of 48 children who all started GH treatment (randomized to 1 or 2 mg/m^2/day) in early puberty with additional GnRHa treatment for 2 years, we found similar FSIGT test results during the entire treatment period in children treated with GH 1 mg/m^2/day and those treated with 2 mg/m^2/day, reassuringly confirming our hypothesis that treatment with GH 2 mg/m^2/day from start of puberty until AH does not result in a more impaired insulin sensitivity. These findings are in line with studies using oral glucose tolerance tests in SGA children treated with GH only (8, 10, 11, 30). The 2mg group had a higher IGF-I SDS at AH than the 1mg group (1.3 vs 0.8 SDS, P=0.042), but Si, AIR, and DI were not significantly different between the GH-dose groups.

In our subgroup, the decline in Si during the first year of combined GH/GnRHa treatment was counterbalanced by a compensatory increase in AIR. This demonstrates that the β-cells can compensate for the decline in insulin sensitivity by increasing their insulin secretion, indicating normal β-cell function. Indeed, β-cell function did not change throughout treatment. At AH, insulin sensitivity was significantly lower than at start, which is in line with previous findings in SGA children treated with GH until AH (8, 30). From start to AH, our patients transitioned through puberty, and it is well known that healthy children show a decrease in insulin sensitivity, an increase in insulin secretion, and a decrease in glucose disposition index during puberty (31), which leads to a lower insulin sensitivity in healthy postpubertal adolescents than in prepubertal children (22, 32, 33). Thus, the lower insulin sensitivity than baseline in our patients might be explained by the fact that they are postpubertal.

Because both GH and GnRHa treatment can independently reduce Si (5-9), we also determined the effects on glucose homeostasis of GnRHa treatment without GH. After 3 months of GnRHa treatment, insulin sensitivity and β-cell function were similar to baseline, indicating that short-term GnRHa treatment does not affect insulin sensitivity. This is in contrast to findings with oral glucose tolerance tests in girls with CPP after 1 year of GnRHa treatment (6) but is supported by our finding that at AH, insulin sensitivity and β-cell function in children treated with GH/GnRHa were similar to those in children treated with GH only, suggesting that additional GnRHa for 2 years does not impair insulin sensitivity.

Our data are unique because the method we used, the FSIGT test with Tolbutamide, is a gold standard for measuring insulin sensitivity and β-cell function like the euglycemic-hyperinsulinemic clamp (34). The addition of Tolbutamide increases the reliability of the measurement of Si by providing a second surge of insulin instead of merely the initial fall in blood glucose which is, to some extent, due to glucose equilibrating into its full distribution volume and not to the action of insulin. The FSIGT test is more invasive, labor-intensive, and costly than other measurements of insulin sensitivity, for instance,

the oral glucose tolerance test and HOMA-IR, but the results are more accurate. A limitation of this study is the absence of FSIGT tests at start of treatment in all children. This would have given us the opportunity to study the development of insulin sensitivity and β-cell function from start of treatment until AH. Furthermore, it will be interesting for future studies to measure insulin sensitivity also at discontinuation of 2 years of GnRHa treatment. Because of the invasiveness of FSIGT tests, we decided not to perform an FSIGT test at that time point.

Our research group previously published AH results for this cohort. Height SDS at start of puberty was significantly lower in children treated with GH/GnRHa, but AH was similar (Table 2). This finding indicates that children who are shorter at start of puberty (<140 cm) with a poor AH expectation benefit from additional GnRHa treatment. The double GH dose of 2 mg/m^2/day during puberty resulted in a significantly better height gain SDS in boys (2).

In conclusion, our study is the first report showing that 2 years of GnRHa treatment in addition to GH treatment results in a similar insulin sensitivity and β-cell function at AH compared with GH treatment only in children born SGA, indicating that combined GH/GnRHa treatment does not have long-term negative effects on glucose homeostasis in early adulthood. When started in early puberty, a GH dose of 2 mg/m^2/day results in a similar insulin sensitivity from childhood into early adulthood as a GH dose of 1 mg/m^2/day.

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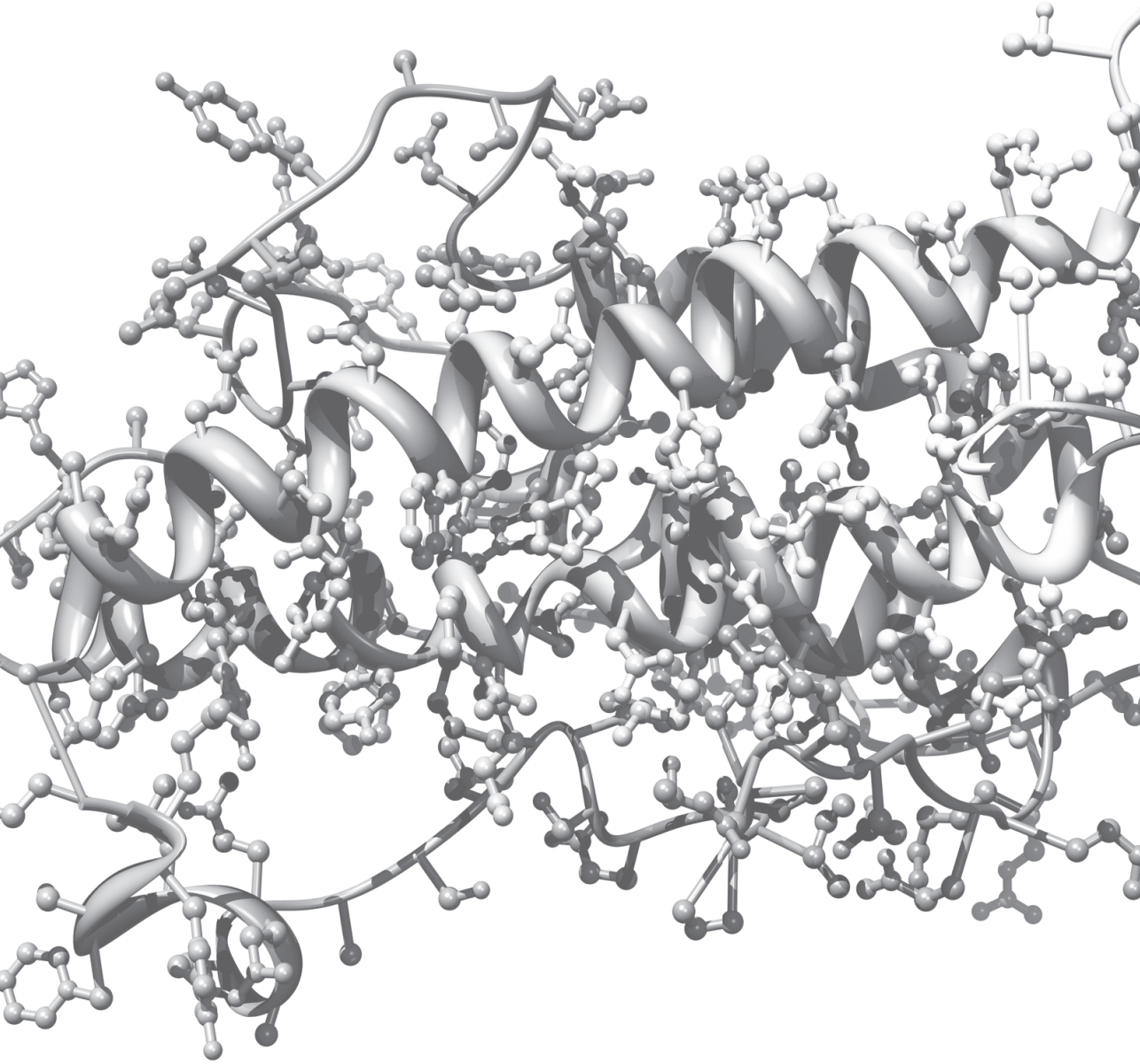

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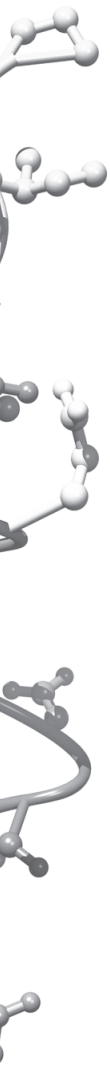

Puberty and pubertal growth in GH-treated SGA children: Effects of 2 years of GnRHa versus no GnRHa

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ABSTRACT

Context Most studies on puberty in children born small for gestational age (SGA) report height and age at onset of puberty. Growth hormone (GH) treated SGA children with an adult height (AH) expectation below -2.5 SDS at onset of puberty can benefit from an additional 2 years of GnRH analog (GnRHa) treatment. There are no data on puberty and growth after discontinuation of GnRHa treatment in GH-treated SGA children.

Objective This study aimed to investigate the effects on puberty and pubertal growth of 2 years GnRHa vs no GnRHa in GH-treated SGA children.

Methods This was a GH trial involving 76 prepubertal short SGA children (36 girls) treated with GH. Thirty-two children received additional GnRHa for 2 years. Pubertal stages were 3-monthly assessed according to Tanner.

Results Age, bone age, and median height at pubertal onset were lower in girls and boys in the GH/GnRHa group compared with the GH group. In girls and boys treated with GH/GnRHa, pubertal duration after stop of GnRHa treatment was shorter than pubertal duration in those with GH only (40.9 vs 46.7 months, P=0.044; 50.8 vs 57.5 months, P=0.006; respectively). Height gain from onset of puberty until AH, including height gain during 2 years of GnRHa treatment, was 25.4 cm in girls and 33.0 cm in boys, which was 6.6 cm more than girls and boys treated with GH only. AH was similar in children treated with GH/GnRHa compared with those with GH only.

Conclusions GH-treated SGA children who start puberty with an AH expectation below -2.5 SDS and are treated with 2 years of GnRHa have a shorter pubertal duration after discontinuation of GnRHa compared with pubertal duration in children treated with GH only. Height gain from onset of puberty until AH is, however, more due to adequate growth during 2 years of GnRHa treatment resulting in a similar AH as children treated with GH only.

INTRODUCTION

Height and age at onset of puberty, as well as the magnitude and duration of pubertal growth are important determinants of adult height, explaining 15-20% of adult height (1). Most studies on puberty in children born small for gestational age (SGA) report height and age at onset of puberty but not pubertal duration. Study results are difficult to compare due to the use of various definitions for the milestones of puberty, but most authors seem to agree that puberty in short SGA children starts within the normal range but relatively early for their short stature (2-6).

In children born SGA with persistent short stature, growth hormone (GH) is an approved therapy for increasing adult height (7-9). GH-treated SGA children with an expected adult height (AH) <−2.5 SDS at onset of puberty benefit from additional treatment with a gonadotropin-releasing hormone analog (GnRHa) for 2 years from onset of puberty to increase AH (10). There are, however, no data on puberty and pubertal growth after discontinuation of GnRHa treatment in GH-treated SGA children.

Based on our clinical experience, we expected that GH-treated SGA children treated with an additional 2 years of GnRHa from onset of puberty would show an accelerated pubertal progression after discontinuation of GnRHa resulting in less pubertal growth than children treated with GH only. However, due to the additional height gain during postponement of puberty by 2 years of GnRHa treatment, we expected AH and AH SDS to be similar in children treated with combined GH/GnRHa compared with those treated with GH only.

METHODS

Subjects

In the Dutch SGA study, children could start GH treatment from the age of 8 years, either being prepubertal or in early puberty. The present study group consisted of 76 short SGA children (36 girls), a subgroup of the total Dutch SGA study, who were prepubertal at start of GH treatment and were followed until AH. Body composition, glucose homeostasis, blood pressure, lipid levels, and AH have been reported for the total Dutch SGA study (10-12). Pubertal development in the present study group has never been published. Children were included when they met the following criteria; 1) birth length and/or birth weight SDS for gestational age less than −2.0 (13); 2) current height less than −2.5 SDS; 3) prepubertal stage at start of GH treatment (Tanner stage I); 4) well-documented growth data from birth to start of treatment; and 5) normal karyotype in all girls. None of the children were GH deficient according to normal serum IGF-I levels (IGF-I level >-2 SDS) and stimulation tests (GH peak >7.7 ng/ml) or overnight GH profiles.

Children started with daily sc somatropin treatment when prepubertal and were treated until AH. A height of less than 140 cm at onset of puberty was used to identify

children with an AH expectation of less than −2.5 SDS, based on Dutch reference values (14, 15). These children received 2 years of GnRHa treatment (leuprolide acetate depots, 3.75 mg sc every 4 weeks) from onset of puberty in addition to GH treatment (GH/GnRHa group). During GnRHa treatment, puberty was sufficiently suppressed in all children, both clinically and by GnRHa-stimulating tests or overnight gonadotropin profiles (16, 17). Children who started puberty with a height above 140 cm were treated with GH only (GH group). Children were treated with GH 1 mg/m^2/day (~0.033 mg/kg/day) until onset of puberty. At onset of puberty, they were randomly assigned to treatment with either GH 1 or 2 mg/m^2/day after stratification for sex, pubertal stage, and parental height (one or two parents with a height <−2 SDS vs both parents with a height >−2 SDS). Every 3 months, the GH dose was adjusted to calculated body surface area. Figure 1 shows the treatment regimen during the study. Seventy-one children reached AH (defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months, and bone age [BA] was ≥15 years in girls and ≥17 years in boys), or near AH (defined as height velocity between 0.5 and 2 cm during the last 6 months and adult pubertal stage). Five children did not reach adult height; one boy was still growing and four children dropped out for the following reasons: lack of motivation despite ongoing catch-up growth (n=2), social problems (n=1), emigration (n=1). Data of these five children were used until the highest pubertal stage attainment during the study.

The 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.

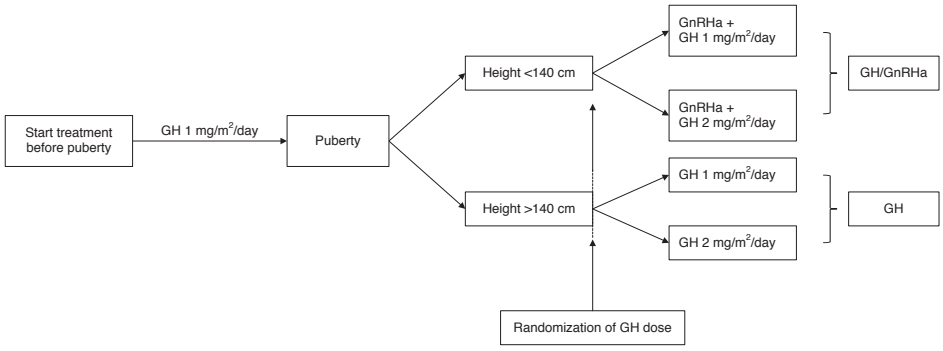

Figure 1. Flowchart of treatment regimen of prepubertal children of the Dutch SGA study.

Measurements

Height and weight were determined at start and every 3 months by the same physicians. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd.). Weight was measured to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior). Body mass index (BMI) was calculated (kilograms per square meter, rounded to the nearest tenth). Height, weight, and BMI were transformed into SDS for sex and chronological age according to Dutch references (14), using Growth Analyzer Research Calculation Tools (https://growthanalyser.org). Radiographs of the left hand and wrist were taken annually. BA was determined at start of GH treatment and yearly thereafter by one investigator (M.v.d.S.) according to Greulich and Pyle (18).

Pubertal development

Pubertal stages were assessed according to the method of Tanner and Whitehouse (19) at each 3-monthly visit, allowing quite precise determination of pubertal onset, which was defined as breast development stage II for girls according to Tanner (19) and a testicular volume equal or more than 4 ml for boys as determined by means of a Prader orchidometer. End of GnRHa treatment, and thus restart of puberty, was defined as 4 weeks after the last GnRHa injection. At each 3-monthly visit, girls were asked whether and when they had their menarche.

We defined several periods during pubertal development to compare pubertal duration between children treated with GH/GnRHa and those treated with GH only. In girls, Period 1 was defined as the period between onset of puberty (M2) and menarche. In boys, Period 1 was defined as the period between onset of puberty (a testicular volume of 4 ml) and a testicular volume of 16 ml. Because we wanted to be certain that there was central puberty, we performed a GnRH-analog test after the appearance of the first clinical signs (M2 in girls and a testicular volume of 4 ml in boys). This resulted in a delay between onset of puberty and start of GH/GnRHa treatment during which pubertal development progressed. Period 1 in girls and boys treated with GH/GnRHa was therefore divided in two separate periods; onset of puberty until start of GnRHa treatment (period 1A) and restart of puberty after stop of GnRHa until menarche in girls or until a testicular volume of 16 ml in boys (period 1B). In girls, Period 2 was defined as the period between menarche and AH. In boys, Period 2 was defined as the period between a testicular volume of 16 ml and AH.

Statistical analyses

We used the same definitions for pubertal milestones as the Fourth Dutch National Growth Study (1997), which served as reference for age and height at onset of puberty and age at menarche of normal-statured children born appropriate for gestational age (controls) (14). Statistical analyses were performed with SPSS version 21. Distribution

of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Data are expressed as median (interquartile range). Differences between the groups were calculated using Mann–Whitney *U* tests. P-values < 0.05 were considered statistically significant.

RESULTS

Onset of puberty

Table 1 lists the clinical data of all 76 prepubertal children compared with Dutch references. All children started GH treatment 1 mg/m^2/day when prepubertal. Median GH treatment duration until onset of puberty was 2.1 years (1.0-2.8) in girls and 2.7 years (1.6-3.9) in boys.

Girls in the GH/GnRHa group started puberty at a similar age compared with Dutch references, whereas girls in the GH group started puberty significantly older compared with references. Age and BA at pubertal onset were significantly younger in girls in the GH/GnRHa group compared with girls in the GH group. As expected, median height in centimeters at pubertal onset was significantly lower in girls in the GH/GnRHa group (134.6 cm) than in the GH group (143.1 cm; P<0.001), but their height SDS at onset of puberty was similar. BMI SDS at pubertal onset was similar between groups.

Boys in the GH/GnRHa group started puberty at a similar age compared with Dutch references, whereas boys in the GH group started puberty significantly older compared with references. Age and BA at pubertal onset were significantly younger in boys in the GH/GnRHa group compared with boys in the GH group. As expected, median height in centimeters at pubertal onset was significantly lower in boys in the GH/GnRHa group (137.0 cm) than in the GH group (143.4 cm; P<0.001), but their height SDS at onset of puberty was similar. BMI SDS at pubertal onset was similar between groups.

Bone maturation

At onset of puberty, BA was significantly younger in girls and boys treated with GH/GnRHa than those treated with GH only (Table 1). BA at onset of puberty was similar in children who were randomly assigned to receive either 1 or 2 mg/m^2/day. In the GH/GnRHa group, the ratio ΔBA/ΔCA during 1 year of GnRHa treatment with sufficient suppression of puberty was 0.3 years in girls and 0.5 years in boys. In the GH group, the ratio ΔBA/ΔCA during 1 year after onset of puberty was 1.0 year in girls and 0.75 years in boys. For girls, the ratio ΔBA/ΔCA was significantly lower in the GH/GnRHa group compared with the GH group (P=0.003), whereas in boys the ratio ΔBA/ΔCA was not significantly different (P=0.119).

Table 1. Clinical characteristics of 76 prepubertal GH-treated SGA children versus Dutch references

Characteristic	GH/GnRHa	GH	P-value [b]	GH/GnRHa	Dutch references [c]
Start of GH					
Girls					
Age, years	10.1 (6.9 to 11.1)	9.9 (9.7 to 10.7)	0.837		
Height, cm	123.4 (109.0 to 128.0)	124.9 (120.1 to 127.1)	0.334		
Height, SDS	-3.0 (-3.4 to -2.9)	-3.0 (-3.5 to -2.7)	0.219		
Boys					
Age, years	9.0 (6.7 to 11.0)	10.1 (9.1 to 11.3)	0.065		
Height, cm	116.0 (108.5 to 128.6)	122.9 (117.4 to 131.2)	**0.039**		
Height, SDS	-3.3 (-3.7 to -2.7)	-2.9 (-3.2 to -2.6)	0.151		
Onset of Puberty				**Restart Puberty**	
Girls	19	17		19	2266
GH dose (1 vs 2 mg/m^2/day)	8 / 11	10 / 7	0.317	8 / 11	
Age, years	11.5 (10.3 to 12.0)	12.4 (12.0 to 12.8) [a]	**0.001**	13.9 (12.8 to 14.5)	10.7
BA, years	10.8 (10.5 to 11.0)	11.3 (11.0 to 12.0)	**0.003**	12.0 (11.5 to 12.3)	
BA delay, years	-0.8 (-1.3 to 0.1)	-0.8 (-1.7 to -0.6)	0.248	-1.6 (-2.5 to -1.0)	
Height, cm	134.6 (130.3 to 136.1)	143.1 (140.3 to 145.5)	**<0.001**	148.9 (144.4 to 153.8)	
Height, SDS	-2.6 (-3.0 to -1.9)	-2.1 (-2.8 to -1.7)	0.257		
BMI, SDS	-0.6 (-1.3 to -0.3)	-1.0 (-1.6 to -0.3)	0.496	-0.6 (-1.0 to 0.1)	
Boys	13	27		12	2524
GH dose (1 vs 2 mg/m^2/day)	8 / 5	12 / 15	0.311	7 / 5	
Age, years	11.4 (10.9 to 12.8)	13.0 (12.6 to 13.5) [a]	**<0.001**	13.6 (13.2 to 14.8)	11.5
BA, years	10.5 (10.0 to 11.4)	12.3 (11.4 to 12.8)	**0.001**	12.6 (12.3 to 13.4)	
BA, years	-0.7 (-2.4 to 0.03)	-1.0 (-1.3 to -0.2)	0.919	-1.1 (-1.7 to -0.4)	
Height, cm	137.0 (134.7 to 138.2)	143.4 (141.2 to 146.7)	**<0.001**	152.2 (149.5 to 153.3)	
Height, SDS	-2.2 (-2.8 to -1.7)	-2.2 (-2.5 to -1.7)	0.955		
BMI, SDS	-1.3 (-2.1 to -0.2)	-0.8 (-1.7 to -0.3)	0.718	-1.0 (-2.3 to -0.1)	

Data are expressed as median (IQR), unless written otherwise. Bold text indicates a significant P-value.

[a] P<0.001 compared with Dutch references.

[b] P-value: comparison between GH/GnRHa group and GH group.

[c] Data of 4th Dutch National Growth study (14).

Pubertal duration

Because we wanted to be certain that there was central puberty, we performed a GnRH-analog test after the appearance of the first clinical signs of puberty (M2 in girls and a testicular volume of 4 ml in boys). In girls and boys treated with GH/GnRHa, this resulted in a delay between onset of puberty and start of GnRHa treatment during which pubertal development progressed. The median delay was 3.4 months (1.6-4.8) in girls and 3.6 months (1.5-5.0) in boys. During these months, puberty progressed and for that reason this period (1A) was added to the pubertal duration after stop of GnRHa treatment (1B) (see Figure 2, A and B; period 1 [A+B]).

Figure 2A shows the pubertal duration in girls. In girls treated with GH/GnRHa, period 1A+1B lasted 21.3 months (15.0-26.2), which was not significantly different from period 1 in girls treated with GH only, which lasted 17.8 months (10.4-27.6) (P=0.466). Period 2, menarche until AH, was 21.6 months (16.3-29.0) in girls treated with GH/GnRHa, which was shorter than the 27.8 months (20.3-31.8) in those treated with GH only (P=0.047). Time from onset of puberty until AH, period 1(A and B)+2, was 40.9 months (33.7-48.5) in girls treated with GH/GnRHa, which was shorter than the 46.7 months (41.1-58.6) in girls treated with GH only (P=0.044). Median age at menarche was significantly older in

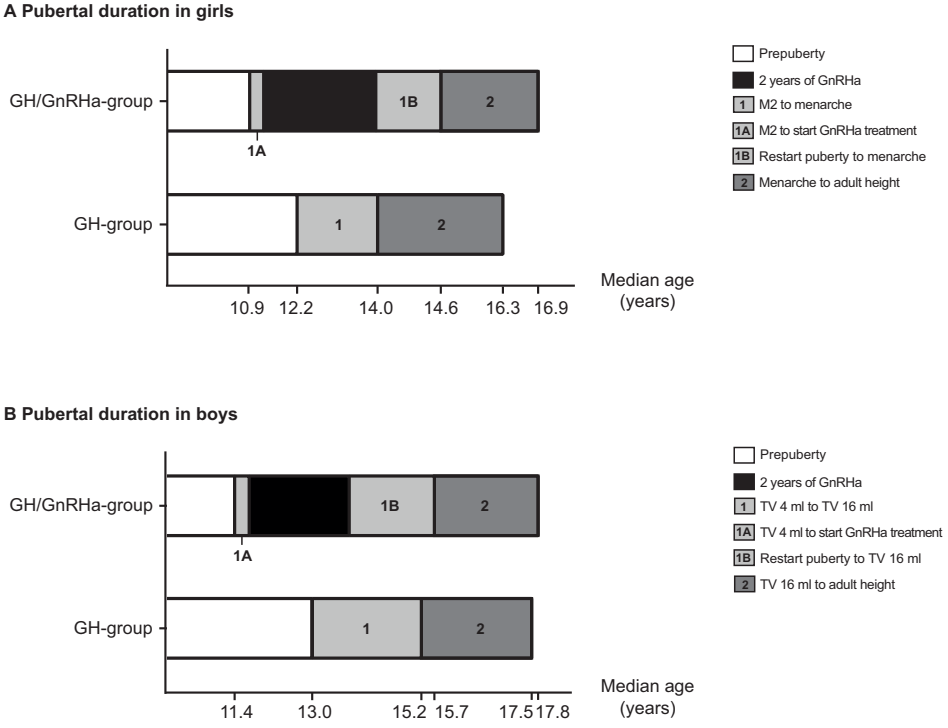

Figure 2. Pubertal duration.
Abbreviations: M2, breast development stage II according to Tanner; TV, testicular volume.

girls treated with GH/GnRHa compared with those treated with GH only (14.6 vs 14.0 years, P=0.001). Girls in both groups had their menarche at an older age compared with the median age of 13.15 years in healthy Dutch references (P<0.001 and P=0.004, respectively). There was no significant difference in pubertal duration between the two GH-dose groups.

Figure 2B shows the pubertal duration in boys. In boys treated with GH/GnRHa, period 1A+1B until a testicular volume of 16 ml, lasted 24.4 months (16.7-29.2) which was not significantly different from period 1 in boys treated with GH only which lasted 27.1 months (21.6-39.8) (P=0.111). Period 2, from a testicular volume of 16 ml to AH, was 23.9 months (19.3-33.9) in boys treated with GH/GnRHa and 27.3 months (21.3-33.1) in those treated with GH only (P=0.887). Time from onset of puberty until AH, period 1(A and B)+2, was 50.8 months (47.4-53.6) in boys treated with GH/GnRHa, which was shorter than the 57.5 months (50.9-62.1) in boys treated with GH only (P=0.006). There was no significant difference in pubertal duration between the two GH-dose groups.

Growth from onset of puberty to AH

Figure 3A shows the growth from onset of puberty to AH in girls. At onset of puberty, the median height of girls in the GH/GnRHa group was 134.6 cm (130.3-136.1), which was significantly lower than the median height of girls in the GH group, which was 143.1 cm (140.3-145.5) (P<0.001). During 2 years of GnRHa treatment, the median height gain in girls was 12.7 cm (11.1-13.7). At restart of puberty, 4 weeks after discontinuation of GnRHa treatment, median height was 148.9 cm (144.4-153.8) in girls treated with GH/GnRHa and their median height gain during period 1A+1B until menarche was 9.0 cm (7.9-11.1), which was not significantly different from the median height gain of 9.7 cm (7.2-17.6) in period 1 in girls treated with GH only (P=0.398). Median height gain in period 2 from menarche until AH was 3.9 cm (3.2-4.4) in girls treated with GH/GnRHa, which was significantly less than the 5.6 cm (4.4-8.5) gain in girls in the GH group (P=0.002). Median height gain during period 1(A+B)+2 until AH was 13.3 cm (11.1-14.3) in girls treated with GH/GnRHa and 18.8 cm (13.8-23.6) in girls treated with GH only (P=0.003). The median total height gain from onset of puberty until AH, including the height gain during GnRHa treatment, was 25.4 cm (24.4-26.4) in girls treated with GH/GnRHa, which was 6.6 cm more than in girls treated with GH only who gained 18.8 cm (13.8-23.6) (P=0.001). Girls with GH/GnRHa treatment were shorter at pubertal onset than those treated with GH only but they reached a similar median AH (160.4 vs 162.7 cm; P=0.217) and AH SDS (-1.6 vs -1.2; P=0.217). In girls treated with GH/GnRHa and those treated with GH only, there was no significant difference in pubertal growth between the two GH-dose groups.

Figure 3B shows the growth from onset of puberty to AH in boys. At onset of puberty, the median height of boys in the GH/GnRHa group was 137.0 cm (134.7-138.2), which was significantly lower than the median height of boys in the GH group, which was 143.4

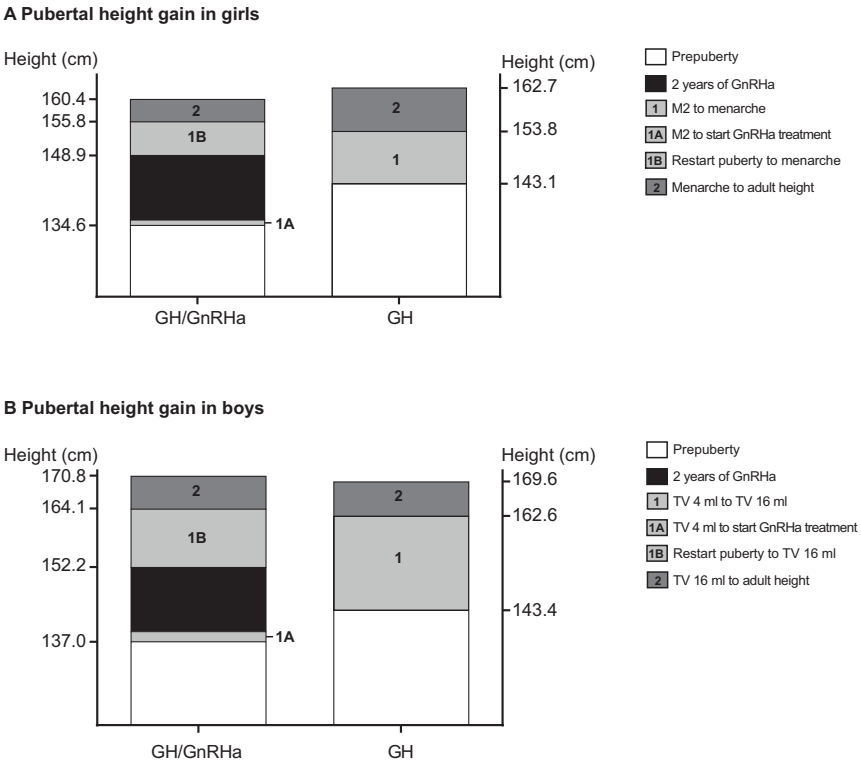

Figure 3. Pubertal height gain.
Abbreviations: M2, breast development stage II according to Tanner; TV, testicular volume.

cm (141.2-146.7) (P<0.001). During 2 years of GnRHa treatment, the median height gain in boys was 13.1 cm (11.1-14.4). At restart of puberty, 4 weeks after discontinuation of GnRHa treatment, median height was 152.2 cm (149.5-153.3) in boys treated with GH/GnRHa and their median height gain during period 1A+1B until a testicular volume of 16 ml was 13.5 cm (11.1-17.7), which was significantly less than the median height gain in period 1 in boys treated with GH only who gained 18.8 cm (16.5-21.8) (P=0.020). Median height gain in period 2 from a testicular volume of 16 ml until AH was 6.1 cm (2.8-7.9) in boys treated with GH/GnRHa, which was not significantly different from the median height gain of 7.7 cm (4.0-9.4) in period 2 in boys treated with GH only (P=0.354). Median height gain during period 1(A+B)+2 until AH, was 21.0 cm (19.4-22.9) in boys treated with GH/GnRHa and 26.4 cm (22.6-29.6) in boys treated with GH only (P=0.001). The median total height gain from onset of puberty until AH, including the height gain during GnRHa treatment, was 33.0 cm (29.9-37.4) in boys treated with GH/GnRHa, which was 6.6 cm more than in boys treated with GH only who gained 26.4 cm (22.6-29.6) (P=0.001). Boys with GH/GnRHa treatment were shorter at pubertal onset than those treated with GH only but they reached a similar median AH (170.8 vs 169.6 cm; P=0.884)

and AH SDS (-1.9 vs -2.0; P=0.960). In boys treated with GH/GnRHa and those treated with GH only, there was no significant difference in pubertal growth between the two GH-dose groups.

