# Small for gestational age

## Adult metabolic and cardiovascular health and long-term safety of growth hormone treatment

door

Wesley Jim Goedegebuure

The studies in this thesis were investigator-initiated studies, supported by an independent research grant from Novo Nordisk, The Netherlands and Denmark, and Netherlands Organisation for Scientific Research (NWO).

Publication of this thesis was financially supported by

ISBN/EAN: 978-94-6423-413-8

Cover: Janine Goedegebuure en Kim Meuwissen
Lay-out: Dennis Hendriks || ProefschriftMaken.nl
Printing: ProefschriftMaken.nl

# Small for gestational age

**Adult metabolic and cardiovascular health and long-term safety of growth hormone treatment**

Proefschrift

Ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

Prof. dr. F.A. van der Duijn Schouten

en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

17 september 2021 om 13:00 uur

door

Wesley Jim Goedegebuure
geboren te Berkel en Rodenrijs

**Erasmus University Rotterdam**

| | |
|---|---|
| **Promotor:** | Prof. dr. A.C.S. Hokken-Koelega |
| **Co-promotor:** | Dr. M. van der Steen |
| **Overige leden:** | Prof. dr. A.J. van der Lelij<br>Prof. dr. F. Chiarelli<br>Prof. dr. S. Cianfarani |

Vanaf de maan gezien, zijn we allen even groot
*Seen from the moon, we are all equally tall*
(Multatuli, 1879)

# Table of contents

# Chapter 1

## General introduction

# Introduction



For almost 30 years, our research group and others have investigated the efficacy and safety of biosynthetic growth hormone (GH) and gonadotropin-releasing hormone agonist (GnRHa) in children born small for gestational age (SGA) with persistent short stature. The children who started treatment back then are now adults, and the knowledge about the long-term effects of these treatments has markedly increased.

Because being born with a lower birth weight leads to an increased risk for age-associated diseases in later life, there have been concerns about the health of adults born SGA. The main aims of the studies described in this thesis were, therefore, to investigate various parameters associated with health in later life in subjects born SGA and the long-term safety of growth hormone treatment of SGA born subjects during 12 years after GH-cessation, in comparison with appropriate, age-matched controls.

We also investigated insulin sensitivity, body composition, serum lipid levels, kidney function, health-related quality of life and psychosocial functioning in adults born SGA, and the effects of GH and GnRHa treatment on these parameters. In a subgroup of young adults born SGA with persistent short stature caused by the Silver-Russell syndrome (SRS), investigated gonadal function and pubertal development.

**Small for gestational age**

SGA refers to the size of an infant at birth and is defined as birth weight or birth length of at least two standard deviation scores (SDS) below the mean for gestational age [1,2]. SGA infants may be born either full-term or preterm. By definition, 2.3% of all infants are born SGA. Accurate measurement of birth weight and length along with gestational dating are essential for determining SGA birth in children.

Intrauterine growth retardation (IUGR) refers to inappropriate growth during a certain period of gestation, based on two ultrasound measurements. Therefore, IUGR does not mean that a child is born SGA (for example a child with IUGR in late gestation can have a normal size at birth), and SGA birth does not necessarily mean that IUGR occurred (for example when a short length was present from the beginning of gestation) (*Figure 1: SGA & IUGR*). IUGR usually results from a pathological process (e.g., disease of the mother, placental defects), while some mild cases of SGA can be constitutionally small infants without any pathology. IUGR is the condition in which there is an adjustment to a poor environment. This is of great influence as this causes an adaptive process to the child, with long-term consequences on metabolic and cardiovascular health.

The etiologies of SGA birth are heterogeneous as it can be caused by maternal, fetal or demographic factors (*Table 1: factors causing SGA*) [3]. SGA might be caused by constitutionally small children, which has similar characteristics of a child born AGA. In a significant proportion of cases, however, the reason for SGA birth remains unclear.

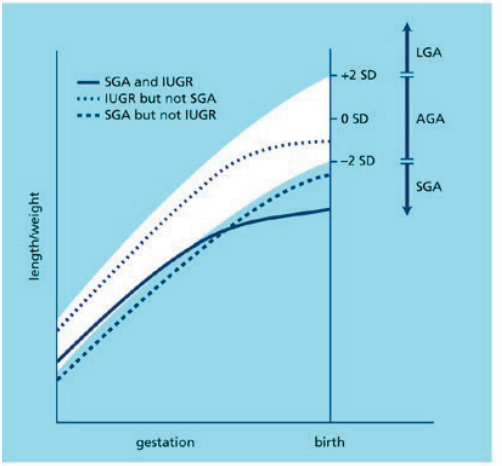


**Figure 1: Fetal growth chart showing different intrauterine growth patterns.**

**Table 1: Factors associated with reduced fetal growth [3]**

| | |
|---|---|
| **Maternal factors** | |
| Medical conditions | Pre-eclampsia<br>Acute or chronic hypertension<br>Abnormality of the uterus<br>Severe chronic disease<br>Therapeutic drugs |
| Social conditions | Maternal nutrition<br>Age at delivery <16 or >35 years<br>Drug use (smoking, alcohol, illicit drugs) |
| Placental factors | Single umbilical artery |
| **Fetal factors** | |
| Chromosomal disorders | Down syndrome<br>Turner syndrome |
| (Epi)genetic disorders | Silver-Russell syndrome<br>3M-syndrome |
| Intrauterine infections | Toxoplasmosis<br>Rubella<br>Cytomegalovirus |
| Inborn errors of metabolism | |
| Congenital defects | |
| **Demographic factors** | |
| Ethnicity | |
| Maternal and paternal short stature | |
| Previous delivery of SGA infant | |

## Normal growth, puberty and gonadal function

### Postnatal growth

Several factors influence postnatal growth, including hormones, genetics, and the physical, emotional and social environment. The growth hormone axis (GH-axis) is the primary hormonal axis involved in human growth (*Figure 2: GH axis*) [4]. 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 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.

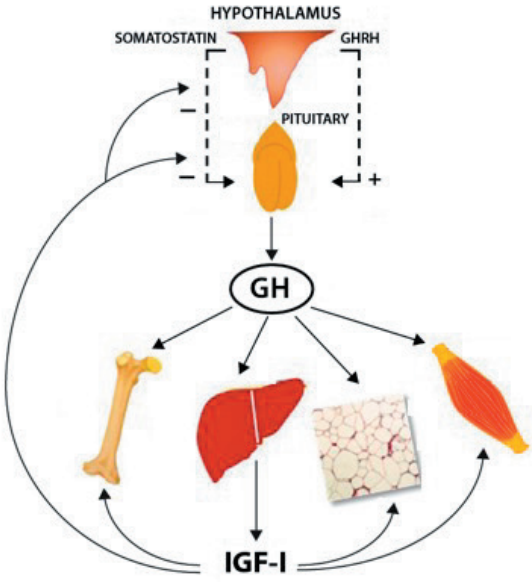


**Figure 2: Physiology of the GH-IGF-I axis.** (adapted from Kumar et al. [5]) GHRH = GH-releasing hormone; GH = Growth hormone; IGF-I = insulin-like growth factor I.

### Pubertal development and pubertal growth

Puberty is the period of transition from childhood to adolescence, marked by the development of secondary sexual characteristics, the pubertal growth spurt, epiphyseal maturation and behavioral changes [6]. The median age of pubertal onset in the Dutch population is 10.7 years for girls and 11.5 years for boys [7]. 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 >10mL. Height and age at onset of puberty and the magnitude and duration of pubertal growth, explain 15-20% of adult height [8].

Although reported study results are difficult to compare due to various definitions of puberty milestones, most authors seem to agree that short children born SGA have a normal pubertal onset and development, but relatively early for their short stature [9-14]. Moreover, subjects born SGA have a shorter pubertal growth duration, resulting in a smaller pubertal growth spurt than appropriate gestational age (AGA) born subjects.

**Gonadal function**

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: HPG-axis*). Due to an increase in frequency and amplitude of gonadotropin-releasing hormone (GnRH) pulses in the hypothalamus, the secretion of gonadotropins (i.e., follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) by the pituitary rises.

In males, FSH stimulates the Sertoli cells to produce inhibin B, a marker of spermatogenesis. LH stimulates the Leydig cells to produce testosterone, necessary for the development of male characteristics and to continue the process of spermatogenesis. In females, FSH and LH are secreted in a cyclic manner and stimulate the ovaries to produce estrogen and progesterone. Ovarian function can be difficult to evaluate because menstrual cycles do not always indicate ovulation. Since Anti-Müllerian Hormone (AMH) is exclusively produced by the ovaries, independent of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool [15,16]. Previous studies have shown no negative effect of SGA birth on male and female gonadal function [17-20].

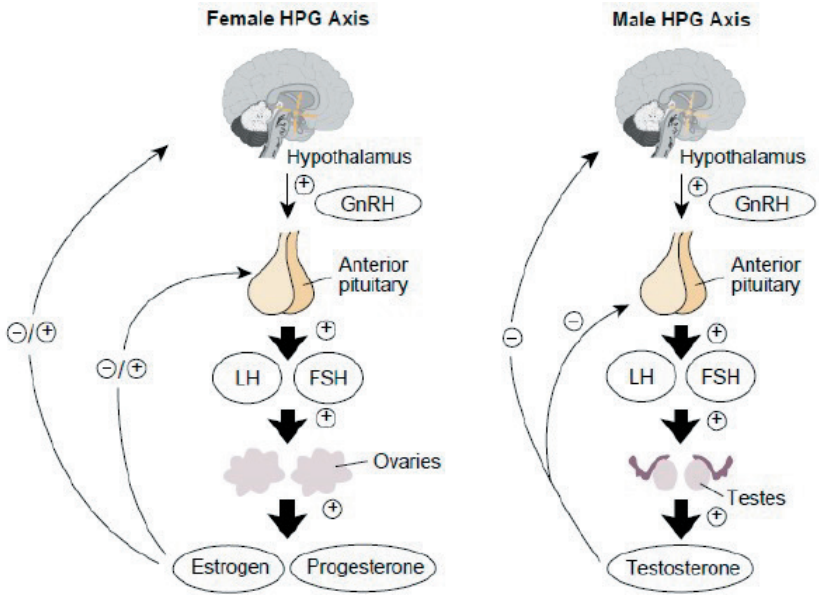


**Figure 3: Male and female hypothalamic-pituitary-gonadal axis.** (adapted from Kong et al. [21])

**Short stature in children born SGA**

Most children born SGA show catch-up growth in the first two years of life, but 10% of SGA children fail to show sufficient catch-up growth and remain short, with a height <-2 SDS [22,23]. Short stature is one of the most common medical concerns in childhood, and SGA birth is a significant risk factor for short stature, accounting for approximately 20% of all cases [24].

The causes of insufficient catch-up growth after SGA birth are not well understood. Several factors influence postnatal growth, such as (epi)genetics and physical, emotional, and social environmental factors. Disturbances in the GH/insulin-like growth factor (IGF)-axis *(Figure 2: GH-axis)* have also been suggested to play an essential role in the insufficient catch-up growth after SGA birth [25-27].

Despite adult height being one of the most heritable human traits [28], the (epi)genetic nature of short stature in SGA children is still largely unknown. Genome-wide association studies have identified genes contributing to the variation in height, but variations in these genes only have a small effect in the general population [29]. Mutations in one gene can also cause short stature, for example IGF1-receptor mutations, 3M syndrome and SHOX deficiency. Furthermore, epigenetic changes (i.e., aberrations in regions that control the imprinting of genes) can lead to short stature, which is the case in Silver-Russell syndrome (SRS).

**Silver-Russell syndrome**

SRS is a rare disorder, with an estimated incidence of 1 in 30,000 to 1 in 100,000 live-born infants per year [30]. Children with SRS are short and almost always born SGA. They show various dysmorphic features, such as a relative macrocephaly, a triangular-shaped head with frontal bossing, clinodactyly, and asymmetry of the face or body [31-33]. Severe feeding difficulties can be present, especially during infancy and early childhood. SRS is primarily a clinical diagnosis. The clinical scoring system with the highest sensitivity (98%) and best negative predictive value (89%) is the Netchine-Harbison clinical scoring system (*Table 2*) [34]. SRS is diagnosed when 4 or more of the clinical criteria are present.

When remaining untreated, mean adult height is around -4 SDS (i.e. 155 cm for men and 145 cm for women), which causes a significant handicap in adulthood [33]. Our study group has shown that SRS children respond similarly to GH treatment as non-SRS children born SGA [35,36]. Therefore, children with SRS are nowadays treated with GH.

There is very little information in the literature regarding pubertal progression and potential reproductive issues in SRS patients. Previous studies have shown that boys with SRS are at an increased risk of genital abnormalities such as cryptorchidism and hypospadias [37,38]. In girls with SRS, an association has been described with Mayer-Rokitansky-Kuster-Hauser syndrome, a disorder characterized by hypoplasia or aplasia of the uterus and upper part

of the vagina [39,40]. Pubertal development and gonadal function, however, have never been evaluated in SRS patients.

**Table 2: Netchine-Harbison clinical scoring system [34].**

| Clinical criteria | Definition |
|---|---|
| SGA | Birth weight and/or birth length ≤-2 SDS for gestational age |
| Postnatal growth failure | Height at 24±1 months ≤-2 SDS or height >2 SDS below mid-parental target height |
| Relative macrocephaly at birth | Head circumference at birth ≥1.5 SDS above birth weight and/or length SDS |
| Protruding/prominent forehead | Forehead projecting beyond the facial plane on a side view as a toddler (1-3 years) |
| Body asymmetry | Leg length discrepancy (LLD) of ≥0.5 cm or arm asymmetry or LLD<0.5 cm with at least two other asymmetrical body parts (one non-face) |
| Feeding difficulties and/or low BMI | BMI ≤-2 SDS at 24 months or current use of a feeding tube or cyproheptadine for appetite stimulation |

**Management of short stature**

Growth assessment requires accurate measurements of height and weight over time, parental height measurement, 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.

**GH treatment in short children born SGA**

Recombinant GH has been used in short children born SGA (including those with SRS) since 1989. Since 2005, GH treatment is licensed and reimbursed for short SGA children. GH treatment aims to achieve an adult height within the normal range or target height range of the child. Various clinical trials have shown that GH treatment with a dose of 1 mg/m$^2$/day (~0.33 mg/kg/day) improves growth rate, and leads to a significant improvement of adult height in children born SGA [41-43].

The GH-induced growth response, however, is highly variable [44]. Several studies have been conducted to determine clinical predictors for growth response to GH treatment [45-49]. Patient characteristics found to be related with adult height were: age and height SDS at start of GH treatment, target height SDS, GH dose, bone age delay at the start of treatment and baseline insulin-like growth factor binding protein-3 (IGFBP-3) SDS, together explaining approximately 40% of the variability in adult height.

**GnRHa treatment in short children born SGA**

Nowadays, GnRHa treatment is used for postponement of puberty in children with precocious puberty and nowadays also in short children born SGA with a relatively early onset of puberty,

thereby extending the prepubertal period of growth. Additional GnRHa treatment for 2 years at the start of puberty improves adult height in GH-treated children born SGA with a relatively early puberty with an expected adult height of less than -2.5 SDS [50].

**Long-term metabolic and cardiovascular health in subjects born SGA**

Several epidemiological studies showed associations between small size at birth and subsequent catch-up in weight, and the risk for cardiovascular diseases (CVD) and diabetes mellitus type 2 (DM2) in later life [51-55]. Both preterm birth and poor fetal growth can lead to small birth size. The exact mechanisms underlying the associations between fetal and early life growth and health later in life are mostly unknown. One of the hypotheses is the fetal origin hypothesis by Barker et al., postulating that events during pregnancy leading to fetal malnutrition could result in permanent metabolic changes in the fetus (i.e. reprogramming) [56]. These metabolic changes are beneficial during fetal life, but this reprogramming might result in diseases in adulthood.

In previous studies, our research group showed that accelerated weight gain during infancy is associated with determinants of adult diseases in early adulthood, such as insulin resistance, unfavorable body composition, higher adverse serum lipids and higher blood pressure [57-60]. These unfavorable metabolic and cardiovascular health parameters could lead to adult disease, and longitudinal changes in adulthood should, therefore, be investigated. Studies investigating detailed longitudinal metabolic and cardiovascular health in adults born SGA compared to adults born AGA at an age beyond 30 years, either with or without short stature, were lacking.

**Long-term safety of GH treatment in subjects born SGA**

Besides the positive effects on linear growth, GH has well-documented lipolytic and anabolic effects. Long-term GH treatment, therefore results in lower fat mass and higher lean body mass [61-65]. GH treatment has, however, also insulin-antagonistic effects which leads to a decrease in insulin sensitivity and a compensatory increase in insulin secretion [63,66-69]. Insulin resistance plays an important role in the pathogenesis of metabolic and cardiovascular disease [70,71]. During long-term GH treatment in SGA children, blood pressure SDS and cholesterol levels decreased and became lower than in untreated short SGA children [62,63,72-74]. After cessation of GH treatment, insulin sensitivity, body composition and blood pressure returned to similar levels at age 21 years in previously GH-treated adults compared to untreated age-matched short adults born SGA and normal statured AGA, whereas LBM and serum lipid levels remained lowest in GH-treated adults born SGA [61,75]. Follow-up beyond early adulthood was, however, lacking [72,76,77].

The Safety and Appropriateness of Growth Hormone treatments in Europe (SAGhE) study, a large European population study investigating long-term GH safety, reported increased

cardiovascular mortality in GH-treated adults born SGA [78,79]. Although this increase was mostly explained by higher mortality in the French cohort, authors advised to closely monitor metabolic and cardiovascular health following GH treatment. The SAGhE study, however, was limited by the absence of an untreated control group of age-matched adults born SGA to adjust for the adverse effects of SGA birth.

As insulin resistance, metabolic and cardiovascular disease might develop over a more extended period, it is important to investigate the long-term changes after GH-cessation in insulin sensitivity, body composition, central adiposity, serum lipid levels and blood pressure in adulthood. Therefore, it is essential that longitudinal studies are continued into adulthood and that metabolic and cardiovascular health parameters in GH-treated adults are compared with untreated age-matched adults born SGA or AGA.

**Long-term safety of additional 2 years of GnRHa treatment**

In patients with central precocious puberty (CPP), gonadotropin-releasing hormone agonist (GnRHa) treatment led to a decrease in insulin sensitivity, an increase in weight and fat mass and a decrease in bone mineral density (BMD) [80-91]. However, studies were performed retrospectively in children with CPP, and most studies evaluated only the changes during GnRHa treatment. In our study of SGA children, no adverse effects were found during and after 2 years of GnRHa treatment in addition to GH treatment until adult height attainment [92-95].

Studies on GnRHa treatment in children with different diagnoses showed potential cognitive and psychosocial effects. Firstly, cognitive functioning at the cessation of GnRHa treatment in CPP patients tended to be lower in the group receiving 2 years of GnRHa treatment [96,97]. Secondly, GnRHa treatment has been linked to a suppressed reward system, causing subsequent depressive emotions when used for endometriosis and during fertility treatment in adult women [98]. Lastly, the postponement of puberty might negatively affect problem behavior and school skills [99].

Studies on the long-term safety of postponing puberty with GnRHa treatment regarding metabolic health, bone mineralization, cognition, health-related quality of life (HRQoL) and psychosocial functioning in young adults born SGA following GH-cessation at adult height attainment were, however, lacking.

## Aims of the thesis

This thesis presents a detailed description of the studies performed to improve the knowledge about the long-term effects of SGA birth, GH treatment alone and with the addition of 2 years of GnRH analogue treatment and consequently the care for children born SGA. The study populations consisted of young adults born SGA treated with either GH only (Appendix A, B and D) or with combined GH/GnRHa treatment (Appendix B), and age-matched untreated adults born SGA or AGA (Appendix C and D). The aims of the studies described in this thesis are presented below.

**Gonadal function and pubertal development in children with Silver-Russell syndrome**
Very little information existed on gonadal function and pubertal development in patients with SRS. We, therefore, analyzed progression of puberty and gonadal function in children and adolescents with SRS until early adulthood.

**Kidney function and blood pressure at 5 years after cessation of GH treatment**
Glomerular filtration rate, blood pressure and microalbuminuria were investigated during 5 years after cessation of GH treatment to assess the long-term effects of GH treatment kidney function. The data at 5 years after cessation of GH-treated young adults were compared to untreated young adults born SGA and AGA.

**Cognition, psychosocial functioning and health-related quality of life of SGA born adolescents at the cessation of GH treatment, either with or without 2 years of GnRHa treatment**
Cognition, psychosocial functioning and health-related quality of life were investigated at the cessation of GH treatment with or without additional 2 years of GnRHa treatment to assess the long-term effects of GnRHa treatment. To determine the effect of additional GnRHa treatment, the data at cessation of GnRHa/GH-treated young adults were compared to young adults treated with GH only and to age-matched untreated young adults born AGA.

**Metabolic and cardiovascular health at 5 years after cessation of GH treatment, either with or without 2 years of GnRHa treatment**
Body composition, insulin sensitivity, beta-cell function, blood pressure, lipid profile and bone mineral density were investigated during 5 years after cessation of GH treatment with or without additional 2 years of GnRHa treatment to assess the long-term effects of GnRHa treatment on metabolic and cardiovascular health. To determine the effect of additional GnRHa treatment, the data at 5 years after cessation of GnRHa/GH-treated young adults were compared to young adults treated with GH only and age-matched untreated young adults born AGA.

**Metabolic health and body composition during 11 years of follow-up in untreated adults born SGA, either with or without short stature, and AGA**

Body composition, insulin sensitivity, beta-cell function, blood pressure and lipid profile were investigated in untreated adults born SGA and AGA during 11 years in adulthood, either with or without short stature, and compared with age-matched adults born AGA.
To determine the effect of SGA birth and catch-up growth on adult metabolic and cardiovascular health, the data at age 32 years were compared between the three groups.

**Metabolic health and body composition during 12 years of follow-up after GH-cessation in previously GH-treated versus untreated adults born SGA and AGA until age 30 years**

Body composition, insulin sensitivity, beta-cell function, kidney function, blood pressure and lipid profile were investigated during 12 years follow-up after cessation of GH treatment to assess the long-term effects of GH treatment on metabolic and cardiovascular health. To determine the effect of GH treatment during childhood on adult metabolic and cardiovascular health, the data at 12 years after cessation of GH treatment were compared with untreated adults born SGA, either with or without short stature, and with adults born AGA.

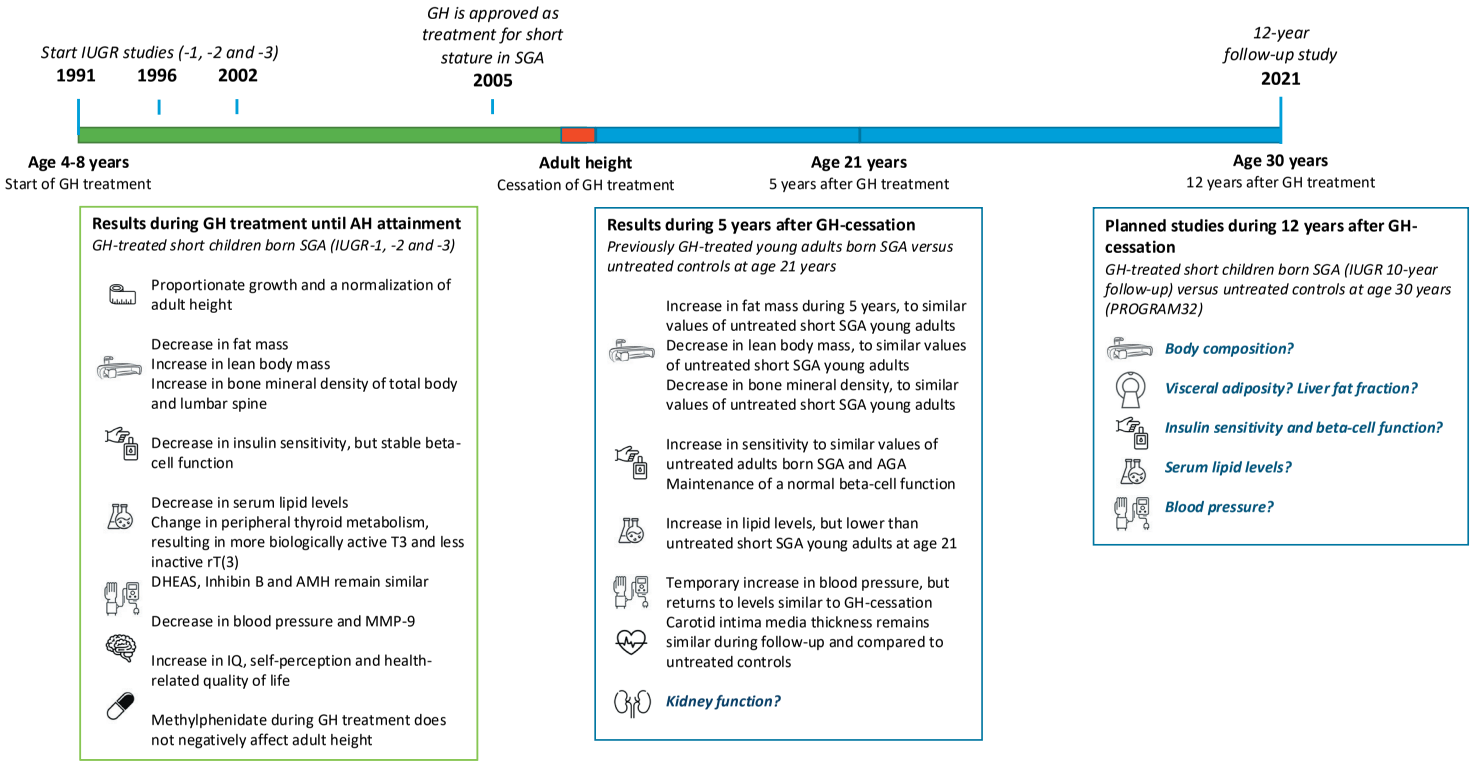


**Figure 4: Overview of the IUGR-1, IUGR-2, IUGR-3 and planned studies.**

# Appendix A: IUGR-1, IUGR-2 and IUGR-3 studies

The first, second and third Dutch GH trials (IUGR-1, IUGR-2 and -3 studies [76,100,101]) included children born SGA with persistent short stature.

**Design**

The IUGR-1 study started in 1991, the IUGR-2 study in 1996 and the IUGR-3 study in 2003. Except of a randomized, GH-controlled study during the first 3 years of the IUGR-2 study, all studies were open-labelled, multicenter studies. Children were treated with a biosynthetic GH with a dose of 1 mg/$m^2$/day. Three-monthly, the GH dose was adjusted to the calculated body surface area.

**Inclusion criteria**

1. Birth length or birth weight SDS for gestational age <-2 SDS [102];
2. 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 for age less than -2.5 SDS, according to Dutch references [103];
5. Height velocity SDS below 0 to exclude children with spontaneous catch-up growth;
6. Prepubertal, defined as Tanner stage I or testicular volume <4 ml in boys;
7. Normal liver, kidney and thyroid functions;
8. Well-documented growth data from birth to start of GH treatment;
9. Informed consent.

**Exclusion criteria**

1. Endocrine metabolic disorders (i.e., diabetes mellitus, diabetes insipidus, hypothyroidism, inborn errors of metabolism or growth hormone deficiency);
2. Celiac disease or other chronic diseases of the major organs;
3. Chromosomal abnormalities or dysmorphic features suggestive of a syndrome, except SRS);
4. Chondrodysplasia;
5. Serious suspicion of psychosocial dwarfism (emotional deprivation);
6. Active malignancy or increased risk of leukemia;
7. Use of medication that might interfere with growth, such as corticosteroids and sex steroids;
8. Expected non-compliance.

## Appendix B: Dutch SGA study

**Design**

The Dutch SGA study was 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). Prepubertal children received 1 mg/m$^2$/day [50]. When 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 sex, pubertal stage and parental height (one or two parents with a height below -2 SDS vs both parents with a height of at least -2 SDS). Because no model is known to predict adult height accurately at the start of puberty, we used a practical, arbitrary cutoff level. A height of less than 140 cm at the start of puberty was used to identify children with an adult height expectation below -2.5 SDS, based on Dutch reference values [7,104]; these children received GnRHa (leuprolide acetate depots, 3.75 mg sc every 4 weeks) for 2 years in addition to GH treatment.

**Inclusion criteria**

1. Birth length or birth weight SDS for gestational age <-2 SDS [102];
2. 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 of 8 years and older;
4. Prepubertal stage (Tanner stage I) or early pubertal stage (breast stage II-III in girls or testicular volume less than 10 mL in boys [105];
5. Height SDS for age less than -2.5 SDS or a predicted adult height less than -2.5 SDS, according to Dutch references [103];
6. Well-documented growth data from birth to start of GH treatment;
7. Informed consent.

**Exclusion criteria**

1. Endocrine metabolic disorders (i.e., diabetes mellitus, diabetes insipidus, hypothyroidism, inborn errors of metabolism or growth hormone deficiency);
2. Celiac disease or other chronic diseases of the major organs;
3. Chondrodysplasia or chromosomal abnormalities or dysmorphic features suggestive of a syndrome, except SRS;
4. Serious suspicion of psychosocial dwarfism (emotional deprivation);
5. Active or treated malignancy or increased risk of leukemia;
6. Use of medication during the previous 6 months that might interfere with growth;
7. Expected non-compliance.

# Appendix C: PROGRAM/PROGRAM32 study

The PROgramming factors for Growth and Metabolism study cohort consisted of healthy adults born either SGA or appropriate for gestational age (AGA), followed from age 21 years (PROGRAM) to age 30 years (PROGRAM32). [18,59]. In these participants, we performed several tests to investigate determinants of metabolic health and cardiovascular diseases (Appendix E).

**Design**

Cohort study to longitudinally investigate determinants of metabolic health and cardiovascular diseases in adults born SGA either with or without postnatal catch-up growth, in comparison with adults born AGA.

**Inclusion criteria**

1. PROGRAM: Chronological age at inclusion 18-24 years;
2. PROGRAM32: Chronological age at inclusion 25-35 years;
3. Neonatal period without signs of severe asphyxia (defined as Apgar score ≤3 after 5 minutes), no serious diseases such as long-term artificial ventilation and oxygen supply, bronchopulmonary dysplasia or other chronic lung diseases;
4. Well-documented growth data;
5. Caucasian;
6. Born singleton;
7. Signed informed consent;
8. Gestational age ≥36 weeks.

**Exclusion criteria**

1. Chromosomal abnormalities or dysmorphic features suggestive of a syndrome, except Silver-Russell syndrome;
2. Any disease, endocrine or metabolic disorder that could have interfered with growth (such as diabetes, GH deficiency, malignancies, severe chronic disease);
3. Treatment that could have interfered with growth (such as radiotherapy or GH treatment);
4. Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood.

## Appendix D: 12-year follow-up SGA study

The 12-year follow-up SGA study included former participants of the IUGR1 and IUGR2 study, with more than 10 years of follow-up after GH-cessation, compared to appropriate, age-matched controls of the PROGRAM/PROGRAM32 study. We performed several tests to investigate determinants of metabolic health and cardiovascular diseases (Appendix E).

**Design**

To investigate the influence of childhood GH treatment on adult health parameters, GH-treated adults born SGA were longitudinally investigated at GH-cessation, and at 5 and 12 years after GH-cessation. In addition, previously GH-treated adults born SGA were compared at around 30 years of age to age-matched untreated adults born SGA, either with or without spontaneous catch-up growth, and untreated adults born AGA.

**Inclusion criteria**

1. Previously GH-treated adults with a follow-up of at least 12 years after GH-cessation;
2. Former participants of the IUGR-1 or IUGR-2 study (Appendix A)
3. Signed informed consent.

**Exclusion criteria**

1. Pregnancy and first 6 months after delivery
2. Childhood GH treatment for less than 4 years.

# Appendix E: Determinants of metabolic and cardiovascular disease

**Body composition by Dual Energy X-ray Absorptiometry (DXA)**

DXA was used to measure body composition and bone mineral density (i.e. fat mass and lean body mass) [106]. The participant needs to lie still for approximately 15 minutes while a scanner slides over the participant. DXA uses X-ray to assess these measures, but the radiation dose is low (about 1/10th of a chest X-ray).

**Frequently Sampled Intravenous Glucose Tolerance (FSIGT) test**

Glucose homeostasis can be assessed by means of an FSIGT test with Tolbutamide [107,108]. The FSIGT test provides the following values regarding glucose homeostasis: Insulin sensitivity: The ability of insulin to increase glucose disposal; Glucose effectiveness: The capacity of glucose to mediate its own disposal; Acute insulin response: An estimate of insulin secretory capacity; Disposition index: A measure of ß-cell function.

The Bergman's minimal model was used to calculate these indicators of glucose regulation, using paired glucose and insulin data obtained by frequent measurements during an FSIGT test with Tolbutamide. This way, early glucose metabolism changes can be assessed, many years before the first symptoms of diabetes mellitus type II occur.

**Blood pressure, kidney function and lipid profile**

Blood pressure, total cholesterol, low-density lipoprotein, high-density lipoprotein, microalbuminuria and glomerular filtration rate were determined to assess the effects of SGA birth and GH treatment on cardiovascular and metabolic disease.

To determine blood pressure, we used resting blood pressure during 30 minutes in supine position. Fasting blood samples were used to evaluate kidney function and serum lipid levels. We used serum creatinine levels to determine glomerular filtration rate. To determine total cholesterol and triglyceride, we used an automated enzymatic method with the CHOD-PAP reagent kit and GPO-PAP reagent kit, respectively and high-density lipoprotein was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics)

**Visceral and subcutaneous adipose tissue and liver fat fraction by MRI**

Visceral and subcutaneous adipose tissue and liver fat fraction were measured by a magnetic resonance imaging (MRI) scan. Adipose tissue measurements were performed by fast-spoiled gradient echo technique to acquire fat-only images in 20-second breath-hold scans on a 3T GE Discovery MR750system (GE Healthcare, Milwaukee, WI, US). The cross-sectional area at the level of L3 was used, using a threshold-based region technique for slight adjustments. Measurements of liver fat fraction were performed using the IDEAL-IQ technique.

## Outline of this thesis

**Chapter 1** Gives an introduction to SGA, metabolic risks of SGA birth and the topics described in this thesis.

**Chapter 2** Describes the pubertal development and gonadal function in subjects with Silver-Russell Syndrome.

**Chapter 3** Presents kidney function and blood pressure in young adults born SGA treated with GH during childhood.

**Chapter 4** Shows the cognition, psychosocial functioning and health-related quality of life after cessation of GH/GnRHa treatment compared to treatment with GH only in young adults born SGA.

**Chapter 5** Describes long-term metabolic and cardiovascular safety of additional 2 years of GnRHa treatment during GH treatment in young adults born SGA.

**Chapter 6** Shows metabolic health and body composition during 11 years of follow-up in untreated adults born SGA and AGA until age 30 years (PROGRAM32 study).

**Chapter 7** Shows the cardiovascular and metabolic safety of childhood GH treatment during follow-up of 12 years after GH-cessation in adult born SGA, also compared to untreated age-matched adults born SGA or AGA at age 30 years.

**Chapter 8** Provides a general discussion about the results of the studies described in this thesis in view of the literature, the clinical implications and suggestions for further research.

**Chapter 9** Summarizes the findings described in this thesis in English and Dutch.

**Chapter 10** Contains a list of abbreviations, a list of publications, PhD portfolio, acknowledgements and curriculum vitae.

## References


1. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab.* 2007;92(3):804-810.
2. Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P, International Small for Gestational Age Advisory B. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics.* 2003;111(6 Pt 1):1253-1261.
3. Bryan SM, Hindmarsh PC. Normal and abnormal fetal growth. *Horm Res.* 2006;65 Suppl 3:19-27.
4. Kumar P, Menon R. New insights into growth hormone's actions on the macrophage: implications for non-growth-related actions of growth hormone. 2013.
5. Kumar PA. New insights into growth hormones actions on the macrophage: implications for non-growth-related actions of growth hormone. *OA Biochemistry.* 2013.
6. Buck Louis GM, Gray LE, Jr., Marcus M, et al. Environmental factors and puberty timing: expert panel research needs. *Pediatrics.* 2008;121 Suppl 3:S192-207.
7. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
8. Carel JC. Management of short stature with GnRH agonist and co-treatment with growth hormone: a controversial issue. *Mol Cell Endocrinol.* 2006;254-255:226-233.
9. Leger J, Levy-Marchal C, Bloch J, et al. Reduced final height and indications for insulin resistance in 20 year olds born small for gestational age: regional cohort study. *BMJ.* 1997;315(7104):341-347.
10. Persson I, Ahlsson F, Ewald U, et al. Influence of perinatal factors on the onset of puberty in boys and girls: implications for interpretation of link with risk of long term diseases. *Am J Epidemiol.* 1999;150(7):747-755.
11. Hokken-Koelega AC. Timing of puberty and fetal growth. *Best Pract Res Clin Endocrinol Metab.* 2002;16(1):65-71.
12. Lazar L, Pollak U, Kalter-Leibovici O, Pertzelan A, Phillip M. Pubertal course of persistently short children born small for gestational age (SGA) compared with idiopathic short children born appropriate for gestational age (AGA). *Eur J Endocrinol.* 2003;149(5):425-432.
13. Boonstra V, van Pareren Y, Mulder P, Hokken-Koelega A. Puberty in growth hormone-treated children born small for gestational age (SGA). *J Clin Endocrinol Metab.* 2003;88(12):5753-5758.
14. Albertsson-Wikland K, Karlberg J. Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl.* 1994;399:64-70; discussion 71.
15. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril.* 2002;77(2):357-362.
16. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod.* 2003;18(2):323-327.

17. Boonstra VH, Weber RF, de Jong FH, Hokken-Koelega AC. Testis function in prepubertal boys and young men born small for gestational age. *Horm Res.* 2008;70(6):357-363.
18. Kerkhof GF, Leunissen RW, Willemsen RH, de Jong FH, Stijnen T, Hokken-Koelega AC. Influence of preterm birth and birth size on gonadal function in young men. *J Clin Endocrinol Metab.* 2009;94(11):4243-4250.
19. Kerkhof GF, Leunissen RWJ, Willemsen RH, et al. Influence of preterm birth and small birth size on serum anti-Mullerian hormone levels in young adult women. *European Journal of Endocrinology.* 2010;163(6):937-944.
20. Lem AJ, Boonstra VH, Renes JS, et al. Anti-Mullerian hormone in short girls born small for gestational age and the effect of growth hormone treatment. *Hum Reprod.* 2011;26(4):898-903.
21. Kong L. Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. *Int J Mol Sci.* 2004.
22. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res.* 1995;38(2):267-271.
23. de Ridder MA, Engels MA, Stijnen T, Hokken-Koelega AC. Small for gestational age children without early catch-up growth: spontaneous growth and prediction of height at 8 years. *Horm Res.* 2008;70(4):203-208.
24. Karlberg J, Albertsson-Wikland K. Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatr Res.* 1995;38(5):733-739.
25. Boguszewski M, Rosberg S, Albertsson-Wikland K. Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. *J Clin Endocrinol Metab.* 1995;80(9):2599-2606.
26. de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL. Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. *Clin Endocrinol (Oxf).* 1994;41(5):621-630.
27. Stanhope R, Ackland F, Hamill G, Clayton J, Jones J, Preece MA. Physiological growth hormone secretion and response to growth hormone treatment in children with short stature and intrauterine growth retardation. *Acta Paediatr Scand Suppl.* 1989;349:47-52; discussion 53-44.
28. Visscher PM, Hill WG, Wray NR. Heritability in the genomics era--concepts and misconceptions. *Nat Rev Genet.* 2008;9(4):255-266.
29. Weedon MN, Lango H, Lindgren CM, et al. Genome-wide association analysis identifies 20 loci that influence adult height. *Nat Genet.* 2008;40(5):575-583.
30. Marsaud C, Rossignol S, Tounian P, Netchine I, Dubern B. Prevalence and management of gastrointestinal manifestations in Silver-Russell syndrome. *Archives of Disease in Childhood.* 2015;100(4):353-358.

31. Russell A. A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and other anomalies (5 examples). *Proc R Soc Med.* 1954;47(12):1040-1044.
32. Silver HK, Kiyasu W, George J, Deamer WC. Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. *Pediatrics.* 1953;12(4):368-376.
33. Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB. Growth and Symptoms in Silver-Russell-Syndrome - Review on the Basis of 386 Patients. *European Journal of Pediatrics.* 1995;154(12):958-968.
34. Azzi S, Salem J, Thibaud N, et al. A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. *J Med Genet.* 2015;52(7):446-453.
35. Smeets CC, Renes JS, van der Steen M, Hokken-Koelega AC. Metabolic Health and Long-Term Safety of Growth Hormone Treatment in Silver-Russell Syndrome. *J Clin Endocrinol Metab.* 2017;102(3):983-991.
36. Smeets CC, Zandwijken GR, Renes JS, Hokken-Koelega AC. Long-Term Results of GH Treatment in Silver-Russell Syndrome (SRS): Do They Benefit the Same as Non-SRS Short-SGA? *J Clin Endocrinol Metab.* 2016;101(5):2105-2112.
37. Bruce S, Hannula-Jouppi K, Peltonen J, Kere J, Lipsanen-Nyman M. Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab.* 2009;94(2):579-587.
38. Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *Journal of Medical Genetics.* 1999;36(11):837-842.
39. Abraham MB, Carpenter K, Baynam GS, Mackay DJ, Price G, Choong CS. Report and review of described associations of Mayer-Rokitansky-Kuster-Hauser syndrome and Silver-Russell syndrome. *J Paediatr Child Health.* 2014.
40. Bellver-Pradas J, Cervera-Sanchez J, Boldo-Roda A, et al. Silver-Russell syndrome associated to Mayer-Rokitansky-Kuster-Hauser syndrome, diabetes and hirsutism. *Arch Gynecol Obstet.* 2001;265(3):155-157.
41. Renes JS, Willemsen RH, Mulder JC, et al. New insights into factors influencing adult height in short SGA children: Results of a large multicentre growth hormone trial. *Clin Endocrinol.* 2015;82(6):854-861.
42. van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: Results of a randomized, double-blind, dose-response GH trial. *J Clin Endocr Metab.* 2003;88(8):3584-3590.
43. Dahlgren J, Wikland KA, T SSGGH. Final height in short children born small for gestational age treated with growth hormone. *Pediatric Research.* 2005;57(2):216-222.

44. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
45. Renes JS, Willemsen RH, Mulder JC, et al. New insights into factors influencing adult height in short SGA children: Results of a large multicentre growth hormone trial. *Clin Endocrinol (Oxf).* 2015;82(6):854-861.
46. Carel JC, Chatelain P, Rochiccioli P, Chaussain JL. Improvement in adult height after growth hormone treatment in adolescents with short stature born small for gestational age: results of a randomized controlled study. *J Clin Endocrinol Metab.* 2003;88(4):1587-1593.
47. de Ridder MA, Stijnen T, Hokken-Koelega AC. Prediction model for adult height of small for gestational age children at the start of growth hormone treatment. *J Clin Endocrinol Metab.* 2008;93(2):477-483.
48. Karlberg JP, Albertsson-Wikland K, Kwan EY, Lam BC, Low LC. The timing of early postnatal catch-up growth in normal, full-term infants born short for gestational age. *Horm Res.* 1997;48 Suppl 1:17-24.
49. Ranke MB, Lindberg A, Board KI. Height at start, first-year growth response and cause of shortness at birth are major determinants of adult height outcomes of short children born small for gestational age and Silver-Russell syndrome treated with growth hormone: analysis of data from KIGS. *Horm Res Paediatr.* 2010;74(4):259-266.
50. Lem AJ, van der Kaay DC, de Ridder MA, et al. Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analog: results of a randomized, dose-response GH trial. *J Clin Endocrinol Metab.* 2012;97(11):4096-4105.
51. Barker DJP, Bull AR, Osmond C, Simmonds SJ. Fetal and Placental Size and Risk of Hypertension in Adult Life. *Brit Med J.* 1990;301(6746):259-262.
52. Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K, Clark PMS. Type 2 (Non-Insulin-Dependent) Diabetes-Mellitus, Hypertension and Hyperlipemia (Syndrome-X) - Relation to Reduced Fetal Growth. *Diabetologia.* 1993;36(1):62-67.
53. Ong KK. Size at birth, postnatal growth and risk of obesity. *Horm Res.* 2006;65 Suppl 3:65-69.
54. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. *BMJ.* 2000;320(7240):967-971.
55. Singhal A. The early origins of atherosclerosis. *Adv Exp Med Biol.* 2009;646:51-58.
56. Barker DJP. The Fetal and Infant Origins of Adult Disease. *Brit Med J.* 1990;301(6761):1111-1111.
57. Kerkhof GF, Willemsen RH, Leunissen RWJ, Breukhoven PE, Hokken-Koelega ACS. Health Profile of Young Adults Born Preterm: Negative Effects of Rapid Weight Gain in Early Life. *J Clin Endocr Metab.* 2012;97(12):4498-4506.
58. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab.* 2008;93(2):445-451.

59. Leunissen RWJ, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and Tempo of First-Year Rapid Growth in Relation to Cardiovascular and Metabolic Risk Profile in Early Adulthood. *Jama-J Am Med Assoc.* 2009;301(21):2234-2242.
60. Leunissen RWJ, Stijnen T, Hokken-Koelega ACS. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol.* 2009;70(2):245-251.
61. Willemsen RH, Arends NJ, Bakker-van Waarde WM, et al. Long-term effects of growth hormone (GH) treatment on body composition and bone mineral density in short children born small-for-gestational-age: six-year follow-up of a randomized controlled GH trial. *Clin Endocrinol (Oxf).* 2007;67(4):485-492.
62. Hokken-Koelega AC, van Pareren Y, Sas T, Arends N. Final height data, body composition and glucose metabolism in growth hormone-treated short children born small for gestational age. *Horm Res.* 2003;60 Suppl 3:113-114.
63. de Kort SW, Willemsen RH, van der Kaay DC, Hokken-Koelega AC. The effect of growth hormone treatment on metabolic and cardiovascular risk factors is similar in preterm and term short, small for gestational age children. *Clin Endocrinol (Oxf).* 2009;71(1):65-73.
64. Richelsen B. Action of growth hormone in adipose tissue. *Horm Res.* 1997;48 Suppl 5:105-110.
65. Mukherjee A, Murray RD, Shalet SM. Impact of growth hormone status on body composition and the skeleton. *Horm Res.* 2004;62 Suppl 3:35-41.
66. de Zegher F, Ong K, van Helvoirt M, Mohn A, Woods K, Dunger D. High-dose growth hormone (GH) treatment in non-GH-deficient children born small for gestational age induces growth responses related to pretreatment GH secretion and associated with a reversible decrease in insulin sensitivity. *J Clin Endocrinol Metab.* 2002;87(1):148-151.
67. Cutfield WS, Jackson WE, Jefferies C, et al. Reduced insulin sensitivity during growth hormone therapy for short children born small for gestational age. *J Pediatr.* 2003;142(2):113-116.
68. Sas T, Mulder P, Aanstoot HJ, et al. Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. *Clin Endocrinol (Oxf).* 2001;54(2):243-251.
69. Kappelgaard AM, Kiyomi F, Horikawa R, Yokoya S, Tanaka T. The impact of long-term growth hormone treatment on metabolic parameters in Japanese patients with short stature born small for gestational age. *Horm Res Paediatr.* 2014;81(4):272-279.
70. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet.* 1992;340(8825):925-929.
71. Facchini FS, Hua N, Abbasi F, Reaven GM. Insulin resistance as a predictor of age-related diseases. *The Journal of clinical endocrinology and metabolism.* 2001;86(8):3574-3578.
72. van Dijk M, Bannink EM, van Pareren YK, Mulder PG, Hokken-Koelega AC. Risk factors for diabetes mellitus type 2 and metabolic syndrome are comparable for previously growth hormone-treated young adults born small for gestational age (sga) and untreated short SGA controls. *J Clin Endocrinol Metab.* 2007;92(1):160-165.

73. Sas T, Mulder P, Hokken-Koelega A. Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. *J Clin Endocrinol Metab.* 2000;85(10):3786-3792.
74. van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Effect of discontinuation of growth hormone treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. *J Clin Endocrinol Metab.* 2003;88(1):347-353.
75. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.
76. Breukhoven PE, Kerkhof GF, van Dijk M, Hokken-Koelega ACS. Long-Term Impact of GH Treatment during Childhood on Body Composition and Fat Distribution in Young Adults Born SGA. *J Clin Endocr Metab.* 2011;96(12):3710-3716.
77. Willemsen RH, Willemsen SP, Hokken-Koelega AC. Longitudinal changes in insulin sensitivity and body composition of small-for-gestational-age adolescents after cessation of growth hormone treatment. *J Clin Endocrinol Metab.* 2008;93(9):3449-3454.
78. Carel JC, Ecosse E, Landier F, et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J Clin Endocrinol Metab.* 2012;97(2):416-425.
79. Savendahl L, Cooke R, Tidblad A, et al. Long-term mortality after childhood growth hormone treatment: the SAGhE cohort study. *Lancet Diabetes Endocrinol.* 2020;8(8):683-692.
80. Chiavaroli V, Liberati M, D'Antonio F, et al. GNRH analog therapy in girls with early puberty is associated with the achievement of predicted final height but also with increased risk of polycystic ovary syndrome. *Eur J Endocrinol.*163(1):55-62.
81. Sorensen K, Mouritsen A, Mogensen SS, Aksglaede L, Juul A. Insulin sensitivity and lipid profiles in girls with central precocious puberty before and during gonadal suppression. *J Clin Endocrinol Metab.* 2010;95(8):3736-3744.
82. Tascilar ME, Bilir P, Akinci A, et al. The effect of gonadotropin-releasing hormone analog treatment (leuprolide) on body fat distribution in idiopathic central precocious puberty. *Turk J Pediatr.* 2011;53(1):27-33.
83. Faienza MF, Brunetti G, Acquafredda A, et al. Metabolic Outcomes, Bone Health, and Risk of Polycystic Ovary Syndrome in Girls with Idiopathic Central Precocious Puberty Treated with Gonadotropin-Releasing Hormone Analogues. *Horm Res Paediatr.* 2017;87(3):162-169.
84. Magiakou MA, Manousaki D, Papadaki M, et al. The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. *J Clin Endocrinol Metab.* 2010;95(1):109-117.
85. Lazar L, Lebenthal Y, Yackobovitch-Gavan M, et al. Treated and untreated women with idiopathic precocious puberty: BMI evolution, metabolic outcome, and general health between third and fifth decades. *J Clin Endocrinol Metab.* 2015;100(4):1445-1451.

86. Aguiar AL, Couto-Silva AC, Vicente EJ, Freitas IC, Cruz T, Adan L. Weight evolution in girls treated for idiopathic central precocious puberty with GnRH analogues. *J Pediatr Endocrinol Metab.* 2006;19(11):1327-1334.
87. Park HK, Lee HS, Ko JH, Hwang IT, Lim JS, Hwang JS. The effect of gonadotrophin-releasing hormone agonist treatment over 3 years on bone mineral density and body composition in girls with central precocious puberty. *Clin Endocrinol (Oxf).* 2012;77(5):743-748.
88. van der Sluis IM, Boot AM, Krenning EP, Drop SL, de Muinck Keizer-Schrama SM. Longitudinal follow-up of bone density and body composition in children with precocious or early puberty before, during and after cessation of GnRH agonist therapy. *J Clin Endocrinol Metab.* 2002;87(2):506-512.
89. Boot AM, De Muinck Keizer-Schrama S, Pols HA, Krenning EP, Drop SL. Bone mineral density and body composition before and during treatment with gonadotropin-releasing hormone agonist in children with central precocious and early puberty. *J Clin Endocrinol Metab.* 1998;83(2):370-373.
90. Saggese G, Bertelloni S, Baroncelli GI, Battini R, Franchi G. Reduction of bone density: an effect of gonadotropin releasing hormone analogue treatment in central precocious puberty. *Eur J Pediatr.* 1993;152(9):717-720.
91. Antoniazzi F, Bertoldo F, Zamboni G, et al. Bone mineral metabolism in girls with precocious puberty during gonadotrophin-releasing hormone agonist treatment. *Eur J Endocrinol.* 1995;133(4):412-417.
92. van der Steen M, Lem AJ, van der Kaay DC, et al. 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. *J Clin Endocrinol Metab.* 2015;100(10):3725-3734.
93. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Insulin Sensitivity and beta-Cell Function in SGA Children Treated With GH and GnRHa: Results of a Long-Term Trial. *J Clin Endocrinol Metab.* 2016;101(2):705-713.
94. Lem AJ, van der Kaay DC, Hokken-Koelega AC. Bone mineral density and body composition in short children born SGA during growth hormone and gonadotropin releasing hormone analog treatment. *J Clin Endocrinol Metab.* 2013;98(1):77-86.
95. Lem AJ, Jobse I, van der Kaay DC, de Ridder MA, Raat H, Hokken-Koelega AC. Health-related quality of life in short children born small for gestational age: effects of growth hormone treatment and postponement of puberty. *Horm Res Paediatr.* 2012;77(3):170-179.
96. Mul D, Versluis-den Bieman HJ, Slijper FM, Oostdijk W, Waelkens JJ, Drop SL. Psychological assessments before and after treatment of early puberty in adopted children. *Acta Paediatr.* 2001;90(9):965-971.
97. Wojniusz S, Callens N, Sutterlin S, et al. Cognitive, Emotional, and Psychosocial Functioning of Girls Treated with Pharmacological Puberty Blockage for Idiopathic Central Precocious Puberty. *Front Psychol.* 2016;7:1053.
98. Frokjaer VG, Pinborg A, Holst KK, et al. Role of Serotonin Transporter Changes in Depressive Responses to Sex-Steroid Hormone Manipulation: A Positron Emission Tomography Study. *Biol Psychiatry.* 2015;78(8):534-543.

99. Waylen A, Wolke D. Sex 'n' drugs 'n' rock 'n' roll: the meaning and social consequences of pubertal timing. *Eur J Endocrinol.* 2004;151 Suppl 3:U151-159.
100. Sas T, de Waal W, Mulder P, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab.* 1999;84(9):3064-3070.
101. van Dijk M, Mulder P, Houdijk M, et al. High serum levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) during high-dose GH treatment in short children born small for gestational age. *J Clin Endocrinol Metab.* 2006;91(4):1390-1396.
102. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74(6):901-910.
103. Fredriks AM, Van Buuren S, Burgmeijer RJF, et al. Continuing positive secular growth change in the Netherlands 1955-1997. *Pediatric Research.* 2000;47(3):316-323.
104. Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965-1997. *Pediatr Res.* 2001;50(4):479-486.
105. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child.* 1976;51(3):170-179.
106. Bachrach LK. Dual energy X-ray absorptiometry (DEXA) measurements of bone density and body composition: promise and pitfalls. *J Pediatr Endocrinol Metab.* 2000;13 Suppl 2:983-988.
107. Bergman RN. Minimal model: perspective from 2005. *Horm Res.* 2005;64 Suppl 3:8-15.
108. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003;5(6):1003-1015.

# Chapter 2

## Gonadal Function and Pubertal Development in Patients with Silver-Russell Syndrome

W.J. Goedegebuure
C.C.J. Smeets
J.S. Renes
Y.B. de Rijke
A.C.S. Hokken-Koelega

*Human Reproduction 2018 , Nov 1;33(11):2122-2130*

## Abstract

**Background:** Males with SRS have an increased risk for genital abnormalities such as cryptorchidism and hypospadias, which could be associated with reproductive problems in later life. In SRS females, an association has been described with Mayer-Rokitansky-Küster-Hauser syndrome, which might compromise their reproductive function.

**Methods:** Longitudinal follow-up study, in thirty-one SRS patients (14 males) and 123 non-SRS patients born at same gestational age (SGA; 65 males). All received growth hormone and 27.3% received additional gonadotropin-releasing hormone analogue treatment (GnRHa).

**Results:** Mean age at onset of puberty was 11.5yrs in SRS males versus 11.6yrs in non-SRS males ($p=0.51$), and 10.5yrs in SRS females versus 10.7yrs in non-SRS females ($p=0.50$). Four of the 14 SRS males had a postpubertal inhibin-B level below the 5th percentile compared to healthy controls, and two of them an FSH above the 95th percentile, indicating Sertoli cell dysfunction. One of them had a history of bilateral cryptorchidism and orchiopexy. All SRS females had AMH, LH and FSH levels within the reference range. Pubertal duration to Tanner stage 5 was similar in SRS and non-SRS. Pubertal height gain was better in SRS patients who additionally received GnRHa ($p<0.01$). Mean age at menarche was 13.1yrs in SRS versus 13.3yrs in non-SRS ($p=0.62$). One SRS female had primary amenorrhea due to Müllerian agenesis.

**Conclusion:** As gonadal function is not affected in females with SRS, it is likely that reproductive function is also not affected. Sertoli cell dysfunction in males with SRS could cause impaired reproductive function and should be assessed during pubertal development.

## Introduction

Silver-Russell syndrome (SRS) is characterized by intrauterine growth retardation, leading to small for gestational age (SGA) birth, postnatal growth failure, feeding difficulties, and several dysmorphic features (i.e., body asymmetry, a triangular face with a prominent forehead and several other minor anomalies) [1-3]. Without treatment, mean adult height (AH) is around −4.2 standard deviation score (SDS), causing a significant handicap in adulthood. Incidence is estimated as 1 in 30 000 to 100 000 life-born infants per year. Overall, SRS is probably more common than some estimates have suggested, but the exact incidence remains unknown [4]. Of all SRS cases, 60% are caused by a loss of methylation in the *ICR1* region of 11p15 (11p15 LOM) [5], and 5-10% by a maternal uniparental disomy of chromosome 7 (UPD(7)mat) [6]. In 30-40%, the genetic cause is unknown, which is referred to as clinical SRS [4]. Nowadays, most SRS patients are treated with growth hormone (GH), which is an effective treatment to improve adult height [7,8].

It is unknown whether SRS patients have reproductive difficulties. Males with SRS have an increased risk for genital abnormalities such as cryptorchidism and hypospadias [9-11], which could be associated with reproductive problems in later life. In SRS females, an association has been described with Mayer-Rokitansky-Küster-Hauser syndrome (MRKH), a disorder characterized by hypoplasia or aplasia of the uterus and upper part of the vagina [12,13]. However, data on gonadal function in SRS are lacking. Since the majority of adults with SRS are not routinely followed up, there is very little information in the literature regarding the natural history of SRS [4,14]. Previous studies have found that onset of puberty is usually within the normal range, but at the younger end of the spectrum, and that adrenarche can be early and aggressive in comparison with children born SGA without SRS [4]. However, there are not many data on pubertal progression in SRS.

In this longitudinal study, we analyzed gonadal function (i.e., serum levels of inhibin B, FSH, LH, anti-Müllerian hormone (AMH) and testosterone) and progression of puberty in SRS patients from childhood until early adulthood. AMH is a marker of immature Sertoli cells in males, and of the follicle pool size in females [15-17], and inhibin B is a marker of the Sertoli cell function in males [18]. We compared these data to those of subjects born small for gestational age (SGA) without SRS, and to age-appropriate reference data. We hypothesized that SRS patients would be younger at onset of puberty and have a faster pubertal progression than their non-SRS SGA counterparts and the healthy reference population. We also hypothesized that gonadal function would not be altered in SRS patients without congenital genital anomalies.

# Methods

**Subjects**

For the present study, we included 31 SRS and 123 non-SRS subjects who participated in a large, multicenter GH trial [19,20]. All subjects were born SGA (birth length and/or birth weight standard deviation score (SDS) <-2.0 for gestational age [21]), received treatment 1 mg GH/$m^2$/day (0.035 mg/kg/day) because of persistent short stature (height <-2.5 SDS [22]), and were prepubertal at onset of GH treatment. Subjects were excluded from analysis of pubertal development when they had not completed puberty by the end of the study, when blood samples were not available, or when they had chromosomal abnormalities or signs of a syndrome except SRS.

The diagnosis SRS was based on the Netchine-Harbison clinical scoring system [23], which comprises the following six factors: 1) prenatal growth retardation (birth length and/or birth weight ≤-2 SDS for gestational age); 2) postnatal growth retardation (height <-2.0 SDS according to national reference [22]; 3) relative macrocephaly at birth (head circumference at birth ≥1.5 SDS above birth length and/or birth weight SDS according to Usher and McLean [21]; 4) prominent forehead; 5) body asymmetry (leg length discrepancy of ≥0.5 cm or arm asymmetry or leg length discrepancy <0.5 cm with ≥2 other asymmetrical body parts (one being a non-face part)); and 6) feeding difficulties during early childhood. Patients were classified as SRS if at least four factors were present. SRS patients were tested for 11p15 LOM and UPD(7)mat, and when negative, also for *CDKN1C* and *IGF2* mutations as previously described [7]. Patients with 4 or more positive criteria of the Netchine-Harbison clinical scoring system but without a known genetic aberration were classified as clinical SRS.

This study was performed according to the Helsinki Declaration and approved by the Medical Ethics Committee of all participating centers. Written informed consent was obtained from all participants and their parents.

**Design**

From onset of GH treatment until AH attainment, all subjects visited the hospital every three months. At each visit, pubertal stage was examined. Blood samples were obtained yearly, always between 8.30 and 9.30 am.

Serum levels of inhibin B (males) and AMH (males and females) were measured in the SRS subjects at three time-points: 1) Prepubertal stage; 2) the first blood sample after onset of puberty and 3) postpubertal stage (i.e., a testicular volume (TV) ≥15 ml in males and ≥one year post menarche in females). At time-point two and three, we also measured serum levels of LH, FSH (males and females) and testosterone (males). For the postpubertal blood sample in females, we only used samples that were drawn before start of oral contraceptives. All

results were compared to those of healthy children and adolescents of the same pubertal stage collected at similar time points [17,18,24,25]. Postpubertal results were compared with GH-treated age-matched non-SRS subjects born SGA.

**Measurements**

Pubertal stage was assessed by an experienced investigator (C.C.J.S and J.S.R) according to the method of Tanner [26], at each three-monthly visit. This allowed adequate determination of pubertal onset, which was defined as persistent breast development stage II according to Tanner for females (M2), and a TV ≥4 ml for males as determined by means of the Prader orchidometer. Precocious puberty was defined as pubertal signs before age of 8 years in females and 9 years in males [27].

SRS and non-SRS subjects with an AH expectation of less than -2.5 SDS at onset of puberty received 2 years of gonadotropin-releasing hormone agonist treatment (GnRHa, leuprolide acetate depots, 3.75 mg sc every four weeks) in addition to GH to postpone puberty [22]. To be certain of central puberty, a GnRHa test was performed before commencement of GnRHa treatment.

AH was defined as the condition when height had not increased more than 0.5 cm during the previous six months and a bone age ≥15 years for females and ≥16.5 years for males. Details regarding genital malformations and surgery before the start of GH treatment were retrieved from medical records.

**Laboratory measurements**

After centrifugation, all samples were kept frozen (-80 °C) until assayed. All hormone concentrations were determined in one endocrine laboratory, Erasmus University Medical Center. Both serum AMH and inhibin B levels were measured by the Gen II ELISA (Beckman Coulter, Inc. Brea, CA, USA). Serum LH and FSH levels were measured by immunometric assays (Immulite 2000XPi, Siemens, Los Angeles, CA, USA). Total serum testosterone was measured using the liquid chromatography-tandem mass spectrometry (LC-MS-MS) method with the CHS™ MSMS Steroids Kit (Perkin Elmer, Turku, Finland). Chromatographic separation and quantification were performed using a Waters® XEVO-TQ-S system equipped with an electrospray ionization (ESI) source. Inter-assay coefficients of variation were 15.1% for AMH, 11.4% for inhibin B, 6.4% for LH, 5.2% for FSH and 6.8% for testosterone, respectively.

**Statistics**

Analyses were performed with SPSS version 21.0. Shapiro Wilk tests and Normal Q-Q-plots were used to determine distribution of variables. Differences between SRS and non-SRS were analyzed by independent-sample *t* tests (continuous data) or chi-squared tests (categorical

data). Subjects who had also received GnRHa treatment were analyzed separately. One-sided *t* tests were used to compare data with those of healthy references. P-values <0.05 were considered statistically significant.

## Results

### Clinical characteristics

Table 1 shows the clinical characteristics of the 31 SRS and 123 non-SRS subjects. Fifteen SRS patients had 11p15 LOM, seven patients an UPD(7)mat and no patients had an *IGF2* or *CDKN1C* mutation. Nine patients had clinical SRS, and these subjects fulfilled the Netchine-Harbison criteria for SRS, including relative macrocephaly. Four SRS males had cryptorchidism during infancy, for which two males underwent orchiopexy. In the other two males, the testes descended spontaneously during early childhood.

In SRS males, mean (SD) age at first blood sampling was 5.7 (2.0) years. Age at onset of puberty was 11.5 (1.0) years in SRS versus 11.6 (0.8) years in non-SRS males (p=0.51). In 28.6% of the SRS males, puberty was postponed by means of two years of GnRHa treatment, versus in 12.5% of the non-SRS males (p=0.13). In SRS females, mean (SD) age at first blood sampling was 6.8 (2.8) years. Age at onset of puberty was 10.5 years in SRS and 10.7 years in non-SRS females (p=0.50). In 52.9% of the SRS females, puberty was postponed by means of two years of GnRHa treatment, versus in 36.2% of the non-SRS females (p=0.22).

**Table 1: Clinical characteristics.**

| | **SRS (n=31)** | **Non-SRS (n=123)** | ***p*-value** |
|---|---|---|---|
| Males (n) | 14 | 65 | |
| Age at first blood sampling (yrs) | 5.7 (2.0) | | |
| Molecular diagnosis (n, %) | | | |
| 11p15 LOM | 8 (57.1) | | |
| mUPD7 | 4 (28.6) | | |
| Clinical | 2 (14.3) | | |
| Age at onset of puberty | 11.5 (0.8) | 11.6 (0.8) | 0.51 |
| GnRHa (n, %) | 4 (28.6) | 8 (12.5) | 0.13 |
| Females (n) | 17 | 58 | |
| Age at first blood sampling (yrs) | 6.8 (2.8) | | |
| Molecular diagnosis (n, %) | | | |
| 11p15 LOM | 7 (41.2) | | |
| mUPD7 | 3 (17.6) | | |
| Clinical | 7 (41.2) | | |
| Age at onset of puberty | 10.5 (1.2) | 10.7 (1.1) | 0.50 |
| GnRHa (n, %) | 9 (52.9) | 21 (36.2) | 0.22 |

Data expressed as mean (SD) or number (%) Abbreviations: 11p15 LOM, loss of methylation in 11p15 region; mUPD7, maternal uniparental disomy of chromosome 7.

**Longitudinal serum levels of reproductive hormones**

Longitudinal serum levels of reproductive hormones in SRS males and females are depicted in Figure 1 and 2, respectively. Postpubertal mean levels were similar in SRS and non-SRS, except for LH levels of females, which were lower in SRS (Table 2). Four of the 14 SRS males (28.6%), all with 11p15 LOM, had an inhibin B level below the 5$^{th}$ percentile (patients 1-4 in Figure 2), indicating Sertoli cell dysfunction. This was a significantly higher proportion than in the non-SRS males (7.1%, p=0.02). Two of the SRS males with a low inhibin B had also an FSH level above the 95$^{th}$ percentile (patient 1 and 3). One of them, with an 11p15 LOM, had a history of cryptorchidism, hypospadias and orchiopexy, and had AMH levels below the 5$^{th}$ percentile, already from a prepubertal age (patient 3). There was also one SRS patient with a low inhibin B level who had a testosterone level below the 5$^{th}$ percentile (patient 4), but FSH and LH levels were within the normal range. All SRS females had AMH, LH and FSH levels within the reference range, with the exception of one SRS female, who had a postpubertal AMH level below the 2.5$^{th}$ percentile (patient 1, Figure 2).

**Table 2: Postpubertal gonadal function in SRS and non-SRS.**

| | **SRS (n=31)** | **Non-SRS (n=123)** | ***p*-value** | **Normal postpubertal range** |
|---|---|---|---|---|
| ***Males*** | | | | |
| Age at blood sampling | 16.5 (2.5) | 16.8 (1.3) | 0.74 | |
| Inhibin B (ng/L) | 193.5 (140) | 218.0 (101.5) | 0.25 | 95-323 |
| FSH (U/L) | 4.1 (3.2) | 4.9 (3.9) | 0.25 | 1.4-7.5 |
| LH (U/L) | 3.6 (1.3) | 4.1 (2.1) | 0.18 | 1.5-6.3 |
| Testosterone (nmol/L) | 14.4 (14.7) | 16.1 (4.4) | 0.35 | 11.3-32.3 |
| AMH (ug/L) | 8.5 (6.5) | 9.0 (6.3) | 0.47 | 3.2-17.9 |
| ***Females*** | | | | |
| Age at blood sampling | 15.1 (1.3) | 15.4 (1.4) | 0.36 | |
| AMH (ug/L) | 3.4 (3.5) | 3.2 (3.5) | 0.64 | 0.7-8.4 |
| FSH (U/L) | 4.4 (4.1) | 5.0 (3.0) | 0.15 | 0.2-9.2 |
| LH (U/L) | 2.6 (2.2) | 3.9 (3.5) | **<0.001** | 0.0-20 |

Data expressed as median (interquartile range). P<0.05 in bold.

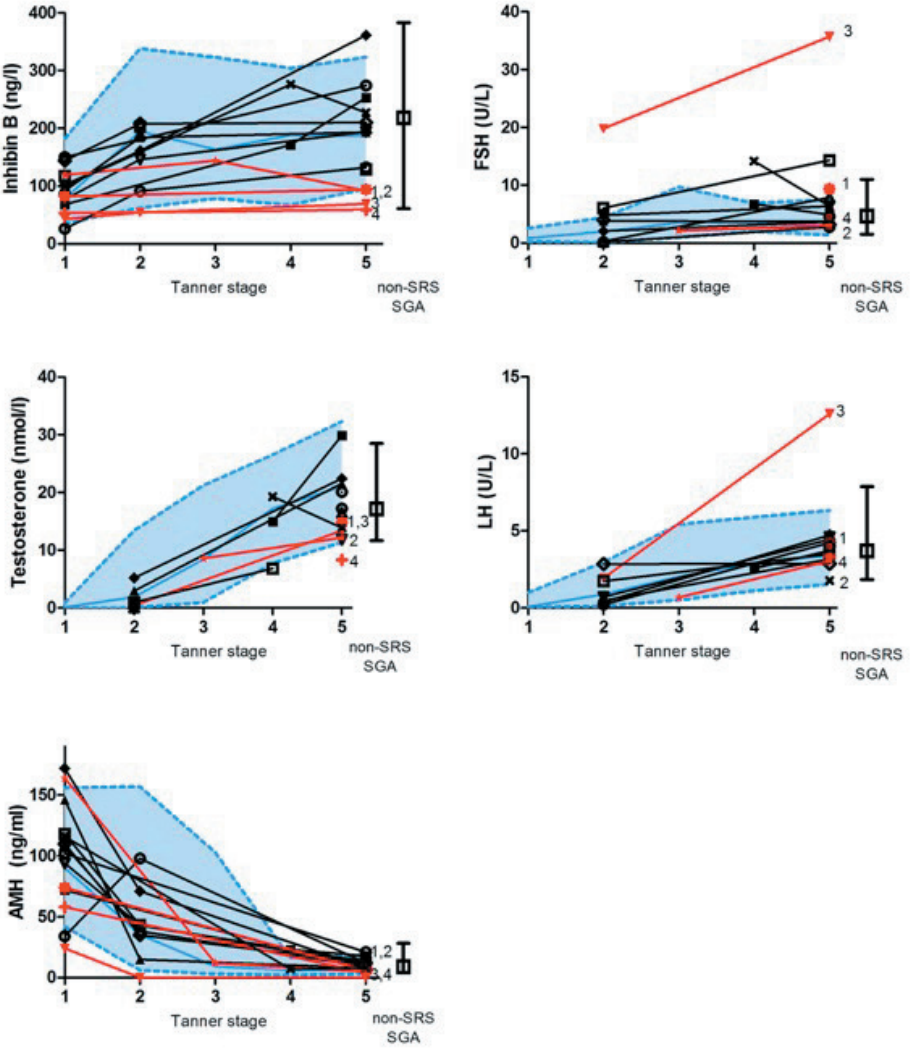


**Figure 1: Serum levels of inhibin B, FSH, LH, testosterone and AMH in SRS and non-SRS males.** Each black line represents the longitudinal measurements of each SRS male, based on Tanner stage (G1-5) at the moment of the measurement. The shaded area with the blue line indicates the median and 5-95$^{th}$ percentile per Tanner stage of the healthy population. The red lines indicate the patients with suspected Sertoli cell dysfunction. The vertical bar represents the median and 5-95$^{th}$ percentile of the postpubertal non-SRS subjects born SGA.

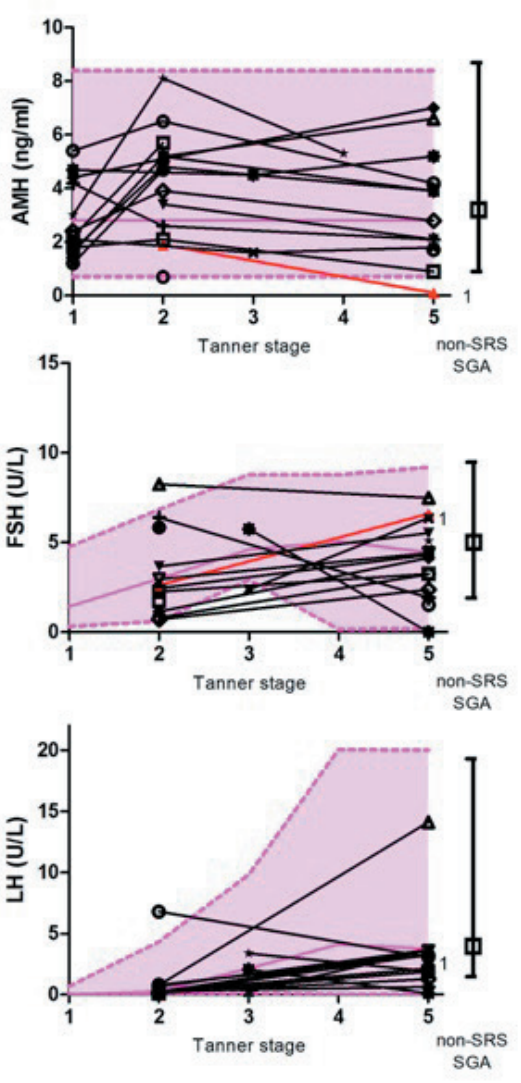


**Figure 2: Serum levels of AMH, FSH and LH in SRS and non-SRS females.** Each black line represents the longitudinal measurements of each SRS female, based on Tanner stage (M1-5) at the moment of the measurement. The shaded area with the pink line indicates the median and 2.5-97.5$^{th}$ percentile per Tanner stage of the healthy population. The red line indicates the patient with AMH levels <2.5$^{th}$ percentile. The vertical bar represents the median and 5-95$^{th}$ percentile of the postpubertal non-SRS subjects born SGA.

**Puberty in SRS versus non-SRS, treated with GH only**

Figure 3 depicts onset and progression of puberty in SRS and non-SRS males and females. In males treated with GH only, mean (SD) age at onset of puberty was 11.8 (0.8) years in both SRS and non-SRS. None of the males had precocious puberty. Progression of puberty from TV 4 ml to TV 15 ml lasted 2.5 (0.7) years in SRS, versus 2.1 (0.8) years in non-SRS (p=0.66). The period from TV 15 to AH attainment lasted 2.2 (1.1) years in SRS, versus 2.9 (0.9) years in non-SRS (p=0.07). Total duration of puberty until AH attainment was 4.6 (1.0) years in SRS versus 4.9 (0.8) years in non-SRS (p=0.35).

In females treated with GH only, mean age at onset of puberty was 11.2 (1.0) years in both SRS and non-SRS. Mean age at menarche was 13.1 (1.1) years in SRS, versus 13.3 (1.1) years in non-SRS (p=0.62). There was one SRS female with primary amenorrhea due to MRKH. The period from menarche to AH lasted 1.8 (1.1) years in SRS versus 2.0 (0.8) in non-SRS (p=0.61). Total duration of puberty until AH attainment was 3.9 (0.9) years in SRS versus 4.1 (0.8) years in non-SRS (p=0.49).

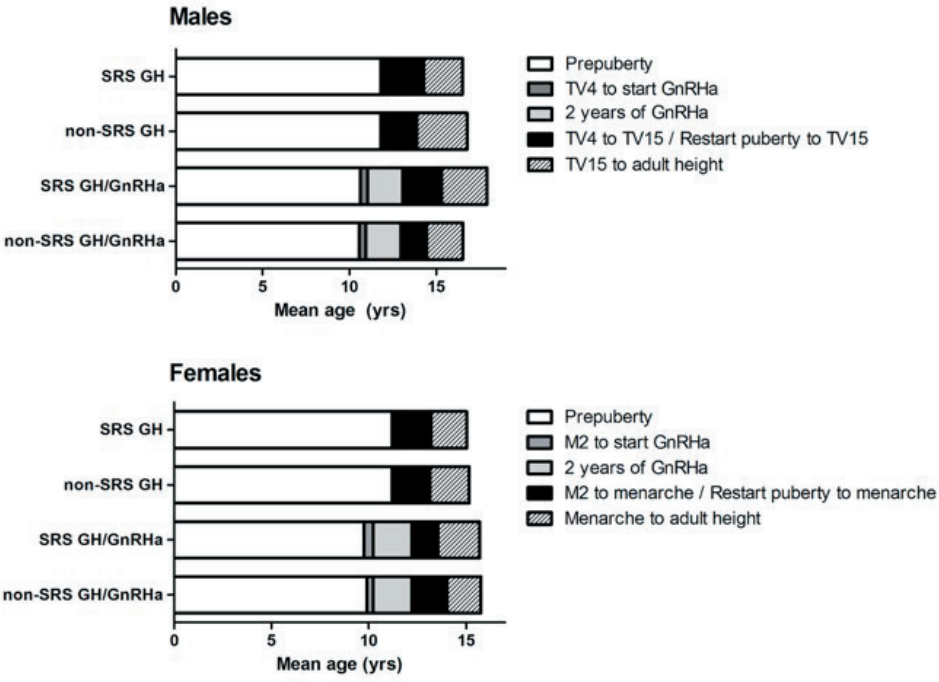


**Figure 3: Duration of puberty in SRS and non-SRS.** Abbreviations: GH, growth hormone; GnRHa, gonadotropin-releasing hormone analogue; M2, breast development stage II according to Tanner; TV, testicular volume in mL.

**Puberty in SRS versus non-SRS, treated with GH and two years of GnRHa**

In males who additionally received two years of GnRHa, mean age at onset of puberty was 10.6 (0.6) years in both SRS and non-SRS (Figure 3). The period from restart of puberty after two years of GnRHa to TV 15 ml lasted 2.3 (0.9) years in SRS versus 1.5 (0.4) years in non-SRS (p=0.19). The period from TV 15 to AH attainment lasted 2.6 (1.0) years in SRS versus 2.1 (0.2) years in non-SRS (p=0.53). Total duration of puberty until AH attainment was 5.2 (0.8) years in SRS versus 3.2 (0.6) years in non-SRS (p<0.01).

In females who were additionally treated with two years of GnRHa, mean (SD) age at onset of puberty was 9.8 (0.9) years in SRS, versus 9.9 (1.0) years in non-SRS (p=0.85). There were two of 17 SRS females with Tanner stage M2 before the age of eight years. Underlying pathology was not found. The period from cessation of GnRHa to menarche lasted 1.3 (0.6)

years in SRS, versus 1.5 (0.4) years in non-SRS (p=0.29). Mean age at menarche was 13.2 (1.1) years in SRS, versus 13.7 (1.1) years in non-SRS (p=0.33). The period from menarche to AH attainment lasted 2.1 (0.9) years in SRS, versus 1.8 (0.6) years in non-SRS (p=0.29). Total duration of puberty until AH attainment was 3.3 (1.0) years in SRS versus 3.3 (0.8) years in non-SRS (p=0.97).



**Table 3: Pubertal growth in SRS and non-SRS.**

| | Height at onset puberty (cm) | AH (cm) | Pubertal height gain (cm)* | *p*-value |
|---|---|---|---|---|
| ***Males*** | | | | |
| SRS GH | 146.6 (5.1) | 169.0 (7.3) | 22.4 (5.3) | 0.12 |
| non-SRS GH | 147.8 (5.1) | 172.9 (5.5) | 25.2 (5.0) | |
| SRS GH/GnRHa | 135.8 (1.2) | 169.5 (3.1) | 33.0 (2.0) | 0.48 |
| non-SRS GH/GnRHa | 139.7 (3.5) | 171.6 (6.1) | 31.4 (3.4) | |
| ***Females*** | | | | |
| SRS GH | 140.4 (7.8) | 156.8 (4.6) | 16.4 (5.6) | 0.89 |
| non-SRS GH | 143.2 (5.1) | 159.6 (5.2) | 16.6 (4.5) | |
| SRS GH/GnRHa | 129.0 (4.7) | 156.9 (1.8) | 27.8 (5.3) | 0.44 |
| non-SRS GH/GnRHa | 132.4 (4.7) | 158.2 (3.5) | 26.1 (4.3) | |

Data expressed as mean (SD), p-values represent pubertal height gain in SRS vs. non-SRS. * Pubertal height gain in GH/GnRHa subjects is including two years of GnRHa treatment. Abbreviations: GH, growth hormone; GnRHa, gonadotropin-releasing hormone analogue.

### Pubertal growth in SRS versus non-SRS, with and without additional GnRHa

In males and females who were treated with GH only, pubertal height gain was similar in SRS and non-SRS (p=0.12 and p=0.89, respectively (Table 3)). In males and females who additionally received two years of GnRHa, total pubertal height gain was similar in SRS and non-SRS (p=0.48 and p=0.44 resp.).

In SRS males who were treated with GH only, pubertal height gain was 22.4 cm versus 33.0 cm in SRS males who additionally received two years of GnRHa (p=0.008). In SRS females who were treated with GH only, pubertal height gain was 16.4 cm, versus 27.8 cm in SRS females who additionally received two years of GnRHa (p=0.004).

## Discussion

This study shows longitudinal data on pubertal progression and gonadal function in SRS patients, compared to a large group of non-SRS patients born SGA and to healthy controls. We found that onset and progression of puberty are similar in SRS and non-SRS subjects born SGA. Sertoli cell dysfunction is more common in males with SRS than non-SRS. Gonadal function does not seem to be impaired in females with SRS.