DISCUSSION

This study presents the long-term effects of 2 years of additional GnRHa treatment on puberty and pubertal growth in GH-treated children born SGA with an AH expectation below -2.5 SDS at onset of puberty. Although children treated with combined GH/GnRHa were shorter at onset of puberty and had a shorter pubertal duration after discontinuation of GnRHa, their total height gain from onset of puberty until AH was greater compared with those treated with GH only due to an adequate growth during 2 years of GnRHa. This resulted in a similar AH as those treated with GH only.

When the option of additional GnRHa treatment is discussed with parents, they often have questions on how much height their child will gain during GnRHa treatment and what to expect after discontinuation of GnRHa treatment; when to expect menarche and how much height gain will occur after menarche. Data to answer these questions were lacking. Our study presents data on puberty and pubertal growth when 2 years of GnRHa treatment is added to GH treatment in SGA children with an AH expectation below -2.5 SDS at onset of puberty. We show that girls and boys grew approximately 13 cm during 2 years of GnRHa treatment. Girls treated with 2 years of additional GnRHa had their menarche approximately 1.5 years after restart of puberty, with a range from 1-2 years, which is in line with findings in girls with central precocious puberty treated with GnRHa (20-22). They grew nearly 10 cm from restart of puberty until AH and reached their AH approximately 3 years after restart of puberty. From menarche until AH, girls treated with GH/GnRHa grew approximately 4 cm compared with nearly 5.5 cm in girls treated with GH only. Boys grew approximately 20 cm from restart of puberty until AH and reached their AH nearly 4 years after restart of puberty. Total growth from onset of puberty until AH was 6.6 cm more in girls and boys treated with GH/GnRHa than in those treated with GH only, which resulted in a similar AH in those treated with GH/GnRHa and GH only. In children treated with GH/GnRHa, total duration from onset of puberty until AH was longer because of the additional GnRHa treatment, which delayed puberty for 2 years. Without the 2 years of GnRHa treatment, pubertal duration was, however, shorter compared with children treated with GH only. The shorter pubertal duration after GnRHa treatment was not due to more progression in bone maturation according to Greulich and Pyle (18), as BA at onset of puberty was significantly younger and BA development during GnRHa treatment was slower in the GH/GnRHa group compared with the GH group.

A possible explanation for the shorter pubertal duration after GnRHa treatment in children treated with GH/GnRHa could be continuing senescence of the growth plate, the progressive loss of function and structural involution of the growth plate, which is growth dependent (23), during GnRHa treatment. When growth plates are more senescent, and have expended more of their growth potential, a shorter exposure to estrogen is sufficient to complete growth plate fusion (24). There are no other studies reporting pubertal duration after discontinuation of GnRHa treatment in SGA children and therefore comparing our results to other studies was not possible.

Adult height in GH-treated children is influenced by pubertal timing and early onset can result in a loss of prepubertal gain in height SDS. Although our study was not designed to evaluate onset of puberty, our findings show that the total group of GH-treated children born SGA did not start puberty at a younger age compared to normal-statured Dutch children born appropriate for gestational age. Children in the GH/GnRHa group started puberty at a similar age as Dutch references, but relatively early for their actual height which is in line with previous studies (2-6). Age at onset of puberty and at menarche was significantly older in the GH group, although within the normal age range for Dutch references (14), which is in line with previous studies in SGA children (25-27). Given that pubertal development in the GH/GnRHa group was delayed for 2 years by additional GnRHa treatment, comparing age at menarche in the GH/GnRHa group to Dutch references would be inappropriate.

This present study was not designed to investigate pubertal postponement by GnRHa vs no postponement in a randomized design. GnRHa treatment in addition to GH depended on absolute height at start of puberty and adult height prediction. Despite this limitation, our study provides pragmatic data on expectations for GH-treated children born SGA in whom additional GnRHa treatment is contemplated and shows a beneficial effect of GnRHa treatment on height which is consisted with previous studies in other populations (28-30).

GnRHa treatment was well tolerated in all children and no adverse effects were reported. Metabolic health, insulin sensitivity, and β-cell function at AH showed similar results in children treated with combined GH/GnRHa and those treated with GH only (11, 12). In the current study, all girls treated with GnRHa reported regular cycles at AH and one pregnancy after AH with normal offspring was reported. Long-term follow-up in girls with central precocious puberty treated with GnRHa also showed that the interruption of the GnRH axis in childhood did not impair reproductive function in adulthood (21). Definitive conclusions on long-term reproductive function in young women born SGA treated with GnRHa can, however, not be made because long-term follow-up data are not yet available.

In conclusion, when GH-treated SGA children with an AH expectation below -2.5 SDS at onset of puberty are treated with 2 years of additional GnRHa treatment, their pu-

bertal duration after discontinuation of GnRHa treatment is shorter compared with the pubertal duration in children with an AH expectation above -2.5 SDS treated with GH only. Although they are shorter at onset of puberty, adequate growth during 2 years of GnRHa treatment leads to a better total growth from onset of puberty until AH resulting in a similar AH as those treated with GH only.

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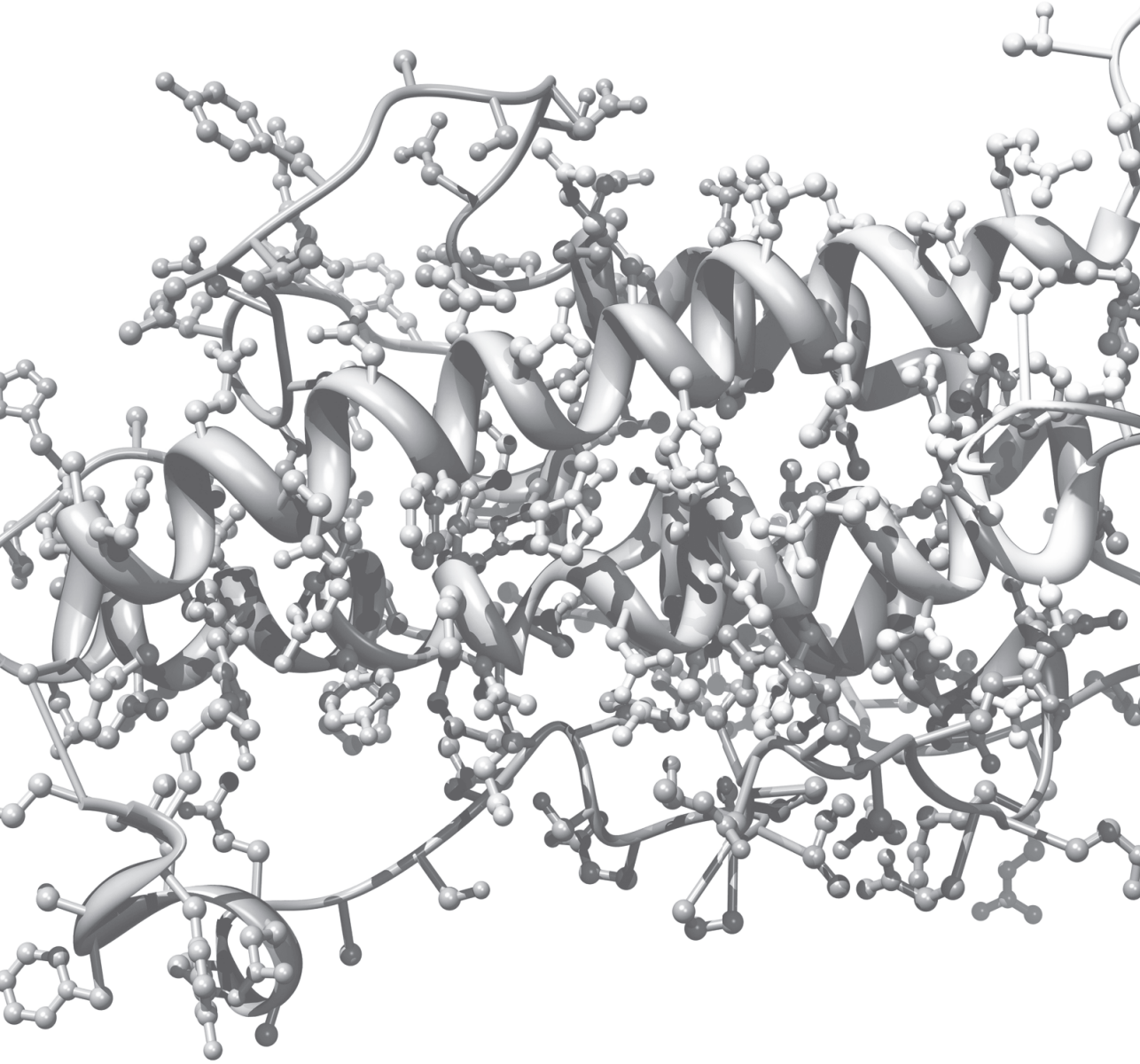
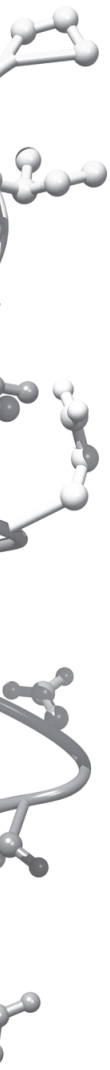


CHAPTER 5



Metabolic health profile in young adults born SGA: A 5-year longitudinal study after cessation of GH treatment

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ABSTRACT

Context Growth hormone (GH) treatment results in a reduction in fat mass (FM) and insulin sensitivity (Si), and an increase in lean body mass (LBM). Only short-term longitudinal changes after cessation of GH treatment in subjects born SGA are available.

Methods 199 previously GH-treated SGA young adults (SGA-GH) were longitudinally followed for 5 years after attainment of adult height: at GH-cessation, and at 6 months, 2 and 5 years thereafter. Data at 5 years after GH-cessation were compared to untreated age-matched controls: 51 untreated short SGA adults (SGA-S), 92 SGA adults with spontaneous catch-up growth (SGA-CU), and 142 adults born appropriate for gestational age (AGA). Body composition was determined by DXA scans. Frequently sampled intravenous glucose tolerance (FSIGT) tests were used to assess Si, acute insulin response (AIR), and β-cell function (DI).

Results FM, trunk and limb fat increased during 5 years after GH-cessation whereas LBM decreased. At 5 years after GH-cessation, FM was higher and LBM lower compared to data at GH-cessation. Si and DI increased and AIR decreased after GH-cessation, but only during the first 6 months. At 5 years after GH-cessation, Si was higher, AIR lower and DI similar to results at GH-cessation. At 5 years after GH-cessation, SGA-GH adults had a similar FM, Si, AIR, and DI, and a lower LBM than untreated SGA-S adults. LBM was lower and FSIGT results similar compared to SGA-CU and AGA adults.

Conclusions This longitudinal study during 5 years after GH treatment shows significant changes in body composition, insulin sensitivity, and β-cell function after GH-cessation, reflecting the loss of GH properties. At 5 years after GH-cessation, FM, insulin sensitivity, and β-cell function of previously GH-treated SGA adults were similar to untreated short SGA adults, indicating that long-term GH treatment in children born SGA has no unfavorable effect on metabolic health in early adulthood.

INTRODUCTION

Approximately 10% of children born small for gestational age (SGA) show insufficient catch-up growth and remain short with a height below -2 SDS (1, 2). Growth hormone (GH) treatment effectively induces catch-up growth and improves adult height (AH) in most short children born SGA (3-6). GH treatment results in a decline in fat mass (FM), an increase in lean body mass (LBM), and a lower insulin sensitivity (7, 8).

Children born SGA have a higher risk to develop diabetes mellitus type 2 in adulthood (9-11) and since GH treatment causes GH-induced insulin resistance (12, 13), concern has been expressed regarding the long-term consequences of GH treatment in children born SGA. French data of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) project suggested that there might be an increased cardiovascular mortality in adults born SGA who were treated with GH during childhood (14). The main limitation of the SAGhE project is, however, the absence of an appropriate control group of untreated SGA patients. To study the effect of GH treatment on the risk of diabetes mellitus type 2, it is important to address the already known increased risk in children born SGA, and compare previously GH-treated subjects born SGA with untreated subjects born SGA.

The primary aim of our study was to investigate the longitudinal changes in body composition and insulin sensitivity after cessation of GH treatment. We, therefore, evaluated body composition and frequently sampled intravenous glucose tolerance test results (FSIGT results) during 5 years after cessation of GH treatment in young adults born SGA. We hypothesized that after cessation of GH treatment, body composition would change in line with the loss of GH properties, i.e. an increase in FM and a decrease in LBM, and that GH-induced insulin resistance would be reversible. We compared the data at 5 years after cessation of GH treatment, to young adults born SGA with persistent short stature who were never treated with GH (SGA-S subjects) to assess the effect of GH treatment during childhood on body composition and insulin sensitivity in early adulthood. We hypothesized that body composition and FSIGT results of previously GH-treated SGA adults would return to levels of untreated SGA-S adults. To evaluate whether GH-induced catch-up growth had a similar effect on adult body composition as spontaneous catch-up growth, we additionally compared the data at 5 years after cessation of GH treatment to young adults born SGA with spontaneous catch-up to a normal stature (SGA-CU subjects).

METHODS

Subjects

The total study group comprised 484 young adults (265 females, 54.8%) of which 199 young adults born SGA (birth weight and/or birth length <-2 SDS) had participated in

a GH trial (103 females, 51.8%). These children started GH treatment when prepubertal, aged 5 to 8 years, with a height SDS below -2.5 and no growth failure caused by other disorders. Children with GH-deficiency (defined as a maximum serum GH <20 mU/l during a GH stimulation test) were excluded. GH 1 mg/m^2/day (~0.033 mg/kg/day) was given daily sc at bedtime (r-hGH Norditropin; Novo Nordisk A/S, Bagsværd, Denmark). Every 3 months, the GH dose was adjusted to the calculated body surface area. GH treatment was discontinued at attainment of AH, defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months and bone age was ≥15 years for girls and ≥16.5 years for boys, or near AH, defined as height velocity between 0.5 and 2 cm during the last 6 months and adult pubertal stage.

At GH-cessation, GH-treated young adults were invited to participate in the current follow-up study evaluating metabolic health at AH while still on GH treatment, and at 6 months, 2 and 5 years after cessation of GH treatment. Some SGA-GH subjects did not participate at every study moment, due to the time-consuming aspect, and some participants had not yet discontinued GH treatment for 2 or 5 years. Data at 5 years after GH-cessation were compared with those of 285 participants of an age-matched healthy young adult study cohort, aged 18 to 24 years: 51 untreated young adults born SGA (birth length <-2 SDS) with persistent short stature (<-2 SDS) (SGA-S), 92 young adults born SGA (birth length <-2 SDS) with catch-up growth and a normal stature (>-1 SDS) (SGA-CU), and 142 young adults born appropriate for gestational age (birth length >-1 SDS) with a normal stature (>-1 SDS) (AGA) (15, 16). SGA-S and SGA-CU young adults were randomly recruited from several hospitals in the Netherlands, where they had been registered because of their small birth size (birth length <-2 SDS) with or without short stature (<-2 SDS). In addition, healthy young adults from schools of different educational levels were randomly asked to participate as AGA controls.

The medical ethics committee of the Erasmus University Medical Centre Rotterdam approved the studies. Written informed consent was obtained from all subjects and, if they were younger than 18 years at cessation of GH treatment, also from their parents or custodians.

Measurements

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd) and weight to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior). Height and weight were expressed as SDS adjusted for chronological age and gender according to Dutch references (17), using Growth Analyzer Research Calculation Tools (https://growthanalyser.org). AH SDS at 5 years after cessation of GH treatment was calculated using references for Dutch adults (21 years) (17).

Body composition

Body composition was measured by one dual-energy X-ray absorptiometry (DXA) scan (Lunar Prodigy, GE Healthcare). Quality assurance was performed daily. For this type of DXA, the intra-assay coefficient of variation has been reported as 0.41-0.88% for fat tissue and 1.57-4.49% for lean body mass (LBM) (18). Total fat mass (FM), LBM, trunk fat, and limb fat were determined.

Insulin sensitivity and β-cell function

Glucose homeostasis was assessed by FSIGT tests with Tolbutamide after an overnight fast (19). Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD Millennium software (20). Si quantifies the capacity of insulin to stimulate glucose disposal and Sg reflects the capacity of glucose to mediate its own disposal. AIR is an estimate of insulin secretory capacity, measured as the area under the curve from 0 to 10 minutes corrected for baseline insulin levels. The DI equals AIR x Si and indicates the β-cell function.

Assays

Fasting glucose levels were determined on an Architect ci8200 system (Abbott). Fasting insulin levels were measured by IRMA (Medgenix, Biosource Europe) with an intra-assay coefficient of variation of 2.1% to 1.5% (6.6–53.3 milligram equivalents [mE]/l) and interassay coefficient of variation 6.5% to 6.1% (14.4–100.4 mE/l). Serum levels of total insulin-like growth factor-I (IGF-I) was expressed as SDS adjusting for age and gender, using reference values for healthy children with normal stature determined in the same laboratory (21).

Statistics

Statistical analyses were performed using SPSS version 23. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics are presented as mean (SD). ANOVA was used to determine differences between subgroups. Because of a skewed distribution, Si, Sg, AIR, and DI were log-transformed. Longitudinal changes in body composition and FSIGT results were analyzed using repeated measurements analysis with an unstructured covariance type. Correlations between IGF-I SDS and FM were determined using Pearson's correlation test. ANCOVA was used for adjusted comparisons between the groups at the age of 21 years. Results were regarded as statistically significant at $P<0.05$.

RESULTS

Clinical characteristics

Table 1 shows the clinical characteristics of the previously GH-treated SGA (SGA-GH) adults at various time points. Mean age at start of GH treatment had been 6.4 (2.0) years and mean GH treatment duration 10.0 (2.3) years. At 5 years after cessation of GH treatment, mean age was 21.3 (1.5) years which was similar to untreated SGA-S, SGA-CU, and AGA adults. AH SDS was -1.6 (0.9) SDS, which was significantly higher in SGA-GH adults compared to SGA-S adults (P<0.001) but significantly lower than in SGA-CU (P<0.001) and AGA adults (P<0.001).

Table 1. Clinical characteristics

	SGA-GH	SGA-S	SGA-CU	AGA
N	199	51	92	142
Sex (male/female)	96 / 103	18 / 33	38 / 54	67 / 75
Gestational age, weeks	36.0 (3.8)	38.1 (3.1) [a]	36.0 (3.4)	36.3 (4.1)
Birth length SDS	-3.2 (1.6)	-3.0 (0.9)	-3.0 (0.8)	0.2 (0.8) [b]
Birth weight SDS	-2.3 (1.2)	-2.1 (0.9)	-2.3 (0.8)	0.3 (1.2) [b]
At start of GH treatment				
Age, years	6.4 (2.0)	N/A	N/A	N/A
Height SDS	-3.0 (0.6)	N/A	N/A	N/A
Weight for Height SDS	-1.5 (1.2)	N/A	N/A	N/A
At adult height				
Age, years	16.4 (1.3)	N/A	N/A	N/A
Height SDS	-1.2 (0.8)	N/A	N/A	N/A
Weight for Height SDS	-0.7 (1.1)	N/A	N/A	N/A
GH duration, years	10.0 (2.3)	N/A	N/A	N/A
At age 21 years (5 years after GH)				
Sex (male/female)	36 / 52	18 / 33	38 / 54	67 / 75
Age, years	21.3 (1.5)	20.9 (1.8)	20.7 (1.7)	20.9 (1.7)
Adult height SDS (21 years)	-1.6 (0.9) [b]	-2.6 (0.5) [b]	-0.2 (0.7) [c]	0.1 (0.8)
Weight for Height SDS	-0.2 (1.4) [c]	0.2 (1.4)	0.2 (1.2)	0.3 (1.0)

Abbreviations: SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age; N/A, not applicable.

Data are expressed as mean (SD).

[a] P<0.02 compared with the other groups.

[b] P<0.001 compared with the other groups.

[c] P≤0.01 compared with AGA.

Longitudinal changes after GH-cessation in SGA-GH adults

Longitudinal changes in body composition

Figure 1 shows the longitudinal changes in body composition after cessation of GH in the SGA-GH adults, expressed as estimated marginal means. During 6 months after GH-

cessation, FM, trunk fat, and limb fat increased significantly (all P<0.001). From 6 months until 2 years and from 2 years until 5 years after GH-cessation, FM, trunk fat, and limb fat showed a constant increase (all P<0.001). At 5 years after GH-cessation, FM, trunk fat, and limb fat were significantly higher than at GH-cessation (all P<0.001). Additional adjustment for age did not change these results.

During 6 months after GH-cessation, LBM declined (P<0.001) and remained similar thereafter, resulting in a lower LBM at 5 years after GH-cessation compared to LBM at GH-cessation. After additional adjustment for age, LBM steadily declined during 5 years after GH-cessation and was lower at 5 years after GH-cessation compared to LBM at GH-cessation (P<0.001).

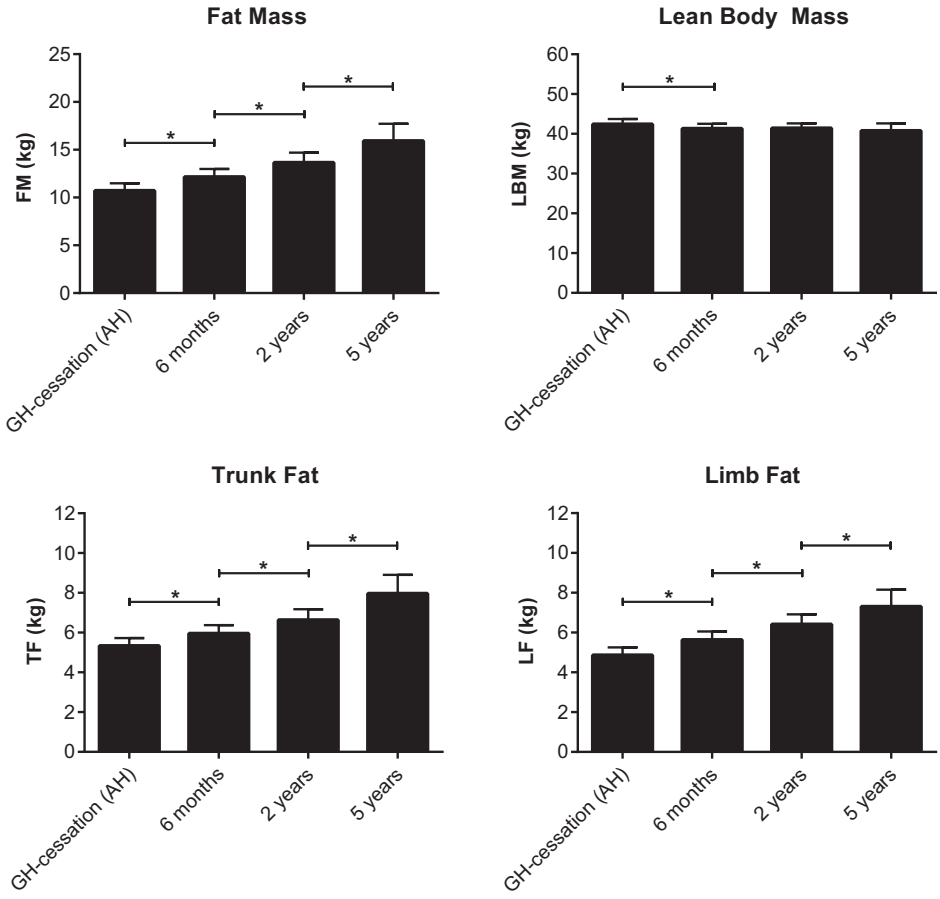

Figure 1. Longitudinal changes of body composition parameters.
Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval.
* P<0.001.

Longitudinal changes in serum IGF-I levels

During 6 months after cessation of GH treatment, IGF-I SDS decreased from 1.2 SDS to -0.1 SDS (P<0.001) and remained similar thereafter. No significant correlations were found between serum IGF-I SDS and the amount of FM at GH-cessation, and at 6 months and 2 and 5 years after GH-cessation.

Longitudinal changes in insulin sensitivity and β-cell function measured by FSIGT

Figure 2 shows the longitudinal changes in Si, Sg, AIR, and DI measured by FSIGT after GH-cessation in the SGA-GH adults, expressed as estimated marginal means. During 6 months after GH-cessation, Si, Sg, and DI increased significantly (P<0.001, P=0.002

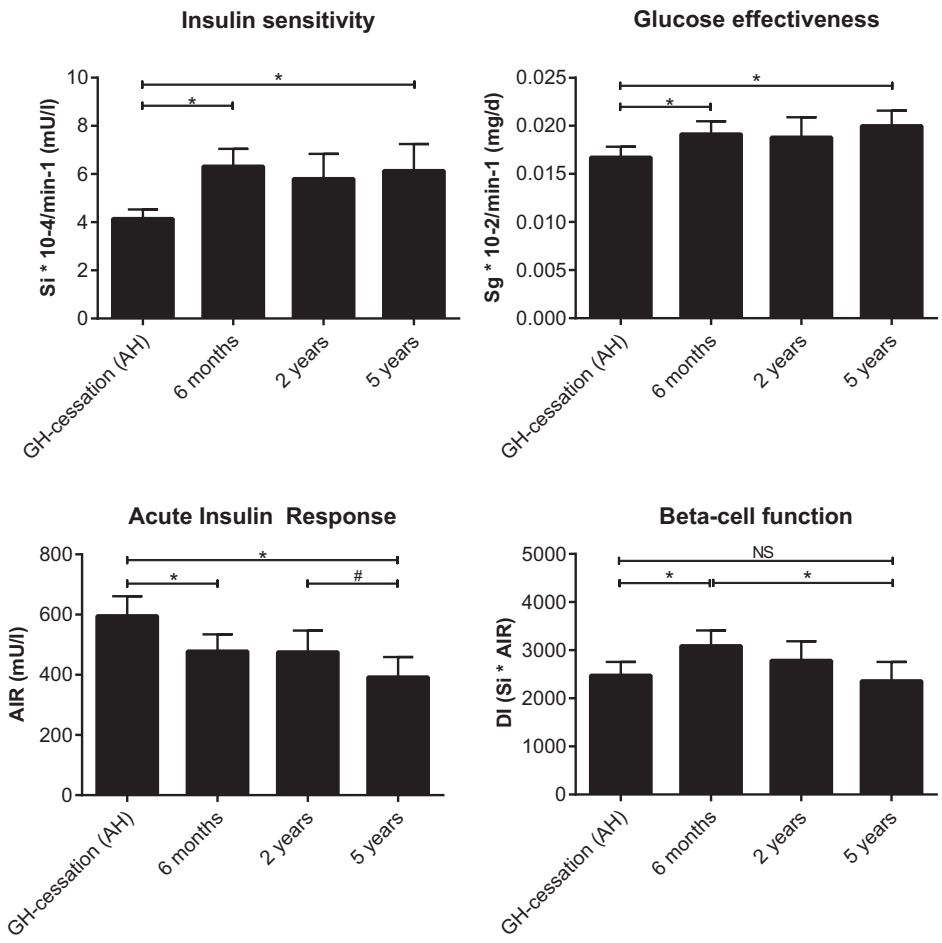

Figure 2. Longitudinal changes of FSIGT results.
Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval.
NS; not statistically significant. * P<0.005, # P<0.05.

and P<0.001, respectively). From 6 months until 2 years and from 2 years until 5 years after GH-cessation, Si, Sg, and DI did not significantly change. AIR decreased during 6 months after GH-cessation (P<0.001), remained similar from 6 months until 2 years and decreased again from 2 years until 5 years after GH-cessation (P=0.033). At 5 years after GH-cessation, Si and Sg were significantly higher (both P<0.001) and AIR was significantly lower (P<0.001) than levels at GH-cessation, whereas DI, the proxy for β-cell function, was similar to DI at GH-cessation. Additional adjustment for FM and age did not change these results.

Comparison between SGA-GH adults and untreated SGA-S, SGA-CU, and AGA adults

Body composition

Since the SGA-GH, SGA-S, SGA-CU, and AGA groups differed in male/female ratio and height, which are important factors for body composition, the comparisons of body composition between groups were adjusted for these factors (Figure 3). At 5 years after

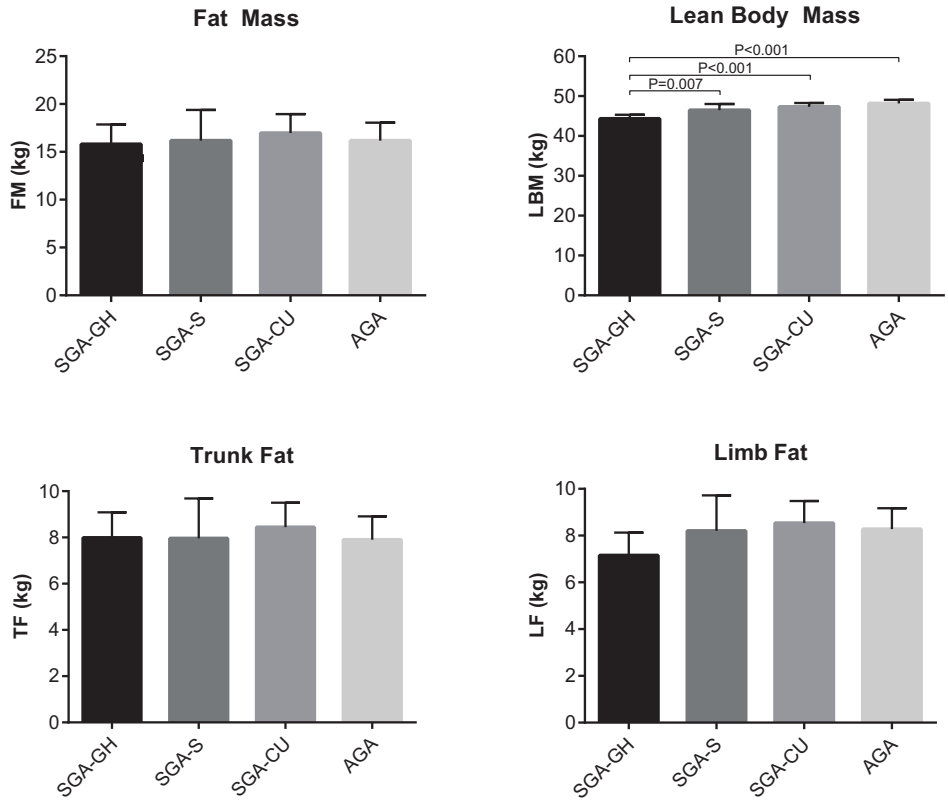

Figure 3. Comparison of body composition between groups.
Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for height and gender. Data of SGA-GH adults are data at 5 years after GH-cessation.

GH-cessation, FM and fat distribution (trunk FM and limb FM) were similar in SGA-GH, SGA-S, SGA-CU, and AGA adults. SGA-GH adults had the lowest LBM (P=0.007 compared to SGA-S, P<0.001 compared to SGA-CU and AGA).

Insulin sensitivity and β-cell function

Because the SGA-GH, SGA-S, SGA-CU, and AGA groups differed in male/female ratio, comparisons in FSIGT results were adjusted for gender. At 5 years after GH-cessation, SGA-GH adults had a similar Si, Sg, AIR, and DI as untreated SGA-S adults (Figure 4). SGA-GH adults had a similar Si, AIR, and DI as SGA-CU and AGA adults, but a higher Sg than SGA-CU and AGA adults (P=0.003 and P=0.018, respectively). SGA-CU adults had the lowest Si and Sg levels with Si being lower compared to AGA (P=0.006) and Sg being lower compared to SGA-GH (P=0.003).

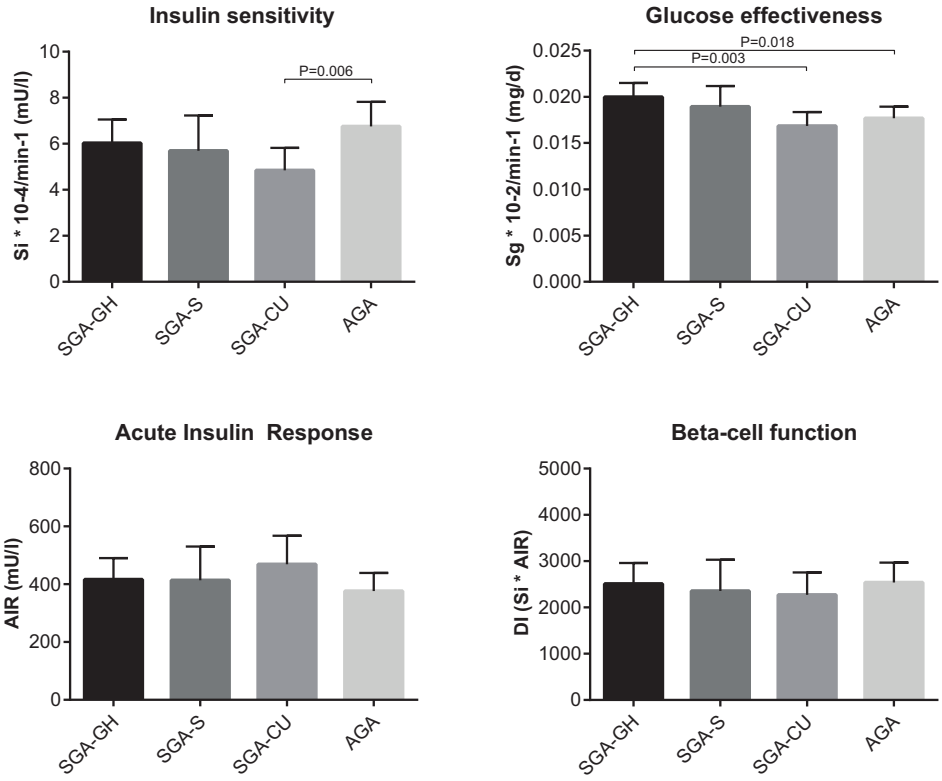

Figure 4. Comparison of FSIGT results between groups.
Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for gender. Data of SGA-GH adults are data at 5 years after GH-cessation.

DISCUSSION

Our longitudinal study during 5 years after cessation of GH treatment is currently the longest and largest follow-up study in 199 GH-treated young adults born SGA. It has been reported that adults treated with GH during childhood for isolated short stature have an increased cardiovascular mortality, raising concerns about the long-term safety of GH treatment (14). We now show that cessation of GH treatment has significant effects on body composition, insulin sensitivity, and β-cell function, reflecting the loss of GH properties. The GH-induced insulin resistance was reversible after cessation of GH treatment, despite the persistent increase in FM. At 5 years after GH-cessation, body composition, insulin sensitivity, and β-cell function of previously GH-treated young adults born SGA were similar to untreated age-matched adults born SGA, with or without catch-up growth, and to those born AGA, except for LBM which was lower in the previously GH-treated SGA adults.

After cessation of GH treatment, FM increased and LBM decreased, which is contrary to the changes that occur during GH treatment. The increase in FM was neither due to increasing age over time nor to lower serum IGF-I levels, indicating that changes reflect the loss of the lipolytic characteristics of GH. Despite the significant increase in FM after cessation of GH, FM at 5 years after GH-cessation, adjusted for gender and height, was similar in previously GH-treated SGA adults and untreated SGA-S, SGA-CU, and AGA adults of similar age. We, therefore, anticipate that the future development of body composition in previously GH-treated SGA adults will be similar to that of untreated short adults born SGA.

During 6 months after cessation of GH treatment, Si, Sg, AIR, and DI (ß-cell function) improved and sustained thereafter, despite the increase in FM. Since Si has a strong correlation with FM, this indicates that the beneficial effect of GH-cessation on Si is greater than the opposite effect of gaining more FM. At 5 years after GH-cessation, Si was higher and AIR was lower compared to levels at GH-cessation, suggesting that GH-induced insulin resistance and increased insulin secretion were fully reversed. Beta-cell function at 5 years after GH-cessation was similar to β-cell function at GH-cessation, which is reassuring since particularly a reduction in ß-cell function relates to an increased risk for diabetes mellitus type 2. We used the FSIGT test with Tolbutamide which is a gold standard for measuring Si and β-cell function like the euglycemic-hyperinsulinemic clamp (22). Because the FSIGT test is more invasive, labor-intensive, and costly than other measurements of Si, such as the oral glucose tolerance test and HOMA-IR, no other studies have performed FSIGT tests in such high numbers of SGA subjects after cessation of GH treatment, making our data unique.

There are no longitudinal data on body composition and insulin sensitivity after cessation of GH treatment, besides our preliminary results in 48 adolescents born SGA during only 6 months after GH-cessation, which showed similar changes (23). We now show that during

the subsequent 5 years after GH-cessation, FM steadily increases and AIR further declines. At 5 years after GH-cessation, FM, Si, Sg, AIR, and DI are similar in previously GH-treated SGA adults and untreated SGA-S adults, indicating that GH treatment does not impair insulin sensitivity on the long-term. We previously showed in another, considerably smaller group of GH-treated SGA subjects, that Si and β-cell function at 6.5 years after GH treatment were similar to untreated SGA-S subjects but there was no comparison with SGA-CU and AGA subjects (24). In the present study, FM, LBM, Si, Sg, AIR, and DI of previously GH-treated SGA adults were also compared to untreated SGA-CU adults to evaluate whether GH-induced catch-up growth had a similar effect as spontaneous catch-up growth. FM was similar but SGA-CU adults had the lowest Si and Sg levels which is consistent with previous findings that accelerated catch-up in weight for length in early life is associated with a higher risk for an unfavorable health profile in adulthood (16, 25-28).

A previous study reported that adults treated with GH during childhood for isolated short stature have an increased cardiovascular mortality but it is unknown whether severely GH-deficient adults in that study were treated with GH into adulthood or stopped GH treatment at AH (14). Other studies reported no deaths due to cardiovascular disease or cancer and indicated that patients without risk factors for cardiovascular disease and malignancies can expect an uneventful course after treatment (29, 30). A limitation of these previous studies was that data of previously GH-treated SGA adults were compared with national reference values and not with a control group of untreated SGA patients. To study the effect of GH treatment on the risk of diabetes mellitus type 2 and cardiovascular diseases, it is important to address the already known increased risk for these diseases in children born SGA, and compare GH-treated adults born SGA with untreated subjects born SGA instead of only with subjects born AGA. Our study now provides these important and long-awaited data which are of interest to healthcare practitioners worldwide.

In conclusion, our follow-up study during 5 years after cessation of GH treatment shows significant longitudinal changes in body composition, insulin sensitivity, and β-cell function reflecting the loss of GH properties. The steady increase in FM during 5 years after GH treatment indicates the loss of GH properties. Reassuringly, the GH-induced changes in insulin sensitivity and β-cell function were fully reversed within 6 months after cessation of GH treatment, despite the increase in FM. At 5 years after GH-cessation, FM, insulin sensitivity, and β-cell function of previously GH-treated SGA adults were comparable to untreated short SGA adults, indicating that long-term GH treatment of SGA children with short stature does not have an unfavorable effect on body composition, insulin sensitivity, and β-cell function in young adulthood.

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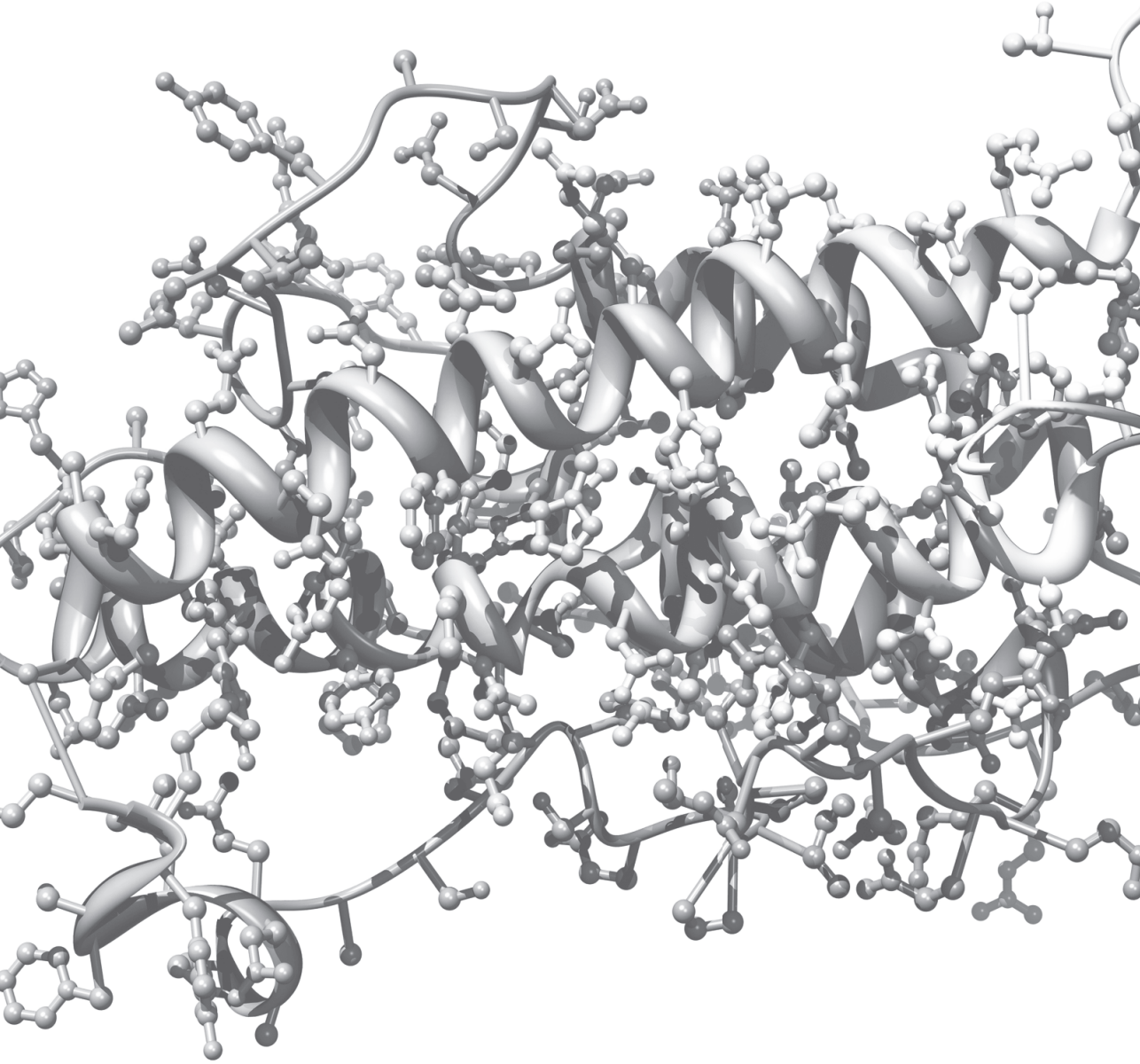
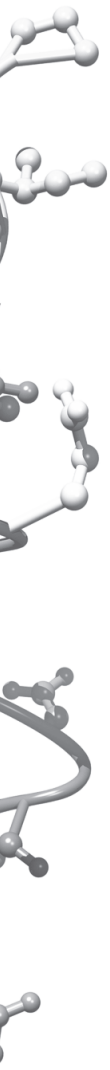


CHAPTER 6



A 5-year longitudinal study after GH cessation on cardiovascular risk factors and cIMT in young adults born SGA

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Submitted

ABSTRACT

Context Growth hormone (GH) treatment results in a decline in blood pressure and lipid levels. Five year longitudinal changes in cardiovascular risk factors and carotid Intima Media Thickness (cIMT) after cessation of GH treatment in subjects born small for gestational age (SGA) are unknown.

Objective To assess longitudinal changes in systolic and diastolic blood pressure (SBP and DBP), fasting lipid levels and cIMT in previously GH-treated young adults born SGA (SGA-GH). To compare SGA-GH subjects at 5 years after GH-cessation to untreated age-matched controls.

Methods 199 SGA-GH adults were longitudinally followed for 5 years after attainment of adult height: at GH-cessation, and at 6 months, 2 and 5 years thereafter. Data at 5 years after GH-cessation were compared to: 51 untreated short SGA adults (SGA-S), 92 SGA adults with spontaneous catch-up growth (SGA-CU), and 142 adults born appropriate for gestational age (AGA).

Results GH-cessation resulted in an increase in SBP and DBP, but only during the first 6 months. SBP and DBP recovered thereafter and were similar at 5 years after GH-cessation compared to levels at GH-cessation. At 5 years after GH-cessation, SGA-GH adults had a similar SBP and DBP as untreated SGA-S adults. Lipid levels increased after GH-cessation in parallel to the increase in fat mass, but remained lower than levels in SGA-S adults. GH-cessation had no effects on cIMT levels and at 5 years after GH-cessation, SGA-GH adults had a similar cIMT as SGA-S adults. SBP, DBP, lipid levels, and cIMT of SGA-GH adults were similar to SGA-CU and AGA adults.

Conclusion This longitudinal study during 5 years after GH-cessation shows that long-term GH treatment in children born SGA does not only improve adult height but has also no unfavorable effects on cardiovascular health in early adulthood and might be beneficial with regard to the lipid profile.

INTRODUCTION

Approximately 10% of children born small for gestational age (SGA) show insufficient catch-up growth and remain short with a height below -2 SDS (1, 2). Growth hormone (GH) treatment effectively induces catch-up growth and increases adult height (AH) in short children born SGA (3-6).

Low birth weight is associated with an increased risk for cardiovascular diseases (CVD) (7). Preliminary data of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) project showed that adults who were treated with GH during childhood for isolated short stature have an increased mortality rate due to cardiovascular disorders(8). Other studies reported no deaths due to CVD (9). A limitation of these studies is the lack of longitudinal data after cessation of GH treatment, and a comparison with an appropriate control group of untreated SGA patients.

The primary aim of the present study was, therefore, to investigate longitudinal changes of cardiovascular risk factors in young adults born SGA during 5 years after cessation of GH treatment. We investigated changes in blood pressure, lipid levels, and carotid intima-media thickness (cIMT) since these are accurate determinants of cardiovascular events in later life (10-14). We hypothesized that after cessation of GH treatment, blood pressure and lipid levels would increase during 6 months, in line with the loss of GH properties, but would remain unchanged during the following years. Our secondary aim was to assess the effect of GH treatment during childhood on blood pressure, lipid levels, and cIMT in early adulthood and we, therefore, compared data of previously GH-treated young adults born SGA (SGA-GH) at 5 years after cessation of GH treatment to age-matched young adults born SGA with persistent short stature who were never treated with GH (SGA-S subjects). We hypothesized that cardiovascular risk factors of previously GH-treated SGA adults would be similar to levels of untreated SGA-S adults. To evaluate whether GH-induced catch-up growth has a similar effect on cardiovascular risk factors as spontaneous catch-up growth, we additionally compared the data at 5 years after cessation of GH treatment to untreated young adults born SGA with spontaneous catch-up to a normal stature (SGA-CU subjects) and with healthy controls born appropriate for gestational age (AGA subjects).

METHODS

Subjects

The total study group comprised 484 young adults (265 females, 54.8%) of which 199 young adults born SGA (birth weight and/or birth length <-2 SDS) had participated in a GH trial (103 females, 51.8%). At start of GH treatment, children were prepubertal, aged 5-8 years, with a height SDS below -2.5 and no growth failure caused by other disorders. Children with GH-deficiency (defined as a maximum serum GH <20 mU/l during a GH

stimulation test) were excluded. GH 1 mg/m^2/day (~0.033 mg/kg/day) was given daily sc at bedtime (r-hGH Norditropin; Novo Nordisk A/S, Bagsværd, Denmark). Every 3 months, the GH dose was adjusted to the calculated body surface area. GH treatment was discontinued at attainment of AH, defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months and bone age was ≥15 years for girls and ≥16.5 years for boys, or near AH, defined as height velocity between 0.5 and 2 cm during the last 6 months and adult pubertal stage.

At GH-cessation, GH-treated young adults were invited to participate in the current follow-up study evaluating cardiovascular risk factors at AH while still on GH treatment, and at 6 months, 2 and 5 years after cessation of GH treatment. Some SGA-GH subjects did not participate at every study moment, due to the time-consuming aspect, and some participants had not yet discontinued GH treatment for 2 or 5 years. cIMT measurements were added to the follow-up protocol for SGA-GH participants at a later stage and therefore numbers for cIMT measurements were lower than for blood pressure and lipid levels (N=70 at GH-cessation, N=60 at 6 months, N=67 at 2 years, and N=57 at 5 years after GH-cessation).

At 5 years after cessation of GH treatment, data of 88 SGA-GH young adults were compared with those of 285 participants of an age-matched healthy young adult study cohort (15, 16): 51 untreated young adults born SGA (birth length <-2 SDS) with persistent short stature (<-2 SDS) (SGA-S), 92 young adults born SGA (birth length <-2 SDS) with catch-up growth and a normal stature (>-1 SDS) (SGA-CU), and 142 young adults born appropriate for gestational age (birth length >-1 SDS) with a normal stature (>-1 SDS) (AGA) (17). SGA-S and SGA-CU young adults were randomly recruited from several hospitals in the Netherlands, where they had been registered because of their small birth size (birth length <-2 SDS) with or without short stature (<-2 SDS). In addition, healthy young adults from schools of different educational levels were randomly asked to participate as AGA controls.

The medical ethics committee of the Erasmus University Medical Centre Rotterdam approved the studies. Written informed consent was obtained from all subjects and, if they were younger than 18 years at cessation of GH treatment, also from their parents or custodians.

Measurements

All participants fasted for 12 hours and abstained from smoking and alcohol for 16 hours. Height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd) and weight to the nearest 0.1 kg on a digital scale (Servo Balance KA-20-150S, Servo Berkel Prior). Height and weight were expressed as SDS adjusted for chronological age and gender according to Dutch references (18), using Growth Analyzer Research Calculation Tools (https://growthanalyser.org). Waist circumference was measured in standing

position by using a nonextensible tape at the midpoint between the iliac crest and the last rib. The mean of two measurements was used for analyses. All participants filled out a questionnaire on alcohol use and smoking.

Blood pressure

Brachial diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured in the supine position after 10 minutes of rest using the non-dominant arm with an automatic device (Accutorr Plus, Datascope Corp.) every 5 minutes for 1 hour and the mean of these 13 measurements was taken to reflect resting blood pressure. Blood pressure was expressed in SDS using sex- and age-matched reference values (19).

carotid Intima Media Thickness (cIMT)

Carotid ultrasonography of both the left and right carotid artery was performed in participants in the supine position with the head tilted slightly to the contralateral side, using the same 7.5 MHz linear array transducer. A longitudinal 1 cm segment of the common carotid artery, proximal to the bifurcation, was identified for measurements and images were transferred to a computer. We used the B-mode of Art Lab, a special intima-media thickness assessment program, to measure the intima media thickness. This measurement was repeated 6 times for each common carotid artery and the mean was calculated. Since cIMT is influenced by gender, height, and gestational age, cIMT analyses were adjusted for these characteristics when comparing SGA-GH adults to untreated control groups.

Assays

Fasting levels of total cholesterol, triglyceride (TG) and high-density lipoprotein (HDLc) were measured. Total cholesterol and TG were determined using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit, respectively (Roche Diagnostics). HDLc was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). The intra-assay variations of measurements of total cholesterol, TG and HDLc were 2.9, 3.3 and 3.9%. Low-density lipoprotein (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = TC – HDLc – 0.45 * TG (20).

Metabolic syndrome

At 5 years after cessation of GH treatment, the various components of metabolic syndrome were assessed in all participants. Revised criteria of the National Cholesterol Education Program (NCEP, Adult Treatment Panel III (ATP III)) were used to determine the presence of metabolic syndrome, defined as having three or more of the following risk factors: 1) abdominal obesity: waist circumference in men >102 cm, in women >88 cm; 2) raised TG levels: ≥1.7 mmol/l; 3) reduced HDLc levels: in men ≤1.03, in women ≤1.3

mmol/l; 4) high blood pressure: ≥130/ ≥85 mm Hg; 5) increased fasting glucose: ≥5.6 mmol/l (21-23).

Statistics

Statistical analyses were performed using SPSS version 23. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics are presented as mean (SD). ANOVA was used to determine differences between subgroups. Because of a skewed distribution, cIMT and lipid levels were log-transformed. Longitudinal changes were analyzed using repeated measurements analysis with an unstructured repeated covariance type. Longitudinal changes were adjusted for age. ANCOVA was used for adjusted comparisons between the groups at the age of 21 years, i.e. 5 years after cessation of GH treatment in the SGA-GH young adults. Comparison of the components of metabolic syndrome was performed using Fisher's exact test. Results were regarded as statistically significant at $P<0.05$.

RESULTS

Clinical characteristics of SGA-GH subjects

Table 1 shows the clinical characteristics of 199 SGA-GH participants at start of GH treatment and at attainment of AH when GH treatment was discontinued and the follow-up study started. Mean age at start of GH treatment had been 6.4 (2.1) years and mean GH treatment duration 10.0 (2.3) years. At 5 years after GH-cessation, mean age was 21.3 (1.5) years. The percentage of participants who smoked increased after cessation of GH treatment; 17.1% at AH, 18.9% at 6 months after GH-cessation, 22.6% at 2 years after GH-cessation and 30.1% at 5 years after GH-cessation.

Longitudinal changes after GH-cessation in SGA-GH adults

Blood pressure

Figure 1A shows the longitudinal changes in SBP and DBP after cessation of GH in the SGA-GH adults, adjusted for age. SBP and DBP increased during 6 months after GH-cessation (both $P<0.001$) and decreased in the 18 months thereafter (both $P<0.001$). From 2 until 5 years after GH-cessation, SBP and DBP remained similar. At 5 years after GH-cessation, SBP and DBP were similar compared to SBP and DBP at GH-cessation. Additional adjustment for percentage of smokers did not change these results.

Mean SBP and DBP SDS remained within the normal range, being 0.17 (0.8) SDS and 0.02 (0.6) SDS (respectively) at GH-cessation, and 0.32 (1.0) SDS and 0.24 (0.7) SDS (respectively) at 5 years after GH-cessation.

Table 1. Clinical characteristics of SGA-GH participants

	At start of GH treatment	At adult height, Start of follow-up study
N	199	199
Sex (male/female)	96 / 103	96 / 103
Age, years	6.4 (2.1)	16.4 (1.3)
GH treatment duration, years	N/A	10.0 (2.3)
Height SDS	-3.0 (0.6) [a]	-1.2 (0.8) [a,c]
Weight for Height SDS	-1.5 (1.2) [a]	-0.7 (1.1) [a]
Body Mass Index	14.0 (1.3)	20.2 (2.4)
Body Mass Index SDS	-1.4 (1.1) [a]	-0.2 (1.0) [b]
Systolic blood pressure, mmHg	103.2 (12.1)	111.1 (9.4)
Diastolic blood pressure, mmHg	58.7 (9.0)	62.0 (6.1)
Systolic blood pressure SDS	0.7 (1.1) [a]	0.17 (0.8) [b]
Diastolic blood pressure SDS	-0.04 (1.0)	0.02 (0.6)
Cholesterol, mmol/l	4.15 (1.2)	3.96 (1.2)
LDLc, mmol/l	2.29 (1.4)	2.02 (1.4)
HDLc, mmol/l	1.36 (1.3)	1.41 (1.3)
TG, mmol/l	0.85 (1.6)	0.92 (1.5)
IGF-I SDS	-0.5 (1.2) [a]	1.2 (0.8) [a]

Abbreviations: N/A, not applicable. Data are expressed as mean (SD). Systolic and diastolic blood pressure were expressed in SDS, using sex- and age-matched reference values (19).
[a] P<0.001 compared with zero SDS.
[b] P<0.02 compared with zero SDS.
[c] P<0.001 compared with -2.0 SDS.

Lipid levels

Figure 1B shows the longitudinal changes in lipid levels after cessation of GH in the SGA-GH adults, adjusted for age. During 6 months after GH-cessation, total cholesterol remained similar, LDLc increased (P=0.016), and TG decreased (P<0.001). In the subsequent 18 months, cholesterol, LDLc, and TG increased (P=0.028, P=0.007, and P=0.006, respectively). From 2 years until 5 years after GH-cessation, cholesterol and LDLc remained similar, and TG increased (P=0.022). HDLc did not significantly change after cessation of GH treatment. At 5 years after GH-cessation, all lipid levels were higher compared to levels at GH-cessation but not significantly (P-values between 0.09 and 0.21).

Pearson correlation test showed that fat mass was a positive determinant for levels of cholesterol, LDLc, and TG (all P<0.001). After additional adjustment for fat mass, the increase in cholesterol, LDLc, and TG from 6 months until 2 years after GH-cessation disappeared.

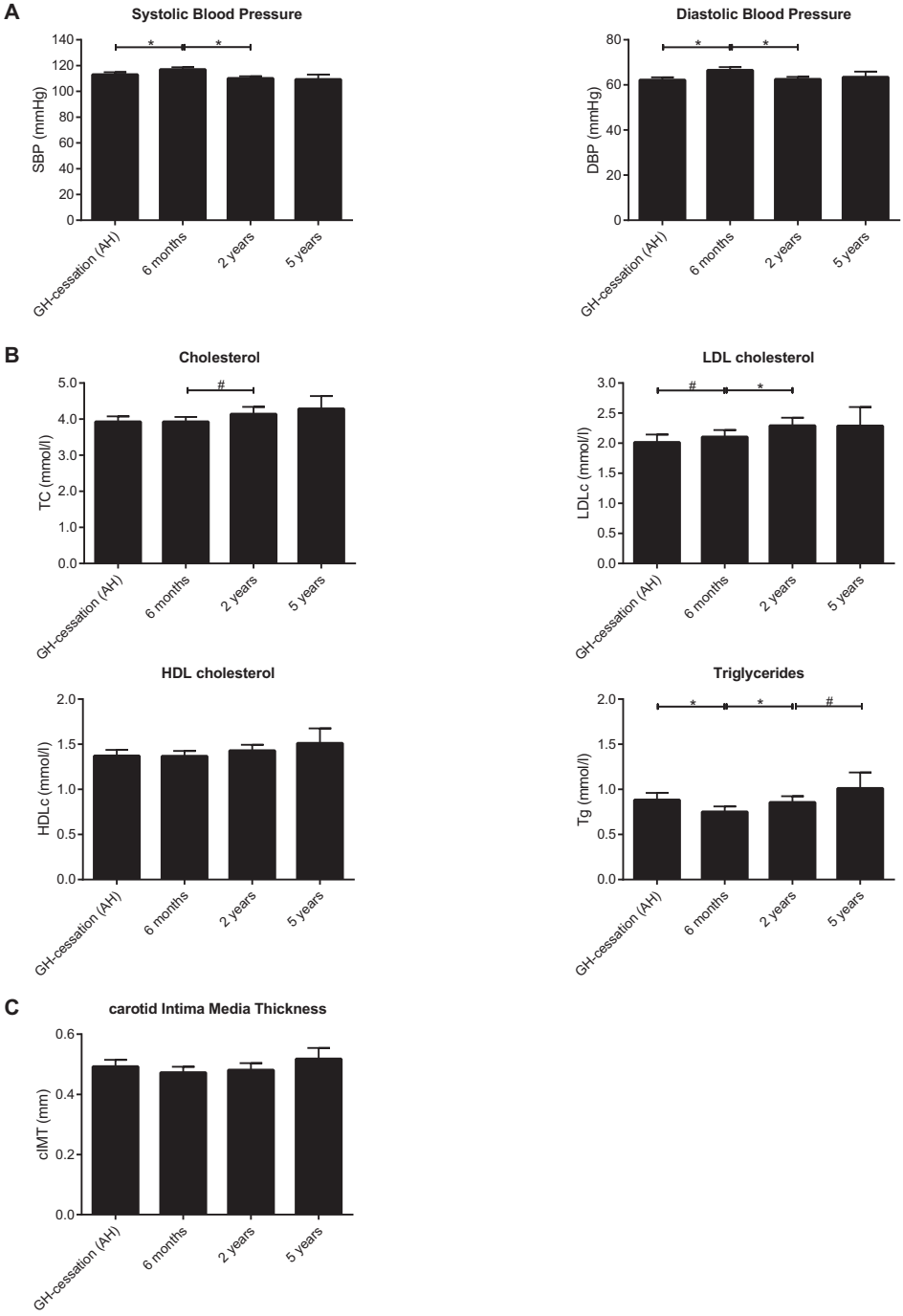

Figure 1. Longitudinal changes after cessation of GH treatment.
Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for age. * P<0.010, # P<0.05.

cIMT

Figure 1C shows the longitudinal changes in cIMT after cessation of GH in the SGA-GH adults, adjusted for age. During 6 months after GH-cessation, and from 6 months until 2 years and 2 years until 5 years after GH, cIMT did not significantly change. cIMT at 5 years after GH-cessation was similar to cIMT at GH-cessation. Additional adjustment for smoking did not change these results.

Comparison between SGA-GH adults and untreated SGA-S, SGA-CU, and AGA adults

At 5 years after cessation of GH, data of SGA-GH adults were compared to untreated controls. Mean age of the SGA-GH adults was 21.3 (1.5) years which was similar to untreated SGA-S, SGA-CU and AGA adults (Table 2). Adult height SDS of the SGA-GH adults was -1.6 (0.9) SDS which was significantly higher compared to SGA-S adults (P<0.001) but significantly lower than in SGA-CU (P<0.001) and AGA adults (P<0.001). The percentage of participants who smoked was similar between the groups. The SGA-CU adults smoked the most cigarettes per day (15 cigarettes), but this was only significantly different compared to AGA adults (7 cigarettes per day, P=0.004).

Table 2. Clinical characteristics of the total study group

	SGA-GH	SGA-S	SGA-CU	AGA
N	88	51	92	142
Sex (male / female)	36 / 52	18 / 33	38 / 54	67 / 75
Gestational age, weeks	36.1 (3.7)	38.1 (3.1) [a]	35.9 (3.5)	36.3 (4.1)
Birth length SDS	-3.3 (1.6)	-3.0 (0.9)	-2.9 (0.8)	0.2 (0.8) [b]
Birth weight SDS	-2.4 (1.2)	-2.1 (0.9)	-2.3 (0.8)	0.3 (1.2) [b]
Smokers, %	30.1	25.5	28.3	23.9
Alcohol users, %	90.0	76.5	77.2	81.7
Age, years	21.3 (1.5)	20.9 (1.8)	20.7 (1.7)	20.9 (1.7)
Adult height SDS (21 years)	-1.6 (0.9) [b]	-2.6 (0.5) [b]	-0.2 (0.7) [c,d]	0.1 (0.8)
Weight for Age SDS	-1.4 (1.5) [e]	-1.4 (1.5) [e]	-0.02 (1.2) [c]	0.2 (0.9) [c]
Body Mass Index	21.4 (3.5) [f]	23.2 (4.4)	22.5 (4.2)	22.4 (3.1)
Body Mass Index SDS	-0.5 (1.4) [a]	0.2 (1.4)	-0.01 (1.3)	0.03 (1.1)
Waist circumference, cm	72.2 (9.0) [e]	75.1 (9.6)	79.2 (11.3)	78.9 (9.3)
Fasting glucose, mmol/l	4.7 (0.4)	4.9 (0.6)	4.7 (0.5)	5.0 (1.0)
Fasting insulin, mU/l	10.0 (5.2)	10.7 (4.8)	10.3 (3.9)	10.0 (5.3)
IGF-I SDS	-0.4 (0.9)	-0.4 (0.8)	-0.3 (0.7)	-0.4 (0.8)

Data are expressed as mean (SD). Abbreviations: SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age.