We longitudinally assessed gonadal function from childhood to early adulthood in SRS patients and compared these data to those of non-SRS subjects born SGA and to those of healthy controls. To our knowledge, this is the first study assessing gonadal function in SRS patients. More than a quarter of the SRS males had a postpubertal inhibin B level below the 5$^{th}$ percentile for healthy references, and two males also had an FSH level above the 95$^{th}$ percentile. Our results imply that Sertoli cell dysfunction is more common in SRS males. One of the SRS males in our cohort had both Sertoli- and Leydig cell dysfunction. He was born with hypospadias and bilateral cryptorchidism, for which he underwent orchiopexy. Both cryptorchidism and orchiopexy are associated with decreased gonadal function [28,29]. In our cohort, four of the 14 SRS males had cryptorchidism, for which two underwent orchiopexy. All of these patients had 11p15 LOM, which suggests increased risk of Sertoli cell dysfunction in this genetic subtype. Although the exact incidence of genital anomalies in SRS males is unknown, our results are in concordance with previous studies, reporting an incidence of cryptorchidism in 19-59% of the SRS males [9-11]. Interestingly, there were SRS males with a normal gonadal function who had a history of cryptorchidism, and on the other hand, three males with Sertoli cell dysfunction without a history of cryptorchidism. Thus, more research is warranted to investigate the etiology of Sertoli cell dysfunction in SRS males, especially those with 11p15 *ICR1* LOM. Moreover, our data show that Sertoli cell dysfunction should also be evaluated in absence of genital anomalies. Larger cohorts with a long follow-up period are needed to confirm our findings.

To study gonadal function in females, we only used blood samples that were drawn before the start of oral contraceptives. In SRS females, we found that LH levels were lower in SRS than in non-SRS. This could be due to the fact that not all blood samples were drawn at the same moment during the menstrual cycle. Unfortunately, data on menstrual cycle length and possible irregularities were lacking. However, all females with SRS had LH, FSH and AMH levels within the reference range. Thus, the follicle pool and gonadal function do not seem to be impaired in SRS females, although there was one female with clinical SRS with primary amenorrhea due to Müllerian agenesis. Previous casereports have shown an association between SRS and MRKH [11-13,30]. In three of these casereports, the patients had SRS based on 11p15 LOM. In one study, describing two SRS patients with MRKH, an association was found between MRKH and the severity of 11p15 LOM [11]. However, our patient with MRKH had clinical SRS, similar as the patient in the case-report of Abraham et al [12]. More research is thus warranted to establish the etiology of the association between SRS and MRKH, and to investigate whether it is more common in certain SRS subtypes. Since the ovaries are unaffected in MRKH, pubertal development and gonadal function are normal in these females, and the diagnosis is usually not made before the age of 16 years, after work-up for primary amenorrhea. Awareness of the association between SRS and MRKH can facilitate timely diagnosis. One female in our study had a postpubertal AMH level below the 2.5$^{th}$ percentile indicating a low follicle reserve. All other SRS females had an AMH level within

the normal range. Apart from the association with MRKH and this low AMH level in our patient, our results are reassuring regarding gonadal function in females with SRS. However, to draw definite conclusions, larger cohorts are needed.



The age at onset of puberty was similar in SRS compared to non-SRS subjects born SGA. The proportion of patients treated with GnRHa to postpone puberty was larger in SRS than in non-SRS, due to the fact that SRS patients were shorter at onset of puberty. We found that SRS patients have the same benefit from additional two years of GnRHa treatment as non-SRS patients, with improved height gain from onset of puberty until AH [31]. We, therefore, suggest to consider additional treatment with two years of GnRHa when SRS children have an expected AH below -2.5 SDS at onset of puberty.

Overall, puberty progressed similarly in SRS and non-SRS. There were two SRS females with Tanner stage M2 before the age of eight years. Underlying pathology was not found, and after two years of GnRHa treatment, their puberty progressed normally. All SRS patients attained an adult Tanner stage. Duration from cessation of GnRHa treatment to AH was significantly longer in SRS males compared to non-SRS males. However, this was based on a small number of SRS patients, and the analysis should be conducted in larger groups to confirm this result. To our knowledge, progression of puberty had never been investigated in SRS patients. Our study shows that SRS patients have a normal pubertal progression. However, we emphasize that more research is warranted.

In conclusion, we show that SRS patients have a similar age at onset of puberty and pubertal progression as non-SRS subjects born SGA. Although gonadal function is on average similar in SRS and non-SRS subjects born SGA and within the normal range, disturbances in Sertoli cell function are more common in SRS males. Gonadal function does not seem to be impaired in SRS females with normal puberty. There is an association with Müllerian agenesis, but larger cohorts are needed to assess the incidence of Müllerian agenesis in SRS. Based on our results, we advise clinicians to assess gonadal function in SRS patients, also when Tanner stage 5 is achieved, particularly in males.

## Acknowledgements

We express our gratitude to all participants and their parents. We acknowledge all research nurses for their contribution to this study, especially J.C. Bruinings-Vroombout. We thank R. van der Wal for analyzing the samples.

## References


1. Russell A. A syndrome of intra-uterine dwarfism recognizable at birth with cranio-facial dysostosis, disproportionately short arms, and other anomalies (5 examples). *Proc R Soc Med.* 1954;47(12):1040-1044.
2. Silver HK, Kiyasu W, George J, Deamer WC. Syndrome of congenital hemihypertrophy, shortness of stature, and elevated urinary gonadotropins. *Pediatrics.* 1953;12(4):368-376.
3. Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB. Growth and Symptoms in Silver-Russell-Syndrome - Review on the Basis of 386 Patients. *European Journal of Pediatrics.* 1995;154(12):958-968.
4. Wakeling EL, Brioude F, Lokulo-Sodipe O, et al. Diagnosis and management of Silver-Russell syndrome: first international consensus statement. *Nat Rev Endocrinol.* 2016.
5. Gicquel C, Rossignol S, Cabrol S, et al. Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome. *Nat Genet.* 2005;37(9):1003-1007.
6. Turner CL, Mackay DM, Callaway JL, et al. Methylation analysis of 79 patients with growth restriction reveals novel patterns of methylation change at imprinted loci. *Eur J Hum Genet.* 2010;18(6):648-655.
7. Smeets CC, Zandwijken GR, Renes JS, Hokken-Koelega AC. Long-Term Results of GH Treatment in Silver-Russell Syndrome (SRS): Do They Benefit the Same as Non-SRS Short-SGA? *J Clin Endocrinol Metab.* 2016;101(5):2105-2112.
8. Binder G, Liebl M, Woelfle J, Eggermann T, Blumenstock G, Schweizer R. Adult height and epigenotype in children with Silver-Russell syndrome treated with GH. *Horm Res Paediatr.* 2013;80(3):193-200.
9. Wakeling EL, Abu Amero S, Alders M, et al. Epigenotype-phenotype correlations in Silver-Russell syndrome. *J Med Genet.* 2010;47(11):760-768.
10. Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet.* 1999;36(11):837-842.
11. Bruce S, Hannula-Jouppi K, Peltonen J, Kere J, Lipsanen-Nyman M. Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab.* 2009;94(2):579-587.
12. Abraham MB, Carpenter K, Baynam GS, Mackay DJ, Price G, Choong CS. Report and review of described associations of Mayer-Rokitansky-Kuster-Hauser syndrome and Silver-Russell syndrome. *J Paediatr Child Health.* 2014(51):555-560.
13. Bellver-Pradas J, Cervera-Sanchez J, Boldo-Roda A, et al. Silver-Russell syndrome associated to Mayer-Rokitansky-Kuster-Hauser syndrome, diabetes and hirsutism. *Arch Gynecol Obstet.* 2001;265(3):155-157.

14. Smeets CC, Renes JS, van der Steen M, Hokken-Koelega AC. Metabolic Health and Long-Term Safety of Growth Hormone Treatment in Silver-Russell Syndrome. *J Clin Endocrinol Metab.* 2017;102(3):983-991.
15. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod.* 2003;18(2):323-327.
16. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC. Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril.* 2002;77(2):357-362.
17. Aksglaede L, Sorensen K, Boas M, et al. Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab.* 2010;95(12):5357-5364.
18. Andersson AM, Juul A, Petersen JH, Muller J, Groome NP, Skakkebaek NE. Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab.* 1997;82(12):3976-3981.
19. van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Effect of discontinuation of growth hormone treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. *J Clin Endocrinol Metab.* 2003;88(1):347-353.
20. Willemsen RH, Arends NJT, Waarde WMBV, et al. Long-term effects of growth hormone (GH) treatment on body composition and bone mineral density in short children born small-for-gestational-age: six-year follow-up of a randomized controlled GH trial. *Clinical Endocrinology.* 2007;67(4):485-492.
21. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74(6):901-910.
22. Fredriks AM, Van Buuren S, Burgmeijer RJF, et al. Continuing positive secular growth change in the Netherlands 1955-1997. *Pediatric Research.* 2000;47(3):316-323.
23. Azzi S, Salem J, Thibaud N, et al. A prospective study validating a clinical scoring system and demonstrating phenotypical-genotypical correlations in Silver-Russell syndrome. *J Med Genet.* 2015;52(7):446-453.
24. Hagen CP, Aksglaede L, Sorensen K, et al. Serum levels of anti-Mullerian hormone as a marker of ovarian function in 926 healthy females from birth to adulthood and in 172 Turner syndrome patients. *J Clin Endocrinol Metab.* 2010;95(11):5003-5010.
25. Sehested A, Juul AA, Andersson AM, et al. Serum inhibin A and inhibin B in healthy prepubertal, pubertal, and adolescent girls and adult women: relation to age, stage of puberty, menstrual cycle, follicle-stimulating hormone, luteinizing hormone, and estradiol levels. *J Clin Endocrinol Metab.* 2000;85(4):1634-1640.
26. Tanner JM, Whitehouse RH. Clinical Longitudinal Standards for Height, Weight, Height Velocity, Weight Velocity, and Stages of Puberty. *Archives of Disease in Childhood.* 1976;51(3):170-179.

27. Mul D, Fredriks AM, van Buuren S, Oostdijk W, Verloove-Vanhorick SP, Wit JM. Pubertal development in The Netherlands 1965-1997. *Pediatr Res.* 2001;50(4):479-486.
28. Lee PA, Coughlin MT. Fertility after bilateral cryptorchidism. Evaluation by paternity, hormone, and semen data. *Horm Res.* 2001;55(1):28-32.
29. Cortes D, Thorup J, Lindenberg S, Visfeldt J. Infertility despite surgery for cryptorchidism in childhood can be classified by patients with normal or elevated follicle-stimulating hormone and identified at orchidopexy. *BJU Int.* 2003;91(7):670-674.
30. Bliek J, Terhal P, van den Bogaard MJ, et al. Hypomethylation of the H19 gene causes not only Silver-Russell syndrome (SRS) but also isolated asymmetry or an SRS-like phenotype. *Am J Hum Genet.* 2006;78(4):604-614.
31. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Puberty and Pubertal Growth in GH-treated SGA Children: Effects of 2 Years of GnRHa Versus No GnRHa. *J Clin Endocrinol Metab.* 2016;101(5):2005-2012.

# Chapter 3

## Glomerular filtration rate, blood pressure and microalbuminuria in adults born SGA: a 5-year longitudinal study after cessation of GH treatment

W.J. Goedegebuure
G.F. Kerkhof
A.C.S. Hokken-Koelega

*Clinical Endocrinology 2019, Dec;91(6):892-898.*

**Abstract**

**Background:** GH treatment increases glomerular filtration rate (GFR), as serum IGF-I stimulates the renin-angiotensin system. Infants born with a low birth weight have a smaller number of nephrons, which causes a lower GFR, a higher blood pressure and a higher albumin-to-creatinine ratio in early adulthood.

**Methods:** 261 young adults born SGA, previously treated with growth hormone (SGA-GH) were longitudinally followed. GFR, based on serum creatinine levels, was determined at cessation of GH treatment and at 6 months, 2 and 5 years thereafter. GFR, blood pressure and urinary albumin-to-creatinine ratio at 5 years after cessation of GH were compared with untreated age-matched controls (56 untreated short subjects born SGA (SGA-S), 118 subjects born SGA with spontaneous catch-up growth (SGA-CU), 135 subjects born appropriate for gestational age (AGA)).

**Results:** GFR decreased significantly only during the first 6 months after cessation of GH treatment, while remaining well within the normal range (124.6 vs. 120.2 mL/min/1.73m$^2$, $p<0.001$). SGA-GH adults had a similar GFR, blood pressure and urinary albumin-to-creatinine ratio as the healthy controls born SGA and AGA.

**Conclusion:** In conclusion, our 5 years longitudinal follow-up study shows a decrease in GFR during 6 months after GH-cessation, but thereafter GFR remained stable and within the normal range. GFR, blood pressure and urinary albumin-to-creatinine ratio at 21 years of age was similar in GH-treated young adults born SGA and untreated controls born SGA or AGA. We conclude that long-term GH treatment in children born SGA has no unfavourable effects on kidney function in early adulthood.

## Introduction

Growth hormone (GH) treatment effectively induces catch-up growth and increases adult height (AH) in children born small for gestational age (SGA) [1-3]. GH treatment increases glomerular filtration rate (GFR), as higher levels of serum insulin-like growth factor 1 (IGF-I) affect renal hemodynamics by stimulating the renin-angiotensin system [4-6]. Also, in GH deficient children, GH treatment has been shown to increase kidney length and total kidney volume [7-10]. It is essential to ascertain longitudinal data after cessation of GH treatment to investigate the possible effects of higher serum IGF-I levels during childhood on GFR in adulthood.



A lower GFR and an increased urinary albumin-to-creatinine ratio are independent predictors of cardiovascular and all-cause mortality risk in the general population and patients with diabetes [11-14]. Infants born with a low birth weight have a smaller number of nephrons, which causes a lower GFR, a higher albumin-to-creatinine ratio and a higher blood pressure in early adulthood [15-20]. A study in young adults, with low birth weight after a gestation of less than 32 weeks, showed a lower GFR and more frequent microalbuminuria compared with those being born appropriate for gestational age (AGA) [21]. A low birth weight can be explained by both prematurity and SGA birth, but the independent effects of gestational age and birth weight on renal function are unknown.

The primary aim of our study was to investigate the longitudinal changes in GFR after cessation of GH treatment. We, therefore, evaluated serum creatinine levels during 5 years after cessation of GH treatment in young adults born SGA (SGA-GH). We hypothesized that after cessation of GH treatment, GFR would decrease in line with the decrease in IGF-I levels, but would remain within the normal range. Our secondary aim was to investigate GFR, blood pressure and urinary albumin-to-creatinine ratio in GH-treated adults born SGA at 21 years of age in comparison with data of age-matched young adults born SGA with persistent short stature (SGA-S), young adults born SGA with spontaneous catch-up growth (SGA-CU) and with healthy controls born appropriate for gestational age (AGA). We hypothesized that GFR, blood pressure and urinary albumin-to-creatinine ratio of SGA-GH young adults would be similar to levels of the untreated SGA-S, SGA-CU and AGA young adults.

# Methods

**Subjects**

The total study group comprised 570 young adults (315 females, 55.3%), of which 261 young adults born SGA (birth weight or birth length < –2 standard deviation scores (SDS) for gestational age) had participated in a Dutch SGA trial (139 females, 53.2%). Participants were recruited between 2002 and 2010. These young adults had no other known cause of short stature than SGA birth, and started GH treatment when prepubertal, with a height at start below -2.5 SDS. Before start of the study, GH status was evaluated using GH stimulation tests. GH deficiency (GHD) was defined as a maximum serum GH level <20 mU/L (<7.7 ng/ml) during two stimulation tests. Patients with GHD were excluded. Treatment with GH 1 mg/$m^2$/day (≈0.033 mg/kg/day) was given daily subcutaneously at bedtime (r-hGH Norditropin; Novo Nordisk A/S, Bagsværd, Denmark). Every three months, the GH dose was adjusted to the calculated body surface area. GH treatment was discontinued at attainment of AH. At GH-cessation, the young adults were invited to participate in the current follow-up study evaluating GFR at AH while still on GH, and at 6 months, 2 and 5 years after GH-cessation.

Data at 5 years after GH-cessation were compared with those of 309 participants of a healthy young adult cohort (PROGRAM study), aged 18 to 24 years [22,23]. The control group comprised 56 untreated young adults born SGA (birth weight and/or birth length <-2 SDS) with persistent short stature (<-2 SDS) (SGA-S), because they had persistent short stature at the same age as the SGA-GH group, when GH treatment was started, 118 young adults born SGA (birth weight and/or birth length <-2 SDS) with a normal stature (>-1 SDS) (SGA-CU), to investigate difference in effects of GH-induced and spontaneous catch-up growth, and 135 young adults born AGA (birth length >-1 SDS) with a normal stature (>-1 SDS) (AGA) as representatives of the general healthy population. SGA-S and SGA-CU were randomly selected from hospitals in the Netherlands, where they had been registered because of small birth size (birth length ≤ -2SDS) with or without short stature (< -2SDS). Healthy young adults from schools of different educational levels were randomly asked to participate as controls born appropriate for gestational age kan weg, vervangen door AGA [23].

Urine samples were collected in 56 SGA-GH young adults and were compared to 181 healthy young adults (37 SGA-S, 62 SGA-CU and 82 AGA), to investigate urinary albumin and creatinine excretion. The SGA-GH subjects had ceased GH treatment due to AH attainment and visited the hospital for their 2- or 5-years follow-up visit.

The Medical Ethics Committee of the Erasmus University Medical Center approved the studies, and we obtained written informed consent from all participants.

**Measurements**

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd, Crymmyth, UK). Birth weight, birth length and height were expressed as SDS adjusted for age and sex, using Growth Analyser Research Calculation Tools (*https://growthanalyser.org*) [24,25]. AH SDS at 5 years after cessation of GH treatment was calculated using references for Dutch adults (21 years). We defined catch-up growth in SDS during childhood as the gain in height SDS from birth to adult height attainment.



**Assays**

Blood and urine samples for measurement of albumin and creatinine were frozen and subsequently stored at -80°C. All measurements were determined in the Same trial laboratory in Erasmus Medical Center. Serum and urine creatinine was measured with a 2-point end assay (Cobas c701 module; Roche), the urine albumin levels were determined with a immunoturbimetric assay (Cobas c501 module; Roche). Before 2013, insulin-like growth factor-I (IGF-I) was measured using an immunometric technique on Immulite 2000 (Siemens Health-care Solutions Diagnostics) with an interassay variation <6.5%. After 2013, IGF-I was measured using the IDS-iSYS (Immunodiagnostic Systems) with an interassay variation <7.5%, with an intra-assay variation <2.1%. Serum levels of total IGF-I were expressed as SDS adjusting for age and sex, using reference values for healthy children with normal stature determined with these assays in the same laboratory.

**Blood pressure**

Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured repeatedly during one hour in the supine position after 10 minutes of rest using the non-dominant arm with an automated device (Accutorr Plus, Datascope Corp., Montvale NJ, USA). The mean of 13 measurements was taken for analysis, to reflect resting blood pressure. Blood pressure was expressed in SDS using sex- and age-matched reference values [26].

**Statistics**

Serum creatinine, patient's age and sex were used to calculate the estimated GFR, according to the CKD-EPI formula [27]. We followed NICE guidelines in the assessment of risk for developing chronic kidney disease [28], using GFR and the calculated albumin-to-creatinine ratio in urine. A moderate risk for developing chronic kidney disease was defined as a GFR value below 60 mL/min/1.73m$^2$ or an albumin-to-creatinine ratio above 3mg/mmol [28].

SPSS version 24.0 was used to perform all analyses. Clinical characteristics are presented as mean (SD). Shapiro Wilk tests and Normal Q-Q-plots were used to determine the distribution of variables. To assess longitudinal changes in GFR, we used repeated measurements analysis with an unstructured covariance type, which takes into account any missing data. As age in years correlated to all GFR scores, we used this as a covariate in all analyses.

Participants were only included in the longitudinal analysis if they had completed at least 3 out of 4 study moments (GH-cessation, 6 months, 2 years and 5 years after). ANCOVA was used for adjusted comparisons between groups at age 21 years. GFR values are corrected for age and sex, blood pressure values are corrected for height and sex. To test differences in the continuous variables between previously GH-treated and control subjects, we used the Mann-Whitney-U test or Kruskal-Wallis test. Correlations between variables were determined by Spearman correlation. P-values <0.05 were considered statistically significant.

**Table 1: Clinical characteristics.**

| | SGA-GH | SGA-S | SGA-CU | AGA |
|---|---|---|---|---|
| Total group (n) | 261 | 56 | 118 | 135 |
| Sex (male/female) | 122/139 | 23/33 | 47/71 | 63/72 |
| Age (years) | 20.9 (1.9) | 20.7 (1.7) | 20.9 (1.7) | 20.9 (1.7) |
| Gestational age (weeks) | 36.6 (3.7) | 38.4 (3.0) ‡ | 36.5 (3.3) | 36.4 (4.1) |
| Birth length SDS | -3.0 (1.4) | -2.9 (0.9) | -2.6 (1.1) | 0.2 (0.9) † |
| Birth weight SDS | -2.2 (1.1) | -2.1 (0.9) | -2.3 (0.8) | 0.5 (1.1) † |
| GH duration (years) | 8.6 (4.3) | N/A | N/A | N/A |
| AH SDS (21 years) | -1.6 (0.8) | -2.5 (0.5) ‡ | -0.2 (0.7) † | 0.2 (0.8) † |
| Urine samples (n) | 56 | 37 | 62 | 82 |
| Sex (male/female) | 31 / 25 | 15 / 22 | 28 / 34 | 39 / 43 |
| Age (years) | 20.7 (2.1) | 20.9 (1.8) | 21.2 (1.7) | 21.0 (1.6) |
| Gestational age (weeks) | 37.9 (3.4) | 38.1 (3.3) | 36.3 (3.2) | 36.8 (3.2) |
| Birth length SDS | -2.7 (1.5) | -3.0 (1.0) | -2.8 (0.9) | 0.2 (0.8) † |
| Birth weight SDS | -1.8 (1.2) | -2.0 (0.9) | -2.3 (0.9) | 0.4 (1.1) † |
| GH duration (years) | 9.1 (3.0) | N/A | N/A | N/A |
| AH SDS (21 years) | -1.5 (1.1) | -2.4 (0.7) ‡ | -0.3 (1.2) † | 0.3 (1.2) † |

Data are expressed as means (SD). Comparison between SGA-GH and young adults without history of growth hormone treatment (SGA-S, SGA-CU and AGA): †p<0.001 compared with SGA-GH; ‡p<0.01 compared with SGA-GH
Abbreviations: SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with persistent short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age; N/A, not applicable SDS, standard deviation scores.

# Results

**Clinical characteristics**

Table 1 shows the clinical characteristics of the SGA-GH adults at 5 years after cessation of GH treatment, and the participants of the age-matched healthy adult cohort (SGA-S, SGA-CU and AGA). At 5 years after cessation of GH treatment, mean (SD) age was 20.9 (1.9) years which was similar in the healthy young adult cohort. Mean duration of GH treatment was 8.6 (4.3) years in the SGA-GH group. AH SDS was -1.6 (0.8) SDS in SGA-GH young adults, which was significantly higher than in SGA-S young adults ($p<0.001$), but significantly lower than in SGA-CU ($p<0.001$) and AGA young adults ($p<0.001$). Urine samples were collected after a mean of 3.9 (1.6) years after GH-cessation, at a mean age of 20.7 (2.1) years. The baseline characteristics of the group with urine samples were similar as the total group, only GH duration was significantly longer in the patients who provided urine samples ($p=0.023$).

**Glomerular Filtration Rate (GFR)**

*Longitudinal changes in GFR after cessation of GH treatment in SGA-GH adults*

Figure 1 shows the longitudinal changes in GFR after GH-cessation in the SGA-GH adults, expressed as estimated marginal means. There was an inverse correlation between age and GFR ($r = -0.380$, $p<0.001$). GFR, decreased during the first 6 months after cessation of GH treatment, while remaining well within the normal range (124.6 vs. 120.2 mL/ min/1.73m$^2$, $p<0.001$). From 6 months to 2 years, GFR decreased from 120.2 to 117.7 mL/min/1.73m$^2$ ($p=0.027$) and GFR did not significantly change in the 3 years thereafter. GFR did not correlate to serum IGF-I levels.

*GFR of SGA-GH compared with untreated SGA-S, SGA-CU and AGA adults*

Table 2 and Figure 2 show GFR values in all groups. SGA-GH adults had a similar GFR as the SGA-S, SGA-CU and AGA young adults. No significant correlation was found between GFR and gestational age, birth weight or degree of postnatal catch-up growth. The percentage of participants with a GFR below 90 mL/min/1.73 m² was similar in all groups (SGA-GH: 4%, SGA-S: 2%, SGA-CU: 4%, AGA: 4%). None of the participants had a GFR below 60 mL/ min/1.73m$^2$.

**Blood pressure**

Results on systolic and diastolic blood pressure are shown in Table 2. Systolic and diastolic blood pressure were similar in SGA-GH compared with SGA-S, SGA-CU and AGA. None of the participants had a blood pressure outside the normal range of -2 SDS and +2 SDS. GFR and urinary albumin excretion did not correlate with systolic and diastolic blood pressure.

### Microalbuminuria

*Urinary albumin excretion in SGA-GH compared with untreated SGA-S, SGA-CU and AGA adults*

Urine albumin and urine creatinine were similar in all groups (Table 2 and Figure 2). The calculated albumin-to-creatinine ratio was also similar in all groups (0.03mg/mmol). No significant correlation was found between urinary albumin excretion and gestational age, birth weight or degree of postnatal catch-up growth.

One of the participants of the AGA group had microalbuminuria, defined as an albumin-to-creatinine ratio > 3mg/mmol. This participant had a normal GFR (135.7 mL/min/1.73m$^2$). According to the NICE guidelines, this participant is at higher risk for developing kidney disease, because of microalbuminuria [28].

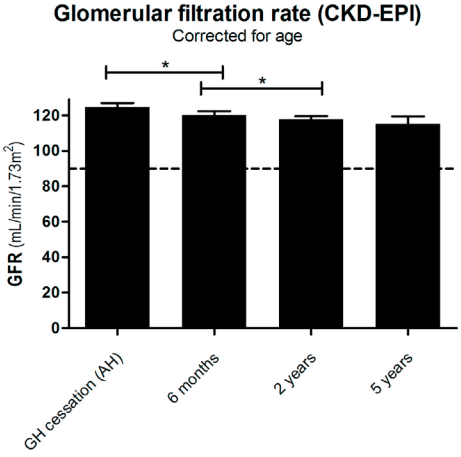


**Figure 1: Longitudinal changes in GFR during 5 years after GH-cessation.**

Bars represent the estimated marginal mean and the upper limit of the 95% confidence interval. Abbreviations: GFR, glomerular filtration rate. Data are for adults born small for gestational age and previously treated with growth hormone, corrected for age.

*p<0.05 was considered significant

**Table 2: Results in all groups at the age of 21 years.**

| | SGA-GH | SGA-S | SGA-CU | AGA | p-value |
|---|---|---|---|---|---|
| GFR (mL/min/1.73m$^2$) | 112 (106-117) | 119 (107-131) | 113 (106-120) | 113 (106-120) | 0.760 |
| Systolic blood pressure (mmHg) | 116 (114-118) | 113 (110-116) | 114 (112-117) | 113 (110-115) | 0.069 |
| Diastolic blood pressure (mmHg) | 67 (66-69) | 66 (63-69) | 68 (66-70) | 67 (65-68) | 0.498 |
| ***Urine*** | | | | | |
| Albumin (mg/dL) | 0.6 (0.0-2.0) | 0.7 (0.0-2.1) | 1.0 (0.6-2.2) | 1.4 (0.6-2.2) | 0.697 |
| Creatinine (mmol/L) | 14.0 (12-16) | 15.7 (14-18) | 16.1 (15-18) | 16.9 (16-18) | 0.142 |
| ACR (mg/mmol) | 0.07 (0.0-0.1) | 0.05 (0.0-0.1) | 0.06 (0.0-0.1) | 0.09 (0.0-0.1) | 0.781 |
| Urine ACR >1mg/mmol | 1 (1%) | 0 | 0 | 2 (2%) | 0.551 |

GFR and blood pressure data are expressed as estimated marginal means (95% CI). GFR values are corrected for age and sex, blood pressure values are corrected for height and sex. P-value represents the between group differences, p<0.05 was considered significant. Abbreviations: SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with persistent short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age; ACR, albumin-to-creatinine ratio; GFR, glomerular filtration rate estimated with the CKD-EPI formula; SDS, standard deviation scores.

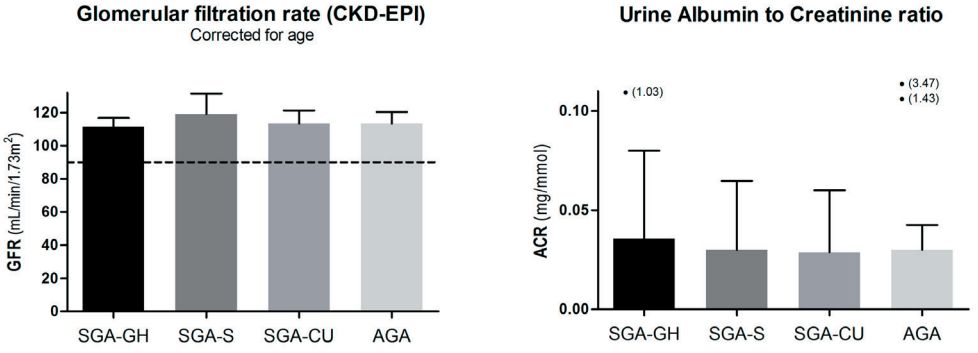


**Figure 2: Distribution of kidney function levels between groups.**

Data are expressed as means with the upper limit of the 95% confidence interval. Abbreviations: GFR, Glomerular filtration rate; ACR, Albumin-to-creatinine ratio; SGA-GH, previously GH-treated adults born SGA; SGA-S, untreated adults born SGA with persistent short stature; SGA-CU, adults born SGA with spontaneous catch-up growth; AGA, adults born appropriate for gestational age. •(…) Albumin-to-creatinine ratio above 1mg/mmol, with absolute value between parentheses.

## Discussion

To the best of our knowledge, our longitudinal study is the first to report GFR after cessation of GH treatment in previously GH-treated adults born SGA. It has been reported that GH treatment increases GFR, which has raised concerns about the long-term effects of GH treatment on kidney function. We now show that GFR remains well within the normal range after cessation of GH treatment and that GFR decreases during the first 6 months after GH-cessation. At 5 years after GH-cessation, previously GH-treated SGA young adults have a similar GFR, blood pressure and urinary albumin excretion as age-matched untreated young adults born either SGA or AGA. At young adult age, birth weight and birth length corrected for gestational age did not correlate with risk factors for developing kidney disease, such as a lower GFR, higher blood pressure or a higher albumin-to-creatinine ratio.

GFR remained well within the normal range, on average 115.2 mL/min/1.73m$^2$ at 5 years after GH-cessation. The higher GFR at cessation, while still on GH treatment, was probably caused by a stimulation of the renin-angiotensin system due to the higher serum IGF-I levels, which has also been described in an earlier study in patients with GH deficiency [5]. However, we did not find a correlation between serum IGF-I levels and GFR in the GH-treated young adults born SGA, which might be explained by the lack of variability in serum IGF-I levels and GFR in this population. Also, in patients with a renal graft, studies have shown that GH treatment did not lead to hyperfiltration or a decline in renal function [29,30]. Most importantly, our findings show that long-term GH treatment has no long-lasting unfavourable effects on GFR in early adulthood.

Urinary albumin-to-creatinine ratio was similar in previously GH-treated young adults born SGA and untreated short SGA young adults. During follow-up after cessation of GH treatment, none of the GH-treated subjects had microalbuminuria. Only one young adult born AGA had mild microalbuminuria, with a GFR well within normal range (135.7 mL/min/1.73m$^2$). We did not find a correlation between birth weight corrected for gestational age and urinary albumin excretion, which is in line with the findings of another study [31]. In a population of young adults born before 32 weeks of gestational age, however, low birth weight was correlated with an increased risk of microalbuminuria at the age of 19 years [21], but this might be explained by the increased risk of the premature birth per se, due to their lower nephron number [17,32]. The fact that we did not find this correlation is likely due to the low number of children below 32 weeks of gestation in our study.

Lower estimated GFR values (<60 mL/min/1.73m$^2$) and a higher albumin-to-creatinine ratio (>10mg/mmol) are associated with an increased risk of cardiovascular mortality [11-14]. None of the SGA-GH participants in our study fulfilled these criteria. Longer follow-up studies on renal function in GH-treated adults should be conducted before a definite conclusion can be drawn. Also systolic and diastolic blood pressure were similar in the SGA-GH subjects compared with the healthy young adults born SGA or AGA. These results are reassuring and support the cardiovascular safety of GH treatment [33,34].

In conclusion, our 5 years longitudinal follow-up study shows a decrease in GFR during 6 months after GH-cessation, but thereafter GFR remained stable and within the normal range. IGF-I levels were not associated with the decrease in GFR values in the first 6 months. The comparison at 5 years after GH-cessation of GH-treated young adults born SGA to a control group of young adults born either SGA or AGA shows similar values in GFR, blood pressure and urinary albumin excretion. Our results show that long-term GH treatment in children born SGA has no unfavourable effects on kidney function in early adulthood.

## Acknowledgements

We express our gratitude to all participants and their parents. We acknowledge the work of research nurses J. Bontenbal-van de Wege, J.C. Bruinings-Vroombout, N. Khieroe and E. Lems during the course of this study.

## References


1. Dahlgren J, Wikland KA, Swedish Study Group for Growth Hormone T. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57(2):216-222.
2. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
3. Maiorana A, Cianfarani S. Impact of growth hormone therapy on adult height of children born small for gestational age. *Pediatrics.* 2009;124(3):e519-531.
4. Bach LA, Hale LJ. Insulin-like growth factors and kidney disease. *American Journal of Kidney Diseases.* 2015;65(2):327-336.
5. Böger RH, Skamira C, Bode-Böger SM, Brabant G, von zur Muhlen A, Frolich JC. Nitric oxide may mediate the hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. *Journal of Clinical Investigation.* 1996;98(12):2706-2713.
6. Kumar PA, Brosius FC, Menon RK. The Glomerular Podocyte as a Target of Growth Hormone Action: Implications for the pathogenesis of diabetic nephropathy. *Current diabetes reviews.* 2011;7(1):50-55.
7. Kamenicky P, Mazziotti G, Lombes M, Giustina A, Chanson P. Growth hormone, insulin-like growth factor-1, and the kidney: pathophysiological and clinical implications. *Endocr Rev.* 2014;35(2):234-281.
8. Feld S, Hirschberg R. Growth hormone, the insulin-like growth factor system, and the kidney. *Endocr Rev.* 1996;17(5):423-480.
9. Ece A, Çetinkaya S, Ekşioğlu S, et al. Kidney growth and renal functions under the growth hormone replacement therapy in children. *Renal failure.* 2014;36(4):508-513.
10. Grunenwald S, Tack I, Chauveau D, Bennet A, Caron P. Impact of growth hormone hypersecretion on the adult human kidney. *Ann Endocrinol (Paris).* 2011;72(6):485-495.
11. Bello AK, Hemmelgarn B, Lloyd A, et al. Associations among estimated glomerular filtration rate, proteinuria, and adverse cardiovascular outcomes. *Clin J Am Soc Nephrol.* 2011;6(6):1418-1426.
12. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* 2010;375(9731):2073-2081.
13. Matsushita K, Coresh J, Sang Y, et al. Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. *Lancet Diabetes Endocrinol.* 2015;3(7):514-525.
14. Nichols GA, Deruaz-Luyet A, Hauske SJ, Brodovicz KG. The association between estimated glomerular filtration rate, albuminuria, and risk of cardiovascular hospitalizations and all-cause mortality among patients with type 2 diabetes. *J Diabetes Complications.* 2018;32(3):291-297.

15. Xu R, Zuo L. Low birthweight and chronic kidney disease. *Nephrology (Carlton).* 2010;15 Suppl 2:18-22.
16. Hughson M, Farris AB, 3rd, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int.* 2003;63(6):2113-2122.
17. Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. *American journal of kidney diseases.* 1994;23(2):171-175.
18. Hallan S, Euser AM, Irgens LM, Finken MJ, Holmen J, Dekker FW. Effect of intrauterine growth restriction on kidney function at young adult age: the Nord Trondelag Health (HUNT 2) Study. *Am J Kidney Dis.* 2008;51(1):10-20.
19. Vikse BE, Irgens LM, Leivestad T, Hallan S, Iversen BM. Low birth weight increases risk for end-stage renal disease. *J Am Soc Nephrol.* 2008;19(1):151-157.
20. Rodriguez-Soriano J, Aguirre M, Oliveros R, Vallo A. Long-term renal follow-up of extremely low birth weight infants. *Pediatr Nephrol.* 2005;20(5):579-584.
21. Keijzer-Veen MG, Kleinveld HA, Lequin MH, et al. Renal function and size at young adult age after intrauterine growth restriction and very premature birth. *Am J Kidney Dis.* 2007;50(4):542-551.
22. Kerkhof GF, Breukhoven PE, Leunissen RW, Willemsen RH, Hokken-Koelega AC. Does preterm birth influence cardiovascular risk in early adulthood? *J Pediatr.* 2012;161(3):390-396 e391.
23. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301(21):2234-2242.
24. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
25. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74(6):901-910.
26. Rosner B, Prineas RJ, Loggie JM, Daniels SR. Blood pressure nomograms for children and adolescents, by height, sex, and age, in the United States. *The Journal of pediatrics.* 1993;123(6):871-886.
27. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
28. Carville S, Wonderling D, Stevens P, Guideline Development G. Early identification and management of chronic kidney disease in adults: summary of updated NICE guidance. *Bmj.* 2014;349:g4507.
29. Fine RN, Yadin O, Nelson PA, et al. Recombinant human growth hormone treatment of children following renal transplantation. *Pediatr Nephrol.* 1991;5(1):147-151.
30. Hokken-Koelega AC, Stijnen T, de Ridder MA, et al. Growth hormone treatment in growth-retarded adolescents after renal transplant. *Lancet.* 1994;343(8909):1313-1317.
31. Yudkin JS, Martyn CN, Phillips DI, Gale CR. Associations of micro-albuminuria with intra-uterine growth retardation. *Nephron.* 2001;89(3):309-314.

32. Rodriguez MM, Gomez AH, Abitbol CL, Chandar JJ, Duara S, Zilleruelo GE. Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. *Pediatr Dev Pathol.* 2004;7(1):17-25.
33. van der Steen M, Kerkhof GF, Smeets CCJ, Hokken-Koelega ACS. Cardiovascular risk factors and carotid intima media thickness in young adults born small for gestational age after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(12):975-985.
34. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.

# Chapter 4

## Cognition, health-related quality of life and psychosocial functioning after GH/GnRHa treatment in young adults born SGA

W.J. Goedegebuure
M. van der Steen
J.L. de With
A.C.S. Hokken-Koelega

*Journal of Clinical Endocrinology and Metabolism 2018 , Nov 1;103(11):3931-3938.*

## Abstract

**Background:** Children born small for gestational age (SGA) with a poor adult height expectation benefit from treatment with growth hormone (GH) and additional gonadotropin-releasing hormone analogue (GnRHa). As both SGA birth and GnRHa-treatment might negatively influence cognition, health-related quality of life (HRQoL) and psychosocial functioning, we assessed these outcomes at adult height (AH).

**Methods:** A randomized, dose-response GH study until AH involving 99 adolescents born SGA, of whom 61 children received additional 2 years of GnRHa-treatment. At AH, the Wechsler Adult Intelligence Scale and TNO-AZL Adults Quality of Life questionnaire were administered to the study group. Additionally, the study group and 67 adolescents born SGA (19 GnRHa) from a second study group completed the Self-Perception Profile of Adolescents and Child/Adolescent Behaviour Checklist at AH. Scores in GH-treated young adults with GnRHa-treatment (GH/GnRHa-group) were compared with GH-treated adolescents without GnRHa treatment (GH-group) and a reference population.

**Results:** Mean age (SD) at AH was 17.5 (1.2) and 17.4 (1.4) years in the GH/GnRHa and GH-group, respectively. Intelligence quotient scores were similar in GH/GnRHa and GH-group (96.33 vs. 92.47). HRQoL was similar between both groups, and also when compared to reference population, but GH/GnRHa-group had a significantly lower perception of cognitive functioning. Self-perception and problem behaviour was similar in the GH/GnRHa and GH-group. AH did not correlate with HRQoL, self-perception and problem behaviour.

**Conclusion:** Combined GH/GnRHa treatment has no long-term negative effects on cognition, HRQoL, self-perception and problem behaviour in early adulthood, compared to GH treatment only.

## Introduction

Being born small for gestational age (SGA) has been associated with problems in health-related quality of life (HRQoL), behaviour and cognitive development [1-6]. In short SGA children without sufficient catch-up growth, long-term continuous recombinant growth hormone (GH) treatment leads to an improvement in adult height (AH) [7-9]. Additional gonadotropin-releasing hormone analogue (GnRHa) treatment for 2 years at start of puberty improves AH in both boys and girls who start GH treatment at onset of or in early puberty with an expected AH of less than -2.5 standard deviation score (SDS) [10].



Gonadotropin-releasing hormone influences structures outside of the pituitary region, in the hippocampus and other limbic structures [11-13]. GnRHa treatment may therefore have cognitive effects, as has been shown during 2 years of GnRHa treatment in precocious puberty. Cognitive functioning at cessation of GnRHa treatment tended to be lower in the group receiving 2 years of GnRHa treatment [14,15]. It is, however, unknown whether GnRHa treatment affects long-term cognitive functioning.