[a] P<0.04 compared with the other groups.
[b] P<0.001 compared with the other groups.
[c] P<0.001 compared with SGA-GH and SGA-S.
[d] P=0.010 compared with AGA.
[e] P<0.001 compared with SGA-CU and AGA.
[f] P<0.05 compared with SGA-S.

Blood pressure

Since the SGA-GH, SGA-S, SGA-CU, and AGA adults differed in male/female ratio and height, which are important factors for blood pressure, the SBP and DBP comparisons between groups were adjusted for these factors (Figure 2A). At the age of 21 years, i.e. 5 years after cessation of GH treatment in the SGA-GH adults, SBP and DBP were similar in SGA-GH and SGA-S adults. SGA-GH adults had a similar SBP but a lower DBP compared to SGA-CU and AGA adults (P=0.002 and P=0.016, respectively). Adjustment for the number of cigarettes smoked per day, resulted in a similar DBP in all groups.

At 5 years after cessation of GH, mean SBP was 0.32 (1.0) SDS in SGA-GH, 0.22 (1.1) SDS in SGA-S, 0.36 (1.0) SDS in SGA-CU, and 0.24 (0.8) SDS in AGA adults. Mean DBP SDS was 0.24 (0.7) SDS in SGA-GH, 0.49 (0.8) SDS in SGA-S, 0.53 (0.7) SDS in SGA-CU, and 0.42 (0.6) SDS in AGA adults.

Lipid levels

Because the SGA-GH, SGA-S, SGA-CU, and AGA adults differed in male/female ratio, the lipid comparisons between groups were adjusted for gender. At the age of 21 years, i.e. 5 years after cessation of GH treatment, the SGA-GH adults had lower cholesterol and LDLc levels than the untreated SGA-S adults (P=0.003 and P<0.001, respectively) (Figure 2B). SGA-GH adults tended to have higher HDLc levels than SGA-S adults (P=0.050), and TG levels were similar (data not shown). SGA-GH adults had similar cholesterol, LDLc, and TG levels as SGA-CU and AGA adults. SGA-GH had higher HDLc levels than SGA-CU adults (P=0.045, data not shown). Of the 4 groups, untreated SGA-S adults had the highest cholesterol and LDLc levels compared to SGA-GH, SGA-CU, and AGA adults (Figure 2B).

cIMT

Without adjustments, cIMT was similar between SGA-GH, SGA-S, SGA-CU, and AGA adults. Adjustment for gender, height, and gestational age did not change these results (Figure 2C). Pearson correlation tests showed that SBP, cholesterol, and LDLc were positively related to cIMT (P=0.002, P=0.039, and P=0.006, respectively). After additional adjustment for SBP, cholesterol or LDLc, cIMT remained similar between SGA-GH, SGA-S, SGA-CU, and AGA adults.

Metabolic syndrome at the age of 21 years in SGA-GH adults, and untreated SGA-S, SGA-CU, and AGA adults

The presence of metabolic syndrome according to the ATP III criteria could be investigated in 77 SGA-GH adults, 47 SGA-S adults, 85 SGA-CU adults, and 137 AGA adults (Table 3). The percentage of participants with abdominal obesity, high TG levels, low HDLc levels, high SBP or DBP, or high fasting glucose was not significantly different

between the groups. Of all participants, 46.0% (159/346) had one or more risk factors for metabolic syndrome. According to the ATP III criteria, 2 of 77 SGA-GH adults (2.6%) had three or more risk factors for metabolic syndrome compared with 3 of 47 untreated SGA-S adults (6.4%), but this difference did not reach statistical significance. The percentage of adults having three or more risk factors for metabolic syndrome tended to be higher in SGA-S adults compared with AGA adults (6.4% vs 0.7%, P=0.053).

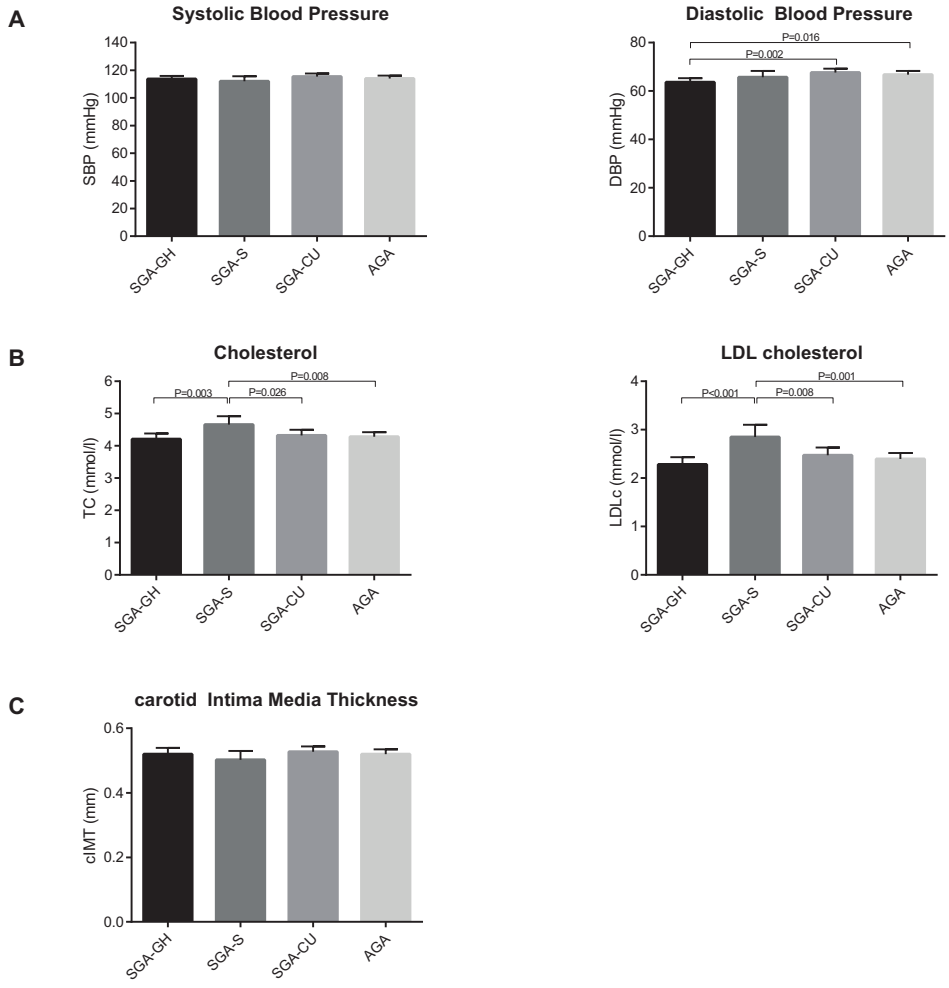

Figure 2. Comparison between groups.

A. Comparison of SBP and DBP, data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for gender and height.
B. Comparison of lipid levels, data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for gender.
C. Comparison of cIMT, data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for gender, height, and gestational age.

Table 3. Metabolic syndrome components, according to ATP III criteria

Symptoms	SGA-GH	SGA-S	SGA-CU	AGA
Abdominal obesity	4/77 (5.2%)	2/47 (4.3%)	9/85 (10.6%)	13/137 (9.5%)
High TG levels	5/77 (6.5%)	6/47 (12.8%)	7/85 (8.2%)	11/137 (8.0%)
Low HDLc levels	21/77 (27.3%)	17/47 (36.2%)	30/85 (35.3%)	36/137 (26.3%)
High blood pressure	4/77 (5.2%)	6/47 (12.8%)	7/85 (8.2%)	6/137 (4.4%)
High fasting glucose	5/77 (6.5%)	5/47 (10.6%)	2/85 (2.4%)	9/137 (6.6%)
One or more symptoms	31/77 (40.3%)	23/47 (48.9%)	41/85 (48.2%)	64/137 (46.7%)
Three or more symptoms	2/77 (2.6%)	3/47 (6.4%) [a]	2/85 (2.4%)	1/137 (0.7%)

Abbreviations: SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age.

[a] P=0.053 compared with AGA adults.

DISCUSSION

Our longitudinal study during 5 years after cessation of GH treatment is currently the longest and largest follow-up study in 199 GH-treated young adults born SGA. We show that cessation of GH treatment has significant effects on blood pressure, but only during the first 6 months after GH-cessation. The changes in lipid levels after cessation of GH treatment were related to the increase in fat mass. Cessation of GH treatment had no effects on cIMT levels. At 5 years after cessation of GH, previously GH-treated SGA young adults had a similar blood pressure and cIMT, and a more beneficial lipid profile, compared to untreated age-matched short SGA young adults who had higher cholesterol and LDLc levels. The percentage of participants with metabolic syndrome at the age of 21 years was not significantly different between previously GH-treated SGA young adults and untreated short SGA young adults.

Atherosclerosis, the main cause of CVD, is a chronic inflammatory condition with adverse remodeling of various arterial walls resulting in hypertension and a higher IMT. Before start of GH treatment, short children born SGA had a higher SBP compared to peers born AGA but SBP and DBP decreased during long-term GH treatment which is in line with previous studies (24). We show that SBP and DBP, adjusted for age, increased in the first 6 months after cessation of GH treatment, reflecting the loss of GH properties. Fortunately, this increase was followed by a decrease in the subsequent 18 months after which SBP and DBP remained similar until 5 years after GH treatment. At 5 years after GH-cessation, SBP and DBP were around zero SDS and not significantly different from levels at GH-cessation. Studies on changes in cIMT during GH treatment in children and adults with GH deficiency showed no change or even a decrease in cIMT (25-31). Only one study reported cIMT after 4 years of GH treatment in 31 children born SGA and found no association between GH treatment and changes in cIMT (32). To the best of our knowledge, our study is the first to report cIMT after cessation of GH treatment in previ-

ously GH-treated adults born SGA and shows that cIMT, adjusted for age, did not change after cessation of GH treatment. At 5 years after GH-cessation, previously GH-treated SGA adults had a similar SBP, DBP, and cIMT as untreated SGA-S adults. These findings indicate that long-term GH treatment has no long-lasting unfavorable effects on blood pressure and cIMT, thereby contradicting the hypothesis that there is a link between GH treatment and vascular diseases (8, 33). This is supported by the fact that levels of matrix metalloproteinase-9, which play a role in the development of atherosclerosis (34), decrease during GH treatment, in parallel to the decrease in blood pressure (35). Since our participants were relatively young and CVD might not yet be clinically apparent, we cannot conclude on the long-term risk for CVD. Epidemiological data, however, indicate that a cIMT value above 1 mm at any age is associated with a significantly increased risk for myocardial infarction and/or cerebrovascular disease, and none of the previously GH-treated SGA adults and other participants in our study had a cIMT above 1 mm at any time point during the study (36).

Previous reports showed that cholesterol and LDLc significantly decreased during GH treatment (24, 37, 38), indicating that GH treatment has a beneficial effect on lipid metabolism. Our data show that this beneficial effect on lipid metabolism sustains after cessation of GH treatment since previously GH-treated SGA adults had lower cholesterol and LDLc levels than untreated SGA-S adults, despite the increase in lipid levels after GH-cessation which was related to the increase in fat mass after GH-cessation. These findings are reassuring, since elevated lipid levels contribute to a higher risk for CVD (39).

Evidence indicates that, on the long-term, adults with metabolic syndrome have an elevated risk for CVD-attributed mortality (39, 40). The previously GH-treated SGA adults showed no increased risk for metabolic syndrome. In our study, the percentage of adults with metabolic syndrome was highest in the untreated SGA-S adults, which was borderline significant compared to AGA adults. Thus, GH treatment does not increase the risk for metabolic syndrome, at least until 5 years after cessation of treatment.

To evaluate whether GH-induced catch-up versus spontaneous catch-up growth to a normal stature has a similar effect on blood pressure, lipid levels, and cIMT, we also compared these outcomes in previously GH-treated SGA adults to those of SGA-CU adults of a similar age. DBP was significantly higher in SGA-CU adults, whereas SBP, lipid levels, and cIMT were similar. This might be due to the catch-up in weight for length in early life (15, 41) but can also be due to a higher number of cigarettes smoked per day in SGA-CU adults compared to previously GH-treated SGA adults, since smoking is positively related to blood pressure. After adjustment for the number of cigarettes smoked per day, DBP was similar between previously GH-treated SGA and SGA-CU adults.

A limitation of previous studies on the long-term mortality after GH treatment such as the preliminary data of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) project (8, 9), is the lack of an appropriate control group of untreated

SGA patients. To study the effect of GH treatment on the risk for CVD, it is particularly important to determine the presence of risk factors for these diseases in GH-treated adults born SGA compared to untreated adults born SGA. Our study now provides such data.

We previously reported the longitudinal changes in body composition, insulin sensitivity, and β-cell function during 5 years after cessation of GH treatment in the same population and demonstrated that fat mass steadily increased after GH-cessation while the GH-induced changes in insulin sensitivity and β-cell function were fully reversed (42). Body composition, insulin sensitivity, and β-cell function at 5 years after GH treatment had reached the same values as in untreated short SGA young adults (42). Together with our present findings that blood pressure and cIMT were similar, and lipid levels lower in previously GH-treated SGA young adults compared to untreated short SGA young adults, we can now conclude that long-term GH treatment in children born SGA does not only improve adult height but has also no unfavorable effects on metabolic and cardiovascular health in early adulthood and might be beneficial with regard to the lipid profile. Longer-term follow-up is important to evaluate if these effects sustain over a longer period.

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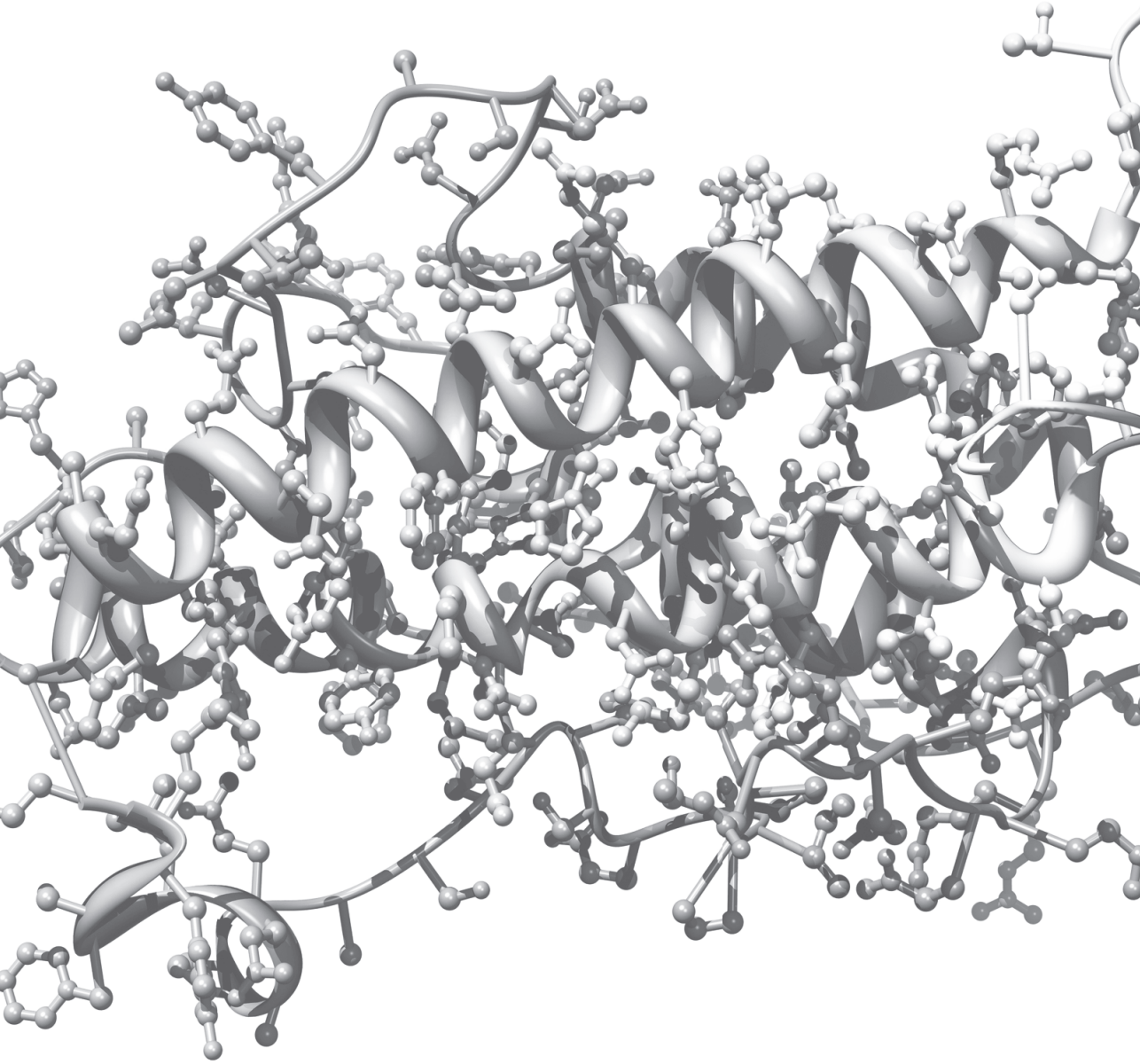
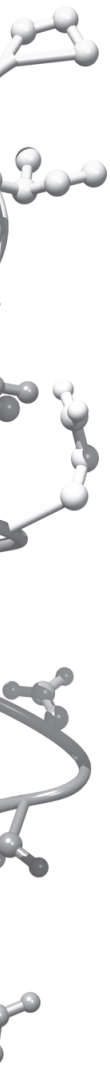


CHAPTER 7



ACAN gene mutations in short children born SGA and response to growth hormone treatment

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ABSTRACT

Background Some children born small for gestational age (SGA) show advanced bone age (BA) maturation during growth hormone (GH) treatment. ACAN gene mutations have been described in children with idiopathic short stature and advanced BA.

Objective To determine presence of ACAN gene mutations in short SGA children with advanced BA and to assess the response to GH treatment.

Methods BA assessment in 290 GH-treated SGA children and ACAN-sequencing in 29 children with an advanced BA of ≥0.5 year compared with calendar age.

Results ACAN gene mutations were found in 4/29 SGA children with advanced BA (13.8%). Mutations were related to the characteristics: midface hypoplasia (P=0.003), joint problems (P=0.010), and broad great toes (P=0.003). Children with advanced BA having none or only 1 of these characteristics had no ACAN gene mutation. Of children with advanced BA and 2 of these characteristics, 50% had an ACAN gene mutation. Of children with advanced BA and 3 of these characteristics, 100% had an ACAN gene mutation. All GH-treated children with an ACAN gene mutation received additional GnRHa treatment for 2 years from onset of puberty. At adult height, 1 girl was 5 cm taller than her mother and 1 boy was 8 cm taller than his father with the same ACAN gene mutation. In boys, bone maturation was delayed by aromatase inhibitor treatment.

Conclusion This study expands the differential diagnosis of genetic variants in children born SGA and proposes a clinical scoring system for identifying subjects most likely to have an ACAN gene mutation. ACAN-sequencing should be considered in children born SGA with persistent short stature, who show advanced bone age and midface hypoplasia, joint problems, or broad great toes. Our findings suggest that children with an ACAN gene mutation benefit from GH treatment with 2 years of GnRHa treatment.

INTRODUCTION

Children born small for gestational age (SGA) comprise a heterogeneous group with a broad spectrum of clinical characteristics (1, 2). Although several factors have been identified in the etiology of children born SGA, the etiology remains unidentified in up to 40% of cases. Approximately 10% of children born SGA remain short and are therefore treated with growth hormone (GH) to increase adult height (3-7). Adult height (AH) is one of the most heritable human traits (8), but only a small number of genetic mutations (<1%), explaining short stature in children born SGA, has been found (9-11). Uncovering the genetic etiology of short stature is important for health prognosis, genetic counseling, and treatment options.

Some children born SGA have an accelerated bone age (BA) maturation during childhood, resulting in early closure of the epiphyseal growth plates and cessation of growth at a young age with a disappointing short AH. Heterozygous mutations in the ACAN gene have been identified in children with idiopathic short stature and advanced BA (12, 13). Various clinical characteristics have been described in affected individuals: advanced BA, early growth cessation, short stature (i.e. height below -2.5 SDS), midface hypoplasia, flat nasal bridge, prognathism, posteriorly rotated ears, broad forehead, broad great toes, short thumbs, brachydactyly, joint problems, exaggerated lumbar lordosis, and genu valgum (12-14). Due to the variance in clinical characteristics, the identification of appropriate patients for genetic testing remains a challenge. We, therefore, assessed BA in 290 GH-treated children born SGA, and performed genetic testing in 29 children suspected for mutations in the ACAN gene due to advanced BA. Phenotypic characteristics of children with advanced BA were used to develop a scoring system to identify children most likely to test positive for mutations in the ACAN gene, and for distinguishing these children from those not likely to test positive. In addition, the response to GH treatment with additional gonadotropin-releasing hormone analog (GnRHa) for 2 years, and aromatase inhibitor treatment in boys, was evaluated in children with a confirmed mutation in the ACAN gene.

METHODS

Subjects

The study population consisted of 290 children born SGA with persistent short stature, without known genetic abnormalities, who were participating in 2 prospective cohort trials evaluating the effects of GH treatment (15, 16). The inclusion criteria for these GH trials were: 1) birth length and/or birth weight SDS for gestational age less than -2.0 (17); 2) height SDS or predicted AH at start of GH treatment less than -2.5 SDS, based on Dutch references (18); 3) well-documented growth data from birth to start of GH treatment; and 4) an uncomplicated neonatal period, without signs of severe asphyxia

(Apgar score >3 after 5 minutes) or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia.

Reported findings in patients with ACAN gene mutations showed that BA was persistently advanced during childhood. In our study population of 290 children born SGA, radiographs of the left hand and wrist were taken at start of GH treatment and yearly thereafter. BA was assessed according to Greulich and Pyle (19). We selected children for ACAN-sequencing based on an advanced BA of at least 0.5 year at ≥ 2 radiographs prior to pubertal onset (Table 1, group 1). Some of the children in our study population came to medical attention in early puberty and started GH treatment with additional GnRHa for 2 years to postpone puberty and improve AH. Since GnRHa treatment influences bone maturation, and BA before start of combined GH/GnRHa treatment was not known in all children, only the first radiograph at start of treatment was reliable to assess BA in these children. Persistent advanced BA was therefore not a possible selection criteria and selection was based on an advanced BA of at least 0.5 year at start of GH/GnRHa treatment (Table 1, group 2). Based on these criteria, 42 children were selected for ACAN-sequencing. In 13 children, ACAN-sequencing was not possible because they did not want to revisit the hospital after attainment of AH. In total, targeted ACAN-sequencing was performed in 29 children after written informed consent.

The following physical characteristics were assessed by one physician (M.v.d.S.), after instruction by a clinical geneticist, and recorded as present or absent; midface hypoplasia, prognathism, flat nasal bridge, broad forehead, microcephaly defined as head circumference below -2.0 SDS, posteriorly rotated ears, joint problems, broad great toes, brachydactyly, exaggerated lumbar lordosis, and genu valgum.

Sequencing

Amplicon sequencing of ACAN was performed in all 29 participants essentially as previously described (20, 21). The sequences of the primers that were used are available in Supplemental table 1.

Statistical analyses

Statistical analyses were performed using SPSS version 23 (IBM). Birth length and birth weight were transformed into SDS for sex and gestational age (17). Height was transformed into SDS for sex and chronological age according to Dutch references (18), using Growth Analyser Research Calculation Tools (https://growthanalyser.org). AH SDS was calculated using references for Dutch adults (age 21 years) (18). Mann-Whitney U test and Fisher's exact test were used to assess the difference in clinical characteristics between children with and without mutations in the ACAN gene. P-values < 0.05 were considered statistically significant.

RESULTS

ACAN-sequencing was performed in 29 children (13 males, 16 females) with advanced BA, originating from 26 nonrelated Dutch families. Clinical characteristics are summarized in Table 1. Four children, 3 boys, including one dizygotic twin, had a mutation in the ACAN gene.

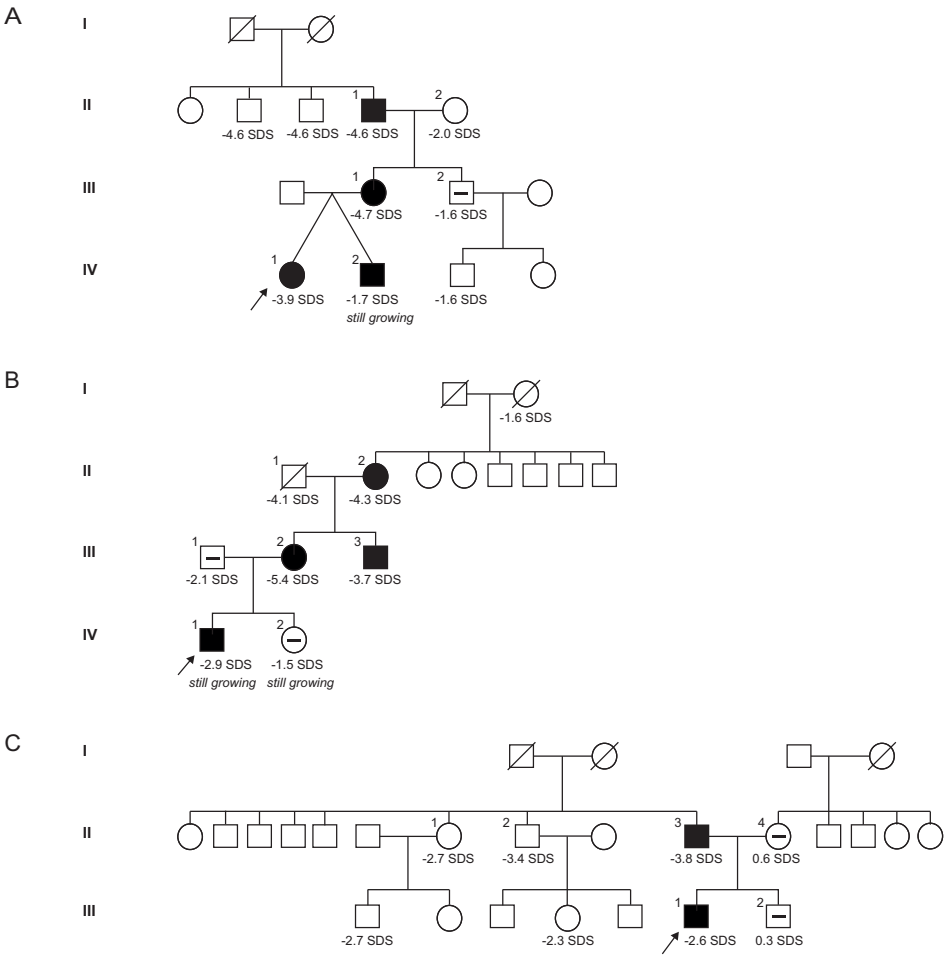


Figure 1. Pedigrees of families with ACAN gene mutations. A, family A. B, family B. C, family C.
Black symbols; patients with a confirmed ACAN gene mutation through ACAN-sequencing.
White symbols with a horizontal line; patients without an ACAN gene mutation through ACAN-sequencing.
White symbols with a slash; deceased patients. White symbols without lines; no ACAN sequencing performed.
Reported height SDS is adult height SDS, in children still growing it represents the current height SDS. When height SDS is not reported, subjects had a height >-1.5 SDS.

Table 1. Clinical characteristics of selected individuals for ACAN-sequencing

Patient	Gender	Age at start GH	Height SDS at start GH	Height SDS father	Height SDS mother	BA-CA (years) at start of GH	BA-CA (years) during GH	Midface hypoplasia	Joint problems	Broad great toes	ACAN mutation
Group 1											
1 (A.IV:1)	female	5.0	-3.7	unknown	-4.7	conform	**+2.0**	**yes**	**yes**	**yes**	**yes**
2	female	6.4	-2.6	-1.6	-3.2	-0.4	**+1.0**	no	no	**yes**	no
3	female	4.2	-2.8	-2.5	-2.4	-1.2	**+1.2**	**yes**	no	no	no
4	male	9.5	-2.9	-2.7	-1.3	**+1.2**	**+2.0**	no	no	no	no
5	male	9.0	-4.2	-2.0	-1.3	conform	**+1.5**	**yes**	no	**yes**	no
6	male	12.8	-2.9	-2.0	-2.0	**+0.5**	**+1.1**	**yes**	no	no	no
7	female	4.1	-3.5	-0.8	-0.4	-0.6	**+1.3**	no	no	no	no
8	male	3.0	-3.6	-0.8	-1.5	conform	**+1.9**	no	no	no	no
9 [a]	female	5.2	-3.2	-1.4	-0.6	conform	**+2.8**	no	no	**yes**	no
10 [a]	male	4.5	-2.7	-1.4	-0.6	**+0.5**	**+1.5**	no	no	no	no
11	female	5.3	-2.2	0.2	-1.0	**+0.7**	**+1.4**	no	no	no	no
12	female	6.0	-2.9	-0.8	-0.1	**+1.1**	**+2.0**	no	no	no	no
13	female	4.3	-3.0	0.6	-0.3	**+0.7**	**+1.1**	no	no	no	no
14 [b]	male	3.7	-3.4	-0.8	-2.0	-0.7	**+1.2**	no	no	no	no
15 [b]	male	3.7	-3.1	-0.8	-2.0	-1.2	**+1.2**	no	no	no	no
16	female	4.7	-2.6	-0.6	-0.4	-0.2	**+1.2**	no	no	no	no
17	male	4.5	-3.3	-1.3	0.5	-0.5	**+1.4**	no	no	no	no
18	male	4.4	-2.5	-1.3	-1.0	-2.4	**+1.4**	no	no	no	no
19	male	5.2	-2.5	0.4	-0.1	-0.2	**+1.1**	no	no	no	no

Table 1. Clinical characteristics of selected individuals for ACAN-sequencing (continued)

Patient	Gender	Age at start GH	Height SDS at start GH	Height SDS father	Height SDS mother	BA-CA (years) at start of GH	BA-CA (years) during GH	Midface hypoplasia	Joint problems	Broad great toes	ACAN mutation
Group 2											
20 (A.IV:2)	male	11.9	-2.4	unknown	-4.7	**+0.6**	influenced by GnRHa treatment	**yes**	**yes**	**yes**	**yes**
21 (B.IV:1)	male	11.7	-2.7	-2.0	-4.9	**+0.8**	influenced by GnRHa treatment	**yes**	**yes**	**yes**	**yes**
22 (C.III:1)	male	12.3	-2.7	-3.8	0.6	**+1.0**	influenced by GnRHa treatment	**yes**	no	**yes**	**yes**
23	female	12.0	-3.1	-3.8	-0.8	**+0.5**	influenced by GnRHa treatment	no	no	no	no
24	female	11.0	-3.0	-2.5	-3.2	**+2.0**	influenced by GnRHa treatment	no	**yes**	no	no
25	female	9.5	-2.2	0.2	-3.1	**+0.7**	influenced by GnRHa treatment	no	no	no	no
26	female	11.3	-2.4	-2.3	-1.4	**+0.7**	influenced by GnRHa treatment	no	no	**yes**	no
27	female	12.2	-2.4	0.7	-2.3	**+0.5**	influenced by GnRHa treatment	**yes**	no	no	no
28	female	9.8	-2.8	-0.2	-2.1	**+1.3**	influenced by GnRHa treatment	no	**yes**	no	no
29	female	11.0	-2.5	-0.9	-2.0	**+0.7**	influenced by GnRHa treatment	no	no	no	no

Patient numbers in bold represent patients with a mutation in the ACAN gene. Abbreviations: BA, bone age; CA, calendar age.

Group 1: persistently advanced bone age of at least 0.5 year at more than 2 radiographs prior to pubertal onset.

Group 2: advanced bone age of at least 0.5 year at start of GH/GnRHa treatment in early puberty.

A.IV:1, proband family A; A.IV:2, twin brother of proband family A; B.IV:1, proband family B; C.III:1, proband family C.

[a] Siblings

[b] Twin brothers

Clinical presentation

Family A

Figure 1A shows the pedigree. The proband (A.IV:1) is a girl, born SGA at 32 6/7 weeks of gestation. She had persistent short stature and was referred to our clinic at the age of 5.0 years with a BA equal to her calendar age (CA) (Table 2). She has midface hypoplasia, broad great toes, and short thumbs. A skeletal survey showed underdeveloped facial bones, no vertebral body deformities. Her twin brother (A.IV:2) was born SGA and referred to our clinic at the age of 11.9 years because of persistent short stature with a BA 0.6 year advanced relative to his CA (Table 2). He has midface hypoplasia, mild posteriorly rotated ears, and broad great toes. Both children were referred to orthopedics because of persistent pain and dysfunction of their knees and elbows and osteoarthritis was diagnosed. Because of recurrent urinary tract infections, the boy was referred to a pediatric urologist and abdominal ultrasound showed an absent left kidney.

Height of their mother (A.III:1) is 140.3 cm (-4.7 SDS) and she has midface hypoplasia, broad great toes, short thumbs, exaggerated lumbar lordosis, osteoarthritis, and partial adrenal insufficiency. The height of the children's maternal grandfather (A.II:1) is 151.6 cm (-4.6 SDS) and the maternal grandmother (A.II:2) is 158.0 cm (-2.0 SDS). The grandfather has midface hypoplasia and had knee and hip replacement surgery at a young age due to osteoarthritis. ACAN-sequencing identified a pathogenic heterozygous nonsense mutation, c1608C>A, in the proband, her twin brother, mother, and maternal grandfather. The maternal uncle (A.III:2) has a height of 173 cm (-1.6 SDS) and no ACAN gene mutation.

Family B

Figure 1B shows the pedigree. The proband (B.IV:1) is a boy, born SGA at 38 6/7 weeks of gestation. He had persistent short stature and was referred to our clinic at the age of 11.7 years with a BA 0.8 years advanced relative to his CA (Table 2). He has midface hypoplasia, mild prognathism, broad great toes, and exaggerated lumbar lordosis. At the age of 14 years, he was referred to orthopedics because of persistent pain in both knees and osteochondritis dissecans was diagnosed based on knee X-rays and magnetic resonance imaging.

Height of his father (B.III:1) is 169.5 cm (-2.1 SDS) and mother (B.III:2) 135.9 cm (-5.4 SDS). His mother has midface hypoplasia, short thumbs, broad great toes, exaggerated lumbar lordosis, and osteoarthritis of her knees for which she had replacement surgery several times. The mother has no nails on most of her digits (hands and feet). The proband has one sister with a height of 156.3 cm (-1.5 SDS) at the age of 14.5 years (B.IV:2). The proband's maternal grandfather (B.II:1) is deceased, his height was 155.0 cm (-4.1 SDS) and he had osteoarthritis of neck and vertebrae. The height of proband's maternal grandmother (B.II:2) is 143.0 cm (-4.3 SDS) and she has midface hypoplasia, short thumbs,

and problems of both knees and neck. The height of proband's maternal uncle (B.III:3) is 158.0 cm (-3.7 SDS). ACAN-sequencing identified a pathogenic heterozygous nonsense mutation, c.7090C>T, in the proband, his mother, his maternal grandmother, and uncle. Besides, ACAN-sequencing identified 2 additional heterozygous missense mutations in the proband's mother and his maternal uncle, c.1973A>G (p.(Asn658Ser)) and c.5419G>A (p.(Gly1807Arg)). The pathogenicity of the second mutation is uncertain. These 2 missense mutations could not be tested in the proband's maternal grandfather since he is deceased. The unaffected father and sister of the proband had no ACAN gene mutation.

Family C

Figure 1C shows the pedigree. The proband (C.III:1) is a boy, born SGA at 41 weeks of gestation. He had persistent short stature and was referred to our clinic at the age of 12.3 years with a BA 1.0 year advanced relative to his CA (Table 2). He has midface hypoplasia, broad great toes, and posteriorly rotated ears, but no joint problems.

Height of his father (C.II:3) is 157.4 cm (-3.8 SDS) and mother (C.II:4) 174.6 cm (0.6 SDS). The proband's father has midface hypoplasia and joint problems of his hips. The proband has one brother (C.III:2) with an AH of 185.0 cm (0.2 SDS). One paternal aunt (C.II:1) has severe knee problems and a height of 153 cm (-2.7 SDS) and one parternal uncle (C.II:2) has a height of 160 cm (-3.4 SDS). ACAN-sequencing identified a pathogenic heterozygous frameshift mutation, c.4762_4765del (p.(Gly1588fs), which leads to an early stopcodon, in the proband and his father. The proband's mother and brother had no ACAN gene mutation. Siblings of the proband's father were not tested for ACAN gene mutations.

Table 2. Clinical characteristics of the 4 children with an ACAN gene mutation

	A.IV:1	A.IV:2	B.IV:1	C.III:1
Birth characteristics				
Gestational age, weeks	32 6/7	32 6/7	38 6/7	41
Birth weight, SDS	-2.1	-0.9	-0.1	-1.8
Birth length, SDS	-4.4	-2.1	-3.0	-3.0
At referral to our clinic				
Age, years	5.0	11.9	11.7	12.3
Bone age, years	5.0	12.5	12.5	13.3
Height, cm	94.5	135.6	131.7	135.4
Height, SDS	-3.7	-2.4	-2.7	-2.7
Weight for Height, SDS	-1.1	2.0	0.7	-0.4
Sitting height to height ratio, SDS	2.2	1.4	1.4	1.6
Head circumference, SDS	-3.0	-1.3	-0.2	-1.6

A.IV:1, proband family A; A.IV:2, twin brother of proband family A; B.IV:1, proband family B; C.III:1, proband family C.

Presence of mutations in the ACAN gene and phenotypic characteristics

Overall, 13.8% of the children with advanced BA had an ACAN gene mutation (4 of 29). Supplemental table 2 provides additional phenotypic information of all 29 children. Children with an ACAN gene mutation differed significantly for presence of midface hypoplasia, joint problems, and broad great toes, from children without an ACAN gene mutation (Table 3; P=0.001). Figure 2 shows a flowchart on the probability of having an ACAN gene mutation. Children with advanced BA and none or only 1 of the additional characteristics midface hypoplasia, joint problems, or broad great toes, had no ACAN gene mutation. Of children with 2 additional characteristics, 50% had an ACAN gene mutation. When 2 additional characteristics were present and at least one parent had a height below -3.5 SDS, the occurrence rate of ACAN gene mutations increased to 100%. All children with advanced BA ≥0.5 year and all 3 additional characteristics had a mutation in the ACAN gene (100%).

In the families with confirmed mutations in the ACAN gene, all affected individuals had midface hypoplasia and broad great toes. Joint problems were present in all affected individuals except in the proband of family C (C.III:2). All affected individuals without GH treatment had a height below -3.5 SDS.

Table 3. Statistical comparison of the clinical characteristics of patients with and without an ACAN gene mutation

	ACAN gene mutation		**No ACAN gene mutation**		**P-value**
N	4		25		
Mean (SD) numbers of factors recorded 'yes'	5.25 (1.0)		1.92 (1.5)		0.001
Characteristics	**N**	**%**	**N**	**%**	
Midface hypoplasia	4	100	4	16	0.003
Broad great toes	4	100	4	16	0.003
Joint problems	3	75	2	8	0.010
Posteriorly rotated ears	2	50	4	16	NS
Brachydactyly	1	25	4	16	NS
Prognathism	1	25	5	20	NS
Microcephaly	1	25	2	8	NS
Exaggerated lumbar lordosis	1	25	2	8	NS
Flat nasal bridge	0	0	6	24	NS
Broad forehead	0	0	5	20	NS
Genu valgum	0	0	4	16	NS

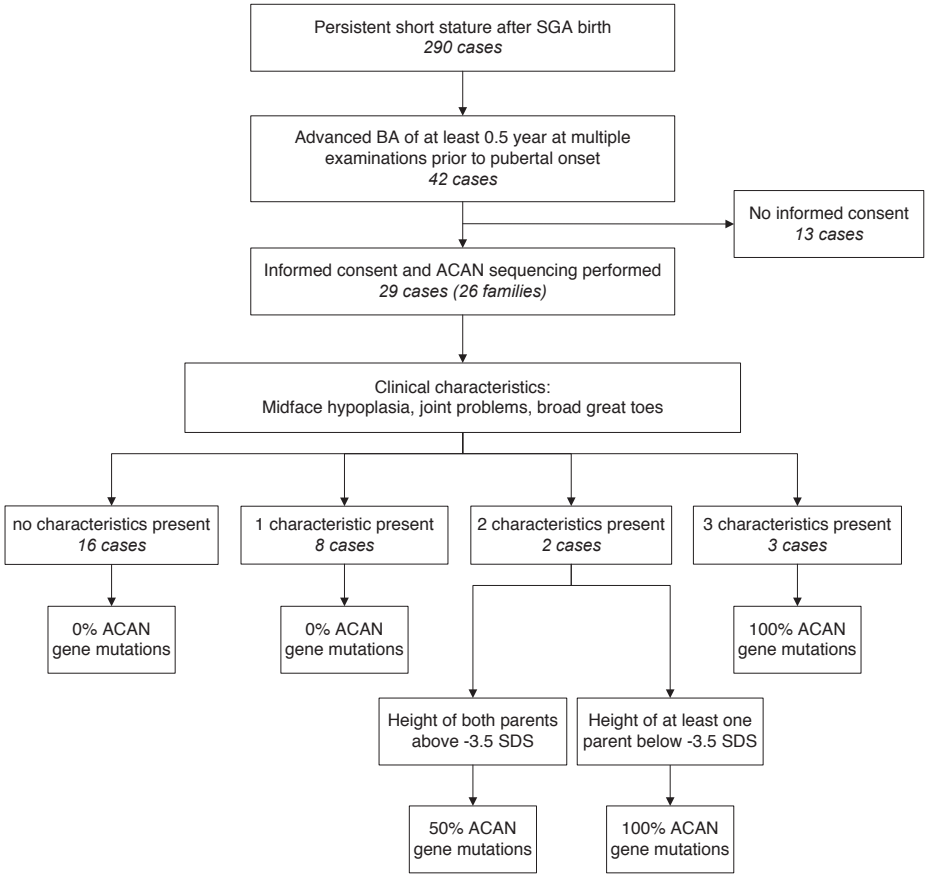


Figure 2. Clinical scoring system for ACAN gene mutations.

Growth response and bone maturation during GH treatment

The 4 children with an ACAN gene mutation were treated with GH based on persistent short stature after SGA birth. Timing of pubertal onset was normal in all 4 children, but relatively early for their short stature. Because of an AH expectation below -2.5 SDS at pubertal onset, they were all treated with 2 years of additional GnRHa treatment from onset of puberty to postpone puberty (leuprolide acetate depots, 3.75 mg sc every 4 weeks). Figure 3 shows the growth charts of the children with an ACAN gene mutation.

The girl of family A started GH treatment at the age of 5 years with a dose of 1 mg/m^2/day (0.033 mg/kg/day) (Figure 3A). From the age of 7 years, her BA was 1.5-2.0 years advanced. She started puberty at the age of 10 years with a height of 132.3 cm (-1.8 SDS) and was additionally treated with GnRHa. During GnRHa treatment, her bone maturation slowed compared with the years before, but her growth rate decreased and GnRHa treatment was therefore discontinued after 1.5 years, while GH treatment was

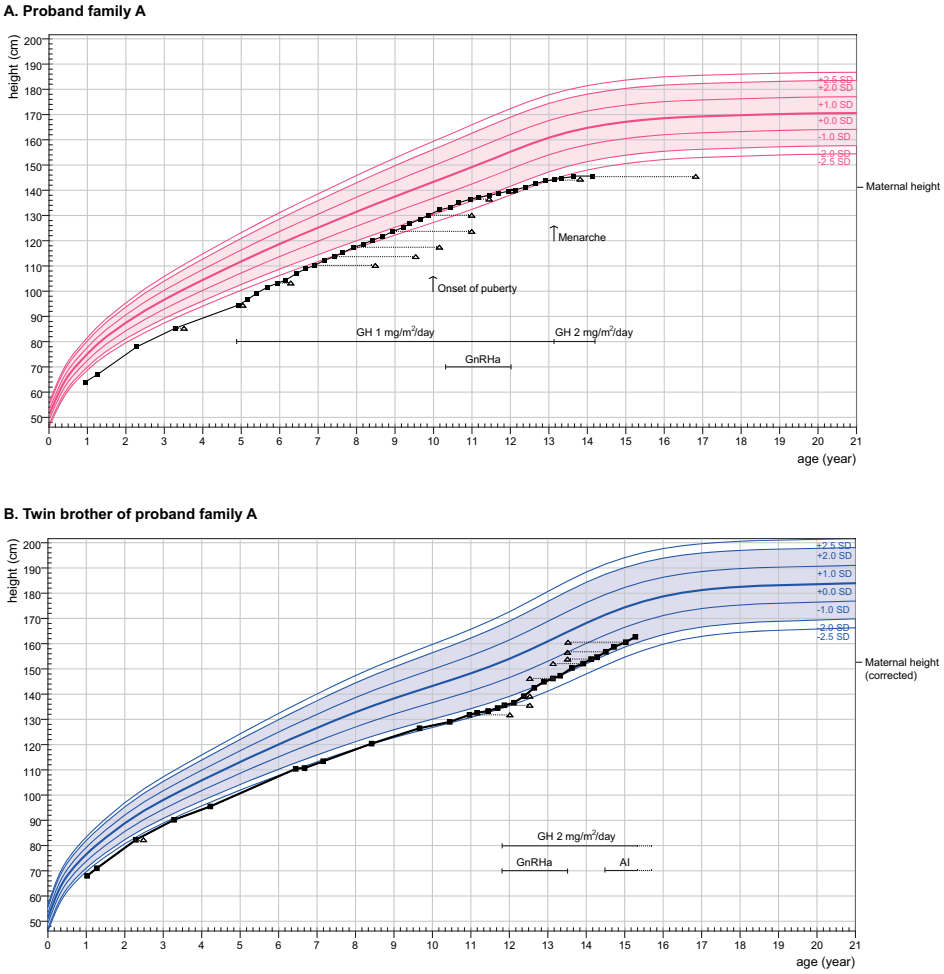
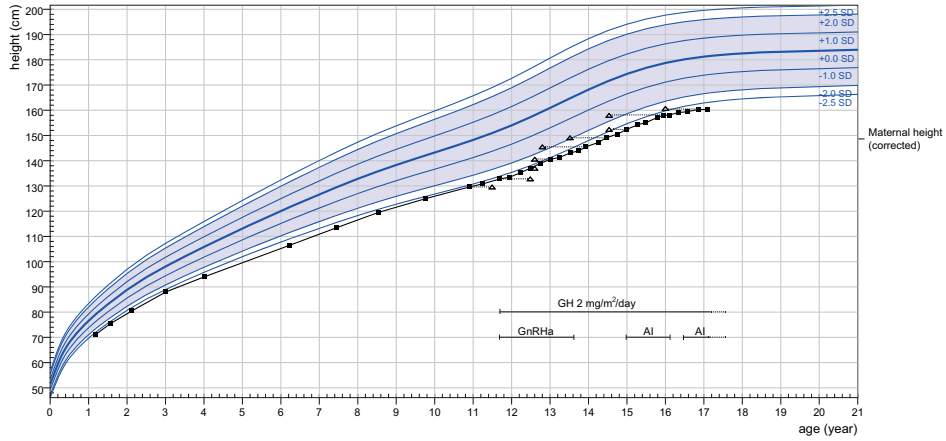


Figure 3. Growth charts of patients with an ACAN gene mutation.
A: proband family A (A.IV:1).
B: brother of proband family A (A.IV:2).
Abbreviations: AI, aromatase inhibitor.
Growth charts according to Growth Analyser 4.0 (18). Triangles represent bone age.

continued. At the age of 13.3 years, the GH dose was increased to 2 mg/m^2/day because of a disappointing pubertal growth spurt. Her growth ceased at the age of 14 years, when BA was 17 years, with a height of 145.5 cm (AH -3.9 SDS) which was 5 cm taller than her mother (AH -4.7 SDS).

The 3 boys all started GH treatment combined with 2 years of GnRHa in early puberty, age 11.5-12.0 years (Figure 3, B-D). Due to the GH trial design, they were randomly assigned to receive either GH 1 mg/m^2/day (boy from family C) or GH 2 mg/m^2/day (boys

C. Proband family B



D. Proband family C

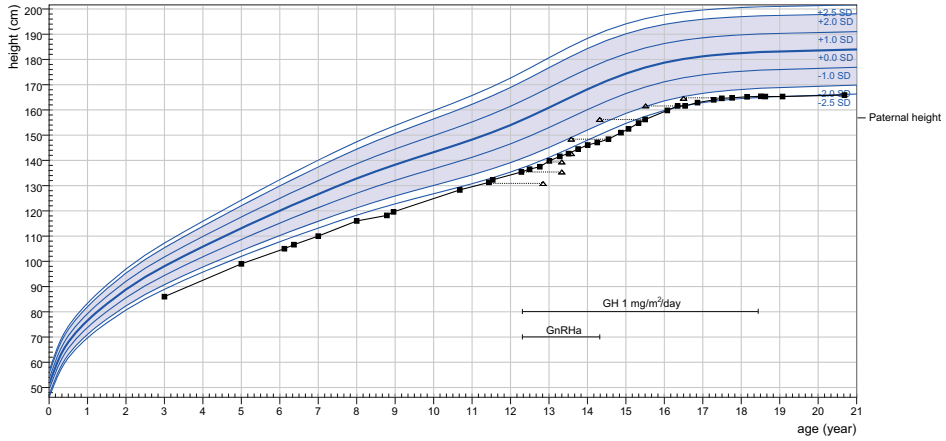


Figure 3. Growth charts of patients with an ACAN gene mutation.
C: proband family B (B.IV:1).
D: proband family C (C.III:1).
Abbreviations: AI, aromatase inhibitor.
Growth charts according to Growth Analyser 4.0 (18). Triangles represent bone age.

from family A and B). Bone maturation was advanced at start of GH treatment but stopped during GnRHa treatment. Because of rapid development of Tanner stage and bone maturation after discontinuation of GnRHa, the boys of family A and B were treated with an aromatase inhibitor (AI) from the age of 14 and 15 years, respectively (Letrozole, 2.5 mg daily). Due to abnormalities in the knee joint, AI treatment was discontinued for 4 months in the boy of family B, during which BA rapidly advanced. AI treatment was restarted after the diagnosis osteochondritis dissecans. Currently, BA is delayed in both

boys (1-1.5 years) while treated with AI. They have not yet reached AH and have a current height of 164.7 cm and 161.3 cm, respectively. In the boy of family C, growth ceased at the age of 18.6 years with a height of 165.3 cm (AH -2.6 SDS) (Figure 3D), which is 8 cm taller than his father (-3.8 SDS).

Treatment had no adverse effects and DXA scans of the total body and lower lumbar spine showed normal bone mineral density in all children.

DISCUSSION

In this study, we performed ACAN-sequencing in 29 short children born SGA with an advanced BA, who participated in prospective GH trials including 290 children born SGA. In 4 children (13.8%), one dizygotic twin, we identified heterozygous genetic mutations in the ACAN gene. Our data show that ACAN gene mutations are related to the presence of midface hypoplasia, joint problems, and broad great toes. ACAN gene mutations were present in 50-100% of the children with advanced BA and at least 2 of these characteristics. We, therefore, suggest that ACAN-sequencing could be considered in children with a combination of advanced BA and at least 2 of these clinical characteristics. When BA is advanced in the absence of these characteristics, a mutation in the ACAN gene is less likely, regardless of parental height. GH treatment with 2 years of additional GnRHa followed by aromatase inhibitor treatment in boys, which successfully delayed bone maturation, was beneficial in all 4 patients with a confirmed mutation in the ACAN gene.

Longitudinal bone growth occurs at the growth plate by forming and remodeling cartilage into bone tissue. The ACAN gene is located on chromosome 15q26 and consists of 19 exons ranging in size from 77-4224 base pairs (22). It encodes for the aggrecan protein, which is a member of the lectican (chondroitin sulfate proteoglycan) family. The encoded protein is a proteoglycan and a critical component of the extracellular matrix in both articular and growth plate cartilaginous tissue, explaining the effects on joints (articular cartilage) and growth (growth plate cartilage) in case of a mutation. Mutations in the ACAN gene result in a broad phenotypic spectrum of nonlethal skeletal dysplasias including spondyloepimetaphyseal dysplasia, spondyloepiphyseal dysplasia (Kimberley type), familial osteochondritis dissecans, and various idiopathic short stature phenotypes (12-14, 20, 23-25). The exact mechanism of how each distinct mutation in the ACAN gene leads to a range of phenotypes is unknown, but in all published studies, affected individuals had short stature and early growth cessation. It is unknown whether all previously reported individuals with ACAN gene mutations had an advanced BA and since we selected on advanced BA, there is a small chance that ACAN gene mutations were present in the children not tested. However, an antiangiogenic function of the aggrecan matrix has been proposed (26) and mutations in the ACAN gene might, therefore, lead to premature or increased invasion of the growth plate by blood vessels and

osteoblasts. This causes ossification of growth cartilage resulting in advanced BA, early epiphyseal fusion and premature growth cessation (27). Since all previously reported individuals with ACAN gene mutations had early growth cessation and short stature, advanced BA could be assumed in these subjects.

We show that having an advanced BA is insufficiently indicative for a mutation in the ACAN gene in children born SGA since only 13.8% of the children with advanced BA had an ACAN mutation. Patients should, therefore, have additional characteristics. Various different characteristics have been described in patients with ACAN gene mutations (12-14, 20, 24, 25), but we only found an association with midface hypoplasia, joint problems, and broad great toes. These characteristics have also been described in other individuals with ACAN gene mutations (12-14). The occurrence of short thumbs was not indicative for ACAN gene mutations when analyzed in our 29 patients. However, all women with an ACAN gene mutation, and none of the men, had short thumbs. This might indicate that phenotypic characteristics of ACAN gene mutations differ in females and males, which is also true for SHOX deficiency in which Madelung deformity is more common in females (28). It could, therefore, be that there are more phenotypic characteristics indicative for mutations in the ACAN gene, which we did not find because of the small number of patients in our study. Nevertheless, this is the first study proposing a clinical scoring system for ACAN-sequencing (Figure 2), showing that advanced BA combined with the presence of midface hypoplasia, joint problems, or broad great toes, has a predictive value of 50-100% of identifying an ACAN gene mutation.

GH treatment with additional 2 years of GnRHa, resulted in an AH of 145.5 cm (-3.9 SDS) in the girl from family A, and an AH of 165.3 cm (-2.6 SDS) in the boy from family C. This is respectively 5 and 8 cm taller than their parent with the same sex and ACAN gene mutation. Another study reported combined GH/GnRHa treatment in a girl aged 11.5 years with a heterozygous ACAN gene mutation in the C-type lectin domain (12). GnRHa treatment successfully blocked bone maturation but both GH and GnRHa treatment were discontinued at the age of 13.5 years, for unknown reasons, and growth ceased shortly thereafter at an AH of 135.8 cm (-4.2 SDS). A case report of GH treatment in a boy with a heterozygous missense mutation in the C-type lectin domain of the ACAN gene, showed that the treated boy was 11.5 cm taller than the mean height of the untreated males with ACAN gene mutations in his family (20, 25). These findings together, suggest that children born SGA who have a mutation in the ACAN gene, benefit from GH treatment with 2 years of additional GnRHa treatment in early puberty. In our study, 2 boys received also aromatase inhibitor treatment. Since these 2 boys have not yet reached their AH, effectiveness of aromatase inhibitor treatment is still uncertain. However, since bone maturation was successfully stopped and bone mineral density remained normal during aromatase inhibitor treatment, this could be considered in boys with an ACAN gene mutation.

Remarkably, accelerated bone maturation did not occur in early childhood but from the age of 7 years in the affected girl and the age of 11 years in the affected boys. This highlights the importance of regularly reevaluating the original diagnosis in children born SGA, who comprise a heterogeneous group with a broad spectrum of clinical characteristics since SGA is a definition and not a diagnosis (1, 2). Being born SGA has been associated with an adverse health profile in young adulthood, and short stature during childhood and adolescence, resulting in a short AH, is associated with reduced quality of life (29). Therefore, identifying underlying causes of being born SGA remains important to alert physicians to potential comorbidities and to improve future treatment options. In most cases, however, no diagnosis is made since only a small number of genetic mutations (<1%) explaining short stature after SGA birth, has been found (9-11).

In conclusion, our study expands the differential diagnosis of genetic variants in short children born SGA and proposes a clinical scoring system for identifying subjects most likely to have an ACAN gene mutation and for distinguishing these subjects from those not likely to test positive. Although the full phenotypic spectrum of this disorder is yet to be elucidated, our findings show that children born SGA with persistent short stature, advanced BA, and at least 2 of the clinical characteristics midface hypoplasia, joint problems, or broad great toes, have a high likelihood of an ACAN gene mutation. Patients with advanced BA without additional characteristics are less likely to have a mutation in the ACAN gene. Our findings suggest that children with an ACAN gene mutation benefit from GH treatment with 2 years of additional GnRHa, and in boys this could be followed by treatment with an aromatase inhibitor, which might further increase adult height. Further research on treatment options is warranted to improve height in children with ACAN gene mutations.

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SUPPLEMENTAL TABLES

Supplemental table 1. Primer sequences

OliogID1	OliogID2	Fragment/Exon	SeqOligoID1	SeqOligoID2
050-547	050-548	15	CTCCTCCATCCCCTCTGC	TGAATGCATCTCTCTCCAGG
050-549	050-550	16	GATGATGAAGAGGCTCCACG	AGAGCCTCACCTGTAGCTGG
050-551	050-552	17	CACAACCAGCATAGGTCATCC	ACGGTGTAGGGTCCCCAG
050-553	050-554	18	GTAGTCTGGGGAGAGCCTGG	GAGGACACCGTCTGGGTG
050-507	050-508	01	TGTGGGACTGAAGTTCTTGG	CAACAGCCAAAAAGTTTGC
050-509	050-510	02	TGACTCTGCTTGACCTCACC	GCACCAGGTTCAGTACCCAG
051-199	051-200	03	CTAACAGGTCTCTCTTCTACC	AGTTAAGTAACTTGGCTGAGG
050-513	050-514	04	CAGCCAGTTCCCTAAGGTCC	ACTAGCTGATGGGCTAGGGC
050-515	050-516	05	GGGAGGAGGATTCAAAGGC	AGGTGTCAGTGGGGAGATGG
050-517	050-518	06	GAGAAGACCCTTACCCAGC	TCCCCTCTTCCCTCTAGGAC
050-519	050-520	07	GAGCCATGCTCATCTCCAG	CCACGTGCCAGGTTAAGTAG
050-521	050-522	08	CCAATATGTCAGGCAGCAGG	TCATTCATTAACCCACAAGG
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050-527	050-528	11	TAAGCGAGAAGGCAAACAGC	GGCACACTGTAGGAAGACAGG
050-529	050-530	12_01	ATTTGAGCTTAAAGTGGGGC	CATCTCCACTGCCTGTGAAG
050-531	050-532	12_02	TCAGAGGAACCATCAGCCTC	GAGGTCTCTAGAACTTCTCC
050-533	050-534	12_03	AGGTTGTAGAGACTTCTGCC	AAGTCCAAGTCTCCAGAAGC
050-535	050-536	12_04	CTGGAAAAGAAGACTTGGTGG	AAGGCTGCCCACTGAGATC
050-537	050-538	12_05	GCACTAGTCAACCCTTTGGC	TCTAAAATGCCAGAAGGCCC
050-539	050-540	12_06	GAATCTGTAACCCAGGCTCC	AGGGGACCCAGAATCTTCTC
050-541	050-542	12_07	TCAGTCCCAGAATCTAGCAG	CCTTCTGTGCTGCAGTAGGC
050-543	050-544	13	TCTTGGAGGCCATGGTAG	GGGGATGAAGAAGTCTGTCC
050-545	050-546	14	GCCATAAGACTGGCAGCATC	CTGCCTTCTGAAAGGTGAGG

Supplemental table 2. Additional phenotypic information of selected individuals for ACAN-sequencing

Patient	Gender	Midface hypoplasia	Joint problems	Broad great toes	Brachydactyly	Prognathism	Flat nasal bridge	Broad forehead	Posteriorly rotated ears	Microcephaly	Exagerrated lumbar lordosis	Genu valgum	ACAN mutation
Group 1													
1 (A.IV:1)	female	**yes**	**yes**	**yes**	**yes**	no	no	no	no	**yes**	no	no	**yes**
2	female	no	no	**yes**	no	no	no	no	no	no	no	no	no
3	female	**yes**	no	no	**yes**	no	**yes**	**yes**	no	no	no	no	no
4	male	no	no	no	no	**yes**	no	no	**yes**	no	no	no	no
5	male	**yes**	no	**yes**	no	**yes**	no	no	no	no	no	no	no
6	male	**yes**	no	no	no	no	no	**yes**	no	no	no	no	no
7	female	no	no	no	no	**yes**	no	no	no	no	no	no	no
8	male	no	no	no	**yes**	no	no	no	no	**yes**	no	no	no
9 [a]	female	no	no	**yes**	no	no	**yes**	no	no	no	no	**yes**	no
10 [a]	male	no	no	no	**yes**	no	**yes**	no	no	no	no	no	no
11	female	no	no	no	no	no	no	no	no	no	no	no	no
12	female	no	no	no	no	no	no	no	no	**yes**	no	no	no
13	female	no	no	no	no	no	no	no	no	no	no	no	no
14 [b]	male	no	no	no	no	no	no	no	no	no	no	**yes**	no
15 [b]	male	no	no	no	no	no	no	no	no	no	no	**yes**	no
16	female	no	no	no	no	no	no	no	no	no	no	no	no
17	male	no	no	no	no	no	no	**yes**	no	no	no	no	no
18	male	no	no	no	no	no	no	no	no	no	no	no	no
19	male	no	no	no	no	no	no	no	no	no	no	no	no

Supplemental table 2. Additional phenotypic information of selected individuals for ACAN-sequencing (continued)

Patient	Gender	Midface hypoplasia	Joint problems	Broad great toes	Brachydactyly	Prognathism	Flat nasal bridge	Broad forehead	Posteriorly rotated ears	Microcephaly	Exagerrated lumbar lordosis	Genu valgum	ACAN mutation
Group 2													
20 (A.IV:2)	male	**yes**	**yes**	**yes**	no	no	no	no	**yes**	no	no	no	**yes**
21 (B.IV:1)	male	**yes**	**yes**	**yes**	no	**yes**	no	no	no	no	**yes**	no	**yes**
22 (C.III:1)	male	**yes**	no	**yes**	no	no	no	no	**yes**	no	no	no	**yes**
23	female	no	no	no	**yes**	no	**yes**	no	no	no	no	no	no
24	female	no	**yes**	no	no	no	no	**yes**	**yes**	no	no	**yes**	no
25	female	no	no	no	no	no	no	no	no	no	no	no	no
26	female	no	no	**yes**	no	**yes**	no	no	**yes**	no	no	no	no
27	female	**yes**	no	no	no	no	**yes**	no	no	no	no	no	no
28	female	no	**yes**	no	no	**yes**	no	no	no	no	**yes**	no	no
29	female	no	no	no	no	no	**yes**	**yes**	**yes**	no	**yes**	no	no

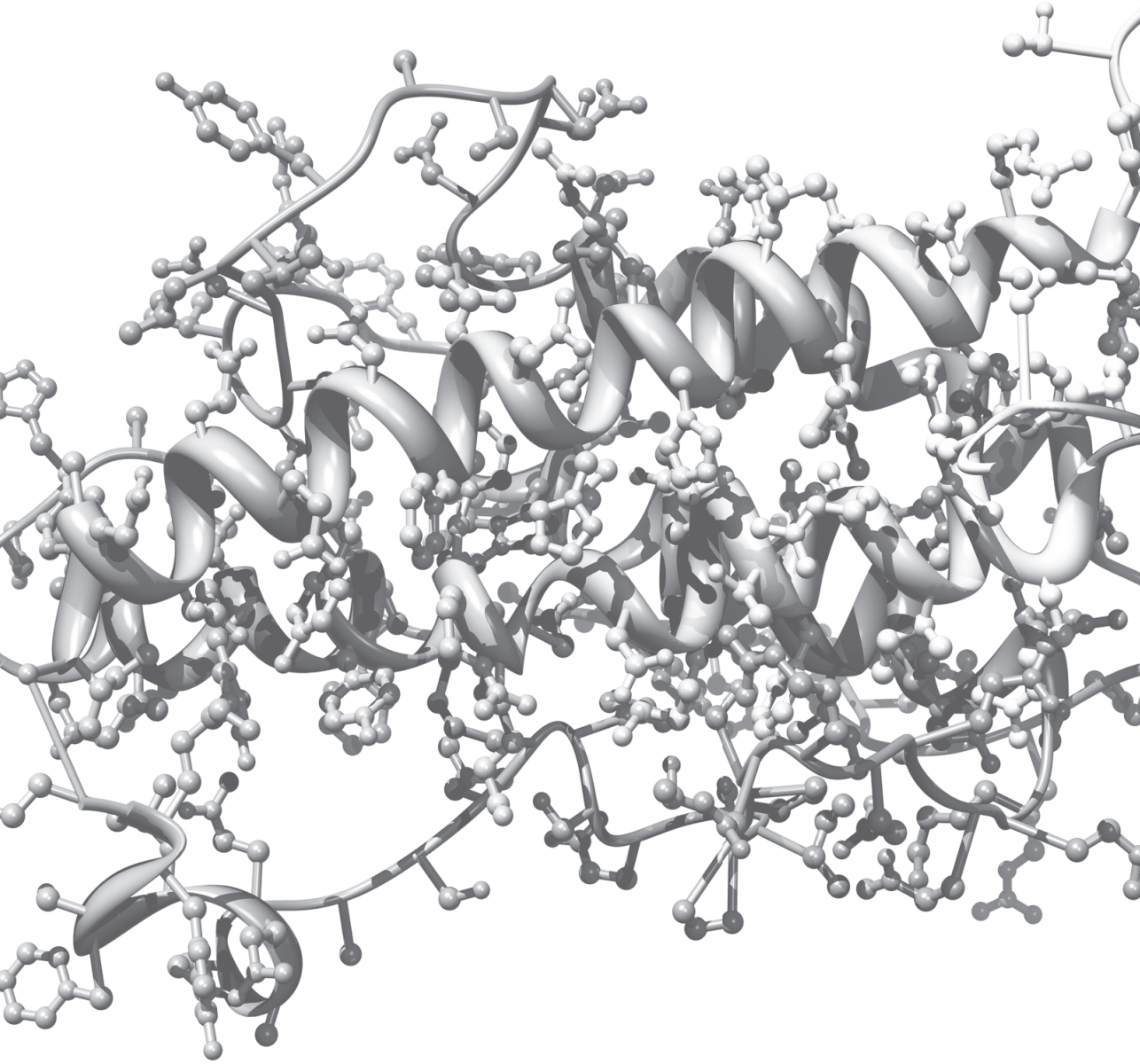
Group 1: persistently advanced bone age of at least 0.5 year at more than 2 radiographs prior to pubertal onset.