Short stature has a negative effect on HRQoL and GH treatment improves HRQoL [16-19]. We have shown that 2 years of GnRHa in addition to GH treatment did not have adverse effects on HRQoL in children born SGA, during 2 years of treatment [20]. The postponement of puberty might, however, negatively affect problem behaviour and school skills later on in life [21]. Furthermore, GnRHa treatment has been linked to a suppressed reward system, causing subsequent depressive emotions when used for endometriosis and during fertility treatment. However, these populations differed greatly from children who receive GnRHa treatment for the purpose of postponement of puberty [15,22]. The long-term effects of pubertal suppression by means of 2 years of GnRHa in addition to GH treatment on HRQoL, self-perception and problem behaviour in early adulthood, are lacking.

The primary objective of this study was to assess cognitive functioning and HRQoL after attainment of AH in subjects who participated in Dutch GH trials involving children born SGA treated with GH, either with or without additional 2 years of GnRHa treatment after onset of puberty. In addition, we tested self-perception and problem behaviour in these subjects, to evaluate psychosocial functioning at AH. We hypothesized that postponement of puberty by 2 years of GnRHa treatment in GH-treated young adults born SGA does not negatively influence cognitive functioning, HRQoL, self-perception and problem behaviour in early adulthood compared to GH treatment only. Additionally, we hypothesized that adult height would positively correlate with HRQoL, self-perception, and negatively with problem behaviour, and that a double GH-dose of 2mg/m$^2$/day (~ 0.067 mg/kg/d) would not influence these outcomes.

# Methods

**Subjects**

The study group consisted of young adults born SGA, who had participated in the Dutch SGA trial (ISRCTN18062389) (study group 1). SGA was defined as birth weight and/or birth length below –2 SDS for gestational age, with a height at start of GH treatment below -2.5 SDS and no endocrine, metabolic or chronic disorders. GH treatment was started at 8 years or above, and continued until attainment of AH. Study group 1 consisted of 99 GH-treated young adults, who either additionally received GnRHa treatment for 2 years (n=61; GH/GnRHa) or had only received GH treatment (n=38; GH). The 2 years of GnRHa treatment was prescribed, when height at start of puberty would result in an expected AH less than -2.5 SDS, based on Dutch references [23]. At start of puberty, subjects were randomly assigned to treatment with either GH 1 or 2 mg/m$^2$/day (~ 0.033 or 0.067 mg/kg/d) after stratification for sex, pubertal stage, and parental height.

For the evaluation of self-perception and problem behaviour only, we added 67 young adults born SGA from the IUGR-3 study (ISRCTN65230311) (study group 2). These subjects started GH treatment at the age of 5-8 years, and 19 subjects received 2 years of GnRHa treatment in addition to GH treatment, if they met the same inclusion criteria used in study group 1. Supplemental figure 1 shows the treatment regimen of both studies [24]. Number of subjects per completed test by the study groups is shown in supplemental Table 1 [25].

The Medical Ethics Committee of the Erasmus University Medical Centre approved both studies. We obtained written informed consent from all subjects and, if they were younger than 18 years, from their parents or guardians. Due to ethical considerations, the medical ethics committee did not allow a randomized untreated short SGA group.

**Measurements**

At start, every three months during GH treatment and at AH, height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd. Crymmyth, UK). Height was transformed into SDS for sex and chronological age according to Dutch references, using Growth Analyser Research Calculation Tools (Growth Analyser B.V., Rotterdam, The Netherlands). AH SDS was calculated using references for Dutch adults, aged 18 years [23].

**Socioeconomic status**

Parents provided information regarding the socioeconomic status (SES), by means of a questionnaire at start of GH treatment. The highest of two education levels (father and mother) was used as socioeconomic indicator to determine SES (categorized as 1 = lowest, 2 = low, 3 = medium and 4 = high) [26].

**Cognitive functioning**

To assess cognitive functioning, the Wechsler Adult Intelligent Scales (WAIS-III) was performed in 86 subjects to obtain the intelligence quotient (IQ). A Wechsler Intelligence Scales for Children (WISC-III) was administered in thirteen subjects who attained AH below 16 years of age [27,28]. All tests were performed by an experienced psychologist in adherence to the standardization instructions of the WAIS-III and WISC-III manual, within a quiet area to minimize distraction during testing.

Both tests consist of 11 subtests (Picture completion, Vocabulary, Digit symbol coding, Comprehension, Symbol search, Similarities, Information, Digit span, Letter-number sequencing, Block design, Matrix reasoning) of which we created the total IQ, performance IQ and verbal IQ, as described by the WAIS-III and WISC-III manuals. [27,28] There is a good correlation between the WAIS-III and WISC-III IQ scores (r=0.88, r=0.78, and r=0.88 for total IQ, performance IQ and verbal IQ, resp.) [29].



**Health-related quality of life**

When subjects had attained AH at 16 years or above, the validated TNO-AZL Adults Quality of Life (TAAQOL) was completed to assess HRQoL [30,31]. The TAAQOL consists of 45 items and contains twelve scales (gross motor function, fine motor function, cognitive functioning, sleep, pain, social functioning, daily activities, sexuality, vitality, positive emotions, depressive emotions and aggressive emotions). Most items consist of two questions: the first question assesses whether a participant has experienced a health status limitation in the last couple of weeks. If present, the second question evaluates the emotional feeling about this limitation. The scale scores are obtained by combining both responses into one HRQoL score per item, adding item scores within scales and transforming crude scale scores to a 0-100 scale. Higher scores indicate a better HRQoL. This questionnaire specifically offers the subjects the possibility to differentiate between their functioning and the way they feel about it.

We excluded the questions about sexuality in our analysis, as most subjects replied they were not able to answer these questions. Thirteen subjects could not complete the TAAQOL questionnaire, because they were not yet 16 years of age.

Based on the data of the Dutch population, Cronbach alphas values ranging from 0.65 to 0.84, indicated that comparisons on group level were justified [32], and the psychometric properties, reliability and validity of this questionnaire were satisfactory [30].

As recommended in the TAAQOL manual, 103 healthy respondents in the same age range of 16-22 years of study group 1 were selected from the total reference group (n=4410) and included in the analyses [30].

**Self-perception**

Subjects of both study groups 1 and 2 completed the validated Dutch version of the Self Perception Profile of Adolescents (CBSA) at attainment of AH [33,34]. This questionnaire comprises seven domains (School skills (SS); Social acceptation (SA); Sports Skills (SP); Physical appearance (PA); Behavioural attitude (BA); Feeling of self-esteem (SE); Friendship (FR)). Each domain consists of five items, which consist of two propositions, to which subjects can indicate which proposition suits them the most. The questionnaire uses a 4-point Likert scale. Results are presented as rank percentiles, compared to a reference population, corrected for level of education and sex. Cronbach alphas values were mostly around 0.80, for each domain. The test-retest reliability correlations of this questionnaire were highly significant (r=0.72-0.76; $P < 0.01$) [34].

**Problem behaviour**

Parents of both study groups 1 and 2 completed the Child/Adolescent Behaviour Check List (CBCL/ABCL) at attainment of AH. The questionnaire consists of 113 questions on specific problem behaviour, scored on a 3-point Likert scale (0 indicating absent behaviour; 2 indicating behaviour is frequently present). This questionnaire is one of the most widely used dimensional rating scales of psychopathology, as it rates behaviour on three main scales (total problems, externalizing problems and internalizing problems) and eight subscales (withdrawn behaviour, somatic complaints, anxious/depressed behaviour, social problems, thought problems, attention problems, delinquent behaviour, and aggressive behaviour) [35]. Raw scores are continuous and can be transformed into standardized T-scores, with a mean of 50 and a standard deviation of 10, compared to a reference population. This questionnaire has been studied extensively in clinical and community populations [36-38].

**Data analysis**

All tests were performed using the statistical package SPSS (version 24.0; SPSS Inc., Chicago, Ill., USA) for Windows. Clinical characteristics are presented as mean (SD) unless stated otherwise. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q plots. Differences in characteristics between the groups were tested with students T-test for continuous variables and $\chi^2$-test for proportions. Non-parametric variables of the TAAQOL questionnaire were tested by means of the Mann-Whitney U test and the Kruskal-Wallis test.

We used the Pearson's correlation and Spearman's Rank Order Correlation (rho) to calculate the strength of the relationship between the outcomes of the questionnaires and adult height. Differences between the 1 and 2 mg/m$^2$/day GH-dose groups in all questionnaires were evaluated using an independent-sample t-test. Results were regarded as statistically significant at a p-value of <0.05.

# Results

### Clinical characteristics

Table 1 shows the clinical characteristics of study group 1 at start and cessation of GH treatment. In total 99 (61 GH/GnRHa, 38 GH) subjects completed the questionnaires at AH, on average 3.48 years after cessation of GnRHa treatment. In the GH/GnRHa and GH groups, mean (SD) age at start of GH treatment was 11.98 (1.72) vs. 11.19 (2.26) years in males and 11.63 (1.05) vs. 10.27 (1.15) years in females (p<0.001), respectively. At cessation of GH treatment, mean (SD) age was 18.35 (0.79) vs. 18.28 (1.19) years in males and 16.88 (1.02) vs. 16.24 (0.93) in females (p=0.009), in the GH/GnRHa and GH groups, respectively. Mean SES was significantly higher in subjects who additionally received GnRHa treatment (p=0.016).



**Table 1: Clinical characteristics.**

| | | **GH/GnRHa** *Means (SD)* | **GH** *Means (SD)* | *p-value* |
|---|---|---|---|---|
| Male/Female | | 24/37 | 22/16 | 0.072 |
| Gestational age (weeks) | | 37.76 (3.27) | 37.64 (2.82) | 0.847 |
| Birth weight (SDS) | | -1.99 (0.89) | -1.83 (0.99) | 0.410 |
| Birth length (SDS) | | -2.72 (1.04) | -2.47 (0.95) | 0.289 |
| Height at start GH-treatment (SDS) | | -3.07 (0.65) | -3.06 (0.55) | 0.930 |
| Age at start GH-treatment in years | *male* | 11.98 (1.72) | 11.19 (2.26) | 0.229 |
| | *female* | 11.63 (1.05) | 10.27 (1.15) | 0.000 |
| Age at stop GH-treatment in years | *male* | 18.35 (0.79) | 18.28 (1.19) | 0.825 |
| | *female* | 16.88 (1.02) | 16.24 (0.93) | 0.009 |
| Age at start GnRHa-treatment in years | *male* | 12.52 (0.34) | | |
| | *female* | 11.74 (0.95) | | |
| Adult height SDS | | -1.70 (0.79) | -1.45 (0.86) | 0.184 |
| Dutch Caucasian ethnicity (%) | | 79.1 | 93.9 | 0.068 |
| Socioeconomic status (%) | | | | 0.016 |
| Lowest | | 0 | 0 | |
| Low | | 24.6 | 39.5 | |
| Medium | | 28.1 | 42.1 | |
| High | | 47.4 | 18.4 | |

P-values<0.05 are considered significant differences between groups. GH=growth hormone, GnRHa=gonadotropin releasing hormone agonist.

### Cognitive functioning

Table 2 shows the mean (SD) scores in the GH/GnRHa and GH group on total, performance and verbal IQ scores in the 99 subjects of study group 1 only. Total IQ scores were not significantly different between the GH/GnRHa group and GH group (96.33 vs. 92.47, resp. (p=0.135)). Also the performance and verbal IQ scores were not significantly different (p=0.280 and p=0.198, resp.). The total IQ, performance IQ and verbal IQ scores were similar between GH-dose groups.

Cognitive functioning correlated significantly with SES (r=0.495, p=0.001), but within each category of SES, cognitive functioning was similar in the GH/GnRHa and GH group.

The GH/GnRHa and GH groups had significantly lower cognitive functioning, when compared to the reference population (94.85 vs. 100.00, resp. (p=0.001)). However, their cognition was still within the normal ranges. Although not significant, total IQ scores were higher in the GH/GnRHa group. This difference disappeared after correction for socioeconomic status (p=0.554).

**Table 2: Cognitive functioning at adult height.**

| | GH/GnRHa (n=61) | | GH (n=38) | | |
|---|---|---|---|---|---|
| | ***Means (SD)*** | ***SD-scores*** | ***Means (SD)*** | ***SD-scores*** | ***p-value*** |
| Verbal | 96.31 (14.09) | -0.25 (0.94) | 92.87 (10.52) | -0.48 (0.70) | 0.198 |
| Performance | 97.15 (13.63) | -0.19 (0.91) | 94.21 (12.12) | -0.39 (0.81) | 0.280 |
| Total | 96.33 (13.47) | -0.24 (0.90) | 92.47 (10.38) | -0.51 (0.69) | 0.135 |

Wechsler Adult Intelligence Scores (WAIS) and Wechsler Intelligence Scores for Children (WISC) was administered to all subjects of the SGA study. Higher scores indicate higher cognitive functioning. GH=growth hormone, GnRHa=gonadotropin releasing hormone agonist.

### Health-related quality of life

Table 3 shows the mean (SD) TAAQOL scores at GH-cessation in the GH/GnRHa and GH groups of study group 1 and of an age-matched reference population.

Subjects who additionally received GnRHa treatment had a significantly higher quality of life regarding positive emotions, when compared to the GH group (76.63 vs. 66.39, resp. (p=0.039)), also after correction for socioeconomic status (p=0.028).

Depressive emotions were similar in both groups, and none of the subjects received antidepressant therapy. No significant correlations with AH were found in all HRQoL categories, in the GH/GnRHa and GH groups. HRQoL scores were similar in both GH-dose groups.

Cognitive functioning, however, was perceived significantly different between the GH/GnRHa group, GH group and reference population (p=0.002). The GH/GnRHa group had a lower perceived cognitive functioning than the reference population (78.42 vs. 87.86, resp. (p=0.002)), while the GH group had a trend towards lower perceived cognitive functioning compared to the reference population (82.76 vs. 87.86, resp. (p=0.176)). There was no significant difference in perception of cognitive functioning between the GH/GnRHa and GH groups (p=0.422).

**Table 3: Health-related quality of life at adult height.**



| | GH/GnRHa (n=56) *Means (SD)* | GH (n=30) *Means (SD)* | GH/GnRHa vs. GH *p-value* | Refs. (n=103) *Means (SD)* | All groups *p-value* |
|---|---|---|---|---|---|
| Gross motor function | 95.23 (12.43) | 95.42 (9.28) | 0.660 | 96.46 (9.54) | 0.735 |
| Fine motor function | 99.19 (2.72) | 98.33 (6.34) | 0.755 | 98.60 (6.78) | 0.771 |
| Cognitive function | 78.42 (21.29) | 82.76 (18.04) | 0.422 | 87.86 (18.46) | **0.002** |
| Sleep | 74.42 (27.10) | 74.78 (22.99) | 0.904 | 82.95 (17.88) | 0.159 |
| Pain | 81.25 (18.69) | 79.53 (21.96) | 0.989 | 86.95 (14.75) | 0.129 |
| Social function | 93.06 (11.24) | 92.86 (11.12) | 0.592 | 91.66 (13.52) | 0.876 |
| Daily activities | 90.45 (12.05) | 88.61 (17.09) | 0.784 | 85.23 (19.34) | 0.566 |
| Vitality | 71.13 (18.21) | 69.54 (15.55) | 0.418 | 69.34 (19.57) | 0.763 |
| Positive emotion | 76.63 (19.00) | 66.39 (25.47) | **0.039** | 74.34 (18.42) | 0.093 |
| Depressive emotion | 85.89 (16.27) | 83.15 (19.64) | 0.539 | 82.61 (15.22) | 0.237 |
| Aggressive emotion | 92.00 (10.54) | 83.91 (19.15) | 0.066 | 89.32 (14.23) | 0.176 |

The TNO-AZL quality of life (TAAQOL) questionnaire was completed by subjects at adult height. Scores are expressed as means (SD). Higher scores indicate better quality of life. Bold p-values are considered significant differences between groups. GH=growth hormone, GnRHa=Gonadotropin releasing hormone agonist, Refs=reference population.

### Self-perception

The self-perception questionnaire was completed by 142 young adults of both study groups 1 and 2. The results are presented in Table 4, with higher scores indicating better self-perception. No significant differences were found between the GH/GnRHa and GH groups. Compared to the reference population, both groups scored significantly better for behavioural attitude, +0.57 SDS for GH/GnRHa and +0.68 SDS for GH (p<0.001). No significant correlations were found between AH and self-perception scores in the GH/GnRHa and GH groups. Self-perception was similar in both GH-dose groups.

**Table 4: Self-perception at adult height.**

| | GH/GnRHa (n=70) | | GH (n=72) | | |
|---|---|---|---|---|---|
| | ***Means (SD)*** | ***SD-scores*** | ***Means (SD)*** | ***SD-scores*** | ***p-value*** |
| School skills | 57.07 (32.85) | +0.18 (1.25) | 57.64 (32.82) | +0.19 (1.09) | 0.921 |
| Social acceptation | 50.55 (33.40) | +0.01 (1.18) | 50.69 (31.82) | +0.03 (1.12) | 0.980 |
| Sports skills | 55.75 (31.21) | +0.14 (1.09) | 47.13 (31.25) | -0.07 (1.09) | 0.111 |
| Physical appearance | 60.06 (27.66) | +0.25 (0.92) | 62.71 (28.02) | +0.32 (0.93) | 0.590 |
| Behavioural attitude | 71.39 (27.15)† | +0.57 (0.95) | 75.06 (26.08)† | +0.68 (1.01) | 0.422 |
| Feeling of self-esteem | 55.39 (28.59) | +0.13 (0.94) | 60.97 (28.53) | +0.28 (0.93) | 0.256 |
| Friendship | 51.36 (27.00) | +0.03 (0.85) | 54.85 (28.59) | +0.12 (0.92) | 0.461 |

Self-perception questionnaire (CBSA) was filled in by subjects, and compared to the reference population provided by the questionnaire. No significant differences were found between groups. †Significantly higher than in reference population used in the questionnaire. GH=growth hormone, GnRHa=Gonadotropin releasing hormone agonist.

## Problem behaviour

The ABCL- and CBCL questionnaires, completed by 136 parents of both study groups 1 and 2, did not show any significant differences between the GH/GnRHa and GH groups and the reference population. Results are presented in Table 5, with lower scores representing less problem behaviour. Total problem scores were similar, being 49.36 and 49.62 in the GH/GnRHa and GH groups, respectively. Externalizing problems and internalizing problems were also similar in both groups. AH did neither significantly correlate with total problem scores (r=-0.33; p=0.714), nor with any other problem behaviour score in the GH/GnRHa and GH groups. At AH, problem behaviour was similar in both GH-dose groups.

**Table 5: Problem behaviour at adult height.**

| | GH/GnRHa (n=69) | | GH (n=67) | | |
|---|---|---|---|---|---|
| | ***Means (SD)*** | ***SD-scores*** | ***Means (SD)*** | ***SD-scores*** | ***p-value*** |
| Withdrawn behaviour | 55.24 (8.28) | +0.52 (0.83) | 56.29 (6.93) | +0.62 (0.69) | 0.126 |
| Somatic complaints | 56.17 (8.19) | +0.62 (0.82) | 57.21 (8.27) | +0.72 (0.83) | 0.794 |
| Depressed behaviour | 53.86 (7.42) | +0.39 (0.74) | 53.89 (5.63) | +0.39 (0.56) | 0.671 |
| Thought problems | 55.26 (7.31) | +0.53 (0.73) | 55.06 (6.78) | +0.51 (0.68) | 0.917 |
| Attention problems | 56.21 (7.99) | +0.62 (0.80) | 55.61 (6.61) | +0.56 (0.66) | 0.556 |
| Rule breaking behaviour | 54.02 (5.10) | +0.40 (0.51) | 53.79 (5.12) | +0.38 (0.51) | 0.309 |
| Aggressive behaviour | 52.91 (6.75) | +0.29 (0.68) | 52.44 (8.21) | +0.24 (0.82) | 0.544 |
| ***Total scores*** | | | | | |
| Externalizing problems | 49.83 (12.66) | -0.02 (1.27) | 51.86 (11.40) | +0.19 (1.14) | 0.348 |
| Internalizing problems | 47.90 (9.92) | -0.21 (0.99) | 47.79 (10.07) | -0.22 (1.01) | 0.952 |
| Total problems | 49.36 (12.27) | -0.06 (1.23) | 49.62 (11.79) | -0.04 (1.18) | 0.905 |

Adult/Child behaviour checklist (ABCL/CBCL) was completed by the parents of the subject and compared to the reference population provided by the questionnaires. Higher scores indicating more problematic behaviour. No significant differences were found between groups. GH=growth hormone, GnRHa=Gonadotropin releasing hormone agonist.

## Discussion

This is the first study reporting cognitive functioning, HRQoL, self-perception and problem behaviour in young adults born SGA who received GH treatment and in addition 2 years of GnRHa treatment, compared to GH treatment only. At AH, full scale IQ, verbal IQ and performance IQ scores were similar in the GH/GnRHa and GH groups. Compared with the reference population, the GH/GnRHa and GH group scored themselves significantly lower in their perception of cognitive functioning. All other categories, however, showed similar results in both groups. At AH, self-perception and problem behaviour were similar in both groups and compared to the reference population. AH did not correlate with HRQoL, self-perception and problem behaviour scores, and all scores were similar between GH-dosage groups.



Cognitive functioning, as measured by the WAIS-III and WISC-III tests, was similar in the GH/GnRHa and GH group. This is a reassuring result, as cognitive function following GnRHa treatment has been subject of concern. Two earlier studies showed a lower cognitive functioning at cessation of GnRHa treatment, when compared to the start of treatment, based on the results of the WAIS-test [14,15]. Our results show that this effect is not present at adult height. The lower scores found in the other studies are most likely due to the delay in psychosocial maturation at time of testing, as GnRHa-treated patients entered puberty later than their peers. Cognitive functioning in the GH/GnRHa and GH groups was below the mean of the reference population, but still within the normal ranges. This is in line with other studies describing lower cognitive functioning in SGA children [39-42]. Socioeconomic status, as defined by parental education levels, had a strong correlation with cognitive functioning. Total IQ scores tended to be higher in the GH/GnRHa group, but when corrected for socioeconomic status, GnRHa treatment did not show a long-term effect on cognitive functioning.

HRQoL is important as a 'patient-reported outcome' as it reflects the subjective perception of health. Young adults of the GH/GnRHa group perceived a lower HRQoL in cognitive functioning compared to the GH group and the reference population, with the GH/GnRHa group scoring lowest, followed by the GH group. This is in contrast to the slightly higher cognitive function in the GH/GnRHa group. As the GH/GnRHa group had a higher SES, it might be that the young adults have underrated their cognitive functioning, because they compared themselves with their better performing parents.

Some studies suggested an increase in depressive emotions following GnRHa treatment for pubertal suppression or other indications [15,22]. Our long-term results are reassuring, as they show that 2 years of GnRHa treatment does not increase depressive emotions in young adults born SGA.

The CBSA questionnaire was created to specifically measure feelings of self-perception in specific domains, as well as global self-perception. These domains of self-perception are of great importance, as they reflect the concerns of young adults about job competence, dating, and close friendships [33,34]. In our study, self-perception scores were similar in the GH/GnRHa and GH-treated groups and also compared with the reference population. Our results are in line with two studies describing no decrease of CBSA scores, during three years of GnRHa treatment [14,43]. To our knowledge, no long-term studies were performed on self-perception in young adults many years after GnRHa treatment.

Internalizing, externalizing and total problem behaviour were not significantly different between young adults in the GH/GnRHa and GH groups and compared with the reference population. Normal behaviour after three years of GH and GnRHa treatment in children with precocious puberty following adoption has been reported [44]. Studies found no difference in problem behaviour in untreated children with short stature after SGA birth or due to ISS, when compared with the reference population [14,15,43]. The comparison with the reference population is likely to overestimate the problem scores in our population, as subjects who visited a mental health professional in the last 12 months or had received extra educational support, were excluded from the reference population [45]. Mean problem scores of our study group were, however, not significantly higher than those of the healthy reference population, which underlines the normal problem behaviour in our study groups.

Subjects who received a double GH-dose of 2mg/m$^2$/day had similar outcomes in cognitive function, HRQoL, self-perception and problem behaviour at AH. We did not find a correlation between AH and HRQoL, self-perception or problem behaviour, which suggests that there is no causal relation between AH and these psychosocial functioning scores. This has also been described in children with untreated short stature [42,46]. However, the lack of correlation in this study might also be explained by the limited variation in adult height in our study groups, as Bannink et al. did report an increase in HRQoL in GH-treated subjects born SGA, in parallel to the increase in height [17].

The growth characteristics were similar in both study groups 1 and 2 [10,47,48]. Our study did not have a randomized placebo group, as it is impossible to give placebo to postpone puberty, as pubertal development will continue during placebo treatment, which will be visible for the patient and the physician. The aim of the present study was to compare GnRHa/GH and GH effects on cognitive functioning and HRQoL and not on growth. The effectiveness and safety of the combined GH/GnRHa treatment on growth have been described in earlier reports about the Dutch SGA study, based on a slightly smaller study population. GnRHa treatment in addition to GH treatment resulted in significantly greater height gain and adult height in early pubertal children with a poor AH. [10]. Results on bone mineral density, body

composition, cardiovascular and metabolic health have been published, and showed that that additional 2 years of GnRHa treatment is effective and safe [49-51].

In conclusion, our study shows that 2 years of GnRHa treatment in addition to GH treatment, results in similar cognitive functioning, health-related quality of life, self-perception and problem behaviour, compared to GH treatment only in young adults born SGA. In contrast to our hypothesis, no significant correlations were found between AH and problem behaviour and self-perception scores. Based on our results, additional GnRHa treatment could be considered at the start of puberty for GH-treated children born SGA with an AH prediction below -2.5 SDS.



## Acknowledgements

We express our gratitude to all children and their parents who participated in this study. We thank J. Bontenbal-van de Wege, C. Bruinings-Vroombout, N. Khieroe and E. Lems, research nurses, for their contribution to the study, and all the collaborating physicians and the paediatricians who referred patients to participate in the studies.

## References


1. Hutton JL, Pharoah PO, Cooke RW, Stevenson RC. Differential effects of preterm birth and small gestational age on cognitive and motor development. *Arch Dis Child Fetal Neonatal Ed.* 1997;76(2):F75-81.
2. Puga B, Puga PG, de Arriba A, Armendariz Y, Labarta JI, Longas AF. Psychomotor and intellectual development (Neurocognitive Function) of children born small for gestational age (SGA). Transversal and longitudinal study. *Pediatr Endocrinol Rev.* 2009;6 Suppl 3:358-370.
3. Smedler AC, Faxelius G, Bremme K, Lagerstrom M. Psychological development in children born with very low birth weight after severe intrauterine growth retardation: a 10-year follow-up study. *Acta Paediatr.* 1992;81(3):197-203.
4. van Wassenaer A. Neurodevelopmental consequences of being born SGA. *Pediatr Endocrinol Rev.* 2005;2(3):372-377.
5. Castanys-Munoz E, Kennedy K, Castaneda-Gutierrez E, et al. Systematic review indicates postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. *Acta Paediatr.* 2017;106(8):1230-1238.
6. Zubrick SR, Kurinczuk JJ, McDermott BM, McKelvey RS, Silburn SR, Davies LC. Fetal growth and subsequent mental health problems in children aged 4 to 13 years. *Dev Med Child Neurol.* 2000;42(1):14-20.
7. Sas T, de Waal W, Mulder P, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab.* 1999;84(9):3064-3070.
8. Dahlgren J, Wikland KA, Swedish Study Group for Growth Hormone T. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57(2):216-222.
9. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
10. Lem AJ, van der Kaay DC, de Ridder MA, et al. Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analog: results of a randomized, dose-response GH trial. *J Clin Endocrinol Metab.* 2012;97(11):4096-4105.
11. Skinner DC, Albertson AJ, Navratil A, et al. Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. *J Neuroendocrinol.* 2009;21(4):282-292.
12. Albertson AJ, Navratil A, Mignot M, Dufourny L, Cherrington B, Skinner DC. Immunoreactive GnRH Type I Receptors in the Mouse and Sheep Brain. *Journal of chemical neuroanatomy.* 2008;35(4):326-333.
13. Hough D, Bellingham M, Haraldsen IR, et al. A reduction in long-term spatial memory persists after discontinuation of peripubertal GnRH agonist treatment in sheep. *Psychoneuroendocrinology.* 2017;77:1-8.

14. Mul D, Versluis-den Bieman HJ, Slijper FM, Oostdijk W, Waelkens JJ, Drop SL. Psychological assessments before and after treatment of early puberty in adopted children. *Acta Paediatr.* 2001;90(9):965-971.
15. Wojniusz S, Callens N, Sutterlin S, et al. Cognitive, Emotional, and Psychosocial Functioning of Girls Treated with Pharmacological Puberty Blockage for Idiopathic Central Precocious Puberty. *Front Psychol.* 2016;7:1053.
16. Christensen TL, Djurhuus CB, Clayton P, Christiansen JS. An evaluation of the relationship between adult height and health-related quality of life in the general UK population. *Clin Endocrinol (Oxf).* 2007;67(3):407-412.
17. Bannink E, Djurhuus CB, Christensen T, Jons K, Hokken-Koelega A. Adult height and health-related quality of life after growth hormone therapy in small for gestational age subjects. *J Med Econ.* 2010;13(2):221-227.
18. Bannink EM, van Pareren YK, Theunissen NC, Raat H, Mulder PG, Hokken-Koelega AC. Quality of life in adolescents born small for gestational age: does growth hormone make a difference? *Horm Res.* 2005;64(4):166-174.
19. van Pareren YK, Duivenvoorden HJ, Slijper FS, Koot HM, Hokken-Koelega AC. Intelligence and psychosocial functioning during long-term growth hormone therapy in children born small for gestational age. *J Clin Endocrinol Metab.* 2004;89(11):5295-5302.
20. Lem AJ, Jobse I, van der Kaay DC, de Ridder MA, Raat H, Hokken-Koelega AC. Health-related quality of life in short children born small for gestational age: effects of growth hormone treatment and postponement of puberty. *Horm Res Paediatr.* 2012;77(3):170-179.
21. Waylen A, Wolke D. Sex 'n' drugs 'n' rock 'n' roll: the meaning and social consequences of pubertal timing. *Eur J Endocrinol.* 2004;151 Suppl 3:U151-159.
22. Frokjaer VG, Pinborg A, Holst KK, et al. Role of Serotonin Transporter Changes in Depressive Responses to Sex-Steroid Hormone Manipulation: A Positron Emission Tomography Study. *Biol Psychiatry.* 2015;78(8):534-543.
23. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
24. Goedegebuure WJ, Van der Steen, M, De With JL, Hokken-Koelega ACS. Supplemental figure 1. Flowchart of treatment regimen. *Figshare digital library.* Deposited July 5, 2018; *https://doi.org/10.6084/m9.figshare.6744038.v1*
25. Goedegebuure WJ, Van der Steen, M, De With JL, Hokken-Koelega ACS. Supplemental Table 1. Number of subjects per test, divided in two study groups. *Figshare digital library* Deposited August 7, 2018; *https://doi.org/10.6084/m9.figshare.6940646.v1*.
26. Sector for Socio-Economic Image CBS. Standard education classification (SOI) *Centraal Bureau voor de Statistiek.* 2014.
27. Wechsler D. Manual of Wechsler Adult Intelligence Scale 3 (WAIS-III). *San Antonio: Harcourt Assessment.* 1997.
28. Wechsler D. Manual of Wechsler Intelligence Scale for Children -Third Edition (WISC-III). *New York: The Psychological Coorporation.* 1991.

29. Tulsky DS, Zhu J, Prifitera A. Chapter 5 - Assessment of Adult Intelligence with the WAIS-III A2 - Goldstein, Gerald. In: Hersen M, ed. *Handbook of Psychological Assessment (Third Edition)*. Amsterdam: Pergamon; 2000:97-129.
30. Bruil J, Fekkes M, Vogels T, Verrips G. TAAQOL manual. *Leiden Center for Child Health and Pediatrics LUMC-TNO.* 2004;90.
31. Bruil J, Fekkes T, Vogels T, Verrips G. TACQoL Manual. *Leiden, Germany: Leiden Center for Child Health and Pediatrics, LUMC-TNO.* 2004.
32. Verrips E, Vogels T, Koopman H, et al. Measuring health-related quality of life in a child population. *The European Journal of Public Health.* 1999;9(3):188-193.
33. Treffers P, Goedhart A, Veerman J, Van den Bergh B, Ackaert L, De Rycke L. Competentie belevingsschaal voor Adolescenten. *Tijdschrift voor Psychiatrie.* 2004;7:468-469.
34. Treffers PDA, Goedhardt A, Veerman J, Van den Bergh B, Ackaert L, De Rycke L. Handleiding competentie belevingsschaal voor adolescenten. *Lisse, The Netherland: Swets & Zeitlinger.* 2002.
35. Verhulst FC VdEJ, Koot HM. Handleiding voor de CBCL 4-18 (Manual for the Child Behavior Check List). 1996.
36. Verhulst FC, Achenbach TM, Akkerhuis GW. Problems reported for clinically referred American and Dutch children. *J Am Acad Child Adolesc Psychiatry.* 1989;28(4):516-524.
37. Achenbach TM, Dumenci L, Rescorla LA. Are American children's problems still getting worse? A 23-year comparison. *J Abnorm Child Psychol.* 2003;31(1):1-11.
38. Achenbach TM. Manual for the child behavior Checklist/4-18 and 1991 profile. 1991.
39. Lee PA, Houk CP. Cognitive and psychosocial development concerns in children born small for gestational age. *Pediatr Endocrinol Rev.* 2012;10(2):209-216.
40. Puga B, Gil P, de Arriba A, et al. Neurocognitive development of children born small for gestational age (SGA). An update. *Pediatr Endocrinol Rev.* 2012;9(4):716-726.
41. Stephen MD, Varni JW, Limbers CA, et al. Health-related quality of life and cognitive functioning in pediatric short stature: comparison of growth-hormone-naive, growth-hormone-treated, and healthy samples. *Eur J Pediatr.* 2011;170(3):351-358.
42. Downie AB, Mulligan J, Stratford RJ, Betts PR, Voss LD. Are short normal children at a disadvantage? The Wessex growth study. *Bmj.* 1997;314(7074):97-100.
43. Visser-van Balen H, Geenen R, Moerbeek M, et al. Psychosocial functioning of adolescents with idiopathic short stature or persistent short stature born small for gestational age during three years of combined growth hormone and gonadotropin-releasing hormone agonist treatment. *Horm Res.* 2005;64(2):77-87.
44. Mul D, Oostdijk W, Waelkens JJ, Schulpen TW, Drop SL. Gonadotrophin releasing hormone agonist treatment with or without recombinant human GH in adopted children with early puberty. *Clin Endocrinol (Oxf).* 2001;55(1):121-129.
45. Sandberg DE, Meyer-Bahlburg HFL, Yager TJ. The Child Behavior Checklist Nonclinical Standardization Samples: Should They Be Utilized as Norms? *Journal of the American Academy of Child & Adolescent Psychiatry.* 1991;30(1):124-134.

46. Voss LD, Bailey BJ, Mulligan J, Wilkin TJ, Betts PR. Short stature and school performance--the Wessex Growth Study. *Acta Paediatr Scand Suppl.* 1991;377:29-31; discussion 32.
47. Renes JS, Willemsen RH, Mulder JC, et al. New insights into factors influencing adult height in short SGA children: Results of a large multicentre growth hormone trial. *Clin Endocrinol (Oxf).* 2015;82(6):854-861.
48. Gardner M, Boshart ML, Yeguez CE, Desai KM, Sandberg DE. Coming Up Short: Risks of Bias in Assessing Psychological Outcomes in Growth Hormone Therapy for Short Stature. *J Clin Endocrinol Metab.* 2016;101(1):23-30.
49. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Insulin Sensitivity and beta-Cell Function in SGA Children Treated With GH and GnRHa: Results of a Long-Term Trial. *J Clin Endocrinol Metab.* 2016;101(2):705-713.
50. van der Steen M, Lem AJ, van der Kaay DC, et al. 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. *J Clin Endocrinol Metab.* 2015;100(10):3725-3734.
51. Lem AJ, van der Kaay DC, Hokken-Koelega AC. Bone mineral density and body composition in short children born SGA during growth hormone and gonadotropin releasing hormone analog treatment. *J Clin Endocrinol Metab.* 2013;98(1):77-86.

# Chapter 5

## Longitudinal study on metabolic health in SGA adults during 5 years after GH with or without 2 years of GnRHa treatment

W.J. Goedegebuure
M. van der Steen
G.F. Kerkhof
A.C.S. Hokken-Koelega

*Journal of Clinical Endocrinology and Metabolism 2020, Aug 1;105(8).*

## Abstract

**Background:** In children born small for gestational age (SGA) with persistent short stature, 2 years of gonadotropin-releasing hormone analogue (GnRHa), in addition to long-term growth hormone (GH) treatment, can improve adult height. We assessed safety on metabolic and bone health of GnRHa/GH treatment during 5 years after cessation of GH.

**Methods:** 363 young adults born SGA, previously treated with combined GnRHa/GH or GH-only, were followed for 5 years after attainment of adult height: at GH-cessation, 2 and 5 years thereafter. Data at 5 years after GH-cessation, at age 21 years, were also compared to 145 age-matched adults born appropriate for gestational age (AGA). Frequently sampled intravenous glucose tolerance (FSIGT) tests were used to assess insulin-sensitivity, acute insulin response and beta-cell function. Body composition and bone mineral density (BMD) was determined by DXA scans.

**Results:** In the GnRHa/GH and GH-only group, fat mass increased during the 5 years after GH-cessation, but the changes in FSIGT results, body composition, blood pressure, serum lipid levels and BMD were similar in both groups. At age 21 years, the GnRHa/GH group had similar fat mass, FSIGT results, blood pressure, serum lipid levels and BMD-total body as the GH-only group and AGA controls, a higher BMD-lumbar spine and lower lean body mass than AGA controls.

**Conclusion:** This study during 5 years after GH-cessation shows that addition of 2 years of GnRHa treatment to long-term GH treatment of short children born SGA has no unfavorable effects on metabolic and bone health in early adulthood.

## Introduction

In children born small for gestational age (SGA) with persistent short stature, treatment with growth hormone (GH) leads to adult height (AH) improvement [1-3]. The Dutch SGA study has shown that postponement of puberty with a gonadotropin-releasing hormone analogue (GnRHa) for 2 years at start of puberty because of an expected AH < −2.5 SDS, can improve AH in children born SGA who start growth hormone (GH) treatment in early puberty [4].

In patients with central precocious puberty (CPP), a decrease in insulin sensitivity, expressed in HOMA-IR, was described during GnRHa treatment [5-8]. Gain in weight and fat mass during treatment with GnRHa was reported, potentially causing obesity in adulthood [9-13]. In studies with central precocious puberty (CPP) patients, a decrease in bone turnover and bone mineral density (BMD) was observed during GnRHa treatment [12,14-16]. However, studies were performed retrospectively in children with CPP and most studies evaluated the changes only during GnRHa treatment. Our study group has shown no negative effects of 2 years of GnRHa treatment in addition to GH treatment until AH attainment [17-19]. Studies on long-term safety of postponing puberty with GnRHa treatment regarding metabolic health and bone mineralization in young adults born SGA following GH-cessation at adult height attainment are lacking.



We performed a longitudinal study during the 5 years after GH-cessation in young adults who were treated with GH until AH, either with or without an additional 2 years of GnRHa after onset of puberty. The primary objective of the study was to assess insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and BMD during the 5 years after GH-cessation. We hypothesized that postponement of puberty by 2 years of GnRHa treatment in GH-treated young adults born SGA would not negatively influence these outcome measures in early adulthood compared to GH treatment only and would result in a similar metabolic and cardiovascular health profile and BMD in both treatment groups. Our secondary objective was to compare both treatment groups at 5 years after GH-cessation with a healthy young adult cohort. We hypothesized that treatment with either GnRHa/GH or GH-only would result in a similar metabolic and cardiovascular health and similar BMD compared with healthy young adults born appropriate for gestational age (AGA).

## Methods

**Subjects**

The study group consisted of 363 young adults born SGA, who had participated in one of three Dutch SGA trials (ISRCTN96883876, ISRCTN65230311 and ISRCTN18062389). SGA was defined as birth weight or birth length below –2 SDS for gestational age, with a height at the start of GH treatment below -2.5 SDS and no endocrine, metabolic or chronic disorders. GH

treatment was started before puberty or in early puberty and continued until attainment of AH. Subjects received GnRHa treatment for 2 years in addition to GH treatment (n=112; GnRHa/GH group) or only GH treatment (n=251; GH group). Two years of GnRHa treatment was prescribed when the expected AH was less than -2.5 SDS at start of puberty, based on Dutch references [20]. A subgroup (n=95) was randomly assigned to treatment with either GH 1 or 2 mg/m$^2$/day (~ 0.033 or 0.067 mg/kg/d) after stratification for sex, pubertal stage and parental height.

Data at 5 years after GH-cessation were compared with those of 145 young adults born AGA (birth length >-1 SDS) with a normal stature (>-1 SDS), aged 18 to 24 years [21,22]. These healthy young adults were recruited from different schools to participate as AGA controls.

The Medical Ethics Committee of the Erasmus University Medical Centre approved the studies. Due to ethical considerations, the Medical Ethics Committee did not allow a randomized untreated short SGA group until adult height. We obtained written informed consent from all subjects and, if they were younger than 18 years, from their parents or guardians.

**Measurements**

At start, every three months during GH treatment and at AH, height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain, Ltd. Crymmyth, UK). Height was transformed into SDS for sex and chronological age according to Dutch references, using Growth Analyser Research Calculation Tools (Growth Analyser B.V., Rotterdam, The Netherlands). AH SDS was calculated using references for Dutch adults, aged 18 years [20]. Weight was measured to the nearest 0.1kg (Servo Balance KA-20-150S). BMI was expressed as SDS adjusted for sex and chronological age, according to Dutch references [20].