Group 2: advanced bone age of at least 0.5 year at start of GH/GnRHa treatment in early puberty.

A.IV:1, proband family A; A.IV:2, twin brother proband family A; B.IV:1, proband family B; C.III:1, proband family C.

[a] Siblings

[b] Twin brothers



CHAPTER 8

General discussion

GENERAL DISCUSSION

Twenty-five years ago, in 1991, our research group initiated the first Dutch study on growth hormone (GH) treatment in children born small for gestational age (SGA) to improve knowledge and care for these children. Since then, several Dutch studies have been initiated to evaluate the effectiveness and safety of GH treatment in children born SGA. GH treatment is most effective when started at a young age (1-3), but some children come to medical attention at an older age, even when pubertal development has started. In 2003, the Dutch SGA study was initiated to investigate the effectiveness of GH treatment in children born SGA who start treatment at the age of 8 years or older. In addition, we evaluated the effectiveness and safety of additional GnRHa treatment for 2 years to improve adult height in children who start puberty with an expected adult height less than −2.5 SDS (height at start of puberty <140 cm), based on Dutch references (4). Furthermore, the effect of GH 1 mg/m^2/day vs 2 mg/m^2/day (~0.033 vs 0.067 mg/kg/day) from pubertal onset until adult height was compared.

Low birth weight is associated with a higher risk of developing metabolic and cardiovascular disease in later life (5-7). Since both GH and GnRHa treatment influence determinants of metabolic and cardiovascular health, evaluating the long-term consequences of these treatments is essential. The effectiveness of GH treatment, with or without additional GnRHa treatment, has been described by previous PhD fellows of the Dutch SGA study (8-12). In the studies presented in this thesis, we investigated the effects of additional GnRHa treatment for 2 years on metabolic health, cardiovascular health, and pubertal development and growth (Chapters 2, 3, and 4). In addition, we assessed the longitudinal changes in metabolic and cardiovascular health during 5 years after cessation of GH treatment, and compared the data of previously GH-treated SGA adults to untreated adults born SGA and adults born appropriate for gestational age (AGA) (Chapters 5 and 6). Furthermore, ACAN gene mutations were investigated as an underlying cause of short stature after SGA birth which might have consequences for growth response and health prognosis (Chapter 7).

In this chapter, our results are discussed in view of current literature. Subsequently, clinical implications of our results are presented and recommendations for future research are provided.

METABOLIC AND CARDIOVASCULAR HEALTH DURING GH TREATMENT WITH OR WITHOUT GNRHA

In 2012, our research group showed that pubertal children born SGA with a poor adult height expectation can benefit from treatment with GH 1 mg/m^2/day combined with 2 years of GnRHa and even more so with a double GH dose of 2 mg/m^2/day (11). Since data on the metabolic effects of combined GH/GnRHa treatment were scarce (8), we

compared the metabolic effects of combined GH/GnRHa treatment with those of treatment with GH only by evaluating longitudinal changes in body composition, blood pressure, lipid levels, insulin sensitivity, and β-cell function until adult height was reached in children born SGA.

In chapters 2 and 3, we demonstrate that body composition, blood pressure, lipid profile, insulin sensitivity, and β-cell function at adult height (~ after 6 years of GH treatment) were similar between children treated with combined GH/GnRHa and those treated with GH only. In children who started combined GH/GnRHa treatment in early puberty, a double GH dose of 2 mg/m^2/day resulted in a lower fat mass and a similar lean body mass, blood pressure, lipid profile, insulin sensitivity, and β-cell function as the standard GH dose of 1 mg/m^2/day.

The metabolic effects of GnRHa treatment were mainly known from studies in children with central precocious puberty (CPP). During GnRHa treatment, sex steroids and stimuli to gain lean body mass are suppressed, which could result in changes in body composition toward relatively more fat mass and less lean body mass. Studies evaluating the effects of GnRHa treatment on fat mass in children with CPP were, however, inconclusive with most studies finding no change in fat mass and some showing a modest decrease (13-20). Other studies reported an increase in fat mass during GnRHa treatment, which returned to baseline values after cessation of treatment (21, 22). In chapter 2, we show an increase in fat mass percentage SDS during 2 years of combined GH/GnRHa treatment in children treated with GH 1 mg/m^2/day, but not in those treated with GH 2 mg/m^2/day. This could indicate that GnRHa treatment has indeed a fat mass enhancing effect which is counterbalanced by the lipolytic effects of GH treatment, particularly when GH is administered in a higher dose, whereas treatment with GH 1 mg/m^2/day appears less sufficient to prevent children from gaining fat mass during GnRHa treatment. The clinical relevance of gaining fat mass during GnRHa treatment depends on the proportion of visceral vs subcutaneous fat, since visceral fat is likely to be more related to a worse metabolic health as opposed to subcutaneous fat. Besides, the clinical relevance also depends on the persistence of the gain in fat mass after cessation of treatment. Since we used a DXA scan to assess fat mass and lean body mass, we could not distinguish between visceral and subcutaneous fat. However, visceral adipose tissue induces specific cytokine changes, such as an increase in proinflammatory cytokines (tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6)) and a decrease in adiponectin, which induces insulin resistance**.** In chapter 3, we show a decrease in insulin sensitivity during combined GH/GnRHa treatment, which might indicate an increase in visceral fat mass during combined GH/GnRHa treatment. Since the decline in insulin sensitivity during combined GH/GnRHa treatment could also be due to the GH treatment, we evaluated insulin sensitivity during 3 months of only GnRHa treatment and found that insulin sensitivity remained similar. Although 3 months of GnRHa treatment might have been

too short to detect significant changes in insulin sensitivity, these findings indicate that short-term GnRHa treatment does not affect insulin sensitivity and that the decline in insulin sensitivity during combined GH/GnRHa was, therefore, due to the GH treatment.

At adult height, when GnRHa treatment had been discontinued for many years, fat mass and insulin sensitivity were similar in adolescents treated with combined GH/GnRHa and those treated with GH only, despite the changes that occurred during combined GH/GnRHa treatment. This indicates that the fat mass enhancing effect of GnRHa treatment is temporarily and disappears after cessation of GnRHa treatment. This is in line with studies in women treated with GnRHa during childhood for CPP (23, 24). One study in girls with CCP showed lower insulin sensitivity at adult height in GnRHa-treated compared to untreated girls (25). In that study, however, HOMA-IR was used to determine insulin sensitivity, which is not comparable with the frequently sampled intravenous glucose tolerance (FSIGT) tests we used. Besides, the girls with lower insulin sensitivity had also a higher body mass index, which could explain the difference in insulin sensitivity between the treatment groups.

Despite the difference in fat mass percentage SDS, insulin sensitivity, acute insulin response, β-cell function, blood pressure and lipid levels were similar in children treated with GH 1 mg/m^2/day and those treated with GH 2 mg/m^2/day, which is in line with previous findings in SGA children who started GH treatment prepubertal (26-30).

Conclusions

In conclusion, our present findings show that there are no long-lasting unfavorable effects of combined GH/GnRHa treatment on metabolic and cardiovascular profile at adult height when compared to GH treatment only. A GH dose of 2 mg/m^2/day started in early puberty, results in a similar metabolic and cardiovascular profile as the standard GH dose of 1 mg/m^2/day.

PUBERTAL DEVELOPMENT AND GROWTH DURING GH TREATMENT WITH OR WITHOUT GNRHA

Clinically, children treated with combined GH/GnRHa seem to have an accelerated pubertal progression after cessation of GnRHa treatment which might result in a shorter pubertal duration and less pubertal growth. There were, however, no data on pubertal development and growth after cessation of GnRHa treatment in GH-treated SGA children. We, therefore, investigated pubertal development and growth in 76 children who started GH treatment prepubertal and were treated until adult height. Children with a poor adult height expectation at onset of puberty were treated with additional 2 years of GnRHa treatment from onset of puberty.

In chapter 4, we show that pubertal duration after cessation of GnRHa treatment was shorter than pubertal duration in those treated with GH only (40.9 vs 46.7 months in girls; 50.8 vs 57.5 months in boys). Bone maturation diminished during GnRHa treatment but was not completely stopped, which is in line with other studies (16, 19, 31-37). This indicates that senescence of the growth plate, the progressive loss of function and structural involution of the growth plate, which is growth dependent (38), continued during GnRHa treatment. When growth plates are more senescent and have expended more of their growth potential, a shorter exposure to estrogen is sufficient to complete growth plate fusion (39), leading to a shorter period of growth after cessation of GnRHa treatment. There are no other studies reporting pubertal duration after discontinuation of GnRHa treatment in SGA children and we, therefore, could not compare our results to other studies.

In children with CPP treated with GnRHa only, a decreased growth rate during GnRHa has been reported, which is lower than the growth rate of prepubertal children (13, 32, 33, 40, 41). Reduced levels of insulin-like growth factor 1 (IGF-I) and GH during GnRHa treatment could explain the diminished growth rate during GnRHa treatment (42). In our study, girls and boys grew approximately 13 cm during 2 years of combined GH/GnRHa treatment, i.e. 6.5 cm/year, which is in line with the annual growth of prepubertal children. This indicates that GH treatment can normalize growth during GnRHa treatment. The average height gain from onset of puberty until adult height was 25.4 cm in girls and 33.0 cm in boys treated with combined GH/GnRHa, which is similar to the average pubertal height gain in healthy children born AGA (4). Adult height was similar in children treated with GH/GnRHa compared with those with GH only. This shows that additional GnRHa treatment has a beneficial effect on adult height in GH-treated SGA children who start their puberty when still short.

In chapters 2 and 3, we show that additional 2 years of GnRHa treatment had no negative effects on metabolic health, insulin sensitivity, and β-cell function (43, 44). Besides, GnRHa treatment was well tolerated in all children and no adverse effects were reported. All girls treated with GnRHa reported regular cycles at adult height and one pregnancy after adult height with normal offspring was reported. Thus, the interruption of the GnRH axis in childhood did not impair reproductive function in adulthood, which is in line with findings in girls with CPP treated with GnRHa only (45).

Conclusions

In conclusion, pubertal duration after cessation of GnRHa treatment is shorter in SGA children with combined GH/GnRHa treatment compared to pubertal duration in children treated with GH only. Due to adequate growth during 2 years of additional GnRHa treatment, height gain from onset of puberty until adult height is more in children treated with combined GH/GnRHa treatment than in children treated with GH only. Thus, although children with GH/

GnRHa treatment started puberty with a shorter stature, they reached a similar adult height as children treated with GH only.

METABOLIC AND CARDIOVASCULAR RISK FACTORS DURING 5 YEARS AFTER CESSATION OF GH TREATMENT

Low birth weight is associated with an increased risk of diabetes mellitus type 2 and cardiovascular diseases (CVD) (5-7, 46). Despite the fact that biosynthetic GH has been used for more than 25 years, there were no longitudinal data on the long-term effects after cessation of GH treatment in young adults born SGA. We, therefore, evaluated longitudinal changes in body composition, insulin sensitivity, β-cell function, blood pressure, lipid levels and carotid intima media thickness (cIMT), during 5 years after cessation of GH treatment and compared these data with age-matched control groups.

In chapters 5 and 6, we demonstrate that fat mass increased continuously and lean body mass decreased. At 5 years after cessation of GH treatment, fat mass was higher and lean body mass lower compared to values at adult height when GH treatment was discontinued, indicating that the lipolytic and anabolic effects of GH treatment on body composition were lost after cessation of treatment. Insulin sensitivity and β-cell function improved during 6 months after cessation of GH treatment and sustained thereafter. The initial increase in blood pressure during the first 6 months after cessation of GH treatment recovered thereafter, and at 5 years after cessation of GH treatment, blood pressure was similar to levels at cessation of GH treatment. Lipid levels increased after GH-cessation in parallel to the increase in fat mass. cIMT levels did not change after cessation of GH treatment.

Preliminary data of the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) project showed that adults treated with GH during childhood for isolated short stature have an increased mortality rate due to cardiovascular disorders, whereas others reported no deaths due to CVD (47-49). A limitation of the SAGhE project is the lack of an appropriate control group of untreated SGA patients, which is necessary to study the effect of GH treatment on the risk of developing diabetes mellitus type 2 and CVD, knowing that subjects born SGA have an increased risk for developing these diseases. We now show that at the age of 21 years, 5 years after cessation of GH treatment, fat mass was similar and lean body mass lower in previously GH-treated young adults compared to untreated short SGA young adults. Since the GH-treated young adults born SGA were the smallest at birth, body composition might have been reprogrammed during fetal and early life, which could explain the lower lean body mass in early adulthood in these subjects. Insulin sensitivity and β-cell function were similar in the previously GH-treated young adults compared to untreated short SGA young adults, indicating that GH treatment during childhood does not impair insulin sensitivity on the

longer-term. Lipid levels increased after GH-cessation in parallel to the increase in fat mass, but remained lower than levels in untreated short SGA young adults, suggesting that the beneficial effect of GH treatment on lipid metabolism sustains after cessation of GH treatment. These findings are reassuring since elevated lipid levels contribute to a higher risk for CVD. Blood pressure and cIMT were similar in the previously GH-treated young adults compared to age-matched untreated short SGA young adults, contradicting the hypothesis that there is a link between GH treatment and vascular diseases (47, 50). We previously showed that levels of matrix metalloproteinase-9, which play a role in the development of atherosclerosis (51), decrease during GH treatment, in parallel to the decrease in blood pressure (52), which supports our current findings. On the other hand, our participants were relatively young and cardiovascular disease might not yet be clinically apparent. Epidemiological data, however, indicate that a cIMT value above 1 mm at any age is associated with a significantly increased risk for myocardial infarction and/or cerebrovascular disease (53), and none of the previously GH-treated young adults born SGA and other participants in our study had a cIMT above 1 mm at any time point during the study.

At 5 years after cessation of GH treatment, previously GH-treated SGA young adults had a similar body composition, insulin sensitivity, blood pressure, and cIMT, and a more beneficial lipid profile, than untreated short SGA young adults. This indicated that GH treatment during childhood has no long-term unfavorable effects on metabolic and cardiovascular profile which is reassuring. However, metabolic and cardiovascular health in late adulthood in both GH-treated and untreated SGA adults remains unknown. Longer-term follow-up of metabolic and cardiovascular risk factors is, therefore, indicated in these predisposed subjects.

Conclusions

Our study shows that the metabolic and cardiovascular risk factors significantly change after cessation of GH treatment due to the loss of GH properties. At 5 years after cessation of long-term GH treatment, however, the metabolic and cardiovascular profiles of previously GH-treated SGA young adults are similar or more beneficial than in untreated young adults born SGA, showing that long-term GH treatment in children born SGA has no unfavorable effects on metabolic and cardiovascular profile in early adulthood.

ACAN GENE MUTATIONS IN SHORT CHILDREN BORN SGA

Some children born SGA show accelerated bone age maturation during childhood, resulting in early closure of the epiphyseal growth plates, cessation of growth at a young age, and extremely short adult stature. The cause of this accelerated bone age maturation was unknown. As heterozygous mutations in the ACAN gene were reported

in children with idiopathic short stature and advanced bone age (54, 55), we wanted to evaluate if ACAN gene mutations could explain the accelerated bone age maturation in some GH-treated children born SGA. Besides, we aimed to develop a clinical scoring system to identify subjects most likely to test positive for an ACAN gene mutation and to assess the effectiveness of GH treatment in children with an ACAN gene mutation.

In chapter 7, we demonstrate the presence of ACAN gene mutations in short SGA children, thereby expanding the differential diagnosis of genetic variants in children born SGA with persistent short stature. The variance in clinical characteristics in patients with confirmed ACAN gene mutations made it difficult to distinguish which children were likely to have an ACAN gene mutation (54-59). We did show that mutations are related to advanced bone maturation in combination with the characteristics midface hypoplasia, joint problems, and broad great toes. Based on advanced bone age and these characteristics, we developed a clinical scoring system which will help physicians identifying children who most likely have an ACAN gene mutation (Figure 1). Advanced bone age in combination with 3 characteristics had a predictive value of 100% on having an ACAN gene mutation. Also, in case of advanced bone age in combination with

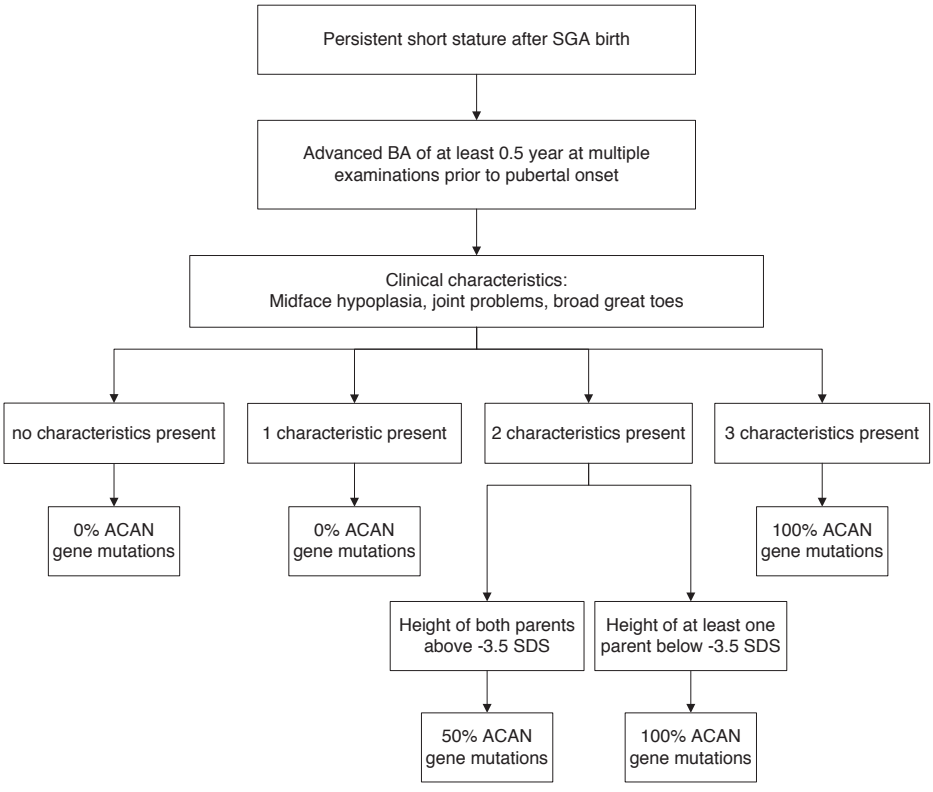


Figure 1. Clinical scoring system for ACAN gene mutations.

2 of these characteristics and at least one extremely short (height <-3.5 SDS) parent, 100% of the children had an ACAN gene mutation. Advanced bone age in combination with 2 characteristics but without short parents, still resulted in the identification of an ACAN gene mutation in 50% of the cases. Children with none or only 1 characteristic did not have an ACAN gene mutation. Our study is the first to describe a clinical scoring system which will likely improve awareness of ACAN gene mutations in short children born SGA. The identification of more children with an ACAN gene mutation will improve our knowledge of these mutations and might, therefore, result in better healthcare and treatment options for these children.

Remarkably, accelerated bone maturation did not occur in early childhood but from the age of 7 years in the affected girl and the age of 11 years in the affected boys. This highlights the importance of regular reevaluations of the original diagnosis in children born SGA.

Since being born SGA is an indication for GH treatment, children with an ACAN gene mutation who are born SGA can be treated with GH. However, one of the European requirements to start GH treatment in children born SGA is a distance to target height of at least 1 SDS (60). Children with an ACAN gene mutation who inherited this mutation from a parent, will most likely have a smaller distance to target height (<1 SDS) because that parent will be very short. They would, therefore, be excluded from GH treatment. A previous study from our research group has shown that this distance to target height criterion is not justified (9). Since a mutation in the ACAN gene results in a skeletal disorder (61), the effectiveness of GH treatment in children with skeletal disorders is uncertain and for many thus questionable. There are no published data on the effectiveness of GH treatment in children with an ACAN gene mutation. Nonetheless, our study shows that the GH/GnRHa-treated SGA adolescents with an ACAN gene mutation had a better adult height SDS than their parent with the same ACAN gene mutation, suggesting that GH treatment with additional 2 years of GnRHa treatment is beneficial for short children born SGA with an ACAN gene mutation. The number of adolescents with an ACAN gene mutation and attainment of adult height was, however, small and therefore definite conclusions can not be drawn.

Conclusions

Our data show that the combination of persistent short stature after SGA birth, advanced bone age, midface hypoplasia, joint problems, and broad great toes, is highly suggestive for an ACAN gene mutation. Although ACAN gene mutations result in skeletal disorders, our data suggest that children born SGA with an ACAN gene mutation can benefit from GH treatment with additional 2 years of GnRHa treatment.

GENERAL CONCLUSIONS AND CLINICAL IMPLICATIONS

GH treatment is an approved and frequently applied growth promoting therapy in children born SGA, which is most effective when started at a young age (1-3). Our research group has shown that GH treatment can still be beneficial when started at an older age, even when pubertal development has started, and that additional 2 years of GnRHa treatment is beneficial in GH-treated SGA children who start puberty with an expected adult height less than -2.5 SDS (11). The studies in this thesis demonstrate that the addition of 2 years of GnRHa treatment in GH-treated SGA children has no unfavorable effects on metabolic and cardiovascular health when compared to GH treatment only. In addition, our research group has shown that the same treatment had no adverse effect on bone mineral density and health-related quality of life in GH-treated SGA children (10, 12). Thus, additional GnRHa treatment to GH treatment appears effective and safe and can, therefore, be considered in SGA children who start puberty with an expected adult height below -2.5 SDS. Based on the data of the Dutch SGA study, adjustment of the treatment guideline for short children born SGA should be considered.

When GH treatment is started in early puberty, a GH dose of 2 mg/m^2/day results in a better adult height in boys and a similar metabolic and cardiovascular profile as the standard GH dose of 1 mg/m^2/day. We do, however, not recommend high GH dosing in all children since concerns have been expressed regarding the possible detrimental effects of persistently relatively high serum IGF-I levels. Nonetheless, our data show that a GH dose of 2 mg/m^2/day could be considered in a small group of short, pubertal SGA children who come to medical attention at such an old age that only a few years of growth remain.

The metabolic and cardiovascular profile of previously GH-treated young adults born SGA is similar to that of untreated young adults born SGA, indicating that long-term GH treatment during childhood does not only improve adult height but has also no unfavorable effects on health. Cessation of GH treatment, however, leads to the loss of GH properties which is disadvantageous. The significant changes in body composition highlight the importance to inform patients to keep a healthy lifestyle and diet when long-term GH treatment is discontinued.

Children born SGA comprise a heterogeneous group with a broad spectrum of underlying causes, including syndromes like Noonan, 3M, Turner, and Bloom, and mutations in the IGF1R gene and the ALS gene. Our study shows that ACAN gene mutations can also be an underlying cause in short children born SGA. We recommend that children with persistent short stature after SGA birth, advanced bone age, midface hypoplasia, joint problems, and broad great toes are tested for ACAN gene mutations. Although ACAN gene mutations result in a skeletal disorder, combined GH/GnRHa treatment improves adult height in those born SGA. Since clinical characteristics tend to change over time, it is important to regularly reevaluate the original diagnosis in children born SGA.

DIRECTIONS FOR FUTURE RESEARCH

Follow-up into adulthood after attainment of adult height is required to investigate the long-term effects of additional GnRHa treatment on metabolic and cardiovascular profile, fertility, bone mineral density, and health-related quality of life, in young adults born SGA. Besides, studies with a follow-up duration beyond 5 years after cessation of GH treatment are needed to finally conclude on the lifetime risk of metabolic and cardiovascular diseases in previously GH-treated adults born SGA, since the development of these diseases might occur later in life.

Up until now, genetic mutations underlying persistent short stature in children born SGA have only been found in a small percentage of patients. Further research into the genetic basis of human stature will increase our knowledge and might explain the variability of the SGA phenotype. This might lead to a more precise prediction of the response to GH treatment, which will ultimately lead to individualized treatment options.

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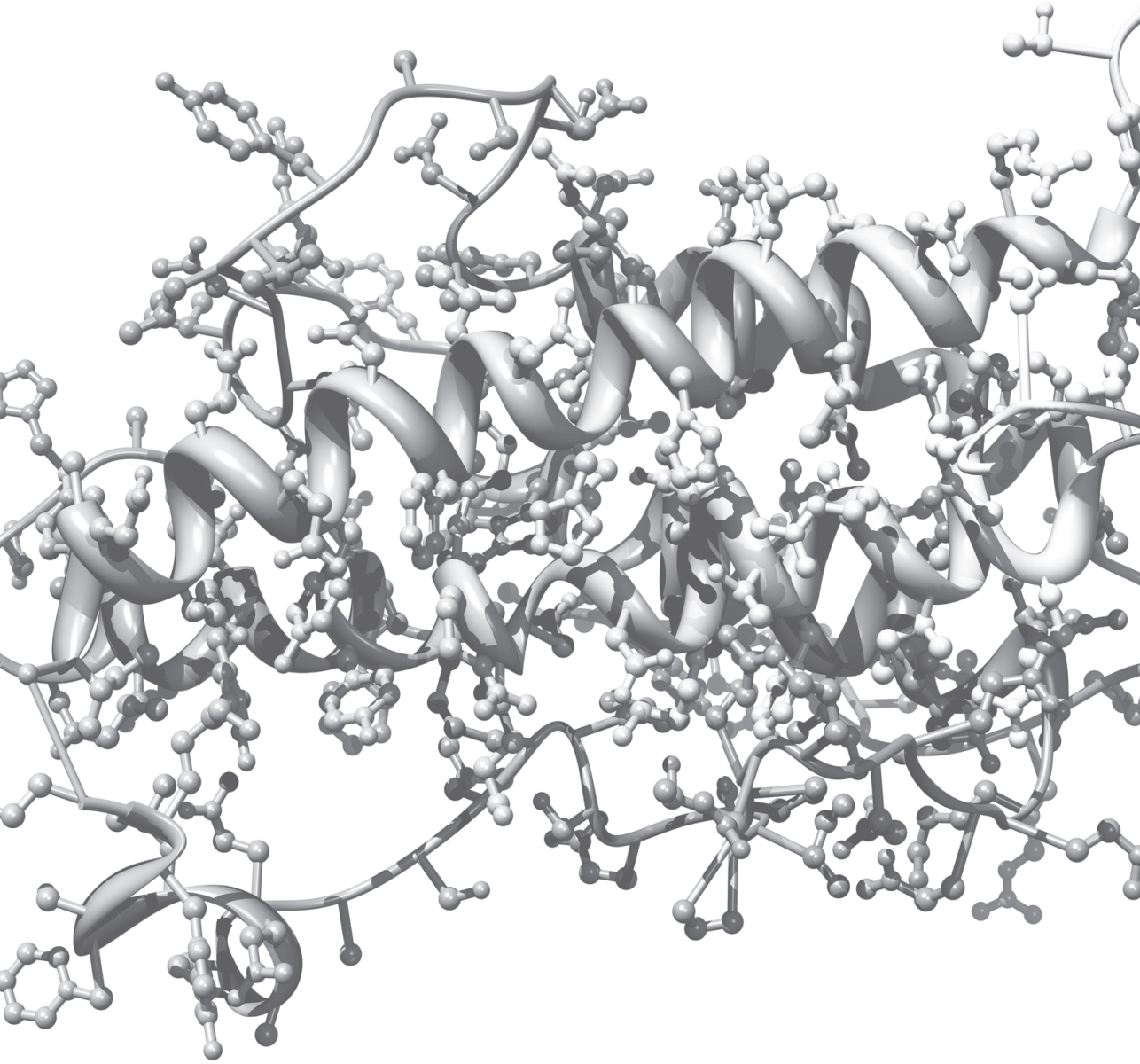
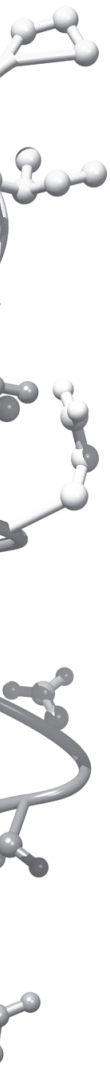


CHAPTER 9



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, the growth hormone (GH) axis, the pituitary hormone axis, and puberty. Treatment options and the reported effects of these treatments in short children born SGA, who come to medical attention around onset of puberty, are discussed. Furthermore, the association between low birth weight and a higher risk of metabolic and cardiovascular disease in later life are described. Finally, the aims of the studies performed and the outline of this thesis are presented.

CHAPTER 2

Pubertal children born SGA with a poor adult height expectation can benefit from treatment with GH 1 mg/m^2/day in combination with 2 years of GnRH analog (GnRHa) and even more so with a double GH dose. We evaluated the long-term effects of combined GH/GnRHa and 2 different GH doses on metabolic health in children born SGA.

At adult height, fat mass percentage SDS, lean body mass SDS, blood pressure SDS, and lipid levels were similar in 64 children treated with combined GH/GnRHa and 43 children treated with GH only. The GH-dose effect on metabolic health was evaluated in a subgroup of 47 children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day ~ 0.033 or 0.067 mg/kg/day) with additional GnRHa for 2 years. Fat mass percentage SDS was lower during treatment with GH 2 mg/m^2/day. There was no GH dose-dependent effect on lean body mass SDS, blood pressure SDS, and lipid profile.

In conclusion, combined GH/GnRHa treatment has no long-term negative effects on metabolic health compared with GH only. Started in early puberty, a GH dose of 2 mg/m^2/day results in a similar metabolic health at adult height and a more favorable fat mass percentage SDS than GH 1 mg/m^2/day.

CHAPTER 3

Pubertal children born SGA with a poor adult height expectation can benefit from treatment with GH 1 mg/m^2/day in combination with 2 years of GnRHa and even more so with a double GH dose. We evaluated the long-term effects of combined GH/GnRHa and 2 different GH doses on insulin sensitivity and β-cell function, measured by frequently sampled intravenous glucose tolerance (FSIGT) tests, in children born SGA.

At adult height, insulin sensitivity and β-cell function were similar in 67 children treated with combined GH/GnRHa and 43 children treated with GH only. The GH-dose effect on metabolic health was evaluated in a subgroup of 48 children who started GH treatment in early puberty (randomized to 1 or 2 mg/m^2/day) with additional GnRHa for 2 years. There was no GH dose-dependent effect on insulin sensitivity and β-cell function.

In conclusion, combined GH/GnRHa treatment has no long-term negative effects on insulin sensitivity and β-cell function compared with GH only. Started in early puberty, a GH dose of 2 mg/m^2/day results in a similar insulin sensitivity and β-cell function at adult height as a GH dose of 1 mg/m^2/day.

CHAPTER 4

GH-treated SGA children with an adult height expectation below -2.5 SDS at onset of puberty, can benefit from additional GnRHa treatment for 2 years. We investigated pubertal development and growth in 76 SGA children who started GH treatment prepubertal. Thirty-two children received additional GnRHa for 2 years.

Age and bone age, but also height at pubertal onset, were lower in girls and boys in the GH/GnRHa-group compared with the GH-group. In girls and boys treated with GH/GnRHa, pubertal duration after stop of GnRHa treatment was shorter than pubertal duration in those treated with GH only (40.9 vs 46.7 months in girls; 50.8 vs 57.5 months in boys). Height gain from onset of puberty until adult height, including height gain during 2 years of GnRHa treatment, was greater during GH/GnRHa treatment with 25.4 cm in girls and 33.0 cm in boys. This was 6.6 cm more than in girls and boys treated with GH only. As a result, children treated with GH/GnRHa reached a similar adult height as those treated with GH only.

In conclusion, GH-treated SGA children who start puberty with an adult height expectation below -2.5 SDS and are treated with additional GnRHa for 2 years, have a shorter pubertal duration after discontinuation of GnRHa compared to pubertal duration in children treated with GH only. Due to adequate growth during 2 years of GnRHa treatment, height gain from onset of puberty until adult height is more in children treated with combined GH/GnRHa treatment than in children treated with GH only. Thus although children with GH/GnRHa treatment started puberty with a shorter stature, they reached a similar adult height as children treated with GH only.

CHAPTER 5

GH treatment results in a reduction in fat mass and insulin sensitivity, and an increase in lean body mass. Only short-term longitudinal changes after cessation of GH treatment

in subjects born SGA were available. We, therefore, assessed longitudinal changes in body composition, insulin sensitivity, acute insulin response, and β-cell function during 5 years after attainment of adult height when GH treatment was discontinued.

In 199 previously GH-treated SGA young adults, fat mass (both trunk and limb fat) gradually increased during 5 years after GH-cessation whereas lean body mass decreased. At 5 years after GH-cessation, fat mass was higher and lean body mass lower compared to levels at GH-cessation. During 6 months after GH-cessation, insulin sensitivity and β-cell function increased and the acute insulin response decreased. At 5 years after GH-cessation, insulin sensitivity was higher, the acute insulin response lower, and β-cell function similar to results at GH-cessation. At 5 years after GH-cessation, previously GH-treated SGA young adults had a similar fat mass, insulin sensitivity, acute insulin response, and β-cell function, and a lower lean body mass as age-matched untreated short SGA young adults. Fat mass, insulin sensitivity, acute insulin response, and β-cell function were similar and lean body mass lower, compared to untreated SGA young adults with spontaneous catch-up growth and young adults born appropriate for gestational age.

In conclusion, fat mass continuously increased after GH-cessation indicating the loss of GH benefits. At 5 years after GH-cessation, however, fat mass, insulin sensitivity, acute insulin response, and β-cell function were similar compared to untreated SGA and AGA controls.

CHAPTER 6

GH treatment influences cardiovascular risk factors. Only short-term longitudinal changes after cessation of GH treatment in subjects born SGA were available. We, therefore, assessed longitudinal changes in systolic and diastolic blood pressure (SBP and DBP), lipid levels, and carotid intima media thickness (cIMT) during 5 years after cessation of GH treatment.

In 199 previously GH-treated SGA young adults, SBP and DBP increased during 6 months after GH-cessation and decreased thereafter. Total cholesterol, LDLc, and triglycerides increased during 2 years after GH-cessation and remained similar thereafter. cIMT did not change after GH-cessation. At 5 years after GH-cessation, SBP, DBP, lipid levels, and cIMT were similar to levels at GH-cessation. At 5 years after GH-cessation, previously GH-treated SGA young adults had a similar SBP, DBP and cIMT, and lower lipid levels than age-matched untreated short SGA young adults. SBP, DBP, lipid levels, and cIMT were similar to untreated SGA young adults with spontaneous catch-up growth and young adults born appropriate for gestational age.

In conclusion, this longitudinal study during 5 years after cessation of GH treatment shows that long-term GH treatment in children born SGA does not only improve adult

height but has also no unfavorable effects on cardiovascular health in early adulthood and might be beneficial with regard to the lipid profile.

CHAPTER 7

Some children born SGA show advanced bone age during GH treatment. ACAN gene mutations had been described in children with idiopathic short stature and advanced bone age. We set out to investigate the presence of ACAN gene mutations in children born SGA with persistent short stature, to develop a clinical scoring system, and to assess the response to GH treatment in children with ACAN gene mutations.

Bone age was assessed in 290 GH-treated SGA children and ACAN-sequencing was performed in 29 children with an advanced bone age of ≥0.5 year compared to calendar age. ACAN gene mutations were found in 4/29 SGA children with advanced bone age (13.8%). Mutations were related to the characteristics: midface hypoplasia, joint problems, and broad great toes. Of children with advanced bone age and 3 of these characteristics, 100% had an ACAN gene mutation. Of children with advanced bone age and 2 of these characteristics, 50% had an ACAN gene mutation, but in combination with a short parent (<-3.5 SDS) it rose to 100%. Children with advanced bone age having none or only 1 of these characteristics had no ACAN gene mutation. All GH-treated children with an ACAN gene mutation received additional GnRHa treatment for 2 years from onset of puberty. At adult height, 1 girl was 5 cm taller than her mother and 1 boy was 8 cm taller than his father with the same ACAN gene mutation. In boys, bone maturation was delayed by aromatase inhibitor treatment.

In conclusion, this study expands the differential diagnosis of genetic variants in children born SGA. Our data show that the combination of persistent short stature after SGA birth, advanced bone age, midface hypoplasia, joint problems, and broad great toes, is highly suggestive for an ACAN gene mutation. Although ACAN gene mutations result in skeletal disorders, our data suggest that children born SGA with an ACAN gene mutation can benefit from GH treatment with additional 2 years of GnRHa treatment.

CHAPTER 8

In the general discussion we discuss our study results described in this thesis in relation to current literature. We emphasize on clinical implications and give directions for future research.

SAMENVATTING

HOOFDSTUK 1

Dit hoofdstuk beschrijft de definities, prevalentie en mogelijke oorzaken van een kleine lengte en/of laag gewicht bij de geboorte (SGA, small for gestational age). Het geeft achtergrondinformatie over groei, puberteitsontwikkeling en de werking van diverse hormonale assen. De behandelingsmogelijkheden voor te kleine, SGA-geboren kinderen die zich rond de start van de puberteit presenteren, en de effecten van deze behandelingen, worden uiteen gezet. Daarnaast wordt de relatie tussen SGA, diabetes mellitus type 2 en hart- en vaatziekten beschreven. Aan het einde van dit hoofdstuk worden de doelstellingen van de studies en de indeling van dit proefschrift besproken.

HOOFDSTUK 2

SGA-geboren kinderen met een blijvend kleine lengte hebben baat bij behandeling met groeihormoon (GH). Wanneer zij een slechte lengteprognose hebben bij het begin van de puberteit, is additionele behandeling met GnRH-analoga (GnRHa) gedurende 2 jaar effectief, vooral met een dubbele dosis GH. We evalueerden de lange-termijn effecten van gecombineerde GH/GnRHa behandeling op de lichaamssamenstelling, bloeddruk en het lipidenprofiel. Daarnaast vergeleken we de effecten van 2 verschillende GH doseringen (1 vs 2 mg/m^2/dag) tijdens de puberteit.

Bij stop van de GH behandeling waren de vetmassa, spiermassa, bloeddruk en lipiden van 64 kinderen met gecombineerde GH/GnRHa behandeling vergelijkbaar met die van 43 kinderen met alleen GH behandeling. In 47 kinderen werd het effect van verschillende GH doseringen tijdens de puberteit onderzocht. De vetmassa was lager in de kinderen met een hogere GH dosering (2 mg/m^2/dag), maar de spiermassa, bloeddruk en lipiden waren vergelijkbaar in de 2 GH doseringsgroepen.

Deze resultaten tonen aan dat gecombineerde GH/GnRHa behandeling geen negatieve lange-termijn effecten heeft vergeleken met alleen GH behandeling. Wanneer gecombineerde GH/GnRHa behandeling wordt gestart in het begin van de puberteit, leidt een hogere GH dosering (2 mg/m^2/dag) tot een lagere vetmassa dan de standaard GH dosering van 1 mg/m^2/dag.

HOOFDSTUK 3

SGA-geboren kinderen met een blijvend kleine lengte hebben baat bij GH behandeling. Wanneer zij een slechte lengteprognose hebben bij het begin van de puberteit, is additionele behandeling met GnRHa gedurende 2 jaar effectief, vooral met een dubbele

dosis GH. We evalueerden de lange-termijn effecten van gecombineerde GH/GnRHa behandeling op de insulinegevoeligheid en β-cel functie. Daarnaast vergeleken we de effecten van 2 verschillende GH doseringen (1 vs 2 mg/m^2/dag) tijdens de puberteit.

Bij stop van de GH behandeling waren de insulinegevoeligheid en β-cel functie van 67 kinderen met gecombineerde GH/GnRHa behandeling vergelijkbaar met die van 43 kinderen met alleen GH behandeling. In 48 kinderen werd het effect van verschillende GH doseringen tijdens de puberteit onderzocht. De insulinegevoeligheid en β-cel functie waren vergelijkbaar in de 2 GH doseringsgroepen.

Deze resultaten tonen aan dat gecombineerde GH/GnRHa behandeling geen negatieve effecten heeft op de insulinegevoeligheid en β-cel functie vergeleken met alleen GH behandeling. Wanneer gecombineerde GH/GnRHa behandeling wordt gestart in het begin van de puberteit, leidt een hogere GH dosering (2 mg/m^2/dag) tot een vergelijkbare insulinegevoeligheid en β-cel functie als de standaard GH dosering van 1 mg/m^2/dag.

HOOFDSTUK 4

Tot op heden was de puberteitsontwikkeling en -groei van SGA-geboren kinderen na staken van 2 jaar GnRHa behandeling onbekend. We vergeleken daarom de duur van de puberteit en de mate van pubertaire groei van 32 kinderen met gecombineerde GH/GnRHa behandeling (GH/GnRHa-groep) met die van 44 kinderen met alleen GH behandeling (GH-groep).

Leeftijd, botleeftijd en de lengte bij start van de puberteit waren lager in de GH/GnRHa-groep dan in de GH-groep. De duur van de puberteitsontwikkeling na staken van de GnRHa behandeling was korter in de GH/GnRHa-groep dan de puberteitsontwikkeling in de GH-groep. De lengtetoename vanaf start van de puberteit tot aan de volwassen lengte, inclusief de twee jaar waarin GnRHa behandeling werd gegeven, was 25.4 cm in meisjes en 33.0 cm in jongens. Dit was 6.6 cm meer dan de lengtetoename vanaf start van de puberteit tot aan de volwassen lengte in meisjes en jongens van de GH-groep. De volwassen lengte was vergelijkbaar in de GH/GnRHa-groep en de GH-groep.

Concluderend, SGA-geboren kinderen die rondom het begin van de puberteit starten met gecombineerde GH/GnRHa behandeling, hebben een kortere puberteitsontwikkeling na staken van de GnRHa behandeling vergeleken met de puberteitsontwikkeling van kinderen met alleen GH behandeling. De lengtetoename vanaf start van de puberteit tot aan de volwassen lengte is echter groter door de adequate groei tijdens 2 jaar GnRHa behandeling. Ondanks het feit dat kinderen in de GH/GnRHa-groep kleiner waren bij start van de puberteit, bereikten zij hierdoor een vergelijkbare volwassen lengte als kinderen in de GH-groep.

HOOFDSTUK 5

GH behandeling resulteert in een afname van vetmassa en insulinegevoeligheid en een toename van spiermassa. Er waren geen gegevens bekend over de lange-termijn effecten van GH behandeling op de lichaamssamenstelling, insulinegevoeligheid en β-cel functie na staken van de behandeling. Daarom onderzochten we dit in 199, voorheen met GH behandelde, SGA-geboren jongvolwassenen (SGA-GH volwassenen) gedurende 5 jaar na staken van de GH behandeling. De gegevens op 5 jaar na staken van de behandeling werden vergeleken met 51 onbehandelde SGA geboren volwassenen met een te kleine lengte (SGA-S), 92 SGA geboren volwassenen met een normale lengte (SGA-CU) en 142 volwassenen met een normale geboortelengte en –gewicht en een normale volwassen lengte (AGA).

Gedurende 5 jaar na staken van de GH behandeling nam de vetmassa toe en de spiermassa af. De insulinegevoeligheid steeg in de eerste 6 maanden na staken van de GH behandeling en bleef daarna onveranderd. Vijf jaar na staken van de GH behandeling waren de vetmassa en insulinegevoeligheid hoger, de spiermassa en acute insuline respons lager, en de β-cel functie vergelijkbaar met de waarden bij stop van de behandeling. Vijf jaar na staken van de GH behandeling waren de vetmassa, insulinegevoeligheid, acute insuline respons en β-cel functie van SGA-GH volwassenen vergelijkbaar met die van SGA-S, SGA-CU en AGA volwassenen. De spiermassa was lager in SGA-GH volwassenen vergeleken met SGA-S, SGA-CU en AGA volwassenen.

Concluderend tonen onze resultaten een toename in vetmassa na staken van de GH behandeling in overeenstemming met het verlies van de lipolytische werking van GH. Vijf jaar na staken van de GH behandeling zijn de vetmassa, insulinegevoeligheid en β-cel functie van voorheen met GH behandelde SGA-geboren volwassenen vergelijkbaar met die van onbehandelde SGA geboren volwassenen met een te kleine lengte.

HOOFDSTUK 6

GH behandeling beïnvloedt verscheidene cardiovasculaire risicofactoren. Er waren geen gegevens bekend over de lange-termijn effecten van GH behandeling op de bloeddruk, het lipidenprofiel en de dikte van de intima-media van de carotiden (cIMT) na staken van de behandeling. Daarom onderzochten we dit in 199, voorheen met GH behandelde, SGA-geboren jongvolwassenen (SGA-GH volwassenen) gedurende 5 jaar na staken van de GH behandeling. De gegevens op 5 jaar na staken van de behandeling werden vergeleken met 51 onbehandelde SGA geboren volwassenen met een te kleine lengte (SGA-S), 92 SGA geboren volwassenen met een normale lengte (SGA-CU) en 142 volwassenen met een normale geboortelengte en –gewicht en een normale volwassen lengte (AGA).

De systolische en diastolische bloeddruk namen gedurende 6 maanden na staken van de GH behandeling toe, maar daalden daarna. Cholesterol, LDLc en triglyceriden namen gedurende 2 jaar na staken van de behandeling toe, ook na correctie voor de toename in vetmassa, en bleven daarna onveranderd. De cIMT veranderde niet na staken van de GH behandeling. Vijf jaar na staken van de GH behandeling waren de systolische en diastolische bloeddruk, het lipidenprofiel en de cIMT vergelijkbaar met de waarden bij stop van de behandeling. Vijf jaar na staken van de GH behandeling waren de systolische en diastolische bloeddruk en de cIMT van SGA-GH volwassenen vergelijkbaar met die van SGA-S volwassenen maar de lipiden waren lager in SGA-GH volwassenen. De systolische en diastolische bloeddruk, het lipidenprofiel en de cIMT van SGA-GH volwassenen waren vergelijkbaar met die van SGA-CU en AGA volwassenen.

Concluderend toont deze longitudinale studie gedurende 5 jaar na staken van de GH behandeling dat lange-termijn GH behandeling op de kinderleeftijd geen negatieve effecten heeft op cardiovasculaire risicofactoren op de jong volwassen leeftijd.

HOOFDSTUK 7

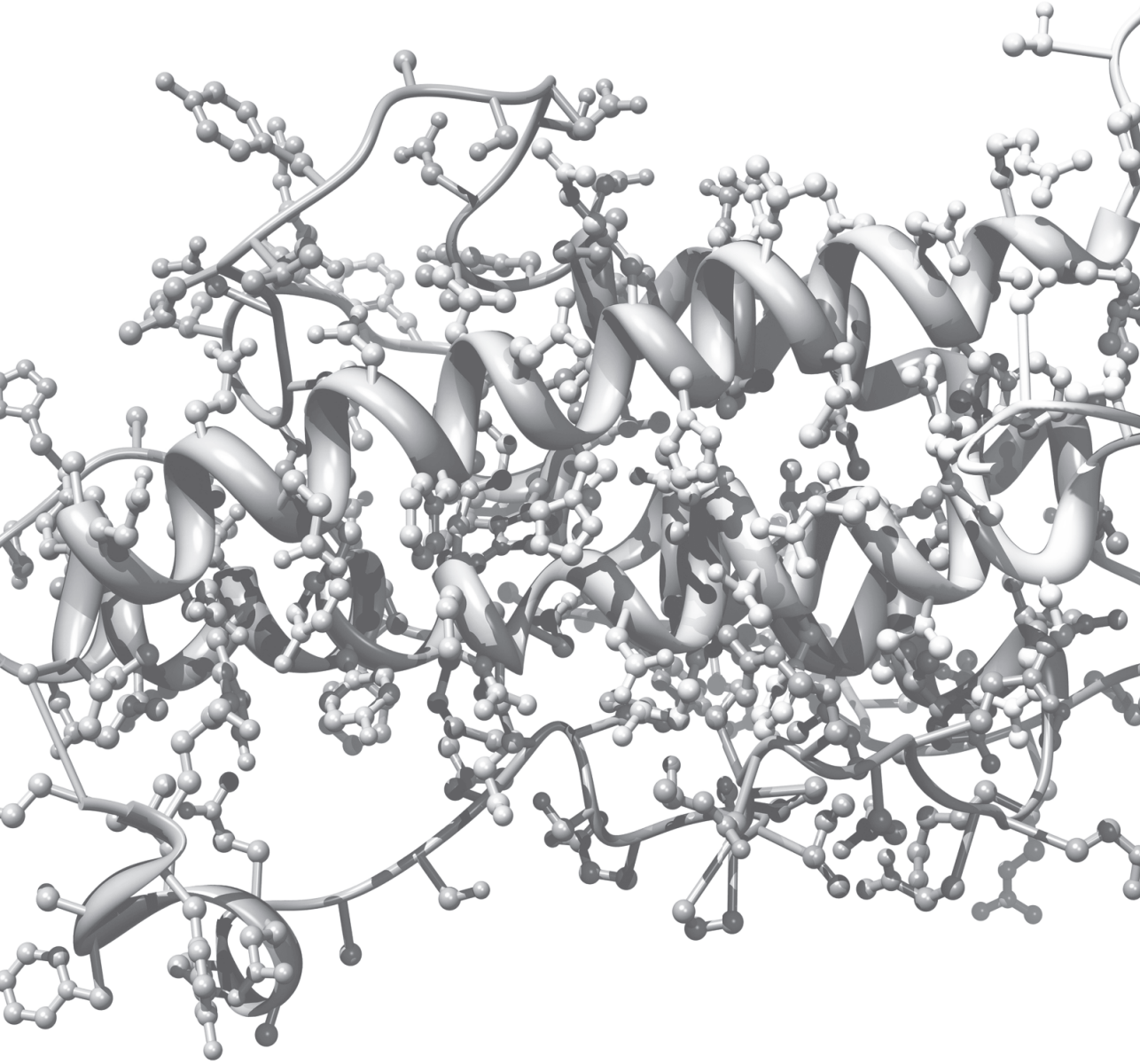
Sommige SGA-geboren kinderen hebben of krijgen een voorlopende botleeftijd tijdens GH behandeling. In kinderen met een idiopathisch kleine lengte en voorlopende botleeftijd werden mutaties in het ACAN gen beschreven. We onderzochten het voorkomen van ACAN gen mutaties in kleine, SGA geboren kinderen, ontwikkelden een klinisch scoringssysteem en beschreven de groeirespons tijdens GH behandeling in kinderen met een ACAN gen mutatie.

De botleeftijd van 290 kinderen met GH behandeling werd beoordeeld en in 29 kinderen met een voorlopende botleeftijd van tenminste 0.5 jaar werd diagnostiek naar het ACAN gen verricht. Bij 4 van de 29 kinderen (13.8%) werd een ACAN gen mutatie gevonden. Mutaties waren gerelateerd aan de karakteristieken: hypoplasie van het middengezicht, gewrichtsklachten en brede, grote tenen. Van de kinderen met een voorlopende botleeftijd en 3 van deze karakteristieken had 100% een ACAN gen mutatie. Van de kinderen met een voorlopende botleeftijd en 2 van deze karakteristieken had 50% een ACAN gen mutatie. Hierbij steeg het percentage positieve bevindingen van 50 naar 100% als tenminste één van de ouders een extreem kleine lengte had (<-3.5 SDS). Kinderen met een voorlopende botleeftijd en geen of slechts 1 van deze karakteristieken hadden geen ACAN gen mutatie. Alle kinderen met een ACAN gen mutatie werden behandeld met GH en gedurende 2 jaar met additionele GnRHa behandeling. Eén meisje en één jongen behaalden een volwassen lengte die respectievelijk 5 en 8 cm langer was dan die van hun ouder met dezelfde mutatie. Behandeling met een aromatase remmer in jongens resulteerde in een vertraging van de botmaturatie.

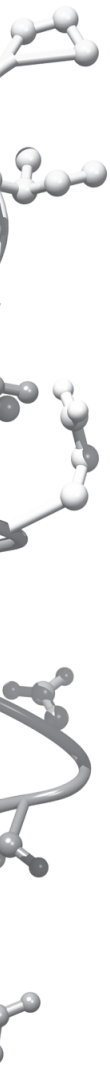
Concluderend laten onze data zien dat ACAN gen mutaties een onderliggende oorzaak kunnen zijn voor een persisterend kleine lengte na SGA geboorte. ACAN gen diagnostiek kan overwogen worden in te kleine, SGA-geboren kinderen met een voorlopende botleeftijd, hypoplasie van het middengezicht, gewrichtsklachten en brede, grote tenen. Kinderen met ACAN gen mutaties hebben baat bij gecombineerde GH/GnRHa behandeling.

HOOFDSTUK 8

In de algemene discussie worden de belangrijkste resultaten van de studies in dit proefschrift besproken, ook in context van de huidige literatuur. Dit hoofdstuk wordt afgesloten met klinische implicaties en suggesties voor toekomstig onderzoek.



CHAPTER 10



25 years of GH treatment in SGA

WHAT HAVE WE LEARNED FROM 25 YEARS OF GROWTH HORMONE TREATMENT IN SGA?

For many years, pediatricians have been anxious that GH treatment deteriorates the known increased risk of metabolic problems in children born SGA. After more than 25 years of experience with GH treatment in short children born SGA, the time has come to sum up the results. This chapter provides an overview of the most important findings of the Dutch GH trials, thereby showing that, besides the well-known effects of GH treatment on growth, GH treatment affects much more than meets the eye.

GH treatment

In the Dutch IUGR-1, IUGR-2, and IUGR-3 study, children born SGA started GH treatment at a young age before puberty and were treated exclusively with GH. Figure 1 shows a schematic timeline of the most important findings during GH treatment and after cessation of treatment. An extensive number of parameters has been studied and Table 1 and 2 summarize the results in more detail.

The effects during treatment

The effects of GH treatment in children born SGA were thoroughly evaluated (Figure 1 and Table 1). GH treatment improved adult height in children born SGA. Of the GH-treated SGA children, 98% attained a height within their target height range (1). This represents a height gain of 11-13 cm in girls and 12-14 cm in boys. In children treated with GH, adult height (AH) was 0.6 SDS higher compared with untreated children. When GH treatment was started before puberty, AH results were similar in children treated with the approved GH dose of 1 mg/m^2/day and those treated with a double dose of 2 mg/m^2/day (1). Children with greater spontaneous catch-up growth after birth showed a lower total height gain SDS during GH (2). Besides, circulating baseline free IGF-I and IGFBP-3 were better predictors for adult height in GH-treated SGA children than total IGF-I, or total IGF-I to IGFBP-3 ratio (3). This suggests a possible role for free IGF-I measurement in predicting the effect of GH therapy in short SGA children. Furthermore, short SGA children tended to have lower ALS levels compared to controls, albeit less reduced than IGF-I and IGFBP-3 levels (4). Determination of ALS levels before the start of GH treatment contributed moderately to a more accurate prediction of the growth response to GH treatment.

Long-term GH treatment had no influence on the age at onset and progression of puberty, regardless of GH-dose (GH 1 vs 2 mg/m^2/day) (5). Duration of puberty and pubertal height gain were not different between the GH-dose groups, and GH treatment had no influence on the serum Inhibin B and AMH levels (5-8).

GH treatment improved body composition and cardiovascular profile in children born SGA, resulting in a decrease in fat mass, blood pressure, and serum MMP-9 levels and

lipid levels, and an increase in lean body mass (9-11). Besides, bone mineral density, which was low at start of GH treatment, normalized within 3 years (10, 12). However, GH treatment induced higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance (13). Long-term GH treatment was not associated with disadvantageous changes in adiponectin, resistin, IL-6 and CRP levels, neither during nor after GH treatment (14, 15). Preterm short SGA children had higher TSH levels than controls, although within the normal range (16). FT4 decreased during GH treatment, but was accompanied by an increase in active T3, thereby not resulting in true hypothyroidism (17).

During 8 years of GH treatment, intelligence and psychosocial functioning improved for most children (18-20). Total IQ was significantly below average at start but had significantly increased after 2 years of GH treatment. After 9 years of treatment, total IQ had become similar to levels in Dutch peers.

The effect of cessation of GH treatment

Since we found that GH treatment affected various aspects of the metabolic and cardiovascular profile of subjects born SGA and we know that they are at a higher risk to develop metabolic and cardiovascular diseases in later life, the effects of cessation of GH treatment needed to be evaluated (Figure 1 and Table 2). During the first 6 months after cessation of GH treatment, the pharmacologic effects of GH treatment on body composition, insulin sensitivity, blood pressure and lipid levels were lost (21, 22). After these initial changes, fat mass continued to increase until 5 years after cessation of GH treatment, but lean body mass, insulin sensitivity, and lipids remained similar *(this thesis)*. After the initial increase in blood pressure, both systolic and diastolic blood pressure decreased and were at 5 years after cessation similar to levels at cessation of GH treatment *(this thesis)*. cIMT did not change during 5 years after cessation of GH treatment and was similar at 5 years after GH-cessation compared to age-matched untreated short SGA subjects *(this thesis)*. At 6.5 years after GH treatment, previously GH-treated young adults born SGA showed a similar body composition, insulin sensitivity, and blood pressure, and a more beneficial lipid profile compared to untreated short young adults born SGA (23, 24). This is reassuring since these findings indicate that GH treatment during childhood has no long-term unfavorable effects on metabolic and cardiovascular profile in early adulthood. However, the positive effects of GH treatment are lost and metabolic and cardiovascular health return to similar levels as untreated short SGA young adults. Since the metabolic and cardiovascular health later in adulthood are still unknown in both GH-treated and untreated SGA adults, longer-term follow-up of metabolic and cardiovascular risk factors is indicated in these predisposed subjects.

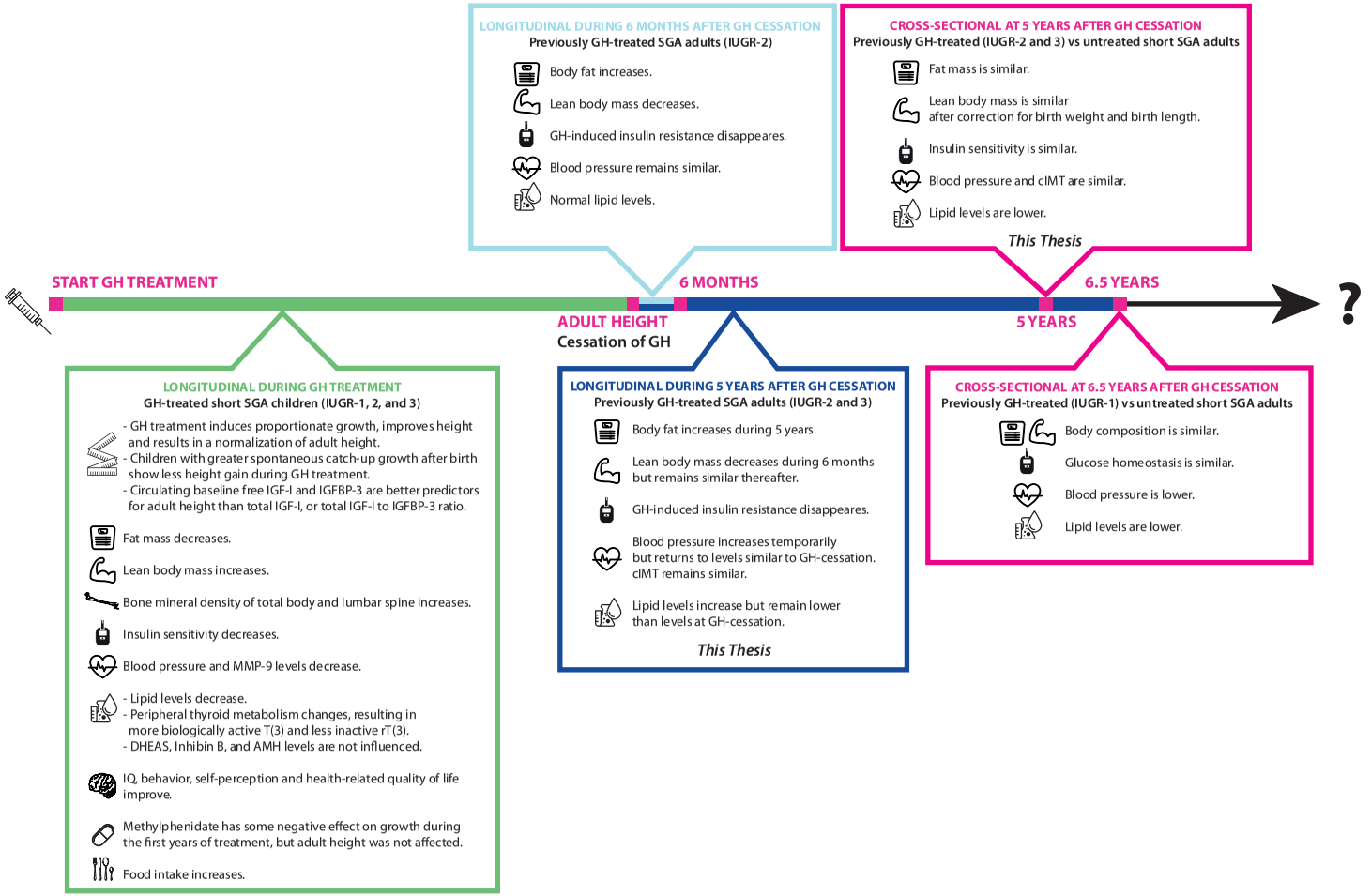


Figure 1. Results of the IUGR-1, IUGR-2 and IUGR-3 studies.