**Insulin sensitivity and beta-cell function**

Glucose homeostasis was assessed by a frequently sampled intravenous glucose tolerance test (FSIGT) with Tolbutamide after an overnight fast (Cutfield WS et al. 1990). Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD Millennium software (Boston RC et al. 2003). Si quantifies the capacity of insulin to stimulate glucose disposal, and Sg reflects the capacity of glucose to mediate its 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 beta-cell function.

**Body composition and bone mineral density**

Total fat mass (FM), lean body mass (LBM), trunk fat (TF), limb fat (LF), bone mineral density of the total body ($BMD_{TB}$) and the lumbar spine ($BMD_{LS}$) was measured by a dual-energy x-ray absorptiometry (DXA) scan, on the same machine (Lunar Prodigy, GE Healthcare, Chalfont

St Giles, UK). Daily quality assurance was performed. The intra-assay coefficient of variation was 0.41-0.88% for fat tissue, 1.57-4.49% for LBM, 0.64% for $BMD_{TB}$ and 1.04% for the bone mineral density of the lumbar spine $BMD_{LS}$ [23,24].

In all subjects with short stature, true $BMD_{LS}$ is underestimated by the areal presentation and should be corrected for bone size by calculating the bone mineral apparent density ($BMAD_{LS}$) [25]. $BMAD_{LS}$ was calculated as follows: $BMAD_{LS} = BMD_{LS} * [4/(\pi * width)]$, with the width as the mean width of the second to fourth lumbar vertebral body. Because $BMD_{TB}$ and $BMAD_{LS}$ are dependent on age and gender, SDS were calculated, based on age- and gender-matched reference values from the Dutch population [26,27].



**Blood pressure**

After 10 minutes of rest, diastolic blood pressure (DBP) and systolic blood pressure (SBP) were measured repeatedly during half an hour in supine position, using the non-dominant arm with an automated device (Accutorr Plus, Datascope Corp., Montvale NJ, USA). The mean of 7 measurements was taken for analysis, to reflect resting blood pressure. Blood pressure values were adjusted for sex and height. Systolic and diastolic blood pressure values were expressed in SDS according to sex- and age-matched reference values [28].

**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).

Total cholesterol (TC) and triglyceride (TG) were measured using an automated enzymatic method with the CHOD-PAP reagent kit and with the GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein cholesterol (HDLc) was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein (LDLc) was calculated using the Friedewald formula: LDLc (mmol/l) = TC – HDLc – 0.45 * TG.

**Data analysis**

Statistical analyses were performed using SPSS version 25. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics are presented as means (SD); the Student's t-test was used to determine differences between subgroups. Because of a skewed distribution, Si, Sg, AIR and DI were log-transformed. Longitudinal changes in FSIGT results, body composition, blood pressure, serum lipid levels and BMD results were analyzed using repeated measurements analysis, with an unstructured covariance matrix. We used sex as a covariate, for the initial analysis of

longitudinal changes in FSIGT test results, body composition, lipid levels and BMD results. For longitudinal analyses on blood pressure, we adjusted for sex and age. ANCOVA was used for comparisons between the groups at the age twenty-one years, with sex as covariate for all variables. Body composition, blood pressure, $BMD_{TB}$ and $BMAD_{LS}$ were additionally adjusted for height. Results were regarded as statistically significant at $p<0.05$.

## Results

**Baseline characteristics**

Table 1 shows the clinical characteristics of all participants at start of GH treatment, at adult height (AH) and 21 years of age (5 years after GH-cessation). In total, 363 subjects (112 GnRHa/GH, 251 GH) participated in the study. Mean (SD) age at start of GnRHa treatment was 12.1 (1.0) years in boys and 11.2 (1.1) years in girls. In the GnRHa/GH and GH groups, mean age at start of GH treatment was 9.7 (3.1) vs 7.4 (2.8) years ($p<0.001$), respectively. At the attainment of AH, mean age in boys was 18.1 (0.9) vs 17.4 (1.2) years ($p=0.004$) and 16.4 (1.2) vs 15.7 (0.9) years ($p<0.001$) in girls, in the GnRHa/GH and GH groups, respectively.

At 5 years after GH-cessation, mean age was similar in the GnRHa/GH and GH group (22.4 (2.1) vs 21.9 (1.6) years, resp.). The GnRHa/GH group was significantly older than the AGA group (20.8 (2.7) years). The GnRHa/GH group had a different sex distribution (males 33%) when compared with the GH group (males 56%) and AGA group (males 46%) ($p=0.03$, $p=0.05$, resp.).

**GnRHa/GH versus GH only group during 5 years after cessation of GH**

*Insulin sensitivity and beta-cell function*

Table 2 and Figure 1a show the longitudinal changes in Si, Sg, AIR and DI after GH-cessation in the GnRHa/GH and GH group. Changes in Si, Sg, AIR and DI were similar in the GnRHa/GH and GH groups during the 5 years after GH-cessation ($p=0.39$, $p=0.07$, $p=0.79$, $p=0.92$, resp.). Additional adjustment for age did not change these results.

In the *GnRHa/GH group*, Si increased significantly during the first 2 years after GH-cessation, while the Sg and AIR decreased significantly (all $p<0.001$). In the following 3 years, Si, Sg and AIR remained similar. DI remained similar during the 5 years after GH-cessation. Similarly, in the *GH group,* during the first 2 years after GH-cessation Si increased, and both Sg and AIR decreased (all $p<0.001$). In the following 3 years, Si, Sg, and AIR remained similar. DI also remained similar during the 5-year follow-up period.

At 5 years after GH-cessation, the GnRHa/GH group had a similar Si, AIR and DI and a significantly lower Sg ($p=0.049$) than the GH group (Table 3). None of the participants in the GnRHa/GH group and GH group had glucose intolerance or developed type 2 diabetes, up to 5 years after GH-cessation.

**Table 1: Baseline characteristics.**

| | Study group | Comparison groups | |
|---|---|---|---|
| | **GnRHa/GH** *Means (SD)* | **GH** *Means (SD)* | **AGA** *Means (SD)* |
| N | 112 | 251 | 145 |
| Male/Female | 37/75†* | 140/111 | 67/78 |
| Gestational age, weeks | 36.8 (3.8) | 36.4 (3.7) | 36.6 (4.1) |
| Birth length SDS | -2.8 (1.3)* | -3.1 (1.5) | 0.2 (0.8) |
| Birth weight SDS | -2.2 (1.4)* | -2.2 (1.1) | 0.3 (1.2) |
| Age at start GnRHa, years | 11.5 (1.2) | N/A | N/A |
| Age at start GH, years | 9.7 (3.1)† | 7.4 (2.8) | N/A |
| BMI at start puberty, SDS | -1.0 (1.0) | -0.8 (1.0) | N/A |
| ***At adult height*** | | | |
| Age, years | 17.0 (1.3) | 16.7 (1.4) | N/A |
| Height SDS | -1.7 (0.8) | -1.5 (0.8) | N/A |
| BMI SDS | -0.2 (1.1) | -0.1 (1.0) | N/A |
| SBP SDS | 0.2 (0.9)† | 0.0 (0.8) | N/A |
| DBP SDS | 0.6 (0.7)† | 0.1 (0.7) | N/A |
| GH duration, years | 7.1 (2.3)† | 9.3 (2.6) | N/A |
| $BMD_{TB}$ SDS | -0.7 (0.9) | -0.5 (1.0) | N/A |
| $BMAD_{LS}$ SDS | -0.2 (1.2) | -0.2 (1.0) | N/A |
| ***At adult height*** | | | |
| Male/Female | 16/35†* | 70/72 | 67/78 |
| Age, years | 22.4 (2.1)* | 21.9 (1.6) | 20.8 (2.7) |
| Height SDS | -1.5 (0.8)* | -1.4 (0.8) | 0.3 (0.8) |
| BMI SDS | -0.3 (1.2) | -0.3 (1.4) | 0.0 (1.1) |
| $BMD_{TB}$ SDS | -0.3 (0.9)† | -0.6 (1.0) | -0.3 (0.8) |
| $BMAD_{LS}$ SDS | -0.2 (1.1)† | -0.4 (0.8) | -0.5 (1.0) |

Values are presented as means (SD). GH, growth hormone; GnRHa, gonadotropin-releasing hormone agonist; SDS, standard deviation score; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
†p<0.05 compared to GH;
*p<0.05 compared to AGA

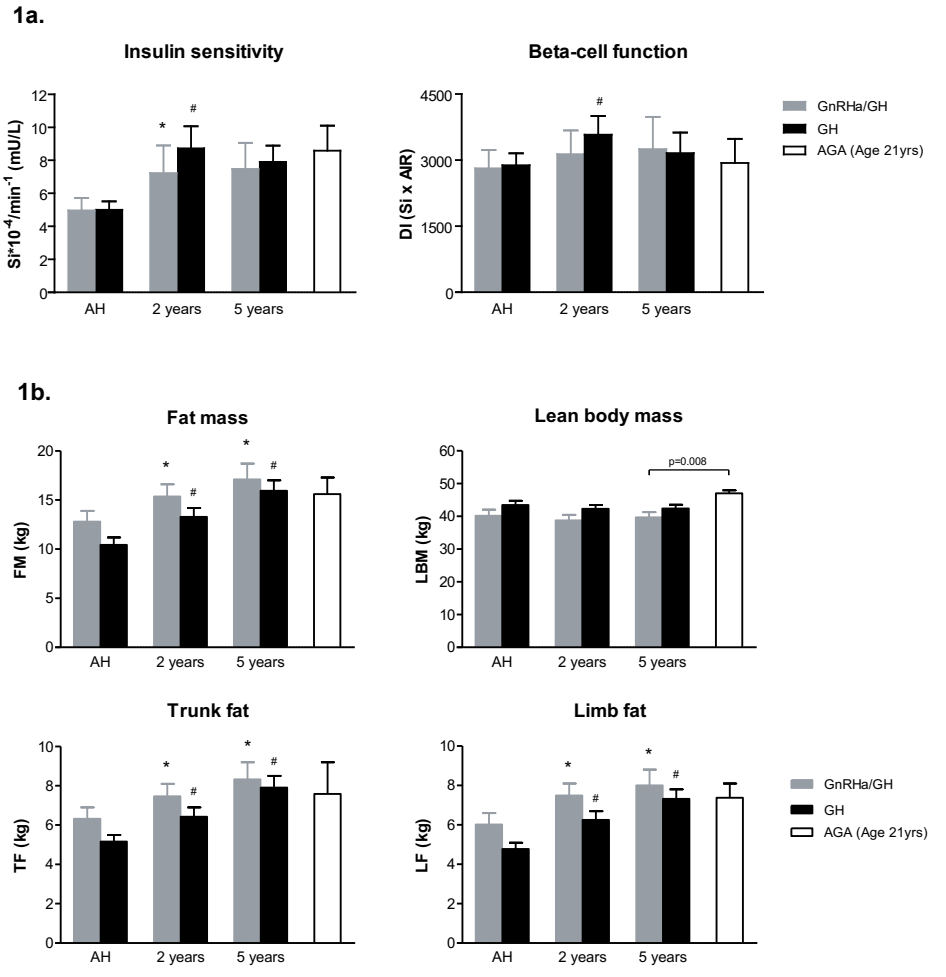


**Figure 1:** Longitudinal changes during 5 years after GH-cessation in FSIGT results (Figure 1a) and body composition (Figure 1b). Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for sex. P-values for the comparison between groups at 5 years after GH-cessation were depicted if p-values <0.05. Abbreviations: AH, Adult Height; SI, Insulin Sensitivity; DI, Disposition Index; AIR, Acute Insulin Response; GH, Growth Hormone; GnRHa, Gonadotropin-releasing Hormone Agonist; AGA, Appropriate for Gestational Age.

*P-value <0.05 compared to previous visit in GnRHa/GH-treated young adults

# P-value <0.05 compared to previous visit in GH-treated young adults

## Body composition

Table 2 and Figure 1b show the longitudinal changes in body composition after GH-cessation in the GnRHa/GH and GH group, measured by DXA-scan. Changes in FM, LBM, TF and LF were similar in the GnRHa/GH group and GH group during the 5-year follow-up period (p=0.26, p=0.08, p=0.24, p=0.10, resp.). Additional adjustment for age did not change the results.

In the *GnRHa/GH group*, FM, LF and TF increased consistently and significantly during the 5 years after GH-cessation (all p<0.001), whereas LBM remained similar during this follow-up period. Also, in the *GH group*, FM, LF and TF increased consistently and significantly, while LBM remained similar during this 5-year follow-up period.

At 5 years after GH-cessation, FM, LBM, TF and LF were similar in the GnRHa/GH and GH group (Table 3). Mean (SD) BMI SDS was -0.3 (1.2) in the GnRHa/GH group, which was similar in the GH group (-0.3 (1.4), p=0.90).

**Blood pressure**

Table 2 and Figure 2a show the longitudinal changes in SBP and DBP in the GnRHa/GH and GH groups. The GnRHa/GH group had higher SBP and DBP values at GH-cessation, but changes in SBP and DBP during the 5 years after GH-cessation were similar in both groups (p=0.84, p=0.43, resp.).



In the *GnRHa/GH group,* SBP and DBP remained similar and within normal range (from 115.6 to 116.4 mmHg, p=0.06 and from 68.9 to 70.0 mmHg, p=0.27, resp.) during 5 years after GH-cessation. In the *GH group,* both SBP and DBP remained well within the normal range but increased significantly during the 5-year follow-up period (from 112.6 to 116.0 mmHg and from 64.0 to 66.3 mmHg, resp., both p<0.001).

At 5 years after GH-cessation, SBP and DBP were still well within the normal range in both groups. The GnRHa/GH group had similar SBP (119.5 mmHg) and higher DBP (71.0 mmHg) when compared with the GH group (117.6 and 67.7 mmHg; p=0.40 and p=0.01, resp.) (Table 3).

**Serum lipid levels**

Table 2 and Figure 2b show the longitudinal changes in serum lipid levels in the GnRHa/GH and GH groups. Changes in TC, HDLc, LDLc and TG during the 5 years after GH-cessation were similar in both groups (p=0.39, p=0.71, p=0.40, p=0.18, resp.). Additional adjustment for age and FM did not change these results.

In the *GnRHa/GH group,* TC, HDLc and LDLc increased significantly (p<0.001, p=0.008, p<0.001, resp.), while TG remained similar during the 5 years after GH-cessation (p=0.50). In the *GH group,* TC and LDLc also increased significantly (p<0.001), but HDLc and TG remained similar during this 5-year follow-up period (p=0.26, p=0.98, resp.).

At 5 years after GH-cessation, the GnRHa/GH and GH group had similar serum lipid levels (TC: p=0.46; HDLc: p=0.38; LDLc: p=0.36; TG: p=0.10).

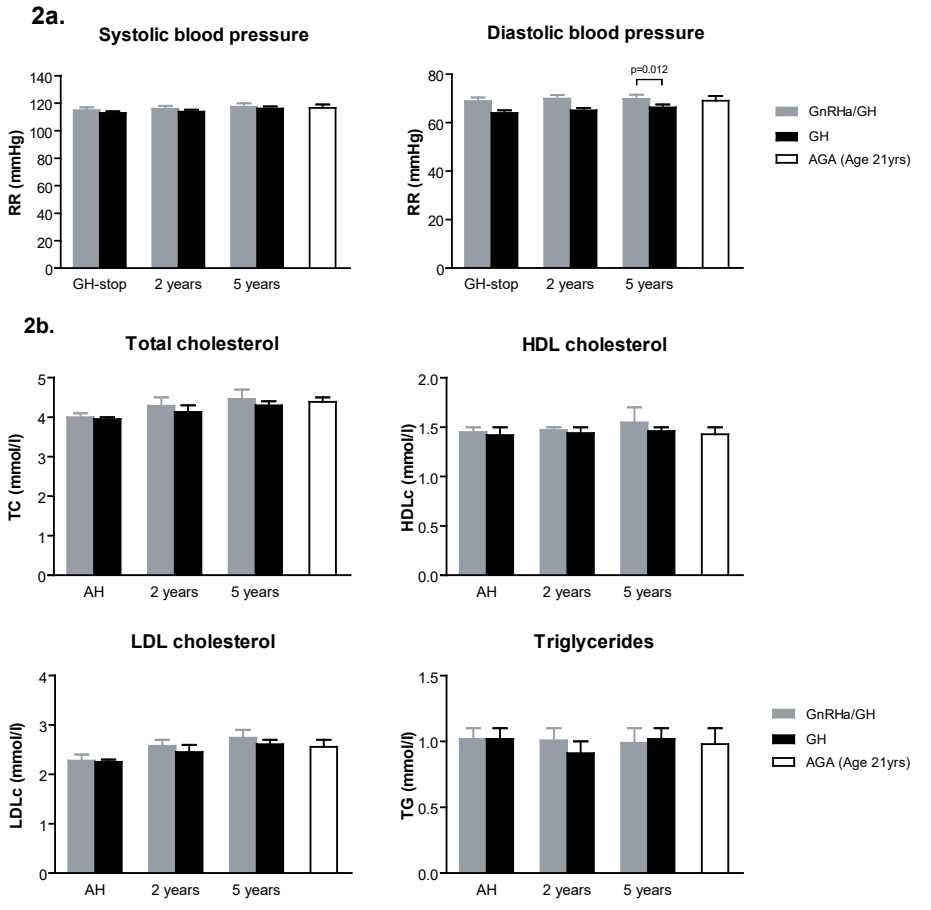


**Figure 2:** Longitudinal changes during 5 years after GH-cessation in blood pressure (Figure 2a) and serum lipid levels (Figure 1b). Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for sex. P-values for the comparison between groups at 5 years after GH-cessation were depicted if p-values <0.05. Abbreviations: AH, Adult Height; HDL, High-Density Lipoprotein; LDL, Low-Density Lipoprotein; GH, Growth Hormone; GnRHa, Gonadotropin-releasing Hormone Agonist; AGA, Appropriate for Gestational Age.

## Bone mineral density

Table 2 shows the longitudinal changes in $BMD_{TB}$ SDS and $BMAD_{LS}$ SDS in the GnRHa/GH and GH groups. Changes in $BMD_{TB}$ SDS and $BMAD_{LS}$ SDS during the 5 years after GH-cessation were similar in both groups (p=0.182 and p=0.081, resp.).

In the *GnRHa/GH group,* $BMD_{TB}$ SDS increased significantly (p=0.009) and $BMAD_{LS}$ remained similar (p=0.89). In the *GH group,* both the $BMD_{TB}$ and $BMAD_{LS}$ remained similar (p=0.33 and p=0.85, resp.) during the 5 years after GH-cessation.

At 5 years after GH-cessation, the GnRHa/GH group had a similar $BMD_{TB}$ and $BMAD_{LS}$, compared to the GH group (-0.29 vs -0.60 SDS, p=0.170 and -0.26 vs -0.44, p=0.053, resp.).

**Table 2: Longitudinal data during 5 years after GH-cessation.**

| | | GnRHa/GH | | GH | | Repeated MM |
|---|---|---|---|---|---|---|
| **Outcome** | **Study moment** | **EMM** | **95% CI** | **EMM** | **95% CI** | ***p-value*** |
| Insulin sensitivity (mU/L) | *AH* | 4.963 | 4.20 – 5.72 | 5.008 | 4.49 – 5.52 | 0.391 |
| | *2 yrs after AH* | 7.222 | 5.53 – 8.91 | 8.721 | 7.37 – 10.07 | |
| | *5 yrs after AH* | 7.471 | 5.88 – 9.06 | 7.902 | 6.90 – 8.90 | |
| Glucose effectiveness (mg/dL) | *AH* | 0.019 | 0.018 – 0.021 | 0.018 | 0.017 – 0.019 | 0.072 |
| | *2 yrs after AH* | 0.021 | 0.019 – 0.022 | 0.021 | 0.020 – 0.023 | |
| | *5 yrs after AH* | 0.019 | 0.016 – 0.022 | 0.021 | 0.020 – 0.023 | |
| Acute insulin response (mU/L) | *AH* | 765.1 | 659.0 – 871.1 | 679.7 | 608.3 – 751.1 | 0.794 |
| | *2 yrs after AH* | 639.0 | 537.9 – 740.1 | 570.1 | 495.3 – 644.9 | |
| | *5 yrs after AH* | 587.7 | 473.4 – 702.1 | 555.3 | 480.3 – 630.2 | |
| Disposition index | *AH* | 2815.1 | 2404 – 3227 | 2880.6 | 2604 – 3157 | 0.921 |
| | *2 yrs after AH* | 3131.3 | 2589 – 3673 | 3574.6 | 3146 – 4002 | |
| | *5 yrs after AH* | 3248.3 | 2516 – 3980 | 3159.4 | 2694 – 3625 | |
| Fat mass (in kg) | *AH* | 12.80 | 11.7 – 13.9 | 10.44 | 9.7 – 11.2 | 0.256 |
| | *2 yrs after AH* | 15.35 | 14.1 – 16.6 | 13.29 | 12.4 – 14.2 | |
| | *5 yrs after AH* | 17.11 | 15.5 – 18.7 | 15.94 | 14.9 – 17.0 | |
| Lean body mass (in kg) | *AH* | 40.18 | 38.4 – 42.0 | 43.44 | 42.2 – 44.7 | 0.075 |
| | *2 yrs after AH* | 38.80 | 37.2 – 40.4 | 42.30 | 41.2 – 43.4 | |
| | *5 yrs after AH* | 39.66 | 38.0 – 41.3 | 42.38 | 41.3 – 43.5 | |
| Trunk fat (in kg) | *AH* | 6.32 | 5.8 – 6.9 | 5.16 | 4.8 – 5.5 | 0.235 |
| | *2 yrs after AH* | 7.46 | 6.8 – 8.1 | 6.43 | 6.0 – 6.9 | |
| | *5 yrs after AH* | 8.33 | 7.4 – 9.2 | 7.90 | 7.3 – 8.5 | |
| Limb fat (in kg) | *AH* | 6.02 | 5.5 – 6.6 | 4.77 | 4.4 – 5.1 | 0.096 |
| | *2 yrs after AH* | 7.49 | 6.9 – 8.1 | 6.25 | 5.8 – 6.7 | |
| | *5 yrs after AH* | 8.00 | 7.2 – 8.8 | 7.32 | 6.8 – 7.8 | |
| SBP (mmHg) | *AH* | 114.94 | 112.9 – 116.9 | 112.85 | 111.5 – 114.2 | 0.837 |
| | *2 yrs after AH* | 116.01 | 114.0 – 118.1 | 113.90 | 112.5 – 115.3 | |
| | *5 yrs after AH* | 117.39 | 114.8 – 119.9 | 116.09 | 114.5 – 117.7 | |
| DBP (mmHg) | *AH* | 68.87 | 67.4 – 70.4 | 64.03 | 63.0 – 65.1 | 0.426 |
| | *2 yrs after AH* | 69.87 | 68.4 – 71.4 | 65.07 | 64.1 – 66.1 | |
| | *5 yrs after AH* | 69.77 | 67.9 – 71.6 | 66.30 | 65.1 – 67.5 | |
| TC (mmol/L) | *AH* | 4.00 | 3.9 – 4.1 | 3.95 | 3.9 – 4.0 | 0.391 |
| | *2 yrs after AH* | 4.29 | 4.1 – 4.5 | 4.13 | 4.0 – 4.3 | |
| | *5 yrs after AH* | 4.46 | 4.3 – 4.7 | 4.30 | 4.2 – 4.4 | |
| HDLc (mmol/L) | *AH* | 1.45 | 1.4 – 1.5 | 1.42 | 1.4 – 1.5 | 0.724 |
| | *2 yrs after AH* | 1.47 | 1.4 – 1.5 | 1.44 | 1.4 – 1.5 | |
| | *5 yrs after AH* | 1.55 | 1.4 – 1.7 | 1.46 | 1.4 – 1.5 | |
| LDLc (mmol/L) | *AH* | 2.28 | 2.2 – 2.4 | 2.25 | 2.2 – 2.3 | 0.395 |
| | *2 yrs after AH* | 2.58 | 2.4 – 2.7 | 2.45 | 2.3 – 2.6 | |
| | *5 yrs after AH* | 2.74 | 2.6 – 2.9 | 2.61 | 2.5 – 2.7 | |
| TG (mmol/L) | *AH* | 1.02 | 0.9 – 1.1 | 1.02 | 1.0 – 1.1 | 0.184 |
| | *2 yrs after AH* | 1.01 | 0.9 – 1.1 | 0.91 | 0.8 – 1.0 | |
| | *5 yrs after AH* | 0.99 | 0.9 – 1.1 | 1.02 | 1.0 – 1.1 | |

Results of repeated measurements analysis, presented as estimated marginal means with 95% CI, and p-values for repeated measurements in the 0 – 5-year follow-up. All variables were corrected for sex; blood pressure was additionally adjusted for age. Abbreviations: EMM, estimated marginal mean; CI, confidence interval; GH, growth hormone; GnRHa, gonadotropin-releasing hormone agonist.

**GnRHa/GH versus AGA group at 21 years of age**

*Insulin sensitivity and beta-cell function measured by FSIGT*

The GnRHa/GH group had a similar Si, Sg, AIR and DI as the AGA group (Table 3, Figure 1a). None of the participants of the GnRHa/GH, GH or AGA group had diabetes mellitus at 21 years of age.

*Body composition*

FM, TF and LF were similar in the GnRHa/GH and AGA group, but LBM was lower in the GnRHa/GH group (p=0.002) (Table 3, Figure 1b). The GnRHa/GH group had a similar mean BMI SDS as the AGA group (-0.3 (1.2) vs 0.0 (1.1), p=0.08). Only 1 participant of the GnRHa/GH group (0.8%) had a BMI above 2 SDS, which did not significantly differ from the GH group (7 participants, 2.5%) and AGA group (4 participants, 2.8%).

*Blood pressure*

The GH/GnRHa group had similar SBP and DBP compared with the AGA group (p=0.17, p=0.23, resp.) (Table 3, Figure 2a). The number of participants with a high SBP (above 140 mmHg) or high DBP (above 90 mmHg) at age 21 years of age, did not differ between the GnRHa/GH, GH, and AGA group. High SBP: GnRHa/GH: 2 (3.9%); GH: 5 (3.5%); AGA: 4 (2.8%), and high DBP: GnRHa/GH: 2 (3.9%); GH: 1 (0.7%); AGA: 0 (0%).

*Serum lipid levels*

The GnRHa/GH group had similar serum lipid levels as the AGA group (Table 3, Figure 2b). Additional adjustment for body fat did not change the results.

*Bone mineral density*

The GnRHa/GH group had a similar $BMD_{TB}$ SDS (p=0.60) and a higher $BMAD_{LS}$ compared to the AGA group (-0.26 vs -0.58 SDS, p=0.009) (Table 3).

**Effect of treatment with 2 versus 1 mg GH/$m^2$/day at 21 years of age**

A subgroup of participants (n=95) was randomly assigned to receive either 2 or 1 mg GH/m2/day from start of puberty until GH-cessation (data not shown). At 5 years after GH-cessation, those treated with 2 mg GH/$m^2$/day had a significantly higher LBM (p=0.04) than those treated with GH 1 mg GH/$m^2$/day. In the participants who had received 2 mg GH/$m^2$/day, FM and TF were lower, and LBM was higher compared to those who were treated with 1 mg GH/$m^2$/day (p=0.06, p=0.09, p=0.07, resp.). FSIGT results, limb fat, blood pressure, serum lipid levels and BMD were similar in both GH-dose groups.

**Table 3: Comparison between the groups at age 21 years.**

| | GnRHa/GH *EMM (95%CI)* | GH *EMM (95%CI)* | *p-value** | AGA *EMM (95%CI)* | *p-value‡* |
|---|---|---|---|---|---|
| ***Glucose metabolism*** | | | | | |
| Glucose effectiveness | 0.019 (0.017 – 0.022) | 0.021 (0.020 – 0.023) | **0.049** | 0.018 (0.016 – 0.020) | 0.888 |
| Insulin sensitivity | 8.03 (5.9 – 10.1) | 8.01 (6.7 – 9.3) | 0.228 | 8.585 (7.1 – 10.1) | 0.766 |
| Acute insulin response | 509.6 (386.3 – 633.1) | 572.0 (497.1 – 646.6) | 0.373 | 441.3 (350.4 – 532.1) | 0.885 |
| Disposition index † | 3171.7 (2428 – 3915) | 3217.8 (2768 – 3667) | 0.791 | 2935 (2388 – 3483) | 0.887 |
| ***Body composition*** | | | | | |
| Fat mass (kg) | 16.36 (14.0 – 18.7) | 16.76 (15.3 – 18.3) | 0.756 | 15.61 (14.0 – 17.3) | 0.649 |
| Lean body mass (kg) | 44.89 (43.7 – 46.1) | 44.18 (43.42 – 44.94) | 0.283 | 47.05 (46.2 – 47.9) | **0.008** |
| Trunk fat (kg) | 8.19 (6.9 – 9.5) | 8.33 (7.5 – 9.2) | 0.851 | 7.59 (6.5 – 9.2) | 0.494 |
| Limb fat (kg) | 7.60 (6.5 – 8.7) | 7.70 (7.0 – 8.4) | 0.860 | 7.38 (6.6 – 8.1) | 0.771 |
| ***Blood pressure*** | | | | | |
| Systolic (mmHg) | 119.5 (116 – 122) | 117.6 (116 – 120) | 0.259 | 116.6 (114 – 119) | 0.167 |
| Diastolic (mmHg) | 71.0 (69 – 73) | 67.7 (66 – 71) | **0.012** | 69.0 (67 – 71) | 0.226 |
| ***Serum lipid levels*** | | | | | |
| TC (mmol/L) | 4.40 (4.2 – 4.6) | 4.30 (4.2 – 4.6) | 0.460 | 4.39 (4.3 – 4.5) | 0.977 |
| HDLc (mmol/L) | 1.53 (1.4 – 1.6) | 1.46 (1.4 – 1.6) | 0.382 | 1.43 (1.4 – 1.5) | 0.117 |
| LDLc (mmol/L) | 2.70 (2.5 – 2.9) | 2.59 (2.5 – 2.7) | 0.357 | 2.56 (2.4 – 2.7) | 0.257 |
| TG (mmol/L) | 0.91 (0.8 – 1.0) | 1.03 (1.0 – 1.2) | 0.101 | 0.98 (0.9 – 1.1) | 0.356 |

Results of the ANCOVA analysis between GnRHa/GH, GH and AGA at 21 years of age. All variables were corrected for sex; body composition was additionally adjusted for height, blood pressure was additionally adjusted for height. Abbreviations: EMM, estimated marginal mean; CI, confidence interval; GH, growth hormone; GnRHa, gonadotropin-releasing hormone agonist; AGA, appropriate for gestational age.
*P-value for the comparison between GnRHa/GH-treated and GH-treated young adults born SGA;
‡ P-value for the comparison between GnRHa/GH-treated young adults born SGA and young adults born AGA.
†A measure of β-cell function, calculated as insulin sensitivity × acute insulin response.

## Discussion

This longitudinal study during the 5 years after discontinuation of GH treatment is currently the longest follow-up study in a large group of young adults born SGA who were treated during childhood with 2 years of GnRHa in addition to GH treatment. We show that 2 years of GnRHa treatment in addition to GH treatment does not change the metabolic health profile, in terms of insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and BMD. At 21 years of age, the GnRHa/GH group had a similar metabolic health profile and bone mineral density of the total body as the GH-only and AGA group and a higher bone mineral density of the lumbar spine compared to the AGA group.

Our study shows that insulin sensitivity and beta-cell function increased similarly during the first 2 years after GH-cessation in both GnRHa-treated young adults and those treated with GH only. We also found that the GnRHa/GH group had similar FSIGT results as healthy young adults born AGA. A cross-sectional retrospective study by Lazar et al. also showed no

metabolic derangements in GnRHa-treated female adults with central precocious puberty (CPP) aged 30-50 years [10]. Several studies during GnRHa treatment in children with CPP showed lower insulin sensitivity, expressed as HOMA-IR [5-8]. This might be explained by the difference in population, as early puberty also increases the risk of diabetes [17]. Our study shows that the addition of 2 years of GnRHa treatment during childhood does not influence insulin sensitivity and beta-cell function in young adults born SGA.

We found no higher prevalence of obesity and a similar fat mass in the GnRHa/GH-treated young adults, compared with GH-only and healthy young adults born AGA. Several studies in CPP children described an increase in weight and BMI during GnRHa treatment, which could negatively influence metabolic health and cause a higher prevalence of obesity in adulthood [9-13]. However, studies in SGA and CPP children have also shown that an increase in BMI during GnRHa treatment did not lead to a higher rate of obesity at AH [10,17,29]. Furthermore, earlier pubertal timing has been associated with higher BMI and a higher prevalence of diabetes and metabolic disease, thus it is questionable whether the described alterations in metabolic health are due to CPP rather than GnRHa treatment. Earlier pubertal timing has been associated with higher BMI and a higher prevalence of diabetes and metabolic disease [30]. Our results show that 2 years of GnRHa treatment in addition to GH treatment during childhood does not have an adverse effect on body composition at the age of 21 years. This strengthens the hypothesis that the previously reported adverse effect on body composition in patients with CPP could be rather due the natural course of body composition after CPP than due to GnRHa treatment.

Our findings show that changes in SBP and DBP during 5 years after cessation of GH were similar in the GnRHa/GH and GH groups. Also, the GnRHa/GH-treated young adults had a similar SBP and DBP as healthy young adults born AGA. High blood pressure was only present in 3 participants of the GnRHa/GH group, which was similar in the GH-only and AGA group. Two case reports have described transient arterial hypertension during GnRHa treatment [31,32]. Both cases showed that blood pressure returned to normal values after cessation of GnRHa treatment. In our study, none of the participants had to cease their GnRHa treatment because of high arterial blood pressure. Our results show that in the GnRHa/GH group, both SBP and DBP remain well within the normal range and are similar to the GH group and young adults born AGA.

Serum lipid levels changed similarly in the GnRHa/GH and GH groups during 5 years after GH-cessation. At 21 years of age, the GnRHa/GH-treated young adults had similar serum TC, HDLc, LDLc and TG levels as GH-treated young adults born SGA and healthy young adults born AGA. This is in line with other studies performed during GnRHa treatment in CPP children, reporting no changes in lipid levels [6,33]. Our results in young adults show that serum lipid levels are not different due to 2 years of GnRHa treatment during childhood.

We have previously reported that the control group of young adults born SGA treated with GH-only, had a similar metabolic health profile as untreated young adults born SGA [34,35]. As the results in the present study are similar between the GnRHa/GH and GH groups, it is likely that metabolic health after 2 additional years of GnRHa treatment to GH treatment is also similar to untreated young adults born SGA.

During 5 years after GH-cessation, $BMD_{TB}$ and $BMAD_{LS}$ changed similarly in the GnRHa/GH and GH group and both groups had a similar $BMD_{TB}$ and $BMAD_{LS}$ at 5 years after GH-cessation. This is in line with studies in subjects with central precocious puberty, describing a similar BMD at adult height in GnRHa-treated subjects and controls [9,19,36,37]. Our findings show that 2 years of GnRHa treatment in addition to GH treatment has no negative effects on BMD in young adults born SGA.



In conclusion, our longitudinal follow-up study in young adults born SGA during the 5 years after GH-cessation shows that the changes in insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and BMD were unaffected by the addition of 2 years of GnRHa treatment for postponement of puberty. At 21 years of age, insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and BMD were similar in GnRHa/GH-treated compared to GH-treated young adults born SGA and untreated young adults born AGA. These results show that the addition of 2 years of GnRHa treatment to long-term GH treatment of short children born SGA does not have an unfavorable effect on metabolic health and bone mineralization at the age of 21 years.

## Acknowledgements

We express our gratitude to all children and their parents who participated in this study. We thank J. Bontenbal-van de Wege, C. Bruinings-Vroombout, N. Khieroe and E. Lems, research nurses, for their contribution to the study, and all the collaborating paediatric-endocrinologists of the Dutch Advisory Group on GH Therapy and the paediatricians who referred patients to participate in this study. We acknowledge the investigator-initiated independent research grants provided by Novo Nordisk BV and Pfizer BV, The Netherlands. The PROGRAM/PREMS study was financially supported by Netherlands Organisation for Scientific Research (ACSH-K received the ASPASIA-award, grant number 015 000 088).

## References


1. Sas T, de Waal W, Mulder P, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab.* 1999;84(9):3064-3070.
2. Dahlgren J, Wikland KA, Swedish Study Group for Growth Hormone T. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57(2):216-222.
3. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
4. Lem AJ, van der Kaay DC, de Ridder MA, et al. Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analog: results of a randomized, dose-response GH trial. *J Clin Endocrinol Metab.* 2012;97(11):4096-4105.
5. Chiavaroli V, Liberati M, D'Antonio F, et al. GNRH analog therapy in girls with early puberty is associated with the achievement of predicted final height but also with increased risk of polycystic ovary syndrome. *Eur J Endocrinol.*163(1):55-62.
6. Sorensen K, Mouritsen A, Mogensen SS, Aksglaede L, Juul A. Insulin sensitivity and lipid profiles in girls with central precocious puberty before and during gonadal suppression. *J Clin Endocrinol Metab.* 2010;95(8):3736-3744.
7. Tascilar ME, Bilir P, Akinci A, et al. The effect of gonadotropin-releasing hormone analog treatment (leuprolide) on body fat distribution in idiopathic central precocious puberty. *Turk J Pediatr.* 2011;53(1):27-33.
8. Faienza MF, Brunetti G, Acquafredda A, et al. Metabolic Outcomes, Bone Health, and Risk of Polycystic Ovary Syndrome in Girls with Idiopathic Central Precocious Puberty Treated with Gonadotropin-Releasing Hormone Analogues. *Horm Res Paediatr.* 2017;87(3):162-169.
9. Magiakou MA, Manousaki D, Papadaki M, et al. The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. *J Clin Endocrinol Metab.* 2010;95(1):109-117.
10. Lazar L, Lebenthal Y, Yackobovitch-Gavan M, et al. Treated and untreated women with idiopathic precocious puberty: BMI evolution, metabolic outcome, and general health between third and fifth decades. *J Clin Endocrinol Metab.* 2015;100(4):1445-1451.
11. Aguiar AL, Couto-Silva AC, Vicente EJ, Freitas IC, Cruz T, Adan L. Weight evolution in girls treated for idiopathic central precocious puberty with GnRH analogues. *J Pediatr Endocrinol Metab.* 2006;19(11):1327-1334.
12. van der Sluis IM, Boot AM, Krenning EP, Drop SL, de Muinck Keizer-Schrama SM. Longitudinal follow-up of bone density and body composition in children with precocious or early puberty before, during and after cessation of GnRH agonist therapy. *J Clin Endocrinol Metab.* 2002;87(2):506-512.

13. Park HK, Lee HS, Ko JH, Hwang IT, Lim JS, Hwang JS. The effect of gonadotrophin-releasing hormone agonist treatment over 3 years on bone mineral density and body composition in girls with central precocious puberty. *Clin Endocrinol (Oxf).* 2012;77(5):743-748.
14. Boot AM, De Muinck Keizer-Schrama S, Pols HA, Krenning EP, Drop SL. Bone mineral density and body composition before and during treatment with gonadotropin-releasing hormone agonist in children with central precocious and early puberty. *J Clin Endocrinol Metab.* 1998;83(2):370-373.
15. Saggese G, Bertelloni S, Baroncelli GI, Battini R, Franchi G. Reduction of bone density: an effect of gonadotropin releasing hormone analogue treatment in central precocious puberty. *Eur J Pediatr.* 1993;152(9):717-720.
16. Antoniazzi F, Bertoldo F, Zamboni G, et al. Bone mineral metabolism in girls with precocious puberty during gonadotrophin-releasing hormone agonist treatment. *Eur J Endocrinol.* 1995;133(4):412-417.
17. van der Steen M, Lem AJ, van der Kaay DC, et al. 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. *J Clin Endocrinol Metab.* 2015;100(10):3725-3734.
18. van der Steen M, Lem AJ, van der Kaay DC, Hokken-Koelega AC. Insulin Sensitivity and beta-Cell Function in SGA Children Treated With GH and GnRHa: Results of a Long-Term Trial. *J Clin Endocrinol Metab.* 2016;101(2):705-713.
19. Lem AJ, van der Kaay DC, Hokken-Koelega AC. Bone mineral density and body composition in short children born SGA during growth hormone and gonadotropin releasing hormone analog treatment. *J Clin Endocrinol Metab.* 2013;98(1):77-86.
20. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
21. Kerkhof GF, Breukhoven PE, Leunissen RW, Willemsen RH, Hokken-Koelega AC. Does preterm birth influence cardiovascular risk in early adulthood? *J Pediatr.* 2012;161(3):390-396 e391.
22. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301(21):2234-2242.
23. Johnson J, Dawson-Hughes B. Precision and stability of dual-energy X-ray absorptiometry measurements. *Calcif Tissue Int.* 1991;49(3):174-178.
24. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P. The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res.* 2004;12(12):1925-1929.
25. Kroger H, Vainio P, Nieminen J, Kotaniemi A. Comparison of different models for interpreting bone mineral density measurements using DXA and MRI technology. *Bone.* 1995;17(2):157-159.
26. Boot AM, Bouquet J, de Ridder MA, Krenning EP, de Muinck Keizer-Schrama SM. Determinants of body composition measured by dual-energy X-ray absorptiometry in Dutch children and adolescents. *Am J Clin Nutr.* 1997;66(2):232-238.