GH treatment with or without additional GnRHa treatment

In the Dutch SGA study, children born SGA started treatment at an older age. They were treated with GH until AH and in children who had already started puberty with an AH prediction below -2.5 SDS, additional GnRHa treatment for 2 years was given. Besides, the effects of two different GH-doses during puberty (GH 1 mg/m^2/day vs 2 mg/m^2/day) were investigated. Figure 2 shows a schematic timeline of the most important findings during treatment and Table 3 summarizes the results in more detail.

The effects of GH treatment vs GH/GnRHa treatment

Our data show that children who start GH treatment in early puberty can still have an impressive catch-up growth and GH treatment should therefore not be withheld (25). GH-treated SGA children who started puberty with an AH expectation below -2.5 SDS and were treated with 2 years of GnRHa, had a shorter pubertal duration after cessation of GnRHa compared with pubertal duration in children treated with GH only (26). However, height gain from onset of puberty until AH was greater due to adequate growth during 2 years of GnRHa treatment. This resulted in a similar AH as children treated with GH only (26). Children with an AH prediction of less than -2.5 SDS at start of puberty, therefore, benefit from additional treatment with GnRHa for 2 years to postpone puberty (27).

Addition of 2 years of GnRHa treatment did not adversely affect body composition, metabolic profile, bone mineral density and quality of life (28-31). At AH, children treated with combined GH/GnRHa treatment had a similar fat mass, lean body mass, blood pressure, lipid levels and insulin sensitivity as those treated with GH only (30, 31).

Since children with an AH expectation less than -2.5 SDS at start of puberty benefitted from additional treatment with GnRHa for 2 years, and the metabolic profile at AH of children treated with GH or combined GH/GnRHa was similar, additional GnRHa treatment can safely be considered in children who start puberty with an AH expectation less than -2.5 SDS.

The effects of GH treatment 1 mg/m^2/day vs 2 mg/m^2/day

When GH treatment is started in early puberty, only a few years of growth remain. The optimal GH dose during puberty and/or postponement of puberty was unknown. We found that boys treated with a higher GH dose of 2 mg/m^2/day during puberty, reached a better adult height compared with boys treated with a GH dose of 1 mg/m^2/day (27). A GH dose of 2 mg/m^2/day resulted in a similar metabolic and cardiovascular profile as GH 1 mg/m^2/day (30, 31). During 2 years of combined GH/GnRHa treatment, a GH dose of 2 mg/m^2/day resulted in a more favorable fat mass percentage than GH 1 mg/m^2/day (30, 32). We do, however, not recommend high GH dosing in all children since concerns have been expressed regarding the possible detrimental effects of persistently relatively high serum IGF-I levels. Nonetheless, our data show that a GH dose of 2 mg/m^2/day could

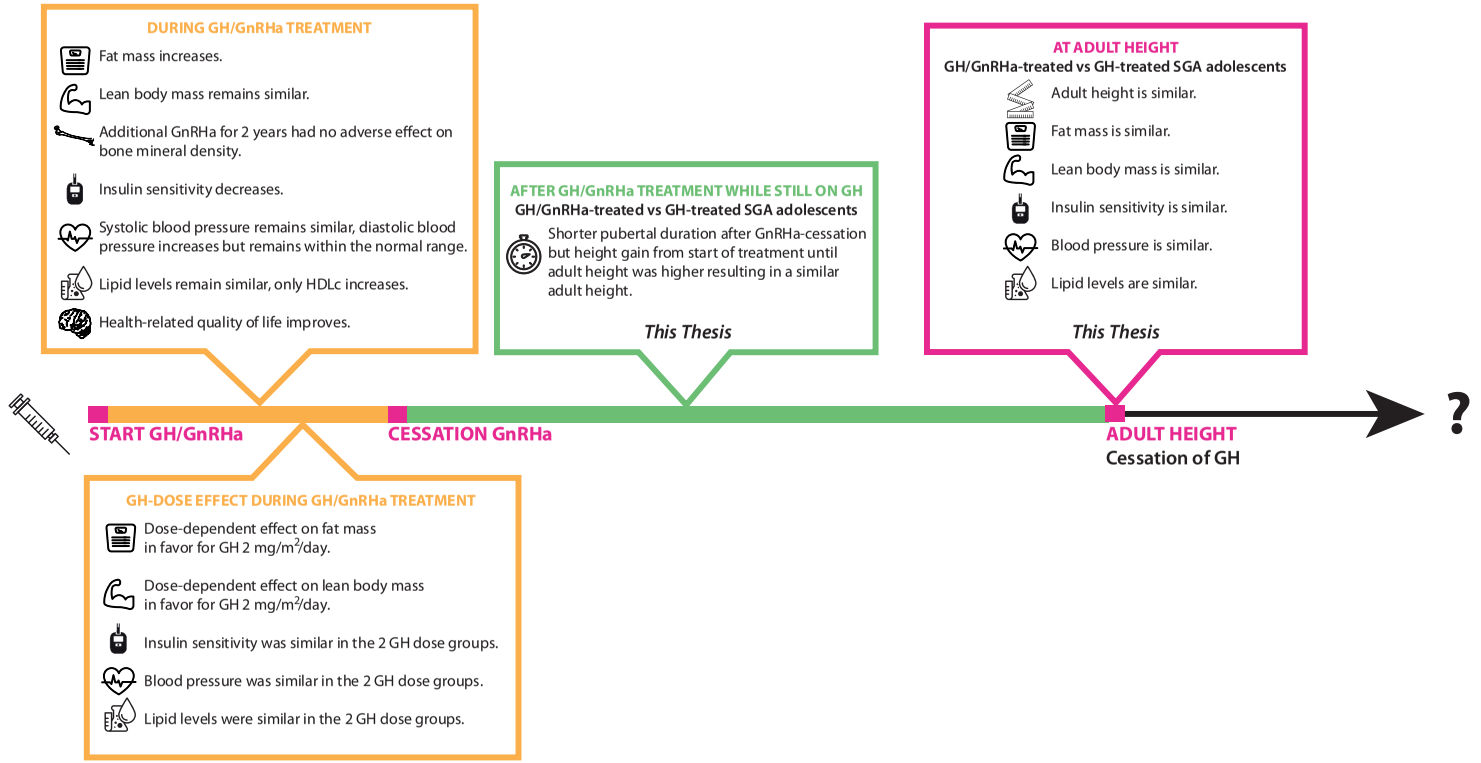


Figure 2. Results of the SGA study.

be considered in a small group of short, pubertal SGA children who come to medical attention at such an old age that only a few years of growth remain.

Genetics

Short children born SGA comprise a heterogeneous group. Bloom syndrome should be tested for in children with consanguineous parents, dysmorphic features (particularly resembling Silver Russell syndrome), skin abnormalities, and/or serum IGF-I levels greater than 2.5 SD score during standard GH treatment with normal IGF binding protein-3 levels (33). Besides, ACAN gene mutations should be tested for in children born SGA with persistent short stature, advanced bone age and midface hypoplasia, joint problems or broad great toes *(this thesis)*.

25 YEARS OF GH TREATMENT IN SGA

In conclusion, 25 years of experience with GH treatment in subjects born SGA has taught us that, besides the well-known effects of GH treatment on growth, GH treatment affects much more than meets the eye. Based on our studies, GH treatment has several beneficial effects and is recommended in short SGA children who are otherwise at increased risk for short stature as adults. Additional GnRHa treatment for 2 years at start of puberty is recommended in children who start puberty with an expected AH below -2.5 SDS. In Europe, the approved GH dose for children who start GH treatment before puberty is 1 mg/m^2/day (~0.033 mg/kg/day). Since long-term effects of GH treatment and safety outcomes were similar between children treated with 1 or 2 mg/m^2/day during puberty, a double GH dose of 2 mg/m^2/day (~0.067 mg/kg/day) could be considered in short, pubertal SGA children who come to medical attention at such an old age that only a few years of growth remain and who do not respond sufficiently to a GH dose of 1 mg/m^2/day.

GH treatment not only improves AH and psychosocial functioning, it has also no negative effects on metabolic profile and cardiovascular risk factors until at least 5-6 years after its cessation. Although we have evaluated the long-term effects of GH for 5-6 years after cessation of treatment, the long-term effects of combined GH/GnRHa treatment after attainment of adult height still need to be evaluated. Besides, it remains very important to perform longer-term follow-up research in these patients, who have an intrinsic risk to develop adult-onset diseases, to elucidate the development of health and disease in adulthood. Future studies will also need to investigate whether GH-treated SGA patients show relatively greater personal and overall life achievements in adulthood.

Table 1. Summary of the results of the IUGR-1, IUGR-2 and IUGR-3 studies during GH treatment

Anthropometrics		
Height	GH treatment led to a normalization of adult height.	(1)
	Children with greater spontaneous catch-up growth after birth showed a lower total height gain SDS during GH.	(2)
	Height normalized during GH treatment.	(12, 34)
Body proportions	GH treatment induced a proportionate growth resulting in a normalization of height, sitting height, weight, head circumference, arm span, hand, tibia and foot size, in relation to height.	(35, 36)
Growth factors		
	Circulating baseline free IGF-I and IGFBP-3 were better predictors for adult height in GH-treated SGA children than total IGF-I, or total IGF-I to IGFBP-3 ratio. This suggests a possible role for free IGF-I measurement in predicting the effect of GH therapy in short SGA children.	(3)
	Short SGA children tended to have lower ALS levels compared to controls, albeit less reduced than IGF-I and IGFBP-3 levels. ALS might be involved in glucose homeostasis. Determination of ALS levels before the start of GH treatment contributed moderately to a more accurate prediction of the growth response to GH treatment.	(4)
	Treatment with 2 mg GH/m^2/day during 6 months resulted in higher GH, IGF-I and IGF-BP3 levels compared to 1 mg GH/m^2/day.	(37)
	In conditions in which IGF-I levels are low, such as young age and in short SGA children, IGFBP-3 proteolytic activity is increased to ensure IGF-I bioavailability.	(38)
Puberty		
	Long-term GH treatment had no influence on the age at onset and progression of puberty, regardless of GH-dose (1 vs 2 mg GH/m^2/day). Duration of puberty and pubertal height gain were not different between the GH-dose groups.	(5)
	Small size at birth had no effect on serum DHEAS levels before the age of 9 yr. The incidence of premature pubarche was comparable with the normal population. GH treatment for 1 year had no effect on serum DHEAS levels.	(6)
	Being born SGA did not impair Sertoli cell function. GH treatment did not result in different Inhibin B and AMH levels.	(7)
	Serum AMH levels in prepubertal short SGA girls were similar to healthy controls, indicating that the follicle pool is not compromised due to SGA birth. GH treatment had no effect on AMH levels in short SGA girls.	(8)
Body composition		
	During long-term continuous GH treatment, BMI normalized without overall changes in subcutaneous fat (skinfolds measurements) compared with age-matched references.	(9)
	Fat percentage SDS decreased and lean body mass SDS remained similar during 6 years of GH treatment.	(10)
Cardiovascular risk factors		
Blood pressure	Systemic blood pressure decreased during 6 years of GH treatment.	(9)
Lipid levels	The atherogenic index decreased during 6 years of GH treatment.	(9)
Plasma matrix metalloproteinase-9 (MMP-9) levels	GH treatment had a positive, lowering effect on both MMP-9 levels and systolic BP SDS.	(11)

Table 1. Summary of the results of the IUGR-1, IUGR-2 and IUGR-3 studies during GH treatment (continued)

Anthropometrics		
Inflammatory markers and adipocytokines	Long-term GH treatment was not associated with disadvantageous changes in adiponectin, resistin, IL-6 and CRP levels, neither during nor after GH treatment.	(14, 15)
Bone Mineral Density (BMD)		
	BMAD SDS increased during 6 years of GH treatment.	(10)
	BMD and BMAD normalized during 3 years of GH treatment.	(12)
Glucose Homeostasis		
	Continuous GH treatment during 6 years had no adverse effects on glucose levels (Oral Glucose Tolerance Tests). However, GH treatment induced higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance.	(13)
Thyroid hormone levels		
	Preterm short SGA children had higher, although within the normal range, TSH levels than controls. The level of TSH did not correlate with gestational age, birth weight SDS or birth length SDS. FT4 decreased during GH treatment, but was neither associated with an increase in TSH nor did it affect the response to GH treatment.	(16)
	Puberty and GH treatment both induced 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 induced altered peripheral thyroid metabolism but did not result in thyroid dysfunction.	(17)
Psychological aspects		
	During GH treatment, IQ, behavior, and self-perception significantly improved from scores below average to scores comparable to Dutch peers. In addition, children whose height over time became closer to that of their peers showed less problem behavior.	(18)
	GH-treated SGA adolescents had a better Quality of Life (QoL) than untreated adolescents born SGA, according to the disorder-specific questionnaire. Since the generic CHQ did not reveal such differences, a disorder-specific questionnaire for measuring QoL in children treated for short stature is preferable.	(19)
	GH treatment in short children born SGA without signs of persistent catch-up growth was associated with significant improvement in Health-Related QoL and normalization of adult height.	(20)
Genetics		
	Short children born SGA comprise a heterogeneous group. Bloom syndrome should be tested for in children with consanguineous parents, dysmorphic features (particularly resembling Silver Russell syndrome), skin abnormalities, and/or IGF-1 levels greater than 2.5 SD score during standard GH treatment with normal IGF binding protein-3 levels.	(33)
	Polymorphic variation in the IGFBP3 promoter region was correlated with IGFBP-3 levels, spontaneous growth and response to GH treatment in short SGA children.	(39)
Remaining areas		
	Short SGA children had a lower food intake than age-matched controls which increased during GH treatment.	(40)
	Methylphenidate had some negative effect on growth during the first years in short SGA children treated with GH, but adult height was not affected.	(41)

Table 2. Summary of the results of the IUGR-1, IUGR-2 and IUGR-3 studies after cessation of GH treatment

Metabolic and cardiovascular health profile		
During 6 months after GH-cessation	During 6 months after cessation of GH treatment, the GH-induced insulin insensitivity disappeared (Oral Glucose Tolerance Tests), the beneficial effect of GH on blood pressure did not change, and most adolescents had normal lipid levels.	(21)
	During 6 months after cessation of GH treatment, longitudinal data from frequently sampled intravenous glucose tolerance (FSIGT) tests showed that the GH-induced lower insulin sensitivity increased and became similar to that of AGA controls. Cessation of GH treatment was, however, also associated with an increase in percent body fat and with a decrease in lean body mass, without changes in fat distribution.	(22)
During 5 years after GH-cessation	During 5 years after cessation of GH treatment, significant changes in body composition, insulin sensitivity and β-cell function reflected the loss of GH properties. Fat mass increased, lean body mass decreased and the GH-induced insulin resistance fully recovered. At 5 years after GH-cessation, FM, insulin sensitivity and β-cell function of previously GH-treated young adults born SGA were similar to untreated short young adults born SGA, indicating that long-term GH treatment in children born SGA has no unfavourable effect on metabolic health in early adulthood.	***This thesis***
	During 5 years after GH-cessation, blood pressure temporarily increased, lipid levels increased and cIMT remained unchanged. At 5 years after GH-cessation, blood pressure and cIMT were similar, and lipid levels lower in previously GH-treated young adults born SGA compared to untreated short young adults born SGA. Long-term GH treatment in children born SGA does not only improve adult height but has also no unfavourable effects on metabolic and cardiovascular health in early adulthood and might be beneficial with regard to the lipid profile.	***This thesis***
At 6.5 years after GH-cessation	At 6.5 years after cessation of long-term GH treatment, previously GH-treated young adults born SGA had a similar glucose homeostasis, BMI, waist circumference, IGF-I and IGFBP-3 levels as untreated short young adults born SGA. Systolic and diastolic blood pressure and serum cholesterol were lower in GH-treated young adults. These data are reassuring because they suggest that long-term GH treatment does not increase the risk for diabetes mellitus type 2 and metabolic syndrome in young adults.	(23)
	At 6.5 years after cessation of long-term GH treatment, previously GH-treated young adults born SGA had a similar body composition and fat distribution as untreated short young adults born SGA. GH-induced catch-up growth had no unfavorable effect on fat mass and fat distribution compared with spontaneous catch-up growth. However, SGA adults in general might have a different body composition than healthy AGA controls.	(24)

Table 3. Summary of the results of the SGA study during GH treatment with or without GnRHa

Adult height	
Excluding children with a distance to target height < 1 SDS from GH treatment is not justified.	(25)
When started in adolescence, GH treatment significantly improved adult height in short SGA children, particularly with GH 2 mg/m^2/day during puberty. When SGA children were short at the start of puberty, they benefitted from combined GH/GnRHa treatment.	(27)
Puberty	
GH-treated SGA children who start puberty with an adult height expectation below -2.5 SDS and are treated with 2 years of GnRHa had a shorter pubertal duration after discontinuation of GnRHa compared with pubertal duration in children treated with GH only. Height gain from onset of puberty until adult height was, however, more due to adequate growth during 2 years of GnRHa treatment resulting in a similar adult height as children treated with GH only.	(26) ***This Thesis***
Metabolic and cardiovascular profile	
During 2 years of combined GH/GnRHa treatment, GnRHa did not adversely affect body composition and metabolic profile of short SGA children who came under medical attention at the onset of puberty. There was a dose-dependent effect on fat mass SDS), percentage trunk fat, lean body mass SDS, and GH and IGF1 levels in favor of treatment with GnRHa and the higher GH dose of 2 mg/m^2/day.	(32)
At adult height, metabolic and cardiovascular parameters were similar in adolescents treated with combined GH/GnRHa treatment and those with GH only. Started in early puberty, a GH dose of 2 mg/m^2/day resulted in a more favorable fat mass percentage than GH 1 mg/m^2/day.	(30, 31) ***This Thesis***
Bone Mineral Density (BMD)	
During GH treatment, BMD 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.	(28)
Psychological aspects	
HRQoL improved in prepubertal and pubertal short SGA children during GH treatment. Additional GnRHa treatment had no adverse effect on the HRQoL gain. Disorder-specific questionnaires were particularly appropriate to evaluate HRQoL in children treated for short stature.	(29)
Genetics	
Mutations in the ACAN gene can be the cause of advanced bone age maturation in GH-treated children born SGA. ACAN-gene sequencing should be considered in children born SGA with persistent short stature, who show advanced bone age and midface hypoplasia, joint problems or broad great toes. Children with ACAN gene mutations benefit from GH treatment with 2 years of GnRHa treatment.	***This Thesis***
GH levels during GnRHa only	
Short SGA girls lacked the normal increase in GH levels seen in puberty and had reduced IGF-I and IGFBP-3 levels, which might explain their reduced pubertal growth spurt. GnRHa treatment led to a significant reduction in GH levels. Therefore, combining GnRHa treatment with GH treatment might improve adult height in short SGA girls.	(42)
GnRH agonist test	
GnRHa sufficiently suppressed puberty in girls and boys born SGA during 3 months of treatment. The GnRH agonist test falsely indicated insufficient pubertal suppression in 33% of the girls and 43% of the boys.	(43, 44)

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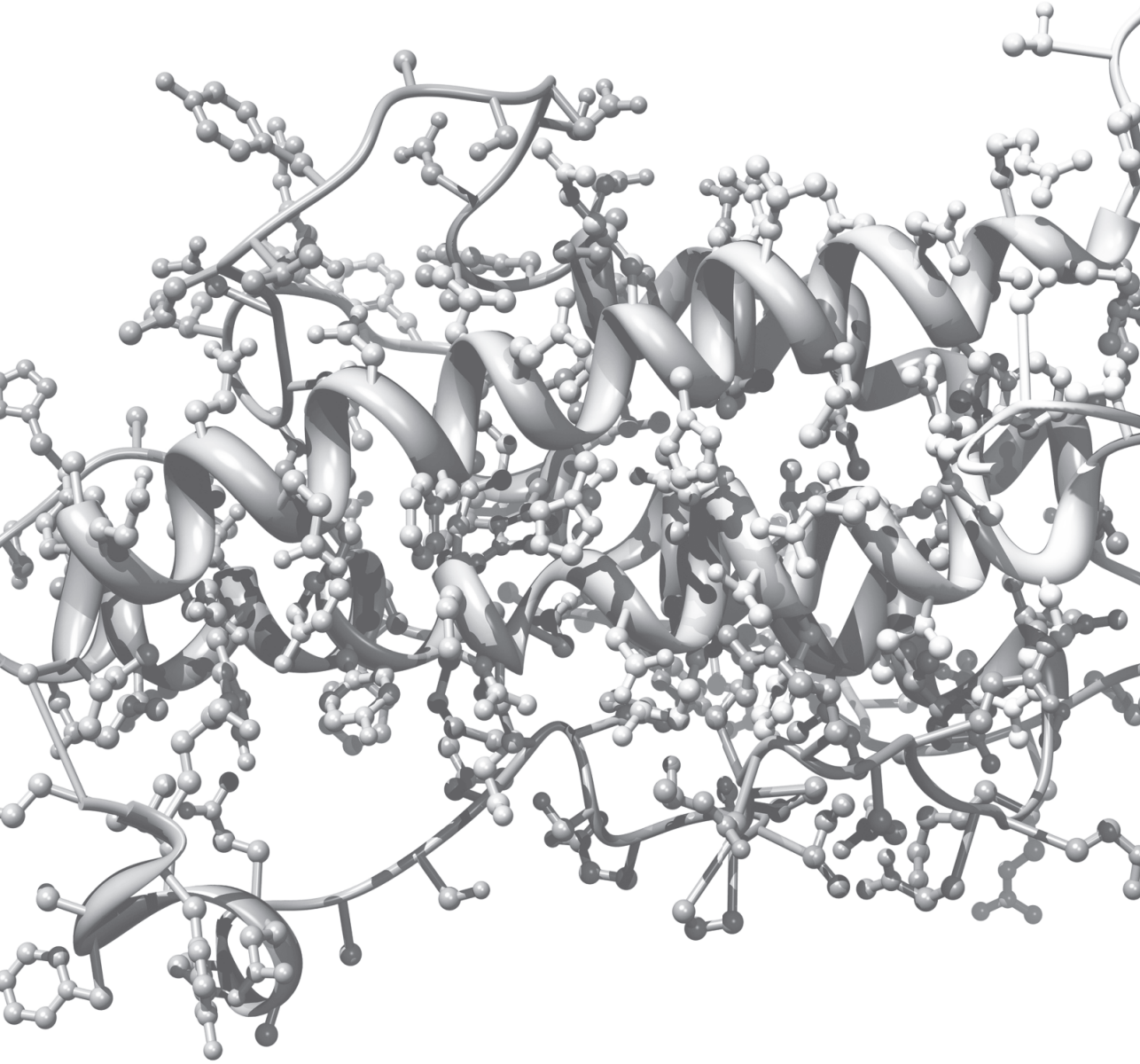
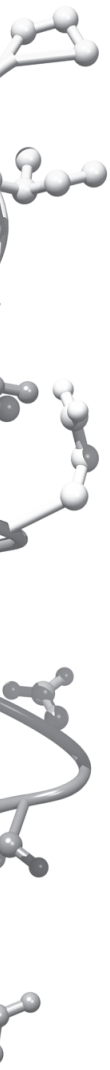


CHAPTER 11



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About the author

LIST OF ABBREVIATIONS

AGA	appropriate for gestational age
AH	adult height
AIR	acute insulin response
ApoA-1	apolipoprotein A-1
Apo-B	apolipoprotein B
ATP III	Adult Treatment Panel III
BA	bone age
BMI	body mass index
BP	blood pressure
CA	calendar age
cIMT	carotid intima media thickness
CPP	central precocious puberty
CVD	cardiovascular disease
DBP	diastolic blood pressure
DI	disposition index
DXA	dual-energy X-ray absorptiometry
FM	fat mass
FM%	fat mass percentage
FSH	follicle stimulating hormone
FSIGT	frequently sampled intravenous glucose tolerance test
GH	growth hormone
GHRH	growth hormone-releasing hormone
GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone analog
HDLc	high density lipoprotein cholesterol
HPG-axis	hypothalamic-pituitary-gonadal axis
HOMA-IR	homeostasis model assessment of insulin resistance
IGF	insulin-like growth factor
IGF-I	insulin-like growth factor-I
IGFBP	insulin-like growth factor binding protein
IGT	impaired glucose tolerance
IUGR	intra-uterine growth retardation
IQR	interquartile range
LBM	lean body mass
LDLc	low density lipoprotein cholesterol
LF	limb fat
LH	luteinizing hormone
M2	breast development stage II according to Tanner

NCEP	National Cholesterol Education Program
QOL	quality of life
SAGhE	Safety and Appropriateness of Growth hormone treatments in Europe
SBP	systolic blood pressure
sc	subcutaneous
SDS	standard deviation score
Sg	glucose effectiveness
SGA	small for gestational age
SGA-CU	born small for gestational age with spontaneous catch-up growth
SGA-GH	previously GH-treated subjects born small for gestational age
SGA-S	born small for gestational age with short adult stature
Si	insulin sensitivity
T2DM	diabetes mellitus type 2
TC	total cholesterol
TF	trunk fat
TG	triglycerides
TH	target height
TV	testicular volume

LIST OF PUBLICATIONS

van der Steen M, Lem AJ, van der Kaay DC, Bakker-van Waarde WM, van der Hulst FJ, Neijens FS, Noordam C, Odink RJ, Oostdijk W, Schroor EJ, Westerlaken C, Hokken-Koelega ACS. Metabolic Health in Short Children Born Small for Gestational Age Treated With Growth Hormone and Gonadotropin-Releasing Hormone Analog: Results of a Randomized, Dose-Response Trial. *The Journal of Clinical Endocrinology & Metabolism, 2015;100(10):3725-34.*

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van der Steen M, Pfundt R, Maas SJWH, Bakker-van Waarde WM, Odink RJ, Hokken-Koelega ACS. ACAN gene mutations in short children born SGA and response to growth hormone treatment. *Journal of Clinical Endocrinology and Metabolism, in press.*

van der Steen M, Smeets CCJ, Kerkhof GF, Hokken-Koelega ACS. Metabolic health profile in young adults born SGA: A 5-year longitudinal study after cessation of GH treatment. *The Lancet Diabetes & Endocrinology, in press*

van der Steen M, Kerkhof GF, Smeets CCJ, Hokken-Koelega ACS. A 5-year longitudinal study after GH cessation on cardiovascular risk factors and cIMT in young adults born SGA. *Submitted*

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	Year	Workload (ECTS)
General courses		
English Biomedical Writing and Communication, MolMed, Erasmus MC	2014	4.0
Research Integrity, Erasmus MC	2014	0.3
Biostatistical Methods I, NIHES, Erasmus MC	2013	5.7
Methodology of research and preparing grant applications, Erasmus MC	2013	0.5
Good Clinical Practice, Erasmus MC	2013	1.0
Specific courses		
Research Management, MolMed, Erasmus MC	2014	1.0
Radiation protection 5A, Erasmus MC	2013	1.0
Basic introduction course on SPSS, MolMed, Erasmus MC	2013	1.0
Photoshop and Illustrator, MolMed, Erasmus MC	2013	0.3
Basic and Translational Endocrinology, Erasmus MC	2012	2.2
PubMed and EndNote, Medical Library, Erasmus MC	2012	0.3
Seminars and Workshops		
Oral presentation, Research Day, Sophia Children's Hospital	2016	1.0
Young investigators day, TULIPS / NVK	2015	0.6
Annual Research Day, Sophia Children's Hospital	2013-2016	1.2
Annual interclinical evening, Erasmus MC	2012-2016	1.5
Weekly Pediatric Endocrinology meetings, Erasmus MC	2012-2016	4.0
International conferences and presentations		
55th Annual Meeting of the ESPE, Paris, France *2 oral presentations*	2016	2.0
54th Annual Meeting of the ESPE, Barcelona, Spain *oral presentation and 2 poster presentations*	2015	2.0
53rd Annual Meeting of the ESPE, Dublin, Ireland *poster presentation*	2014	1.0
9th Joint Meeting of the LWPES/ESPE, Milan, Italy *poster presentation*	2013	1.0
Lecturing		
Dutch Advisory Board Growth Hormone, Pediatric Endocrinology, Utrecht	2015	1.0

Educational Lecture Pfizer BV	2015	1.0
Annual SGA Day (SGA Platform)	2014-2015	1.0
Annual IMC Weekendschool 'Growth and Development', Rotterdam	2015-2016	2.0
Supervising		
Supervising research internship of medical student	2016	4.0
Advising		
Medical advisor SGA Platform	2015-2016	0.5
Research Proposals		
Follow-up study of subjects who participated in the IUGR-1, IUGR-2 and PROGRAM/PREMS studies during childhood and early adulthood. Long term effects of growth hormone many years after discontinuation.	2015	5.0
Miscellaneous		
Growth and Metabolism in Children Born Small for Gestational Age. M. van der Steen, A.C.S. Hokken-Koelega. *Endocrinology and Metabolism Clinics of North America*. Elsevier, June 2016. ISBN 13: 9780323446129	2016	2.0
Board member Sophia Researchers Association	2015-2016	2.0
Peer review of articles for international scientific journals	2015-2016	0.5

ECTS (European Credit Transfer and Accumulation System) are training credits.
One ECTS stands for approximately 28 working hours.

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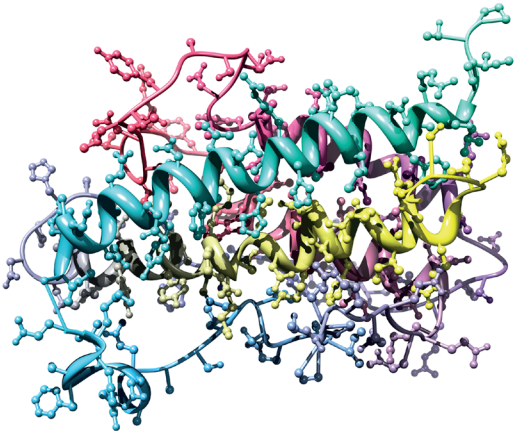
'I am part of one terrific sister act'

Manouk



Growth Hormone Treatment in SGA

More than meets the eye

Growth hormone (GH) treatment effectively induces catch-up growth and improves adult height in short children born small for gestational age (SGA). Besides this visual effect, GH treatment also has several other effects which occur inside the body.

This doctoral thesis presents the effects of GH treatment, with or without additional gonadotropin-releasing hormone analog (GnRHa) treatment, on metabolic and cardiovascular risk factors. The effects of GnRHa treatment, in addition to GH treatment, on pubertal development are described. Further, the long-term effects of discontinuation of GH treatment are assessed in young adults born SGA. Finally, a new genetic cause for persistent short stature after SGA birth is revealed.

The results presented in this thesis illustrate that there is more to GH treatment than meets the eye.