27. van der Sluis IM, de Ridder MA, Boot AM, Krenning EP, de Muinck Keizer-Schrama SM. Reference data for bone density and body composition measured with dual energy x ray absorptiometry in white children and young adults. *Arch Dis Child.* 2002;87(4):341-347; discussion 341-347.
28. Rosner B, Prineas RJ, Loggie JM, Daniels SR. Blood pressure nomograms for children and adolescents, by height, sex, and age, in the United States. *The Journal of pediatrics.* 1993;123(6):871-886.
29. Chiocca E, Dati E, Baroncelli GI, et al. Body mass index and body composition in adolescents treated with gonadotropin-releasing hormone analogue triptorelin depot for central precocious puberty: data at near final height. *Neuroendocrinology.* 2009;89(4):441-447.
30. Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. *Int J Obes (Lond).* 2013;37(8):1036-1043.
31. Sifaki L, Cachat F, Theintz G, Chehade H. Transient Arterial Hypertension Induced by Gonadotropin-Releasing Hormone Agonist Treatment for Central Precocious Puberty. *Front Pediatr.* 2019;7:74.
32. Palma L, Gaudino R, Cavarzere P, Antoniazzi F. Does the risk of arterial hypertension increase in the course of triptorelin treatment? *Journal of pediatric endocrinology & metabolism : JPEM.* 2018.
33. Arcari AJ, Freire AV, Escobar ME, et al. One-year treatment with gonadotropin-releasing hormone analogues does not affect body mass index, insulin sensitivity or lipid profile in girls with central precocious puberty. *Journal of pediatric endocrinology & metabolism : JPEM.* 2019;32(2):181-186.
34. van der Steen M, Kerkhof GF, Smeets CCJ, Hokken-Koelega ACS. Cardiovascular risk factors and carotid intima media thickness in young adults born small for gestational age after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(12):975-985.
35. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.
36. Alessandri SB, Pereira Fde A, Villela RA, et al. Bone mineral density and body composition in girls with idiopathic central precocious puberty before and after treatment with a gonadotropin-releasing hormone agonist. *Clinics (Sao Paulo).* 2012;67(6):591-596.
37. Heger S, Partsch CJ, Sippell WG. Long-term outcome after depot gonadotropin-releasing hormone agonist treatment of central precocious puberty: final height, body proportions, body composition, bone mineral density, and reproductive function. *J Clin Endocrinol Metab.* 1999;84(12):4583-4590.

# Chapter 6

## SGA-born adults with postnatal catch-up have a persistently unfavorable metabolic health profile and increased adiposity at age 32 years

W.J. Goedegebuure
M. van der Steen
C.C.J. Smeets
G.F. Kerkhof
A.C.S. Hokken-Koelega

*Submitted*

## Abstract

**Background:** Twenty-one-year-old adults born small for gestational age (SGA) with catch-up growth during the first year of life had insulin resistance, adverse lipid profile and significantly higher fat mass (FM) than those born appropriate for gestational age (AGA). Would this worsen over the years and further increase their risk of cardiovascular and metabolic diseases in later life?

**Methods:** We longitudinally investigated 287 adults, 170 SGA with catch-up growth (SGA-CU) or persistent short stature (SGA-S) and 117 AGA, at age 21 and 32 years. Insulin sensitivity (Si) and β-cell function was measured by frequently-sampled intravenous glucose tolerance test, body composition by DXA-scan, and abdominal adipose tissue and liver fat fraction by MRI-scan. Also, fasting serum lipid levels and blood pressure were measured.

**Results:** At age 32 years, SGA-CU had lower Si than AGA ($p=0.030$), while SGA-S had similar Si as AGA. FM and trunk fat were higher in SGA-CU than AGA ($p=0.033$, $p=0.024$, resp.), while SGA-S had lower lean body mass than SGA-CU and AGA ($p=0.001$ and $p<0.001$, resp.). SGA-CU had significantly higher levels of adverse lipids than AGA. Beta-cell function, visceral fat, liver fat fraction and blood pressure were similar in all groups. Metabolic health parameters in SGA-CU and SGA-S did not worsen more compared to AGA during 11 years of follow-up. Gain in weight SDS from birth to age 32 years was associated with a higher risk of developing metabolic syndrome at age 32 years.

**Conclusion:** At age 32 years, SGA-CU adults had insulin resistance, higher FM with central adiposity and an adverse lipid profile. Postnatal catch-up growth increases the risk of developing cardiovascular disease and diabetes mellitus type II, therefore accelerated weight gain should be prevented in SGA-born children.

## Introduction

Low birth weight has been associated with adult diseases like diabetes mellitus type II and cardiovascular diseases [1]. The PROgramming factors for GRowth And Metabolism (PROGRAM) study showed that 21-year-old adults born small for gestational age (SGA) with postnatal catch-up had insulin resistance, higher fat mass (FM) and adverse lipid profile, but similar blood pressure compared to those born appropriate for gestational age (AGA) [2-5]. As 90-95% of children born SGA demonstrate catch-up in weight and length [6,7], subjects born SGA might have an increased risk for developing obesity and cardiometabolic diseases in later life. In contrast, young adults born SGA with persistent short stature had similar insulin sensitivity, FM, lipid levels and blood pressure than AGA adults, but lower lean body mass (LBM) [3]. Insulin resistance and reduced β-cell function precede type II diabetes mellitus, and unfavorable body composition, central adiposity, high adverse serum lipid levels, blood pressure and liver fat fraction are components of the metabolic syndrome (MetS) [8,9]. Data on longitudinal metabolic and cardiovascular health in adults born SGA beyond age 30 years, either with or without postnatal catch-up, compared to age-matched adults born AGA did not exist.



For that reason, the PROGRAM32 study was initiated, investigating longitudinal metabolic and cardiovascular health in a large group of adults at age 21 and 32 years. The study population consisted of 32-year-old adults born SGA, with either spontaneous catch-up growth (SGA-CU) or persistent short stature (SGA-S), and adults born AGA with normal adult stature (AGA). Firstly, we investigated differences in metabolic health parameters between groups at age 32 years. We hypothesized that SGA-CU would have lower insulin sensitivity and β-cell function, adverse lipid profile and less favorable body composition, higher abdominal adipose tissue and liver fat fraction than SGA-S and AGA adults. Secondly, we hypothesized that these parameters would worsen from age 21 to 32 years in the SGA groups, and particularly in SGA-CU compared to AGA adults. Lastly, we investigated the associations of birth size, weight gain in standard deviation scores (SDS) from birth to 32 years and lifestyle factors with insulin sensitivity, body composition, central adiposity and components of MetS at age 32 years.

## Methods

### Subjects

The PROGRAM32 study investigated a cohort of 287 adults at age of 21 and 32 years. In order to increase the statistical power of the comparison between the SGA-CU, SGA-S and AGA group, the cut-off values for small birth size and short adult height were set at <-2 SDS and the cut-off values for normal birth size and normal adult height were set at >-1 SDS. This resulted in a study population consisting of three groups: 1) Adults born SGA (birth length

or birth weight <-2SDS) with spontaneous catch-up growth (adult height (AH) >-1SDS) (SGA-CU), 2) Adults born SGA (birth length or birth weight <-2SDS) with persistent short stature (AH <-2SDS) (SGA-S), and 3) Adults born AGA (birth length >-1 SDS) with normal stature (AH>-1SDS) (AGA). Included subjects were Caucasian, born term (gestational age (GA) > 36 weeks) and singleton, with an uncomplicated neonatal period without severe asphyxia, sepsis or long-term complications of respiratory ventilation or oxygen supply. Birth data were obtained from hospital records and primary health care records.

The Medical Ethics Committee of Erasmus University Medical Center approved the PROGRAM32 study, and all participants gave written informed consent.

**Anthropometric measurements**

Height was measured to nearest 0.1 cm using Harpenden stadiometer (Holtain, Ltd. Crymmyth, UK) and weight to nearest 0.1kg (Servo Balance KA-20-150S). Standard deviation (SD)-scores for birth length and birth weight were corrected for gestational age and sex [10]. SD-scores for AH and BMI were corrected for sex and chronological age [11], using Growth Analyser Research Calculation Tools (https://growthanalyser.org).

**Insulin sensitivity and beta-cell function**

Glucose homeostasis was assessed by frequently sampled intravenous glucose tolerance test (FSIGT) with Tolbutamide after an overnight fast [12]. Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD Millennium software [13]. Si stands for insulin sensitivity, and Sg reflects the capacity of glucose to mediate its disposal. AIR estimates insulin secretory capacity and DI equals AIR x Si, indicating beta-cell function [13].

**Assays**

Fasting glucose levels were determined with Architect ci8200 system (Abbott) and fasting insulin by IRMA (Medgenix, Biosource Europe), intra-assay and interassay coefficient of variation being 2.1% and 6.5%, respectively.

Total cholesterol (TC) and triglyceride (TG) were measured by automated enzymatic method with the CHOD-PAP reagent kit and GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein cholesterol (HDLc) was measured using a homogeneous enzymatic colourimetric assay (Roche Diagnostics) and low-density lipoprotein (LDLc) calculated by the Friedewald formula.

**Body composition**

Body composition was measured by DXA scan. All measurements were made with same machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) and software (enCORE

software version 14.1), with daily quality assurance. Intra-assay coefficient of variation was 0.41-0.88% for FM and 1.57-4.49% for LBM [14]. LBM was determined as fat-free mass minus bone mineral content.

**Abdominal visceral and subcutaneous adiposity and liver fat fraction**

Subjects underwent a magnetic resonance imaging (MRI) scan to measure visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and liver fat fraction at age 32 years. Scanning was performed on two 3T GE Discovery MR750systems (GE Healthcare, Milwaukee, US) with identical protocols. VAT and SAT measurements were performed by fast-spoiled gradient echo technique to acquire fat-only images in 20-second breath-hold scans. Cross-sectional area at the level of L3 was used, which correlated with total VAT and SAT volumes ($r>0.95$, $p<0.01$), using threshold-based region technique for slight adjustments [15]. Measurements of liver fat fraction were performed using the IDEAL-IQ technique. The intra-assay and interassay coefficients of variation were calculated for VAT (4.8%, 6.7%, resp.), SAT (0.9%, 4.2%, resp.) and liver fat fraction (9.5%, 16.8%, resp.). All measurements were performed in triplicate by one investigator (WG), and mean of measurements was used for analysis.



**Blood pressure**

After 10 minutes of rest, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured repeatedly during half an hour in supine position, using the non-dominant arm with an automated device (Accutorr Plus, Datascope, Montvale, USA). Mean of 7 measurements was taken for analysis, to reflect resting blood pressure.

**Socioeconomic status and lifestyle factors**

Adults provided information regarding lifestyle factors at age 32 years, by questionnaire. Total yearly income and highest completed education were used to determine socioeconomic status (SES) (1 = low, 2 = medium and 3 = high). The questionnaire assessed smoking (current smoking: light < 6 cigarettes/day, heavy ≥ 6 cigarettes/day, and history of smoking), alcohol consumption (1 = less than 1 unit/week, 2 = less than 1 unit/day, 3 = more than 1 unit/day), illicit drug use (frequency, amount and type of drugs), exercise level (frequency: 1 = less than 1 hour/month, 2 = 1 hour/ 2 weeks, 3 = 1-2 hours/week, 4 = 3-5 hours/week, 5 = more than 5 hours/week), pregnancies, number of deliveries and perception of general health (1 = poor, 2 = good, 3 = very good).

**Metabolic syndrome**

Revised criteria of National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of MetS [16,17]. MetS was defined as having three or more of following risk factors: Abdominal obesity (waist circumference): Men ≥ 102cm, women ≥ 88cm; Serum triglyceride levels: ≥ 1.7 mmol/L; HDLc: Men ≤ 1.03 mmol/L, women ≤ 1.3 mmol/L; Blood pressure: >130 / 85 mmHg; Fasting glucose: ≥ 5.6 mmol/L.

**Data analysis**

Statistical analyses were performed using SPSS version 25. Distribution of variables was determined by Kolmogorov-Smirnov test and normal Q-Q-plots. Clinical characteristics are presented as means (SD), differences between the groups were determined by ANOVA and Student's t-test.

We did a power calculation to calculate the sample size. In a previous study [12], the insulin sensitivity for adults born SGA with catch-up growth was mean 4.9 mU/L (4.0-5.8) and for healthy controls born AGA it was 6.8 mU/L (5.8-7.8), suggesting that healthy controls have an insulin sensitivity that is roughly 35% higher than in individuals born small for gestational age. Assuming equal means and an SD of 3.0, with a non-inferiority test (one-sided, $\alpha$=0.025, t test), a sample size of 47 participants in each group would have a power of 80%.

We used repeated measurement analysis, with an unstructured covariance matrix, to analyze each outcome measured at age 21 and 32, with group, time of measurement and their interaction as determinants. Using these models, we determined differences between the groups at age 32 years and analyze longitudinal changes from age 21 to 32 years. All models were adjusted for sex. Body composition, VAT, SAT, and blood pressure were additionally adjusted for AH SDS. Because of skewed distribution, Si, Sg, AIRg and DI were log-transformed, we transformed mean data used in the figures and tables back to original units.

Multiple regression analyses were performed to determine the association of birth size, catch-up in weight from birth to age 32 years and lifestyle factors with insulin sensitivity, body composition, VAT and SAT at age 32 years. In the first model, we investigated the association of birth size with Si, FM, LBM, VAT and SAT, corrected for age, sex and AH SDS. In the second model, we added adult weight and lifestyle factors (SES, smoking, alcohol, illicit drug use and exercise level). In the third model, we replaced adult weight SDS with adult FM and LBM, to investigate the association between body composition and Si at age 32 years.

Ordinal regression analyses were performed to determine associations of birth weight SDS, birth length SDS, gain in weight SDS from birth to age 32 years, and FM and lifestyle factors at age 32 years with number of MetS components per individual. All regression analyses were adjusted for age and sex. gain in weight SDS from birth to age 32 years and FM SDS were additionally adjusted for AH SDS. Results were regarded as statistically significant at $p<0.05$.

**Table 1: Clinical characteristics.**

| | SGA-S Means (SD) | SGA-CU Means (SD) | AGA Means (SD) | *p*-value |
|---|---|---|---|---|
| Number (Female) | 65 (39) | 105 (62) | 117 (69) | 0.990 |
| At birth | | | | |
| Gestational age, weeks | 39.2 (1.7) | 38.4 (1.6) | 39.6 (1.6) | **<0.001** |
| Birth length, SDS | -2.8 (1.0) * | -2.4 (1.1) * | 0.0 (0.8) | **<0.001** |
| Birth weight, SDS | -2.1 (0.8) * | -2.4 (0.7) * | 0.2 (1.0) | **<0.001** |
| At 21 years | | | | |
| Age, years | 20.7 (1.7) | 21.0 (1.6) | 20.7 (1.7) | 0.433 |
| Height, SDS | -2.4 (0.6) * | -0.1 (0.7) | 0.4 (0.8) | **<0.001** |
| BMI, SDS | 0.1 (1.4) | 0.2 (1.4) | -0.1 (0.9) | 0.210 |
| At 32 years | | | | |
| Age, years | 31.9 (3.2) | 32.6 (2.5) | 33.0 (2.8) | 0.136 |
| Gain in weight SDS (birth to age 32 years) | 1.0 (1.8) | 3.1 (1.3) * | 0.4 (1.2) | **<0.001** |
| BMI, SDS | 0.3 (1.7) | 0.8 (1.1) | 0.4 (1.2) | 0.168 |
| SES (Income) (%) | | | | |
| Low | 31.6% * | 8.2% | 6.5% | **0.002** |
| Middle | 44.7% | 34.7% | 27.4% | |
| High | 23.7% | 57.1% | 66.1% | |
| SES (Education) (%) | | | | |
| Low | 7.9% | 13.5% * | 4.7% | **0.001** |
| Middle | 52.6% * | 30.8% | 21.9% | |
| High | 39.5% | 55.8% | 73.4% | |
| Smoking (%) | | | | 0.057 |
| Light | 2.8% | 13.0% | 10.0% | |
| Heavy | 16.7% | 19.6% | 3.3% | |
| History | 5.2% | 7.5% | 18.4% | |
| Alcohol consumption (%) | | | | |
| <1/week | 47.3% | 43.4% | 32.3% | 0.499 |
| 1-6/week | 50% | 47.1% | 53.8% | |
| >1/day | 2.6% | 9.4% | 13.8% | |
| Illicit drug use (%) | | | | |
| Total | 13.2% | 22.6% | 12.3% | 0.269 |
| Marihuana | 11.1% | 13.0% | 3.3% | |
| Ecstasy | 0% | 15.2% | 10.0% | |
| Cocaine | 0% | 8.7% | 10.0% | |
| Exercise (%) | | | | |
| <1hr/month | 36.8% | 26.4% | 25.0% | 0.709 |
| 1hr/2 weeks | 5.3% | 5.7% | 9.4% | |
| 1-2hrs/week | 34.2% | 24.5% | 31.3% | |
| 3-5hrs/week | 10.5% | 30.2% | 21.9% | |
| >5hrs/week | 5.3% | 13.2% | 12.5% | |
| Pregnancies (% female) | | | | |
| Deliveries (n) | 46.2% | 50% | 60.5% | 0.482 |
| 1 | 2 | 6 | 7 | |
| 2 | 6 | 6 | 10 | |
| >2 | 2 | 1 | 4 | |
| Health perception | | | | |
| Poor | 13.2% * | 1.9% | 3.1% | **<0.001** |
| Good | 39.5% | 60.4% * | 25.0% | |
| Very good | 47.4% | 37.7% | 71.9% | |

Values are presented as means (SD). Abbreviations: SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature; SDS, standard deviation score; BMI, body mass index; SES, Socioeconomic status. *p<0.05 compared to AGA

## Results

**Baseline characteristics**

Table 1 shows the clinical characteristics of 287 participants. Mean age at follow-up visit was 32.6 (2.5) years in SGA-CU, 31.9 (3.2) years in SGA-S and 33.0 (2.8) years in AGA. Gestational age was lower in SGA-CU adults than AGA adults (p=0.043), but similar to SGA-S adults. BMI SDS was similar in all groups.

SGA-CU adults tended to have a lower educational level (p=0.07), but a similar SES based on total yearly income compared to AGA adults. SGA-S adults had a lower educational level (p=0.002) and total annual income (p<0.001) than AGA adults. Tobacco use was higher in SGA-CU adults than AGA adults (p=0.03), but not different between SGA-S and AGA adults. Alcohol consumption, illicit drug use and exercise were similar in SGA-CU, SGA-S and AGA adults. SGA-CU and SGA-S adults had a lower perception of general health than AGA adults at age 32 years (p=0.014, p=0.003, resp.).

Weight gain in SDS from birth to 32 years was significantly higher in SGA-CU adults compared to SGA-S and AGA adults (+ 3.1 SDS vs + 1.0 SDS and + 0.4 SDS, both p<0.001, resp.), and it was also significantly different between SGA-S and AGA adults (p<0.001).

**Metabolic health at age 32 years**

Figure 1 and 2 and Table 2 present metabolic health data at age 21 and 32 years, we describe the differences at age 32 years.

*Insulin sensitivity and beta-cell function*

SGA-CU adults had lower Si, after correction for sex, than AGA adults (p=0.016), whereas SGA-S adults had similar Si as AGA adults. AIRg, β-cell function (DI) and Sg were similar between all groups. Additional correction for FM, AH SDS, SES or lifestyle factors did not change results.

*Body composition*

SGA-CU adults had higher FM and trunk fat than AGA adults, after correction for AH SDS and sex (p=0.033, p=0.024, resp.), while SGA-S and AGA adults had similar FM and trunk fat. Limb fat was similar in all groups.

LBM was similar in SGA-CU and AGA adults, after correction for sex and AH SDS, while SGA-S adults had lower LBM than SGA-CU and AGA adults (p=0.001 and p<0.001, resp.). Additional correction for birth length SDS, SES or lifestyle factors did not change results.

*Abdominal visceral and subcutaneous adiposity and liver fat fraction*
VAT and SAT were similar, after correction for sex, in SGA-CU, SGA-S and AGA adults. Liver fat fraction was similar in SGA-CU, SGA-S and AGA adults. Additional correction for AH SDS, SES or lifestyle factors did not change results.

*Serum lipid levels*
SGA-CU and SGA-S adults had higher total cholesterol, after correction for sex, than AGA adults (p=0.013 and p=0.041, resp.) and LDLc was higher in SGA-CU than AGA adults (p=0.029). Triglycerides were higher in SGA-CU and SGA-S than AGA adults (p=0.027 and p=0.025, resp.). Serum HDLc levels were lower in both SGA-CU and SGA-S than AGA adults (p=0.014 and p=0.016, resp.). Additional correction for FM, SES or lifestyle factors did not change results.

*Blood pressure*
SGA-CU and SGA-S adults had similar SBP as AGA adults, after correction for sex and AH SDS, but there was a trend towards higher SBP in SGA-CU than SGA-S adults (p=0.06). DBP was similar between SGA-CU, SGA-S and AGA adults. Additional correction for SES or lifestyle factors did not change results.

*Metabolic syndrome*
MetS (according to the NECP III criteria) was present in 7.4% of SGA-CU, 7.5% of SGA-S and 4.2% of AGA adults, which was significantly different between groups (p=0.027).

**Longitudinal changes in metabolic health from age 21 to 32 years**
Figure 1 and 2 and Table 2 present metabolic health data in all groups, at age 21 and 32 years.

*Longitudinal changes within the group during 11 years of follow-up*
Si, AIRg and DI did not change significantly in SGA-CU, SGA-S and AGA adults. Sg increased only in SGA-CU adults (p=0.019). FM increased in all groups (SGA-CU: p<0.001, SGA-S: p=0.001 and AGA: p<0.001). Trunk and limb fat increased in all groups (all p<0.001), while LBM remained similar. Total cholesterol increased in SGA-CU adults (p=0.032), tended to increase in SGA-S adults (p=0.06) and remained similar in AGA adults. LDLc tended to increase in SGA-CU adults (p=0.07), but remained similar in SGA-S and AGA adults. Triglycerides increased significantly in SGA-CU and SGA-S adults (p=0.023 and p=0.001), but not significantly in AGA adults, while HDLc remained similar in all groups. SBP increased in SGA-CU, SGA-S and AGA adults (p=0.001, p=0.007 and p=0.001, resp.), as did DBP (p=0.026, p=0.003 and p=0.018, resp.).

*Longitudinal changes between the groups during 11 years of follow-up*
The change in FSIGT results, body composition, serum lipid levels and blood pressure during 11 years of follow-up was similar between SGA-CU, SGA-S and AGA adults.

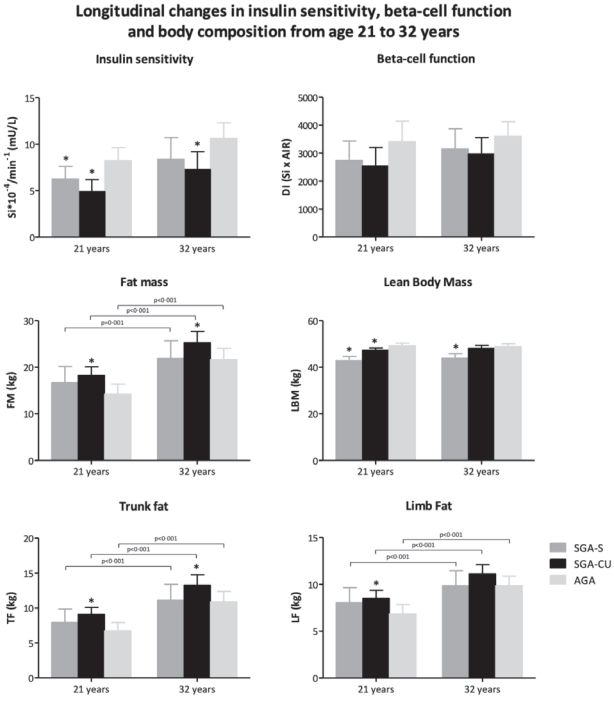


**Figure 1: Longitudinal changes in glucose metabolism and body composition during 11 years.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for sex. P-values for change within groups during 11 years follow-up were depicted if p<0.05. Abbreviations: Si, Insulin Sensitivity; DI, Disposition index; TC, Total cholesterol; FM, Fat mass; LBM, Lean body mass; TF, Trunk fat; LF, Limb fat; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature.

*p<0.05 compared to AGA

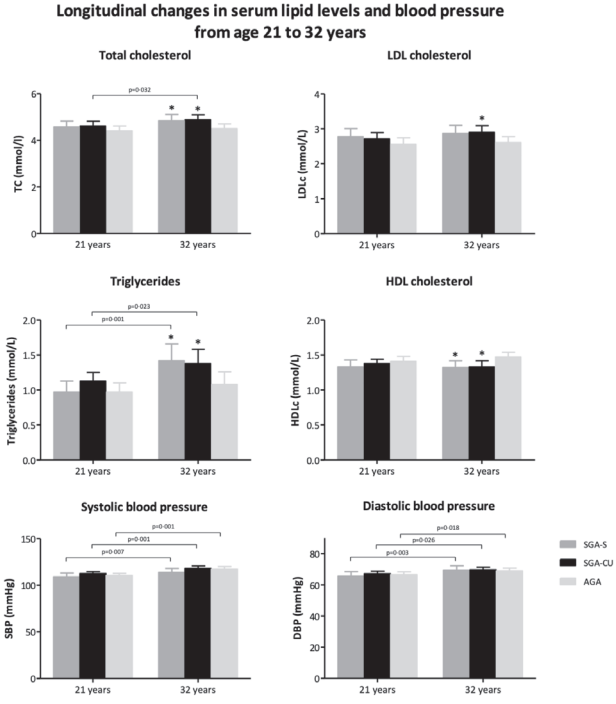


**Figure 2: Longitudinal changes in lipid levels and blood pressure during 11 years.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval, adjusted for sex. P-values for change within groups during 11 years follow-up were depicted if p<0.05. Abbreviations: LDLc, Low-density lipoprotein cholesterol; HDLc, High-density lipoprotein cholesterol; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature/

*p<0.05 compared to AGA

**Table 2: Metabolic and cardiovascular health at 21 and 32 years of age.**

| | 21 years | | | | 32 years | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | SGA-S | SGA-CU | AGA | | SGA-S | SGA-CU | AGA | | |
| **Outcome** | **EMM (95% CI)** | **EMM (95% CI)** | **EMM (95% CI)** | ***p*-value*** | **EMM (95% CI)** | **EMM (95% CI)** | **EMM (95% CI)** | ***p*-value*** | ***p*-value**** |
| ***FSIGT*** [1] | | | | | | | | | |
| **Si** $*10^{-4}/min^{-1}$ (mU/l) | 6.24 (4.89 - 7.59) [b] | 4.89 (3.58 - 6.19) [b] | 8.20 (6.78 - 9.63) | **0,008** | 8.35 (5.99 - 10.70) | 7.28 (5.37 - 9.20) [b] | 10.60 (8.90 - 12.31) | **0,044** | 0,772 |
| **Sg** $*10^{-2}/min^{-1}$ (mg/d) | 0.019 (0.017 - 0.021) | 0.018 (0.016 - 0.020) | 0.018 (0.016 - 0.021) | 0,575 | 0.020 (0.017 - 0.022) | 0.021 (0.019 - 0.023) | 0.021 (0.019 - 0.022) | 0,502 | 0,33 |
| **AIRg** (mU/l) | 680.0 (483.8 - 876.3) | 640.3 (456.8 - 823.8) | 463.1 (259.9 - 666.3) | 0,655 | 579.7 (452.5 - 707.0) | 573.7 (467.8 - 679.5) | 427.9 (331.8 - 524.0) | 0,234 | 0,876 |
| **DI** (Si *AIRg) | 2730.9 (2030 - 3431) | 2536.5 (1873 - 3200) | 3404.4 (2664 - 4145) | 0,404 | 3144.8 (2422 - 3867) | 2962.5 (2377 - 3548) | 3597.6 (3073 - 4123) | 0,236 | 0,985 |
| ***Body composition*** [2] | | | | | | | | | |
| **FM** (kg) | 16.66 (13.15 - 20.17) | 18.22 (16.34 - 20.09) [b] | 14.21 (12.02 - 16.39) | **0,014** | 21.85 (18.04 - 25.66) | 25.23 (22.78 - 27.67) [b] | 21.61 (19.15 - 24.07) | 0,065 | **0,529** |
| **TF** (kg) | 7.93 (6.02 - 9.84) | 9.09 (8.09 - 10.09) [b] | 6.74 (5.57 - 7.92) | **0,005** | 11.13 (8.87 - 13.39) | 13.25 (11.73 - 14.77) [b] | 10.88 (9.39 - 12.37) | 0,053 | **0,686** |
| **LF** (kg) | 8.05 (6.45 - 9.64) | 8.49 (7.62 - 9.36) [b] | 6.85 (5.84 - 7.85) | **0,037** | 9.85 (8.23 - 11.46) | 11.12 (10.13 - 12.10) | 9.86 (8.84 - 10.88) | 0,12 | **0,36** |
| **LBM** (kg) | 42.82 (41.00 - 44.64) [ab] | 47.23 (46.27 - 48.20) | 49.16 (48.04 - 50.28) | **<0.001** | 43.82 (41.85 - 45.79) [ab] | 48.14 (46.90 - 49.39) | 48.84 (47.57 - 50.10) | **0,001** | **0,285** |
| ***MRI results*** [1] | | | | | | | | | |
| **VAT** ($cm^2$) | N/A | N/A | N/A | | 84.1 (64.5 - 103.7) | 87.7 (70.7 - 104.7) | 73.4 (59.8 - 86.9) | 0,387 | |
| **SAT** ($cm^2$) | N/A | N/A | N/A | | 187.2 (149.4 - 225.0) | 197.4 (164.5 - 230.2) | 174.7 (148.6 - 200.9) | 0,56 | |
| **Fat fraction** (%) | N/A | N/A | N/A | | 3.80 (2.66 - 4.94) | 3.37 (2.45 - 4.29) | 2.79 (2.05 - 3.52) | 0,297 | |
| ***Serum lipid levels*** [1] | | | | | | | | | |
| **Cholesterol** (mmol/L) | 4.57 (4.32 - 4.83) | 4.62 (4.42 - 4.82) | 4.40 (4.20 - 4.61) | 0,308 | 4.84 (4.58 - 5.11) [b] | 4.88 (4.66 - 5.10) [b] | 4.50 (4.30 - 4.70) | **0,025** | 0,546 |
| **LDLc** (mmol/L) | 2.78 (2.55 - 3.01) | 2.71 (2.54 - 2.89) | 2.56 (2.38 - 2.74) | 0,268 | 2.87 (2.64 - 3.10) | 2.90 (2.71 - 3.09) [b] | 2.61 (2.44 - 2.78) | 0,059 | 0,642 |
| **Triglycerides** (mmol/L) | 0.97 (0.82 - 1.13) | 1.13 (1.01 - 1.25) | 0.97 (0.85 - 1.10) | 0,127 | 1.42 (1.18 - 1.66) [b] | 1.38 (1.18 - 1.58) [b] | 1.08 (0.90 - 1.26) | **0,029** | 0,129 |
| **HDLc** (mmol/L) | 1.33 (1.23 - 1.43) | 1.38 (1.31 - 1.44) | 1.41 (1.33 - 1.48) | 0,45 | 1.32 (1.22 - 1.42) [b] | 1.33 (1.25 - 1.42) [b] | 1.47 (1.40 - 1.54) | **0,014** | 0,23 |
| ***Blood pressure*** [2] | | | | | | | | | |
| **Systolic** (mmHg) | 109.1 (105.1 - 113.1) | 112.5 (110.4 - 114.6) | 110.4 (108.1 - 112.8) | 0,187 | 113.8 (109.6 - 118.1) | 118.1 (115.4 - 120.7) | 117.3 (114.7 - 120.0) | 0,278 | 0,579 |
| **Diastolic** (mmHg) | 65.48 (62.6 - 68.4) | 67.1 (65.6 - 68.7) | 66.5 (64.8 - 68.3) | 0,594 | 69.4 (66.6 - 72.2) | 69.6 (67.8 - 71.3) | 68.9 (67.2 - 70.7) | 0,874 | 0,604 |
| ***Metabolic syndrome*** | | | | | | | | | |
| **MetS** (%) | 2 | 1,2 | 1,3 | 0,943 | 7,5 | 7,4 | 4,2 | **0,027** | |

sults of repeated measurement analyses, presented as estimated marginal means with 95% CI; Abbreviations: EMM, estimated marginal mean; CI, confidence interval; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature; FSIGT, Frequently-sampled intravenous glucose tolerance test; Si, Insulin sensitivity; Sg, Glucose effectiveness; AIRg, Acute insulin response; DI, Disposition index; FM, Fat mass; TF, Trunk fat; LF, Limb fat; LBM, Lean Body Mass; ; LDLc, Low-density lipoprotein cholesterol; HDLc, High-density lipoprotein cholesterol; N/A, Not available; MetS, Metabolic syndrome classified as 3 or more risk factors based on the revised criteria of the National Cholesterol Educational Program.

[1] Adjusted for sex

[2] Adjusted for sex and adult height SDS

[a] p<0.05 compared to SGA-CU

[b] p<0.05 compared to AGA

* p-value between groups at age 21 or 32 years

** p-value of the difference in change between groups from age 21 to 32 years

**Associations of birth size and adult size with insulin sensitivity, body composition and central adiposity at age 32 years**

The associations between birth size, adult size and lifestyle factors with Si, FM, VAT, SAT and LBM, at age 32 years are shown in Table 3.

*Insulin sensitivity:* Model A shows that birth length SDS and birth weight SDS did not associate with Si at age 32 years. Model B demonstrates that adult weight associated inversely with Si (β: -2.81, p=0.001). As adult weight SDS was adjusted for birth weight SDS, this indirectly demonstrates the association of the change in weight SDS from birth to age 32 years with Si at age 32 years. Subjects with the highest gain in weight SDS from birth to age 32 years had a lower Si. Exercise level, SES and other lifestyle factors were not associated with Si. Model C shows that FM associated with Si (β: -0.28, p<0.0005), whereas LBM did not.

*Fat mass:* Model A shows that birth weight SDS and birth length SDS did not associate with FM at age 32 years. Model B demonstrates that adult weight associated with FM (β: 7.81, p<0.0005). Subjects with the highest gain in weight SDS from birth to age 32 years had higher FM, while female sex was associated with higher FM (β: 4.80, p<0.0005). Higher AH SDS and higher exercise level were associated with lower FM (β: -1.96, p=0.001 and β: -0.73, p=0.002, resp.). SES and other lifestyle factors were not associated with FM.

*VAT and SAT:* Model A shows that birth weight SDS and birth length SDS did not associate with VAT and SAT at age 32 years. Model B demonstrates that adult weight SDS associated with both VAT and SAT (β: 38.20 and 83.82, resp., both p<0.0005). Subjects with higher gain in weight SDS from birth to age 32 years had higher VAT. Both VAT and SAT were associated with sex, with females having lower VAT (β: -37.17, p<0.0005) and higher SAT (β: 63.67, p<0.0005). Higher exercise level associated with a lower VAT at age 32 years (β: -5.14, p=0.030), but was not associated with SAT. Higher AH SDS was associated with lower VAT and SAT, after correction for adult weight SDS (β: -14.00, p=0.010 and β: -32.69, p<0.0005, resp.). SES and other lifestyle factors were not associated with VAT and SAT.

*Lean body mass:* Model A shows that birth weight SDS and birth length SDS did not associate with LBM at age 32 years. Model B demonstrates that adult weight associated with LBM (β: 3.14, p<0.0005). Subjects with the highest gain in weight SDS from birth to age 32 years had higher LBM, while female sex was associated with lower LBM (β: -14.18, p<0.0005). Higher AH SDS and higher exercise level were associated with higher LBM (β: 1.31, p=0.001 and β: 0.56, p<0.0005). Other lifestyle factors were not associated with LBM.

**Table 3: Association between birth size, adult size and lifestyle on insulin sensitivity, body composition and central adiposity at age 32 years.**

| | Insulin sensitivity (*10$^{-4}$/min$^{-1}$ mU/l) | | | | | | Fat mass (kg) | | | | Lean body mass (kg) | | | | Visceral adipose tissue (cm$^2$) | | | | Subcutaneous adipose tissue (cm$^2$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Model A | | Model B | | Model C | | Model A | | Model B | | Model A | | Model B | | Model A | | Model B | | Model A | | Model B | |
| **Variables** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** | **β$^1$** | ***p*-value** |
| Age (yrs) | -0,16 | 0,511 | 0,05 | 0,823 | 0,05 | 0,814 | 0,63 | 0,029 | -0,03 | 0,86 | 0,29 | **0,038** | 0,02 | 0,828 | 3,43 | 0,064 | -0,21 | 0,883 | 10,54 | 0,003 | 2,54 | 0,275 |
| Sex | 1,14 | 0,405 | 0,82 | 0,51 | -0,61 | 0,825 | 4,87 | **0,006** | 4,8 | **<0.0005** | -14,23 | **<0.0005** | -14,18 | **<0.0005** | -41,89 | **<0.0005** | -37,17 | **<0.0005** | 53,3 | **0,01** | 63,67 | **<0.0005** |
| AH SDS | 0,01 | 0,993 | 1,17 | 0,184 | 0,96 | 0,283 | 1,36 | 0,205 | -1,96 | **0,001** | 2,65 | **<0.0005** | 1,31 | **0,001** | 2,08 | 0,75 | -14 | **0,01** | -1,14 | **0,926** | -36,44 | **<0.0005** |
| BL SDS | 0,63 | 0,394 | 0,73 | 0,272 | 0,68 | 0,311 | 0,49 | 0,56 | 0,35 | 0,391 | -0,35 | 0,384 | -0,42 | 0,123 | -5,92 | 0,282 | -6,13 | 0,143 | 6,46 | 0,533 | 5,99 | 0,371 |
| BW SDS | 0,37 | 0,633 | 0,5 | 0,499 | 0,48 | 0,495 | -0,27 | 0,765 | -0,89 | 0,052 | 0,76 | 0,081 | 0,54 | 0,08 | 3,09 | 0,588 | -0,77 | 0,865 | -6,76 | **0,53** | -15,22 | **0,038** |
| Exercise level | 0,06 | 0,887 | 0,08 | 0,814 | -0,03 | 0,943 | -0,58 | 0,233 | -0,73 | 0,002 | 0,63 | **0,008** | 0,56 | <0.0005 | -4,48 | 0,145 | -5,14 | **0,03** | 0,05 | 0,994 | -1,41 | 0,707 |
| SES | -0,02 | 0,975 | -0,66 | 0,235 | -0,69 | 0,223 | -2,04 | **0,006** | -0,47 | 0,198 | -0,33 | 0,347 | 0,31 | 0,195 | -9,19 | 0,06 | -2,7 | 0,471 | -13,78 | 0,133 | 0,47 | 0,938 |
| Smoking | -0,16 | 0,854 | 0,12 | 0,88 | 0,09 | 0,912 | 0,73 | 0,496 | 0,37 | 0,471 | -0,11 | 0,832 | -0,26 | 0,455 | -2,09 | 0,76 | -2,78 | 0,592 | 10,34 | 0,424 | 8,82 | 0,29 |
| Weight SDS | | | -2,81 | **0,001** | | | | | 7,81 | **<0.0005** | | | 3,14 | **<0.0005** | | | 38,2 | **<0.0005** | | | 83,82 | **<0.0005** |
| FM, kg | | | | | -0,28 | **<0.0005** | | | | | | | | | | | | | | | | |
| LBM, kg | | | | | -0,21 | 0,194 | | | | | | | | | | | | | | | | |
| Overall | | NS | | <0.0005 | | <0.0005 | | 0,002 | | <0.0005 | | <0.0005 | | <0.0005 | | 0,001 | | <0.0005 | | NS | | <0.0005 |
| R2 adjusted | | 0 | | 0,18 | | 0,16 | | 0,1 | | 0,79 | | 0,8 | | 0,91 | | 0,14 | | 0,5 | | 0,06 | | 0,61 |

Results of the multiple regression analyses. 1: Refers to the unstandardized regression coefficient; Model A additionally included interaction term BL*AH SDS; Model B additionally included interaction terms BL*AH SDS and BW*AW SDS. P-values below 0.05 are presented in bold. Abbreviations: AH, Adult height; BL, Birth length; BW, Birth Weight; SES, socioeconomic status; SDS, Standard deviation scores; FM, Fat mass; LBM, Lean body mass; NS, not significant.

### Association of birth size and adult size with metabolic syndrome at age 32 years

The association between birth size, adult size and lifestyle factors with components of MetS at age 32 years are shown in Table 4.

Higher birth weight and birth length SDS were both associated with a lower number of MetS components at ag 32 years (odds ratio (OR): 0.69, 95% confidence interval (CI): 0.55 – 0.85) and (OR: 0.74, 95% CI: 0.61 – 0.90), adjusted for age and sex. Thus, per one SDS decrease in birth weight, the odds of having a higher number of MetS components increased with 31%. Higher increase in weight SDS from birth to age 32 years and a higher adult FM were also associated with a higher number of MetS components (OR: 1.75, 95% CI: 1.43 – 2.13 and OR: 1.14, 95% CI: 1.10 – 1.18, resp.). Lower SES based on the educational level was associated with a higher number of MetS components (OR: 0.67, 95% CI: 0.52 – 0.86), but total yearly income, smoking and exercise level were not associated with number of MetS components.

**Table 4: Associations of birth size, gain in weight SDS and adult body composition and lifestyle factors, with the number of MetS components per individual at age 32 years.**

| | Number of components MetS | | |
|---|---|---|---|
| | **Odds ratio** | **95% CI** | ***p*-value** |
| Birth weight (SDS) [1] | **0.69** | **0.55 – 0.85** | **0.001** |
| Birth length (SDS) [1] | **0.74** | **0.61 – 0.90** | **0.003** |
| Gain in weight SDS from birth to 32 years (SDS) [2] | **1.75** | **1.43 – 2.13** | **<0.001** |
| Fat mass (kg) [2] | **1.14** | **1.10 – 1.18** | **<0.001** |
| SES (income) [1] | 0.83 | 0.62 – 1.10 | 0.197 |
| SES (education) [1] | **0.67** | **0.52 – 0.86** | **0.002** |
| Smoking [3] | 1.14 | 0.69 – 1.71 | 0.724 |
| Exercise [3] | 0.86 | 0.71 – 1.04 | 0.109 |
| Alcohol consumption [3] | **0.62** | **0.43 – 0.89** | **0.010** |
| Illicit drug use [3] | 0.72 | 0.30 – 1.66 | 0.471 |

Results of the ordinal regression analysis. Abbreviations: CI, confidence interval; SDS, standard deviation scores; SES, socioeconomic status.
[1] Adjusted for age and sex
[2] Adjusted for age, sex and adult height SDS
[3] Adjusted for age, sex and SES (education)

## Discussion

This longitudinal study during 11 years in adulthood is currently the longest follow-up study investigating metabolic health in a large group of adults born SGA compared to age-matched adults born AGA, using high-quality research tools such as FSIGT, DXA and MRI. This PROGRAM32 study investigated 170 adults born SGA, either with or without postnatal catch-up, compared to age-matched 117 healthy adults born AGA, at age 21 and 32 years. At age 32 years, SGA-CU adults had higher insulin resistance, higher FM and trunk fat and higher adverse serum lipid levels than AGA adults. SGA-S adults had also higher adverse serum lipid levels and a lower LBM than AGA adults, but all other parameters were similar. Thus, the adverse metabolic health profile in SGA-CU adults at age 21 years persisted up to age 32 years, but did not worsen during the 11 years of follow-up.



Insulin sensitivity at age 32 years was lower in SGA-CU adults than in AGA controls while β-cell function was similar, like the results at age 21 years [2,4]. In the total group of adults, more gain in weight SDS from birth to age 32 years was associated with a lower Si at age 32 years. Other studies, using HOMA-IR instead of the FSIGT test, showed also lower insulin sensitivity in adults born SGA aged 22 to 30 years [18,19]. Reassuringly, none of the adults born SGA developed diabetes mellitus type 2, and the β-cell function was similar at age 32 years in all groups, suggesting an appropriate β-cell response to higher insulin resistance in SGA-CU adults.

At age 32 years, SGA-CU adults had a less favourable body composition with higher central fat deposition, similar to findings at 21 years [2,3,5]. In contrast to our hypothesis, the metabolic health parameters did not worsen in the SGA groups during the 11 years of follow-up, but in SGA-CU adults the adverse metabolic health parameters persisted to age 32 years. Our multiple regression analyses in the total cohort also showed that weight gain SDS from birth to age 32 years was associated with higher FM, VAT and SAT at age 32 years. Studies in children reported higher FM and more central fat distribution following SGA birth, especially after postnatal catch-up [20-23]. This tendency of attaining more FM and central fat distribution in adults born SGA is less healthy, as it is strongly associated with glucose intolerance, insulin resistance and hypertension in later life [24,25].

SGA-S adults had lower LBM at age 32 years compared to SGA-CU and AGA adults, after correction for AH SDS and sex. At age 21 years, LBM was also lower in SGA-S adults than SGA-CU and AGA adults [3]. Lower LBM might be caused by poor fetal nutrition or lower fetal IGF-I levels, leading to less muscle mass development [26,27]. As SGA-S adults have lower LBM, but similar FM as AGA adults, the ratio between FM and LBM is less healthy. SGA-S subjects

would, therefore, benefit from maintaining their weight and thus FM at the lower end of the normal range compared to AGA subjects.

Serum lipid levels at age 32 years in SGA-CU and SGA-S adults were less favorable than in AGA adults, with significantly higher cholesterol and LDLc and lower HDLc, similar to findings at age 21 years [28]. These results show that adults born SGA have an adverse lipid profile, independent of postnatal catch-up, which is related to a higher risk for cardiovascular disease [29].

SGA-CU adults tended towards higher SBP than SGA-S adults at age 32 years, comparable to the results found at age 21 years [30]. SGA-S had a similar SBP and DBP as AGA adults and SGA birth seems not associated with an increased risk of high blood pressure at age 32 years.

Birth weight, birth length and adult height were different between groups, as these were part of the inclusion criteria for the three groups. The difference in gestational age was clinically not relevant, as all adults were born at term. SGA-S adults had a lower SES, and SGA-CU adults had more tobacco use compared to AGA adults. We, therefore, additionally corrected analyses for SES and lifestyle factors, which did not change the results. Our total study cohort shows that a higher frequency of physical exercise associates with a lower FM, lower VAT and higher LBM. We, therefore, advise an active lifestyle for all adults and especially for those born SGA, as this would decrease the risk of unfavorable body composition and central adiposity at age 32 years.

Our findings now show that postnatal catch-up growth negatively affects metabolic health and is associated with an increased risk of developing MetS, at age 32 years. Most postnatal catch-up occurs within the first 2 years of life [6]. This postnatal catch-up in height is accompanied by an accelerated gain in weight. Studies have shown that an accelerated gain in weight, especially during the first year of life, is associated with an increaed metabolic risk [2,5]. Based on these findings, children born SGA should be monitored to prevent the development of negative metabolic consequences of catch-up growth during infancy.

Until now, this is the longest follow-up study in a unique and large cohort of adults born SGA either with or without postnatal catch-up and compared to age-matched AGA controls. Although our total study population was relatively large, the proportion of SGA-S adults was smaller due to the low prevalence of SGA-born subjects with persistent short stature (~0.1% of all live-born children [6,7]). This was complicated further, because most children with postnatal growth failure were treated with growth hormone for adult height improvement during the last 25 years. As our study population consisted of 32-year old adults, endpoints such as cardiovascular diseases and type 2 diabetes might not be present at this moment.

We, therefore, used high-quality research tools, such as FSIGT, DXA and MRI, providing a detailed health profile at age 32 years.

In conclusion, our study shows that dults born SGA with postnatal catch-up had higher insulin resistance, an unfavorable body composition and higher adverse serum lipid levels at age 32 years compared to age-matched AGA adults, similar to results at age 21 years. However, metabolic health parameters in adults born SGA did not worsen from age 21 to 32 years. Based on these findings, children born SGA should be monitored to prevent the development of negative metabolic consequences of catch-up growth during childhood.

## Acknowledgements

We want to express our gratitude to all adults who participated in this study. We thank J. Bontenbal-van de Wege, C. Bruinings-Vroombout, N. Khieroe and E. Lems, research nurses, to contribute to the study, Dr. W. Hackeng for performing laboratory analyses, and Prof. dr. A. van der Lugt, M. Van den Ijssel, I. Vanwersch, and D. van Maldegem for assisting with the MRI scans. We acknowledge the investigator-initiated independent research grant provided by Novo Nordisk BV, The Netherlands.

## References


1. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341(8850):938-941.
2. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301(21):2234-2242.
3. Leunissen RW, Stijnen T, Hokken-Koelega AC. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf).* 2009;70(2):245-251.
4. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab.* 2008;93(2):445-451.
5. Kerkhof GF, Hokken-Koelega AC. Rate of neonatal weight gain and effects on adult metabolic health. *Nat Rev Endocrinol.* 2012;8(11):689-692.
6. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res.* 1995;38(2):267-271.
7. Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S. Longitudinal follow-up of growth in children born small for gestational age. *Acta Paediatr.* 1993;82(5):438-443.
8. Cornier MA, Dabelea D, Hernandez TL, et al. The metabolic syndrome. *Endocr Rev.* 2008;29(7):777-822.
9. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365(9468):1415-1428.
10. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74(6):901-910.
11. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
12. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.
13. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003;5(6):1003-1015.
14. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P. The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res.* 2004;12(12):1925-1929.

15. Schweitzer L, Geisler C, Pourhassan M, et al. What is the best reference site for a single MRI slice to assess whole-body skeletal muscle and adipose tissue volumes in healthy adults? *Am J Clin Nutr.* 2015;102(1):58-65.
16. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009;120(16):1640-1645.
17. Graham I. European guidelines on cardiovascular disease prevention in clinical practice: Executive summary. *Atherosclerosis.* 2007;194(1):1-45.
18. Meas T, Deghmoun S, Alberti C, et al. Independent effects of weight gain and fetal programming on metabolic complications in adults born small for gestational age. *Diabetologia.* 2010;53(5):907-913.
19. Balasuriya CND, Stunes AK, Mosti MP, et al. Metabolic Outcomes in Adults Born Preterm With Very Low Birthweight or Small for Gestational Age at Term: A Cohort Study. *J Clin Endocrinol Metab.* 2018;103(12):4437-4446.
20. Faienza MF, Brunetti G, Ventura A, et al. Nonalcoholic fatty liver disease in prepubertal children born small for gestational age: influence of rapid weight catch-up growth. *Horm Res Paediatr.* 2013;79(2):103-109.
21. Breij LM, Kerkhof GF, Hokken-Koelega AC. Accelerated infant weight gain and risk for nonalcoholic fatty liver disease in early adulthood. *J Clin Endocrinol Metab.* 2014;99(4):1189-1195.
22. Levy-Marchal C, Czernichow P. Small for gestational age and the metabolic syndrome: Which mechanism is suggested by epidemiological and clinical studies? *Hormone Research.* 2006;65:123-130.
23. Marcovecchio ML, Gorman S, Watson LPE, Dunger DB, Beardsall K. Catch-Up Growth in Children Born Small for Gestational Age Related to Body Composition and Metabolic Risk at Six Years of Age in the UK. *Horm Res Paediatr.* 2020;93(2):119-127.
24. Lorbeer R, Rospleszcz S, Schlett CL, et al. Correlation of MRI-derived adipose tissue measurements and anthropometric markers with prevalent hypertension in the community. *Journal of Hypertension.* 2018;36(7):1555-1562.
25. Hiuge-Shimizu A, Kishida K, Funahashi T, et al. Absolute value of visceral fat area measured on computed tomography scans and obesity-related cardiovascular risk factors in large-scale Japanese general population (the VACATION-J study). *Ann Med.* 2012;44(1):82-92.
26. Gluckman PD, Harding JE. Fetal growth retardation: underlying endocrine mechanisms and postnatal consequences. *Acta Paediatr Suppl.* 1997;422:69-72.
27. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev.* 2014;94(4):1027-1076.

28. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC. Fat mass and apolipoprotein E genotype influence serum lipoprotein levels in early adulthood, whereas birth size does not. *J Clin Endocrinol Metab.* 2008;93(11):4307-4314.
29. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP, San Antonio Heart S. National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation.* 2004;110(10):1251-1257.
30. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega AC. Effect of birth size and catch-up growth on adult blood pressure and carotid intima-media thickness. *Horm Res Paediatr.* 2012;77(6):394-401.

# Chapter 7

## Childhood growth hormone treatment does not increase metabolic and cardiovascular risk in adults born SGA: A 12-year follow-up study after GH-cessation

W.J. Goedegebuure
M. van der Steen
C.C.J. Smeets
A.C.S. Hokken-Koelega

*Submitted*

## Abstract

**Background:** Childhood GH treatment has been associated with increased cardiovascular mortality and morbidity in adults born small for gestational age (SGA) compared to the general population, but risks were not compared with untreated control groups.

**Methods:** We longitudinally investigated the metabolic health profile in 167 previously GH-treated adults born SGA (SGA-GH) during 12 years after GH-cessation, using frequently-sampled intravenous glucose tolerance test, DXA-scan and MRI-scan. At age 30 years, we compared metabolic health profile of SGA-GH adults to 219 untreated adults: 127 born SGA with either short stature (SGA-S) or spontaneous catch-up to normal stature (SGA-CU) and 92 born appropriate for gestational age (AGA).

**Results:** During 12 years of follow-up, SGA-GH adults maintained a normal beta-cell function and showed an increase in insulin sensitivity, fat mass (FM), total cholesterol and blood pressure to similar levels as SGA-S adults. SGA-GH adults had also similar metabolic and cardiovascular health parameters as AGA adults, except of lower lean body mass and higher adverse serum lipid levels, which were present in all SGA groups. Abdominal adiposity, liver fat fraction and blood pressure were similar between SGA-GH and control groups. At age 30 years, SGA-GH, SGA-S, SGA-CU, and AGA adults had similar metabolic syndrome components (MetS), as described by the National Cholesterol Educational Program (NCEP).

**Conclusions:** At age 30 years, previously GH-treated adults born SGA had a similar metabolic and cardiovascular health profile as untreated adults born SGA or AGA, indicating long-term metabolic and cardiovascular safety of GH treatment for short children born SGA.

## Introduction

In children born small for gestational age (SGA) with persistent short stature, treatment with growth hormone (GH) improves adult height (AH) [1-3]. GH treatment is associated with a decrease in insulin sensitivity [4,5], fat mass (FM), serum lipid levels and blood pressure, and an increase in lean body mass (LBM) [6-8]. Insulin resistance precedes type II diabetes mellitus, and unfavorable body composition, central adiposity, high adverse serum lipid levels, high blood pressure, and liver fat fraction increase the risk of developing metabolic syndrome [9,10]. Since children born SGA have an increased risk of developing metabolic and cardiovascular disease and these diseases develop over a longer time, it is important to investigate the long-term changes in metabolic and cardiovascular profile after GH-cessation compared with appropriate untreated control groups.

Large population studies investigating GH safety, including the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study, reported increased cardiovascular mortality in GH-treated adults born SGA compared to the general population at age 30 years [11-13]. These studies, however, were limited by the absence of an untreated control group of adults born SGA to correct for the inverse association between low birth weight with adult diseases like diabetes mellitus type II and cardiovascular diseases [14].



We, therefore, performed this 12-year follow-up study after cessation of GH treatment in adults born SGA who were treated with GH during childhood (SGA-GH), investigating longitudinal metabolic and cardiovascular health. In addition, we assessed metabolic health parameters in SGA-GH adults at around age 30 years compared to two untreated control groups: untreated adults born SGA with persistent short stature (SGA-S) and adults born AGA with normal adult stature (AGA). We hypothesized that beta-cell function would remain the same during follow-up in SGA-GH adults and that insulin sensitivity, FM, serum lipid levels and blood pressure would increase in SGA-GH adults to a similar level of SGA-S and AGA adults at around age 30 years. Secondly, to evaluate whether GH-induced catch-up growth has a different long-term effect on metabolic health parameters as spontaneous catch-up, we additionally compared results of SGA-GH adults with those of untreated adults born SGA with spontaneous postnatal catch-up growth to a normal adult stature (SGA-CU).

## Methods

### Subjects

The total study group consisted of 386 adults, of which 167 adults born SGA (birth weight or birth length below –2 SDS for gestational age) had been treated with GH during childhood (IUGR-1 or IUGR-2 study). At the start of GH treatment, children were prepubertal, aged 5-8 years, with a height below -2.5 SDS and no endocrine, metabolic or chronic disorders.

GH treatment was continued until the attainment of adult height (AH), defined as height reached when growth velocity had decreased to <0.5 cm during the last 6 months. GH-treated adults were invited to participate in the current follow-up study evaluating metabolic and cardiovascular risk factors at GH-cessation at AH attainment and at 5 and 12 years after that.

At the end of the 12-year follow-up period, SGA-GH adults were compared to three untreated control groups with a similar age *(Ref PROGRAM32)*: 1) Adults born SGA (birth length or birth weight <-2SDS) with persistent short stature ((AH <-2SDS) <-2SDS) (SGA-S), 2) Adults born SGA (birth length or birth weight <-2SDS) with spontaneous catch-up growth to a normal AH (>-1SDS) (SGA-CU) and 3) Adults born AGA (birth length >-1 SDS) with normal AH (>-1SDS) (AGA). Included subjects were Caucasian and had an uncomplicated neonatal period without severe asphyxia (defined as an Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation or oxygen supply. Females were excluded from participation if they were pregnant or until 6 months after delivery.

The Medical Ethics Committee of Erasmus University Medical Center approved the 12-year follow-up study and the PROGRAM32 study, and all participants gave written informed consent.

## Measurements

Height was measured to nearest 0·1 cm using Harpenden stadiometer (Holtain, Ltd. Crymmyth, UK) and weight to nearest 0·1kg (Servo Balance KA-20-150S). Standard deviation (SD)-scores for birth length and birth weight were corrected for gestational age, and sex [15] and SD-scores for AH and BMI were corrected for sex and chronological age [16], using Growth Analyser Research Calculation Tools (https://growthanalyser.org).

**Insulin sensitivity and beta-cell function**

Glucose homeostasis was assessed by frequently sampled intravenous glucose tolerance test (FSIGT) with Tolbutamide after an overnight fast [17]. Insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response (AIR), and disposition index (DI) were calculated using Bergman's MINMOD Millennium software [18]. Si stands for insulin sensitivity, and Sg reflects the capacity of glucose to mediate its disposal. AIR estimates insulin secretory capacity, and DI equals AIR x Si, indicating beta-cell function [18].

**Assays**

Fasting glucose levels were determined with Architect ci8200 system (Abbott) and fasting insulin by IRMA (Medgenix, Biosource Europe), intra-assay and interassay coefficient of variation being 2·1% and 6·5%, respectively.

Total cholesterol (TC) and triglyceride (TG) were measured by an automated enzymatic method with the CHOD-PAP reagent kit and GPO-PAP reagent kit, respectively (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein cholesterol (HDLc) was measured using a homogeneous enzymatic colourimetric assay (Roche Diagnostics) and low-density lipoprotein (LDLc) calculated by the Friedewald formula.

**Body composition**

Body composition was measured by a DXA scan. All measurements were made with the same machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) and software (enCORE software version 14·1), with daily quality assurance. The intra-assay coefficient of variation was 0·41-0·88% for FM and 1·57-4·49% for LBM [19]. LBM was determined as fat-free mass minus bone mineral content.

**Abdominal visceral and subcutaneous adiposity**

Subjects underwent a magnetic resonance imaging (MRI) scan to measure visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and liver fat fraction at age 30 years. Scanning was performed on two 3T GE Discovery MR750systems (GE Healthcare, Milwaukee, US) with identical protocols. VAT and SAT measurements were performed by fast-spoiled gradient echo technique to acquire fat-only images in 20-second breath-hold scans. Cross-sectional area at the level of L3 was used, which correlated with total VAT and SAT volumes ($r>0·95$, $p<0·01$), using threshold-based region technique for slight adjustments [20]. Measurements of liver fat fraction were performed using the IDEAL-IQ technique. The intra-assay and interassay coefficients of variation were calculated for VAT (4·8%, 6·7%, resp.), SAT (0·9%, 4·2%, resp.) and liver fat fraction (9·5%, 16·8%, resp.). All measurements were performed in triplicate by one investigator (WG), and the mean of measurements was used for analysis.

**Blood pressure**

After 10 minutes of rest, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured repeatedly for 30 minutes in supine position, using the non-dominant arm with an automated device (Accutorr Plus, Datascope, Montvale, USA). Mean of 7 measurements was taken for analysis to reflect resting blood pressure.

**Socioeconomic status and lifestyle factors**

Adults provided information regarding lifestyle factors at age 30 years, by questionnaire. Total yearly income and highest completed education were used to determine socioeconomic status (SES) (1 = low, 2 = medium and 3 = high). The questionnaire assessed smoking (current smoking: light < 6 cigarettes/day, heavy ≥ 6 cigarettes/day, and history of smoking), alcohol consumption (1 = less than 1 alcoholic drink/week, 2 = less than 1 alcoholic drink/day, 3 = more than 1 alcoholic drink/day), illicit drug use (frequency, amount and type of drugs), exercise level (frequency: 1 = less than 1 hour/month, 2 = 1 hour/ 2 weeks, 3 = 1-2 hours/

week, 4 = 3-5 hours/week, 5 = more than 5 hours/week), pregnancies, number of deliveries and perception of general health (1 = poor, 2 = good, 3 = very good).

**Metabolic syndrome**

Revised criteria of the National Cholesterol Educational Program (NCEP, Adult Treatment Panel III) were used to determine components of metabolic syndrome (MetS) [21,22]. MetS was defined as having three or more of the following risk factors: Abdominal obesity with a waist circumference in men ≥ 102cm, women ≥ 88cm; Serum triglyceride levels: ≥ 1·7 mmol/L; HDLc: men ≤ 1·03 mmol/L, women ≤ 1·3 mmol/L; Blood pressure: >130 / 85 mmHg; Fasting glucose: ≥ 5·6 mmol/L.

**Data analysis**

Statistical analyses were performed using SPSS version 25. The Kolmogorov-Smirnov test and normal Q-Q-plots determined the distribution of variables. Clinical characteristics are presented as means (SD); Student's t-test was used to determine differences between subgroups.

Longitudinal changes during the 12 years of follow-up were analyzed using repeated measurements analysis, with an unstructured covariance matrix, with sex as a covariate. We used ANCOVA to compare the groups at around age 30 years, with sex and age as a covariate for all variables. Body composition, VAT, SAT, and blood pressure were additionally adjusted for AH SDS. Because of skewed distribution, Si, Sg, AIRg and DI were log-transformed. Results were regarded as statistically significant at $p<0.05$.

**Table 1: Clinical characteristics of 386 participants.**

| | SGA-GH Means (SD) | SGA-S Means (SD) | SGA-CU Means (SD) | AGA Means (SD) | *p*-value |
|---|---|---|---|---|---|
| ***At birth*** | | | | | |
| Number (Female) | 167 (90) | 50 (31) | 77 (43) | 92 (48) | 0.708 |
| Gestational age, weeks | 36.8 (3.8) [a c] | 38.0 (2.9) | 36.6 (3.1) | 38.5 (2.8) | **<0.001** |
| Birth length, SDS | -3.33 (1.6) [b c] | -3.09 (1.2) | -2.63 (1.0) | 0.15 (0.8) | **<0.001** |
| Birth weight, SDS | -2.40 (1.2) [c] | -2.24 (0.9) | -2.30 (0.8) | 0.28 (1.0) | **<0.001** |
| ***At GH-cessation*** | | | | | |
| Number (Female) | 167 (90) * | N/A | N/A | N/A | |
| Age, years | 16.2 (1.3) | N/A | N/A | N/A | |
| GH duration | 8.9 (2.5) | N/A | N/A | N/A | |
| ***At 12 years after cessation or at around age 30 years*** | | | | | |
| Number (Female) | 105 (59) | 50 (31) | 77 (43) | 92 (48) | 0.735 |
| Age, years | 28.6 (3.3) [a b c] | 31.8 (3.3) | 32.6 (2.5) | 32.8 (2.7) | **<0.001** |
| Height, SDS | -1.39 (1.0) [a b c] | -2.15 (0.6) | 0.00 (0.7) | 0.52 (0.8) | **<0.001** |
| ***Lifestyle factors*** | | | | | |
| SES (income) (%) | | | | | **<0.001** |
| Low | 29.0% [b c] | 24.3% | 5.1% | 6.5% | |
| Middle | 49.3% | 43.2% | 32.2% | 25.9% | |
| High | 21.7% | 32.4% | 62.7% | 67.5% | |
| Education (%) | | | | | **<0.001** |
| Low | 21.5% [a b c] | 10.4% | 12.2% | 4.8% | |
| Middle | 44.1% | 50.0% | 32.4% | 21.4% | |
| High | 34.4% | 39.5% | 55.4% | 73.8% | |
| Smoking (%) | | | | | 0.671 |
| Light | 12.2% | 10.0% | 16.9% | 8.8% | |
| Heavy | 11.2% | 10.0% | 10.3% | 4.4% | |
| History | 13.3% | 6.0% | 9.1% | 14.2% | |
| Alcohol use (%) | | | | | 0.151 |
| <1/wk | 36.3% | 32.5% | 42.6% | 29.4% | |
| 1-6/wk | 58.8% | 65.0% | 47.0% | 56.4% | |
| >1/day | 5.0% | 2.5% | 10.3% | 14.1% | |
| Illicit drug use (%) | | | | | 0.207 |
| Total | 14.7% | 10.0% | 21.3% | 11.9% | |
| Marihuana | 9.5% | 8.0% | 10.4% | 3.3% | |
| Ecstasy | 2.9% | 0% | 10.4% | 7.6% | |
| Cocaine | 4.8% | 0% | 6.5% | 4.3% | |
| Exercise (%) | | | | | 0.482 |
| <1h/month | 40.9% | 36.8% | 26.4% | 26.2% | |
| 1h/2wks | 7.5% | 5.3% | 5.7% | 9.2% | |
| 1-2hrs/wk | 23.7% | 34.2% | 24.5% | 32.3% | |
| 3-5hrs/wk | 18.3% | 10.5% | 30.2% | 20% | |
| >5hrs/wk | 9.7% | 5.3% | 13.2% | 10.8% | |
| Pregnancies (% female) | 31.5% | 40.0% | 51.2% | 56.5% | 0.060 |
| Deliveries (n) | | | | | 0.789 |
| 1 | 4 | 2 | 9 | 7 | |
| 2 | 9 | 6 | 9 | 12 | |
| >2 | 1 | 2 | 1 | 5 | |

Values are presented as means (SD). Abbreviations: SGA-GH, small for gestational age treated with growth hormone; SGA-S, small for gestational age with persistent short stature; SGA-CU, small for gestational age with spontaneous catch-up to a normal adult height; AGA, appropriate for gestational age; SDS, standard deviation score; BMI, body mass index; SES, socioeconomic status based on income. * in 38 adults no FSIGT and DEXA-scan was performed at AH attainment

[a] p<0.05 compared to SGA-S; [b] p<0.05 compared to SGA-CU; [c] p<0.05 compared to AGA

**Table 2: Longitudinal metabolic and cardiovascular health in SGA-GH adults during follow-up.**

| Outcome | | EMM (95% CI) | *p*-value (0-12 years) |
|---|---|---|---|
| ***FSIGT results*** [1] | | | |
| Insulin sensitivity *10-4/min-1 (mU/l) | GH-stop | 4.57 (4.12 – 5.03) | |
| | 12-year | 8.27 (6.82 – 9.72) | 0.002 |
| Acute insulin response (mU/l) | GH-stop | 744.5 (666.5 – 822.5) | |
| | 12-year | 520.8 (420.8 – 620.7) | <0.001 |
| Beta-cell function (Si *AIRg) | GH-stop | 3061.0 (2701 – 3421) | |
| | 12-year | 3137.6 (2659 – 3616) | 0.157 |
| Glucose effectiveness *10-2/min-1 (mg/d) | GH-stop | 0.019 (0.017 – 0.021) | |
| | 12-year | 0.021 (0.020 – 0.022) | 0.003 |
| ***Body composition*** [2] | | | |
| FM (kg) | GH-stop | 10.32 (9.52 – 11.12) | |
| | 12-year | 19.42 (18.09 – 20.74) | <0.001 |
| TF (kg) | GH-stop | 5.19 (4.77 – 5.61) | |
| | 12-year | 10.14 (9.33 – 10.94) | <0.001 |
| LF (kg) | GH-stop | 4.89 (4.37 – 5.42) | |
| | 12-year | 8.36 (7.80 – 8.92) | <0.001 |
| LBM (kg) | GH-stop | 43.37 (2.46 – 44.28) | |
| | 12-year | 42.23 (41.27 – 43.19) | 0.001 |
| ***Serum lipid levels*** [1] | | | |
| Total cholesterol (mmol/L) | GH-stop | 3.95 (3.82 – 4.08) | |
| | 12-year | 4.56 (4.34 – 4.77) | <0.001 |
| LDLc (mmol/L) | GH-stop | 2.24 (2.13 – 2.35) | |
| | 12-year | 2.65 (2.49 – 2.81) | <0.001 |
| Triglycerides (mmol/L) | GH-stop | 0.99 (0.91 – 1.07) | |
| | 12-year | 1.21 (1.07 – 1.34) | 0.006 |
| HDLc (mmol/L) | GH-stop | 1.42 (1.36 – 1.49) | |
| | 12-year | 1.33 (1.26 – 1.40) | 0.025 |
| ***Blood pressure*** [2] | | | |
| SBP (mmHg) | GH-stop | 110.7 (107.8 – 113.7) | |
| | 12-year | 118.6 (116.8 – 120.5) | <0.001 |
| DBP (mmHg) | GH-stop | 61.9 (59.9 – 63.8) | |
| | 12-year | 71.5 (70.0 – 73.1) | <0.001 |

Results of repeated measurement analysis, presented as estimated marginal means with 95% CI; Abbreviations: EMM, estimated marginal mean; CI, confidence interval; SGA-GH, Adults born small for gestational age treated with growth hormone; FM, Fat mass; LBM, Lean Body Mass; TF, Trunk fat; LF, Limb fat; HDLc, High-density lipoprotein cholesterol; LDLc, Low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[1] Adjusted for sex and age
[2] Adjusted for sex, height SDS and age

## Results

**Baseline characteristics**

Table 1 shows the clinical characteristics of all 386 adults at birth, at adult height and at around age 30 years. In the 167 SGA-GH adults, mean GH treatment duration had been 8.9 years, mean age at cessation of GH treatment was 16.2 years and follow-up after GH-cessation was 12 years. Mean age at the last follow-up visit was 28.6 years in SGA-GH, 31.8 years in SGA-S, 32.6 years in SGA-CU and 32.8 years in AGA adults ($p<0.001$). Birth length and birth weight were different in all groups, as this was part of the inclusion criteria (all $p<0.001$). AH SDS in SGA-GH (-1.39 SDS) was significantly higher compared to SGA-S adults but significantly lower than in SGA-CU and AGA adults (all $p<0.001$).

SGA-GH had a similar total yearly income and educational level as SGA-S adults but significantly lower than AGA adults (both $p<0.001$).



**Longitudinal changes in previously GH-treated adults (SGA-GH) and comparison with the untreated control groups (SGA-S, SGA-CU and AGA) at age 30 years**

Figure 1 and 2 and Tables 2 and 3 present the change in metabolic health parameters in SGA-GH adults during 12 years after GH-cessation and the comparison to SGA-S, SGA-CU and AGA adults at around age 30 years.

*Insulin sensitivity and beta-cell function*

During the 12 years of follow-up, insulin sensitivity (Si) increased significantly in SGA-GH adults ($p=0.002$), also after adjustment for FM. Correspondingly, AIRg decreased ($p<0.001$), while beta-cell function (DI) remained similar. Sg, the glucose uptake without insulin, increased during follow-up ($p=0.005$). At age 30 years, SGA-GH adults had similar Si, AIRg, DI and Sg as SGA-S, SGA-CU and AGA adults. Diabetes mellitus type II (repeated fasting glucose >7.0mmol/L) was present in 2 SGA-GH adults and 1 AGA adult.

*Body composition*

During 12 years of follow-up, FM, trunk fat, and limb fat increased in SGA-GH adults (all $p<0.001$), while LBM decreased ($p=0.001$). The decrease in LBM was due to a decrease during the first 5 years of follow-up ($p<0.001$) and remained stable thereafter ($p=0.67$).

At age 30 years, SGA-GH adults had a similar FM, corrected for height, age and sex, as SGA-S and AGA adults. SGA-GH had a trend towards lower FM than SGA-CU adults ($p=0.11$). Trunk fat was similar between SGA-GH and SGA-S, SGA-CU and AGA adults. SGA-GH adults had similar limb fat as SGA-S and AGA adults but significantly lower limb fat than SGA-CU adults ($p=0.023$). LBM, corrected for height, age and sex, was similar in SGA-GH and SGA-S adults, but SGA-GH adults had lower LBM than SGA-CU and AGA adults ($p=0.008$ and $p=0.003$, resp.). Additional adjustment for birth length did not change results.

*Serum lipid levels*
During the 12 years of follow-up, there was an increase in total cholesterol (p<0.001), LDLc (p<0.001) and triglycerides (p=0.006), and a decrease in HDLc (p=0.025) in SGA-GH adults. Mean serum lipid levels remained within the normal range.

At age 30 years, SGA-GH had similar total cholesterol and LDLc, corrected for age and sex, as SGA-S and SGA-CU adults, and significantly higher cholesterol and LDLc than AGA adults (p=0.005 and p=0.023, resp.). SGA-GH had a trend towards lower triglycerides than SGA-S adults (p=0.09) and towards higher triglycerides than AGA adults (p=0.09). Triglycerides in SGA-GH and SGA-CU adults were similar.

SGA-GH adults had similar HDLc as SGA-S and SGA-CU adults but, like the other SGA groups, lower HDLc than AGA adults (p=0.005).

*Blood pressure*
During 12 years of follow-up, SGA-GH adults had an increase in SBP and DBP (both p<0.001) while remaining within the normal range.

At age 30 years, SBP and DBP, corrected for age, sex and AH SDS, were similar in SGA-GH, SGA-S, SGA-CU and AGA adults.

*Abdominal adiposity and liver fat fraction by MRI*
At age 30 years, SGA-GH had similar VAT, SAT and liver fat fraction, after correction for age and sex, as SGA-S, SGA-CU and AGA adults.

*Metabolic syndrome*
At age 30 years, MetS (according to the NECP III criteria) was present in 5.8% of SGA-GH, 10.2% of SGA-S, 8.3% of SGA-CU and 4.4% of AGA adults, which was not significantly different between groups.

Regarding the MetS components (Table 3), high waist circumference was less present in SGA-GH than in SGA-S and SGA-CU adults (p=0.04 and p=0.007, resp.). SGA-GH tended towards a lower presence of high triglycerides than SGA-S and AGA adults (both p=0.06) and had a significantly lower presence of high triglycerides than SGA-CU adults (p=0.011). High HDLc was significantly more present in SGA-GH than in AGA adults (p=0.018) and similarly present in SGA-GH, SGA-S and SGA-CU adults. High blood pressure and high fasting glucose levels were similarly present in all groups.

**Table 3: Metabolic and cardiovascular health at around age 30 years.**

| Outcome | SGA-GH EMM (95% CI) | SGA-S EMM (95% CI) | SGA-CU EMM (95% CI) | AGA EMM (95% CI) | *p*-value |
|---|---|---|---|---|---|
| ***Glucose homeostasis*** [1] | | | | | |
| Si *10-4/min$^{-1}$ (mU/l) | 8.32 (6.77 – 9.87) | 7.67 (5.59 – 9.74) * | 6.95 (5.35 – 8.56) * | 9.94 (8.44 – 11.45) | **0.023** |
| AIRg (mU/L) | 476.7 (380.5 – 572.9) | 558.7 (429.9 – 687.4) | 554.8 (455.3 – 654.3) | 464.4 (370.8 – 557.9) | 0.656 |
| Disposition index | 2983.4 (2490 – 3476) | 2930.1 (2271 – 3590) | 2718.9 (2209 – 3229) * | 3467.0 (2988 – 3946) | 0.166 |
| Sg *10-2/min$^{-1}$ (mg/d) | 0.021 (0.020 – 0.023) | 0.020 (0.018 – 0.022) | 0.020 (0.019 – 0.022) | 0.020 (0.019 – 0.022) | 0.688 |
| ***Body Composition*** [2] | | | | | |
| FM (kg) | 20.79 (18.71 – 22.87) | 22.05 (19.04 – 25.06) | 23.55 (21.35 – 25.74) | 21.15 (18.82 – 23.48) | 0.228 |
| TF (kg) | 11.11 (9.84 – 12.37) | 11.78 (9.94 – 13.61) | 12.20 (10.86 – 13.54) * | 10.34 (8.92 – 11.76) | 0.166 |
| LF (kg) | 8.86 (8.00 – 9.72) [b] | 9.43 (8.18 – 10.69) | 10.49 (9.57 – 11.41) | 9.96 (8.99 – 10.94) | 0.152 |
| LBM (kg) | 44.67 (43.54 – 45.80) [b] * | 45.28 (43.64 – 46.92) * | 47.15 (45.95 – 48.35) | 47.65 (46.39 – 48.92) | **0.024** |
| ***Visceral adiposity*** [1] | | | | | |
| VAT (cm$^2$) | 75.0 (62.4 – 87.6) | 87.1 (71.4 – 102.8) | 88.7 (75.6 – 101.8) * | 70.2 (58.9 – 81.6) | 0.107 |
| SAT (cm$^2$) | 164.8 (140.9 – 188.8) | 195.3 (165.5 – 225.2) | 187.9 (163.0 – 212.8) | 166.5 (144.9 – 188.1) | 0.244 |
| Liver fat fraction (%) | 3.15 (2.37 – 3.93) | 3.93 (2.91 – 4.96) | 3.85 (3.04 – 4.66) * | 2.73 (2.03 – 3.44) | 0.104 |
| ***Serum lipid levels*** [1] | | | | | |
| Cholesterol (mmol/L) | 4.75 (4.55 – 4.95) * | 4.84 (4.58 – 5.10) * | 4.64 (4.43 – 4.85) * | 4.33 (4.13 – 4.52) | **0.006** |
| LDLc (mmol/L) | 2.78 (2.61 – 2.95) * | 2.89 (2.67 – 3.11) * | 2.74 (2.56 – 2.92) * | 2.50 (2.33 – 2.66) | **0.018** |
| Triglycerides (mmol/L) | 1.23 (1.08 – 1.39) | 1.46 (1.26 – 1.66) * | 1.35 (1.19 – 1.51) * | 1.04 (0.89 – 1.19) | **0.003** |
| HDLc (mmol/L) | 1.30 (1.23 – 1.37) * | 1.32 (1.23 – 1.42) * | 1.30 (1.22 – 1.37) * | 1.45 (1.38 – 1.52) | **0.007** |
| ***Blood pressure*** [2] | | | | | |
| SBP (mmHg) | 118.1 (115.7 – 120.5) | 116.9 (113.5 – 120.4) | 118.1 (115.6 – 120.5) | 117.8 (115.1 – 120.5) | 0.927 |
| DBP (mmHg) | 70.8 (69.1 – 72.4) | 70.0 (67.6 – 72.3) | 69.6 (67.9 – 71.3) | 69.2 (67.4 – 71.1) | 0.737 |
| ***Metabolic syndrome*** | | | | | |
| MetS | 5.2 % | 10.8 % | 8.3 % | 4.4 % | 0.532 |
| - High waist circumference | 4.8 % [a b] | 14.0 % | 16.9 % | 12.0 % | 0.061 |
| - High blood pressure | 7.7 % | 16.3 % | 13.9 % | 14.3 % | 0.351 |
| - High triglycerides | 12.4 % [b] | 24.0 % * | 27.2 % * | 8.7 % | **0.003** |
| - Low HDLc | 33.3 % * | 42.0 % * | 36.4 % * | 18.5 % | **0.013** |
| - High fasting glucose | 8.6 % | 8.0 % | 3.9 % | 4.3 % | 0.466 |

Results of ANCOVA analysis, presented as estimated marginal means with 95% CI  Abbreviations: EMM, estimated marginal mean; CI; confidence interval; SGA-GH, Adults born small for gestational age treated with growth hormone; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature; Si, Insulin sensitivity; AIRg, Acute insulin response; Sg, Glucose effectiveness;  FM, Fat mass; TF, Trunk fat; LF, Limb fat; LBM, Lean Body Mass; VAT, Visceral adipose tissue; SAT, Subcutaneous adipose tissue; HDLc, High-density lipoprotein cholesterol; LDLc, Low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[1] Adjusted for sex and age  [a] p<0.05 SGA-GH compared to SGA-S  * p<0.05 compared to AGA

[2] Adjusted for sex, height and age  [b] p<0.05 SGA-GH compared to SGA-CU

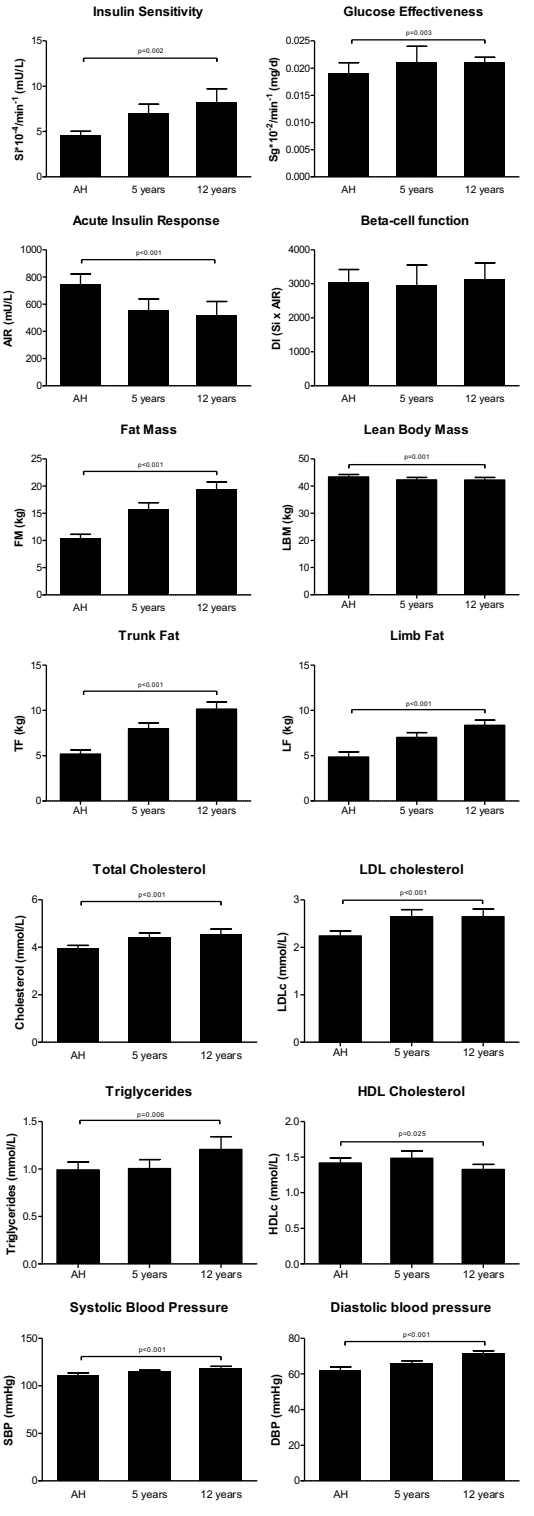


**Figure 1: Longitudinal changes in FSIGT results and body composition during 12 years after cessation of growth hormone treatment.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval. P-values for change within groups during 12 years follow-up were depicted if p<0.05. FSIGT results were corrected for sex and age; body composition results were corrected for sex, age and height. Abbreviations: FSIGT, frequently-sampled intravenous glucose tolerance test; Si, Insulin Sensitivity; Sg, Glucose Effectiveness; AIR, Acute Insulin Response; DI, Disposition Index; FM, Fat Mass; LBM, Lean Body Mass; TF, Trunk Fat; LF, Limb Fat.

*p<0.05 compared to AGA




**Figure 2: Longitudinal changes in serum lipid levels and blood pressure during 12 years after cessation of growth hormone treatment.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval. P-values for change within groups during 12 years follow-up were depicted if p<0.05. Serum lipid levels were corrected for sex and age; blood pressure results were corrected for sex, age and height. Abbreviations: SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; HDLc, High-density Lipoprotein; LDLc, Low-density Lipoprotein.

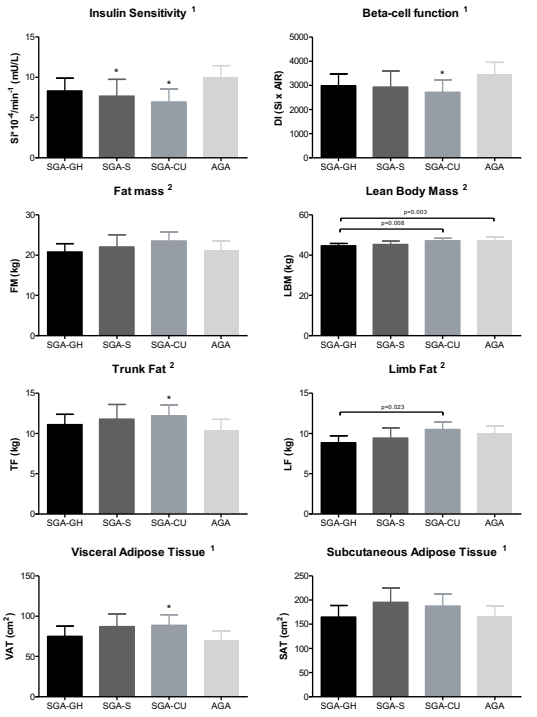


**Figure 3: FSIGT results, body composition and central adiposity at around age 30 years.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval. Abbreviations: VAT, Visceral Adipose Tissue; SAT, Subcutaneous Adipose Tissue; SGA-GH, SGA-GH, Adults born small for gestational age treated with growth hormone; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature.

* p<0.05 compared to AGA
1 Adjusted for sex and age
2 Adjusted for sex, height and age



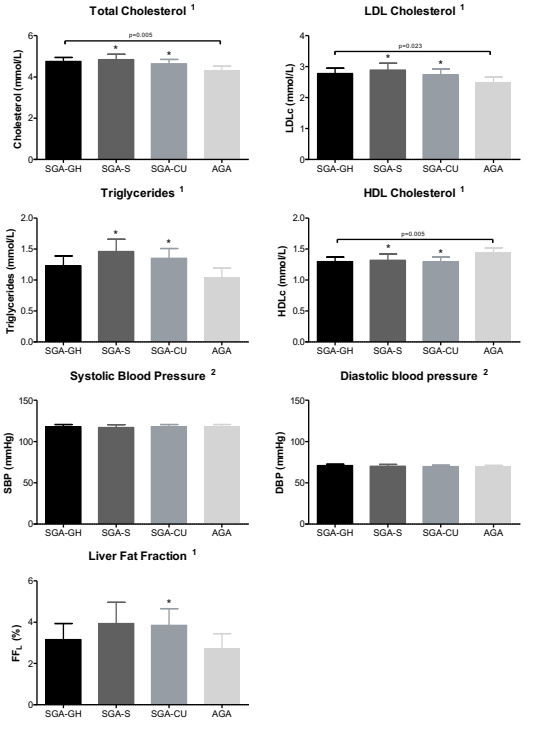


**Figure 4: Serum lipid levels, blood pressure and liver fat fraction at around age 30 years.**

Data are expressed as estimated marginal means with the upper limit of the 95% confidence interval. Abbreviations: HDLc, High-density Lipoprotein Cholesterol; LDLc, Low-density Lipoprotein Cholesterol; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FFL, Liver Fat Fraction; SGA-GH, Adults born small for gestational age treated with growth hormone; SGA-S, Adults born small for gestational age with persistent short stature; SGA-CU, Adults born small for gestational age with spontaneous catch-up growth; AGA, Adults born appropriate for gestational age with a normal adult stature.

* p<0.05 compared to AGA
1 Adjusted for sex and age
2 Adjusted for sex, height and age

## Discussion

Our study is the first 12-year follow-up study after cessation of GH treatment with detailed longitudinal measurements of metabolic and cardiovascular health parameters in previously GH-treated adults born SGA (SGA-GH) and compared with appropriate control groups consisting of untreated short adults born SGA (SGA-S) and adults born AGA with normal stature at age around 30 years. During 12 years after GH-cessation, SGA-GH adults had an increase in insulin sensitivity, fat mass parameters, total cholesterol and blood pressure to similar levels as SGA-S adults at around age 30 years. At that age, SGA-GH had similar insulin sensitivity, beta-cell function, FM, central fat mass, liver fat fraction, serum lipid levels and blood pressure as SGA-S adults. SGA-GH adults had also similar metabolic and cardiovascular health parameters as AGA adults, except of a lower LBM and higher adverse serum lipid levels which were present in all SGA groups.

Large population studies, including the SAGhE, presented higher cardiovascular mortality in previously GH-treated SGA adults at an average age of 30 years [11-13,23]. A Swedish population study also demonstrated increased cardiovascular death in GH-treated adults but showed that this difference disappeared after correction for birth characteristics [24]. These studies, however, did not include untreated adults born SGA as controls and compared cardiovascular events in GH-treated adults to the general population. Our study investigated determinants of cardiovascular and metabolic diseases in previously GH-treated adults born SGA at a similar age as in the SAGhE study in comparison with adequate untreated control groups of adults born SGA and AGA. Our results show that SGA-GH adults had similar metabolic and cardiovascular health determinants as SGA-S and AGA adults at around age 30 years, thus contradicting the previously reported higher metabolic and cardiovascular morbidity risk following childhood GH treatment in subjects born SGA.

There has been concern that the increased insulin resistance resulting from the insulin-antagonistic effects of GH treatment could increase diabetes mellitus type II risk in adulthood [4,5]. Our study shows that Si in SGA-GH adults during 12 years of follow-up increased and had similar values as in the SGA-S, SGA-CU and AGA adults. SGA-GH adults also had similar beta-cell function as SGA-S and SGA-CU adults. Remarkably, both SGA-S and SGA-CU groups had lower Si than AGA adults, indicating a negative influence of SGA birth on Si. In contrast, previously GH-treated SGA adults did not have a lower Si and beta-cell function at around age 30 years.

Body composition changes significantly during GH treatment in children born SGA, with a decrease in FM and an increase in LBM [8]. Our study shows that during the 12 years after GH-cessation, FM, trunk fat, and limb fat increased in SGA-GH adults but to similar values as SGA-S and AGA adults at around age 30 years. The SGA-S and AGA adults also showed an

increase in FM during 11 years in adulthood *(Ref PROGRAM32)*. Because the various groups differed in height, age and sex distribution, we adjusted all group comparisons for these variables. LBM decreased during 12 years of follow-up after GH-cessation, but only during the first 5 years, probably due to the loss of GH-properties [17]. At around age 30 years, LBM in SGA-GH was similar to SGA-S adults, and both were lower than SGA-CU and AGA adults.

Our study is the first to present MRI data on abdominal visceral and subcutaneous adipose tissue and the liver fat fraction in GH-treated adults born SGA. At around age 30 years, SGA-GH adults had a similar VAT, SAT, and liver fat fraction as SGA-S, SGA-CU and AGA adults. In untreated SGA-S and SGA-CU adults, however, there was a tendency of more visceral adiposity compared to AGA adults, with a significantly higher VAT in SGA-CU adults and a trend towards higher liver fat fraction in SGA-S and SGA-CU adults. Higher VAT and higher liver fat fraction are strongly associated with glucose intolerance, insulin insensitivity and hypertension in later life [25-27]. Our results indicate a positive long-term effect of GH treatment resulting in less central fat deposition.



At around age 30 years, SGA-GH adults had similar serum lipid levels as SGA-S and SGA-CU adults. However, SGA-GH, SGA-S, and SGA-CU adults had higher total cholesterol, LDLc and triglycerides, and lower HDLc than AGA adults, indicating higher adverse serum lipid levels in adults born SGA. Higher adverse serum lipid levels are associated with a higher risk for cardiovascular disease [28]. Our study shows that serum lipid levels were higher in all adults born SGA during adulthood, albeit within the normal range and independent of GH treatment. It is important to further investigate the cause of this unfavorable lipid profile in subjects born SGA. One explanation might be that fetal growth restriction leads to fetal and early postnatal re-programming of the lipid metabolism, leading to dyslipidemia when the fetal growth-restricted infant receives high-caloric feeding in an attempt to normalize the size of the SGA infant [14]. It is already known that rapid postnatal weight gain should be avoided in children who are born SGA to prevent an unhealthy metabolic and cardiovascular profile in early adulthood [29].

The SGA-GH adults showed a similar risk for metabolic syndrome as untreated adults born SGA and AGA, according to the NECP III criteria. This is reassuring as these criteria comprise an important clustering of risk factors for cardiovascular and metabolic disease [28].

Birth weight, birth length and adult height were different between groups, as these were part of the inclusion criteria for the three groups. Age was also different between groups, but we corrected all comparisons for age. SGA-GH adults had a lower SES based on income and educational level than SGA-S, SGA-CU and AGA adults, which might be due to the younger age in SGA-GH adults. We, therefore, additionally corrected for SES, but this did not change the results between the SGA-GH adults and control groups.

The PROGRAM32 study reported higher insulin resistance, unfavorable body composition and higher adverse serum lipid levels in SGA-CU adults, showing that SGA-birth and spontaneous catch-up in weight and length negatively influence adult health *(Ref PROGRAM32)*. Our results show that SGA-GH adults are more healthy than SGA-CU adults, indicating that GH-induced catch-up in height is associated with better long-term metabolic health than spontaneous catch-up in weight and height.

Although our study had smaller numbers than published population studies, our study presents a large cohort of previously GH-treated adults born SGA compared to appropriate untreated control groups, with extensive and longitudinal measurements to assess determinants of metabolic and cardiovascular risks. Such detailed measurements during many years of follow-up are not achievable in population studies. The proportion of SGA-S adults was smaller due to the low prevalence of SGA-born subjects with persistent short stature (~0·1% of all live-born children [30]) and the fact that most children with postnatal growth failure during the last 25 years were treated with growth hormone for adult height improvement. As our study population consisted of adults at around age 30 years, endpoints such as cardiovascular diseases and diabetes mellitus type II might not be present at that age. We, therefore, assessed a detailed health profile in this cohort using high-quality research tools, such as FSIGT, DXA and MRI.

In conclusion, our 12-year follow-up study after GH-cessation in previously GH-treated adults born SGA shows an increase in insulin sensitivity, FM, total cholesterol and blood pressure to similar levels as the untreated control groups, and maintenance of a normal beta-cell function and a slight decrease in LBM. Large population studies, including the SAGhE study, suggested an increase in cardiovascular morbidity in GH-treated adults, but detailed cardiovascular and metabolic determinants in our study were similar in GH-treated compared to untreated adults born SGA or AGA at around age 30 years. Our results show that long-term GH treatment in children born SGA has no adverse effects on metabolic and cardiovascular health up to 12 years after GH-cessation.

## Acknowledgements

We would like to express our gratitude to all adults who participated in this study. We thank J. Bontenbal-van de Wege, C. Bruinings-Vroombout, J. Dunk, N. Khieroe and E. Lems, research nurses, for their contribution to the study, W. Hackeng for analysing FSIGT samples and A. van der Lugt, M. Van den Ijssel, I. Vanwersch, and D. van Maldegem for assisting with the MRI scans. This study was financially supported by an independent research grant provided by Novo Nordisk, The Netherlands and Denmark.

## References


1. Sas T, de Waal W, Mulder P, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab.* 1999;84(9):3064-3070.
2. Dahlgren J, Wikland KA, Swedish Study Group for Growth Hormone T. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57(2):216-222.
3. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
4. Heptulla RA, Boulware SD, Caprio S, Silver D, Sherwin RS, Tamborlane WV. Decreased insulin sensitivity and compensatory hyperinsulinemia after hormone treatment in children with short stature. *The Journal of clinical endocrinology and metabolism.* 1997;82(10):3234-3238.
5. Cutfield WS, Wilton P, Bennmarker H, et al. Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet.* 2000;355(9204):610-613.
6. Stanley TL, Grinspoon SK. Effects of growth hormone-releasing hormone on visceral fat, metabolic, and cardiovascular indices in human studies. *Growth Horm IGF Res.* 2015;25(2):59-65.
7. Pasarica M, Zachwieja JJ, Dejonge L, Redman S, Smith SR. Effect of growth hormone on body composition and visceral adiposity in middle-aged men with visceral obesity. *J Clin Endocrinol Metab.* 2007;92(11):4265-4270.
8. Sas T, Mulder P, Hokken-Koelega A. Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. *J Clin Endocrinol Metab.* 2000;85(10):3786-3792.
9. Cornier MA, Dabelea D, Hernandez TL, et al. The metabolic syndrome. *Endocr Rev.* 2008;29(7):777-822.
10. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365(9468):1415-1428.
11. Carel JC, Ecosse E, Landier F, et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J Clin Endocrinol Metab.* 2012;97(2):416-425.
12. Savendahl L, Cooke R, Tidblad A, et al. Long-term mortality after childhood growth hormone treatment: the SAGhE cohort study. *Lancet Diabetes Endocrinol.* 2020;8(8):683-692.
13. Tidblad A, Bottai M, Kieler H, Albertsson-Wikland K, Sävendahl L. Association of Childhood Growth Hormone Treatment With Long-term Cardiovascular Morbidity. *JAMA Pediatr.* 2021;175(2):e205199.
14. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341(8850):938-941.

15. Usher R, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr.* 1969;74(6):901-910.
16. Fredriks AM, van Buuren S, Burgmeijer RJ, et al. Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res.* 2000;47(3):316-323.
17. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.
18. Boston RC, Stefanovski D, Moate PJ, Sumner AE, Watanabe RM, Bergman RN. MINMOD Millennium: a computer program to calculate glucose effectiveness and insulin sensitivity from the frequently sampled intravenous glucose tolerance test. *Diabetes Technol Ther.* 2003;5(6):1003-1015.
19. Guo Y, Franks PW, Brookshire T, Antonio Tataranni P. The intra- and inter-instrument reliability of DXA based on ex vivo soft tissue measurements. *Obes Res.* 2004;12(12):1925-1929.
20. Schweitzer L, Geisler C, Pourhassan M, et al. What is the best reference site for a single MRI slice to assess whole-body skeletal muscle and adipose tissue volumes in healthy adults? *Am J Clin Nutr.* 2015;102(1):58-65.
21. Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009;120(16):1640-1645.
22. Graham I. European guidelines on cardiovascular disease prevention in clinical practice: Executive summary. *Atherosclerosis.* 2007;194(1):1-45.
23. Savendahl L, Maes M, Albertsson-Wikland K, et al. Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. *J Clin Endocrinol Metab.* 2012;97(2):E213-217.
24. Albertsson-Wikland K, Mårtensson A, Sävendahl L, et al. Mortality Is Not Increased in Recombinant Human Growth Hormone-treated Patients When Adjusting for Birth Characteristics. *J Clin Endocrinol Metab.* 2016;101(5):2149-2159.
25. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev.* 2000;21(6):697-738.
26. Lorbeer R, Rospleszcz S, Schlett CL, et al. Correlation of MRI-derived adipose tissue measurements and anthropometric markers with prevalent hypertension in the community. *Journal of Hypertension.* 2018;36(7):1555-1562.
27. Hiuge-Shimizu A, Kishida K, Funahashi T, et al. Absolute value of visceral fat area measured on computed tomography scans and obesity-related cardiovascular risk factors in large-scale Japanese general population (the VACATION-J study). *Ann Med.* 2012;44(1):82-92.

28. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP, San Antonio Heart S. National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation.* 2004;110(10):1251-1257.
29. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301(21):2234-2242.
30. Hokken-Koelega AC, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL. Children born small for gestational age: do they catch up? *Pediatr Res.* 1995;38(2):267-271.

# Chapter 8

## General discussion

# General discussion

In 1991, our research group initiated the first Dutch study on growth hormone (GH) treatment in children born small for gestation age (SGA) with persistent short stature. Since then, several clinical trials have proved that GH treatment effectively improves adult height [1-3]. As low birth weight has been associated with higher risk of diabetes mellitus and cardiovascular diseases [4], long-term safety of GH treatment in children born SGA during and after cessation of treatment has been our focus. Our study group showed that childhood GH treatment in short children born SGA is safe, at least until 21 years of age [5,6]. In the studies presented in this thesis, we extended the follow-up period to 12 years after cessation of childhood GH treatment in short subjects born SGA and investigated the long-term safety of 2 years of gonadotropin-releasing hormone agonist (GnRHa) treatment in addition to childhood GH treatment to further improve adult height.

The first study presented in this thesis investigated the pubertal development and gonadal function in Silver-Russell syndrome (SRS), one of the causes of short SGA (Chapter 2). In the second study, we investigated kidney function and blood pressure in previously GH-treated young adults until 5 years after GH-cessation (Chapter 3). We also investigated the effect of 2 years of GnRHa treatment in addition to GH treatment on cognitive and psychosocial functioning and health-related quality of life (HRQoL) in young adults born SGA, at cessation of GH treatment (Chapter 4) and on metabolic and cardiovascular health and bone mineral density (BMD) comparing subjects treated with GH/GnRHa with those treated with GH only, during 5 years after cessation of GH treatment (Chapter 5). In addition, we longitudinally investigated body composition, central adiposity, metabolic and cardiovascular health in untreated adults born SGA compared to adults born appropriate for gestational age (AGA) until 32 years of age (Chapter 6). Finally, we assessed the metabolic and cardiovascular safety of childhood GH treatment during 12 years after cessation GH treatment in adults born SGA, also compared to age-matched adults born SGA or AGA without childhood GH treatment (Chapter 7).

In the general discussion, the results of the studies are discussed in view of recent literature. Furthermore, the clinical implications are addressed, as well as future directions for research.



# Silver-Russell syndrome

SRS is a rare disorder and one of the causes of short SGA. GH treatment has been approved for children with SRS, as children with SRS benefit the same from GH treatment as subjects born SGA [7,8]. Since most SRS individuals are not routinely followed up after adult height attainment, there was very little information in the literature regarding the long-term natural history of SRS.

**Pubertal development and gonadal function**

One of the issues that had not been evaluated in SRS patients was their pubertal progression and gonadal function. In Chapter 2, we longitudinally assessed gonadal function from childhood to early adulthood and pubertal development in 31 subjects with SRS (14 boys, 17 girls). We compared these data with those of 123 subjects born SGA without SRS and with data of healthy controls.

We showed that children with SRS have an average age at onset of puberty and a similar pubertal progression as children born SGA without SRS. However, two out of seventeen girls had onset of puberty before the age of 8 years, without underlying pathology. We postponed puberty with 2 years of additional GnRHa treatment in nine out of seventeen girls and four out of fourteen boys, if adult height prediction at start of puberty was below -2.5 standard deviation scores (SDS). After cessation of GnRHa treatment, pubertal progression was similar to that of children without SRS. Our results also suggest a similar beneficial effect of GnRHa treatment on adult height in children with and without SRS. GnRHa treatment could, therefore, be considered in children with SRS with an adult height prediction below -2.5 SDS at the start of puberty [9].

We found that girls with SRS had no impairment of the gonadal function. Previous cohorts have described Mayer-Rokitansky-Kuster-Hauser syndrome in females with SRS, a rare disorder characterized by hypoplasia of the uterus and upper part of the vagina [10,11]. In our cohort, one out of 17 girls with SRS had Müllerian agenesis, suggesting that Mayer-Rokitansky-Kuster-Hauser is more prevalent in SRS. However, larger cohorts of SRS females are needed to assess the exact prevalence of Müllerian agenesis.

Our results show that boys with SRS have, however, an increased risk for genital abnormalities such as cryptorchidism and hypospadias. Leydig cell function was similar in boys with SRS as controls. However, more than a quarter of the boys with SRS had a postpubertal serum inhibin B level below the 5$^{th}$ percentile for healthy references. Two out of fourteen boys also had an FSH level above the 95$^{th}$ percentile. All affected males had 11p15 loss of methylation (LOM). Our results suggest an increased risk of Sertoli cell dysfunction in males with SRS compared to males born SGA without SRS, particularly in those with 11p15 LOM. This is in concordance with previous studies, reporting a higher incidence of genital abnormalities in males with SRS due to 11p15 LOM (44-59%) than in those with UPD(7)mat (21-29%) [12-14]. Three cases have been described in the literature about severe under-virilisation and dysplastic testes in males with SRS [15-17]. Genital abnormalities and Sertoli cell dysfunction have predominantly been reported in children with SRS with 11p15 LOM and *IGF2* mutation [18,19], indicating a potential role of *IGF2* in the pathophysiology of genital abnormalities. More research is warranted to investigate the etiology of genital abnormalities and Sertoli cell dysfunction in males with SRS.

**Conclusions and clinical implications**

Both males and females with SRS have on average a similar onset and progression of puberty as GH-treated subjects born SGA without SRS. If puberty starts at an early age or adult height prediction at start of puberty is below -2.5 SDS, 2 years of additional GnRHa treatment could be considered since this improves adult height. Gonadal function in girls with SRS is within the normal range and similar to girls born SGA without SRS. Boys with SRS have, however, an increased risk of genital abnormalities and Sertoli cell dysfunction. A relatively small testicular volume due to the Sertoli cell dysfunction can be a pitfall in boys with SRS as they might have a more progressed puberty than expected based on Tanner stage. This complicates defining the onset of puberty and a reliable adult height prediction. We, therefore, would advise to regularly assess bone age and gonadotropins to accurately monitor pubertal onset in boys with SRS and to evaluate Sertoli cell function during puberty and at adult height attainment. Finally, we emphasize the need for SRS reference centers for providing specialized care for SRS patients and performing further dedicated research in larger cohorts of SRS patients.

## Kidney function after GH treatment in adults born SGA: 5-year follow-up after cessation of GH treatment



Infants born with a low birth weight have a smaller number of nephrons, which is associated with a lower GFR, a higher albumin-to-creatinine ratio and a higher blood pressure in early adulthood [20-25]. GH treatment increases the glomerular filtration rate (GFR), as serum IGF-I stimulates the renin-angiotensin system.

**Glomerular filtration rate, blood pressure and microalbuminuria**

The independent effects of GH treatment and SGA-birth on renal function had not been evaluated before. In Chapter 3, we longitudinally followed 261 young adults born SGA, previously treated with GH (SGA-GH) . GFR, based on serum creatinine levels, was determined at the cessation of GH treatment and at 6 months, 2 and 5 years thereafter. At 5 years after cessation, we compared these data to 309 untreated adults born SGA and AGA at age 21 years.

We found a relatively high GFR at the cessation of GH treatment, probably caused by stimulation of the renin-angiotensin system due to the higher serum IGF-I levels, which has also been described in a study describing patients with GH deficiency [26]. Our study shows that GFR after cessation of GH treatment in previously GH-treated adults born SGA remained well within the normal range, with a small decrease in GFR during the first 6 months after GH-cessation. At 5 years after GH-cessation, previously GH-treated SGA young adults had a similar GFR, blood pressure and urinary albumin excretion as age-matched untreated young adults born either SGA or AGA. Reassuringly, our results indicate that GH treatment has no long-lasting unfavorable effects on GFR in early adulthood.

None of the SGA-GH participants in our study had low estimated GFR values (<60 mL/min/1.73m$^2$) or a high albumin-to-creatinine ratio (>10 mg/mmol), which is reassuring, as this is associated with an increased risk of cardiovascular mortality [27-30]. Systolic and diastolic blood pressure was also similar in SGA-GH adults and age-matched untreated controls born SGA or AGA. These results are in line with previous publications of our study group [5,6].

Birth weight and birth length corrected for gestational age did not correlate with risk factors for developing kidney diseases in our study, at least at young adult age. In a cohort study in young adults born before 32 weeks gestational age, low birth weight was correlated with an increased risk of microalbuminuria at age 19 years [31]. The difference with our findings might be explained by the association of premature birth and a lower nephron number [22,32]. Our results suggest that SGA birth per se does not increase the risk of developing kidney disease in children born at term.

**Conclusions and clinical implications**

We show that GFR values remained well within the normal range during 5 years after cessation of GH treatment, and were similar to untreated SGA and AGA young adults at age 21 years. Our results suggest that GH treatment has no long-lasting unfavourable effects on kidney function and blood pressure. These results are reassuring for children with GH treatment, as these show another aspect of GH treatment safety.

## GnRHa treatment

GnRHa treatment in children postpones puberty, and additional GnRHa treatment for 2 years at the start of puberty improves adult height in GH-treated children born SGA with relatively early puberty resulting in an expected adult height of less than -2.5 SDS [9].

**Cognition, health-related quality of life and psychosocial functioning in young adults born SGA after GH/GnRHa treatment during childhood**

Postponement of puberty for 2 years might influence cognitive functioning, health-related quality of life (HRQoL), problem behaviour and school skills. Chapter 4 presents these findings at adult height attainment in 61 GH-treated young adults born SGA, who were additionally treated with 2 years of GnRHa during childhood. We compared these data to those of 38 young adults born SGA treated with GH only and to healthy references.

Studies during GnRHa treatment for central precocious puberty (CPP) reported a decrease in cognitive functioning [33,34]. Our results at adult height attainment, however, show similar cognitive results in GH-treated subjects born SGA independent of additional 2 years of GnRHa treatment at start of puberty. Therefore, the lower scores found in the other studies are most likely due to the delay in psychosocial maturation at the time of testing, as GnRHa-treated

patients entered puberty later than their peers. The lower cognitive functioning in the SGA subjects treated with GH treatment with or without additional GnRHa treatment might be explained by the association between SGA-birth and lower cognitive functioning [35-38].

The main concern about GnRHa treatment prior to our study was an increase in depressive emotions [34,39]. GnRHa resulted in an estradiol-dependent depressive response in healthy women undergoing short-term sex hormone manipulation with GnRHa, due to serotonin transporter changes [40]. Reassuringly, our long-term results show that 2 years of GnRHa treatment does not result in depressive emotions in young adults born SGA.

To our knowledge, no other study has investigated HRQoL, self-perception and problem behaviour in young adults born SGA following GnRHa treatment. These outcome measures are important as a 'patient-reported outcome' as they reflect the subjective perception of health and psychosocial functioning. Young adults of the GH/GnRHa group perceived lower cognitive functioning than the GH group and the reference population. The GH/GnRHa group scored lowest, followed by the GH group. This lower perception of cognitive functioning in the GH/GnRHa group contrasts with the slightly higher cognitive function in the same group. As the GH/GnRHa group had a higher SES, based on the educational level of the parents, it might be that the young adults underrated their cognitive functioning because they compared themselves with their better-performing parents. Self-perception scores and problem behaviour were described to remain similar during GnRHa treatment [33,34,41,42]. Reassuringly, we show similar self-perception scores and problem behaviour in our study in GH/GnRHa-treated, GH-treated adults and the reference population.



**Conclusions and clinical implications**

We show that 2 years of GnRHa treatment in addition to GH treatment results in similar cognitive functioning, HRQoL, self-perception and problem behaviour in young adults born SGA, compared to young adults born SGA treated with only GH. Values were within the normal range of the general population. Although we did not find negative effects on school performance, the child's perception of GnRHa treatment and school performance during treatment should be monitored during GnRHa treatment, as subjects undergoing GnRHa treatment might behave younger than their peers.

**Metabolic health and bone mineral density in young adults born SGA after GH/GnRHa treatment during childhood**

GnRHa treatment has been associated with a decrease in insulin sensitivity, gain in weight and fat mass (FM) and a decrease in BMD during the treatment. There were, however, no data on the long-term metabolic safety of additional 2 years of GnRHa treatment to GH treatment during childhood in young adults born SGA after GH-cessation at adult height attainment. In Chapter 5, we investigated metabolic health, body composition and BMD of

363 young adults born SGA, previously treated with combined GH/GnRHa or GH-only, until 5 years after attainment of adult height: at GH-cessation, 2 and 5 years thereafter. Data at 5 years after GH-cessation, at age 21 years, were additionally compared to 145 age-matched adults born AGA.

Metabolic effects of GnRHa treatment were predominantly described in populations of children with CPP. Several studies during GnRHa treatment showed a decrease in insulin sensitivity, expressed as HOMA-IR, a decrease in BMD, and an increase in weight and body mass index (BMI) [43-54]. Most of these studies, however, did not comprise long-term follow-up data. In contrast to the results described during GnRHa treatment, our results during 5 years of follow-up after GH-cessation show that insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and BMD are unaffected by the addition of 2 years of GnRHa treatment for postponement of puberty.

Our study was the first to investigate longitudinal metabolic effects in previously GnRHa-treated adolescents born SGA in comparison with an adequate control group of adolescents born SGA treated with GH only and with healthy young adults born AGA. At 21 years of age, the GH/GnRHa group had a similar metabolic health profile and BMD of the total body as the GH-only and AGA group and a higher BMD of the lumbar spine compared to the AGA group. Only one other study had presented metabolic health results during follow-up of GnRHa-treated female adults with CPP, showing no metabolic derangements at age 30-50 years [48]. Our results show that 2 years of GnRHa treatment in addition to GH treatment during childhood does not influence metabolic health at the age of 21 years. These suggests that the previously reported adverse effects on metabolic parameters in patients with CPP were due to the natural course of body composition due to CPP rather than GnRHa treatment.

**Conclusions and clinical implications**

The addition of 2 years of GnRHa treatment to long-term GH treatment in short children born SGA does not have an unfavorable effect on metabolic health and BMD until 5 years after GH-cessation at the age of 21 years. Based on our results, additional GnRHa treatment for 2 years could be considered at the start of puberty for GH-treated children born SGA with an adult height prediction below -2.5 SDS.

## Long-term metabolic health in adults born SGA (PROGRAM32)

Low birth weight has been associated with adult diseases like diabetes mellitus type II and cardiovascular diseases [4]. At age 21 years, the PROgramming factors for GRowth And Metabolism (PROGRAM) study showed that young adults born SGA with postnatal catch-up (SGA-CU) and accelerated weight gain in early life had insulin resistance, adverse lipid profile, significantly higher FM and an increased metabolic risk compared to those born AGA [55-58].

**Metabolic health following accelerated weight gain in early life in adults born SGA at age 32 years**

In Chapter 6, we longitudinally investigated insulin sensitivity, beta-cell function, body composition, abdominal adipose tissue, liver fat fraction, serum lipid levels and blood pressure in 287 adults (65 adults born SGA with persistent short stature (SGA-S), 105 adult born SGA with spontaneous catch-up to a normal adult height (SGA-CU) and 117 AGA adults) from age 21 to 32 years.

We showed that changes during 11 years of follow-up in adulthood were similar in all groups, but the SGA-CU group had an adverse health profile at age 21 and 32 years, compared to SGA-S and AGA adults. Our findings suggest that the adverse metabolic health profile at age 32 years is due to their accelerated weight gain in early childhood [55,56,58-60]. Another study had reported more insulin resistance in adults born SGA, using HOMA-IR to measure insulin sensitivity [61,62]. We found that the adverse lipid profile, unfavorable body composition and higher central adiposity persisted during 11 years of follow-up in SGA-CU adults. This suggests that accelerated weight gain following SGA birth has long-term negative consequences as these factors are all associated with an increased risk of cardiovascular and metabolic diseases in later life [63-66]. Reassuringly, none of the adults born SGA had diabetes mellitus type 2, and during 11 years the beta-cell function remained similar in all groups.



Remarkably, all adults born SGA in our study had higher serum lipid levels, independent of postnatal catch-up growth. One explanation might be that fetal growth restriction leads to fetal and early postnatal reprogramming of the lipid metabolism, when the fetal growth-restricted infant receives high-caloric feeding in an attempt to normalize the size of the SGA infant [4]. This postnatal reprogramming could lead to dyslipidemia in adulthood.

Our findings show that SGA birth followed by accelerated weight gain during the first months of life negatively affects metabolic health at age 32 years, as we also found an increased risk of developing metabolic syndrome (MetS), which is an important clustering of risk factors for cardiovascular and metabolic disease [63]. Weight development in early life should, therefore, be closely monitored to prevent accelerated weight gain, particularly in children born SGA. Our study also showed that a higher frequency of physical exercise was associated with better body composition in the total cohort.

**Conclusions and clinical implications**

SGA-CU adults have a persistently unhealthy metabolic profile, and accelerated weight gain during early life results in an increased metabolic risk. We, therefore, advise to avoid excess gain in weight in early life and particularly in children born SGA. Also, an active lifestyle for all adults is recommended, especially for those born SGA, to decrease the risk of unfavorable body composition and central adiposity at age 32 years.

## Long-term health following GH treatment in adults born SGA

GH treatment effectively induces catch-up growth and increases adult height in children born SGA [1,3,67]. Besides the positive effects on linear growth, GH has well-documented lipolytic, anabolic and insulin-antagonistic effects. As low birth weight has been associated with higher risk of diabetes mellitus and cardiovascular diseases [4], long-term safety of childhood GH treatment in subjects born SGA has been our focus. Our study group showed that childhood GH treatment in short children born SGA is safe, at least until 21 years of age [5,6]. However, large population studies including the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study, presented higher cardiovascular morbidity and mortality in previously GH-treated SGA adults [68-71], but these studies did not have an appropriate control group.

**Metabolic health of previously GH-treated adults born SGA at age 30 years**

Longitudinal data on the long-term effects of GH treatment on adult health after cessation of GH treatment in comparison with appropriate controls were lacking. The study described in Chapter 7 longitudinally investigated changes in body composition, central adiposity, and metabolic health in 167 adults during 12 years after cessation of GH treatment. We compared these data with 219 age-matched controls at age 30 years.

Due to the insulin-antagonistic effects of GH treatment, the primary concern of GH treatment was an increased risk of developing diabetes mellitus type II (DMII) during follow-up. Our results now show that during 12 years of follow-up, SGA-GH adults maintain a normal beta-cell function and show an increase in insulin sensitivity to similar levels as AGA adults at age 30 years, in contrast to the untreated SGA groups (SGA-S and SGA-CU). Our results show that GH treatment has no long-term adverse effects on insulin sensitivity and beta-cell function.

Body composition changed significantly during GH treatment in children born SGA, with a decrease in FM and an increase in LBM [72]. During the 12 years after GH-cessation, FM, trunk fat, and limb fat increased in SGA-GH adults but to similar values as SGA-S and AGA adults at around age 30 years. The PROGRAM32 study showed that the FM also increased in untreated adults born SGA and AGA during 11 years in adulthood. LBM decreased during 12 years of follow-up after GH-cessation, but only during the first 5 years, probably due to the loss of GH-properties [6]. At around age 30 years, LBM in GH-treated adults born SGA was similar to short adults born SGA, and both were lower than adults born AGA.

Adverse serum lipid levels were higher in all adults born SGA, irrespective of GH treatment. This might be due to fetal growth restriction leading to fetal and early postnatal reprogramming of the lipid metabolism, when the fetal growth-restricted infant receives

high-caloric feeding in an attempt to normalize the size of the SGA infant [4]. This postnatal reprogramming could lead to dyslipidemia in adulthood.

Long-term effects of GH treatment were mainly known from large population studies, including the SAGhE study, presenting higher cardiovascular morbidity and mortality in previously GH-treated SGA adults [68-71]. As GH treatment is associated with a decrease in insulin sensitivity [73-75], FM, serum lipid levels and blood pressure, and an increase in LBM, these could influence the risk of developing metabolic and cardiovascular diseases. A limitation of the population studies was the absence of an appropriate control group of adults born SGA, knowing that SGA-birth and subsequent postnatal catch-up influences metabolic and cardiovascular health [4]. A Swedish population study also presented increased cardiovascular death in previously GH-treated adults but showed that this difference disappeared after correction for birth characteristics [76], indicating the importance of correcting for SGA-birth in studies regarding adult metabolic and cardiovascular health. By assessing detailed cardiovascular and metabolic determinants in GH-treated adults born SGA compared to appropriate control groups of untreated adults born SGA and AGA, our study shows that long-term GH treatment in children born SGA has no adverse effects on metabolic and cardiovascular health up to 12 years after GH-cessation. Based on our findings, it is unlikely that childhood GH treatment results in more cardiovascular morbidity and mortality in SGA born subjects at the age of around 30 years, as reported in the SAGhE study.



**Conclusions and clinical implications**

Our study shows that cardiovascular and metabolic health parameters significantly change during 12 years after cessation of GH treatment. However, at age 30 years, metabolic and cardiovascular health parameters were similar between GH-treated adults and untreated adults born SGA with persistent short stature and adults born AGA. Our results indicate that GH treatment is a safe and effective treatment for short children born SGA to induce catch-up to a normal stature.

## General conclusions

Our study group has shown that children with SRS show specific traits and growth patterns compared to children born SGA without SRS. This thesis shows that SRS patients have on average a similar age at onset of puberty and pubertal progression as non-SRS subjects born SGA but that disturbances in Sertoli cell function are more common in SRS males.

In children born SGA with persistent short stature, treatment with GH leads to adult height improvement [1,3,77]. We show that GFR values remained well within the normal range during 5 years after cessation of GH treatment, while GFR values only slightly decreased during the first 6 months. Our results suggest that GH treatment has no unfavourable effects on kidney function and blood pressure on the long-term.

The Dutch SGA study has shown that postponement of puberty with GnRHa for 2 years at the start of puberty because of an expected adult height < −2.5 SDS can improve adult height in children born SGA [9]. The studies in this thesis show that the additional 2 years of GnRHa treatment during childhood does not have adverse effects on metabolic and cardiovascular health, cognitive function, health-related quality of life and psychosocial functioning in adults born SGA. Thus, 2 years of GnRHa treatment appears to be safe and effective and can be considered as treatment of short SGA children.

Adults born SGA with spontaneous postnatal catch-up had higher insulin resistance, unfavorable body composition and higher adverse serum lipid levels than age-matched AGA adults, and accelerated weight gain in early childhood is associated with an increased metabolic risk. Our results highlight the importance of evaluating weight development in early life to prevent accelerated weight gain, particularly in children born SGA.

By assessing detailed cardiovascular and metabolic determinants in previously GH-treated adults born SGA and in appropriate control groups of untreated adults born SGA or AGA, our study shows that long-term GH treatment in children born SGA has no adverse effects metabolic and cardiovascular health up to 12 years after GH-cessation. Based on our findings, it is unlikely that childhood GH treatment results in more cardiovascular morbidity and mortality in SGA born subjects at the age around 30 years, as reported in the SAGhE study. Our results indicate that GH treatment is a safe and effective treatment for short children born SGA to induce catch-up to a normal stature.

## Directions for future research

Firstly, it is essential to investigate the (epi)genetic mechanism of clinical SRS further, as a large percentage of subjects with SRS remain without genetic diagnosis. Metabolic and cardiovascular health beyond age 30 years should be investigated in larger cohorts of adults with SRS, as some case reports showed an increased risk of metabolic disease at a relatively young age (50 years). Our results on gonadal function, particularly in males, warrant more research on reproduction in adults with SRS. To investigate these parameters, it is essential to follow-up subjects with SRS after attainment of adult height. SRS reference centers and international collaboration should be used to enlarge cohorts and increase the current knowledge about SRS in adulthood.

Although gonadal function is unaffected by 2 years of additional GnRHa treatment, it is essential to further investigate the long-term follow-up of reproduction in adults born SGA. Also, long-term follow-up of psychosocial functioning could be investigated after combined GH/GnRHa treatment during childhood.



Further investigations about the effect of food intake in younger and older children born SGA on serum lipid profiles and body composition is clinically highly relevant. It is essential to investigate optimal feeding for infants and children and develop a strategy for a balanced catch-up in weight during infancy and childhood, to prevent negative long-term effects on metabolic health. Also, it is important to monitor children born SGA in order to identify children at risk for overweight and obesity and an adverse metabolic health profile.

It is essential to investigate the long-term effects of GH treatment on cerebral vasculature and brain structures, as a French population study (French part of SAGhE study) reported an increased risk of cerebral bleeding around age 30 years, following childhood GH treatment compared to the general population. Follow-up of GH-treated adults could further focus on health-related quality of life and psychosocial functioning. Lastly, it is essential to investigate the effect of SGA-birth and GH treatment on offspring characteristics, such as birth weight, birth length and metabolic health.

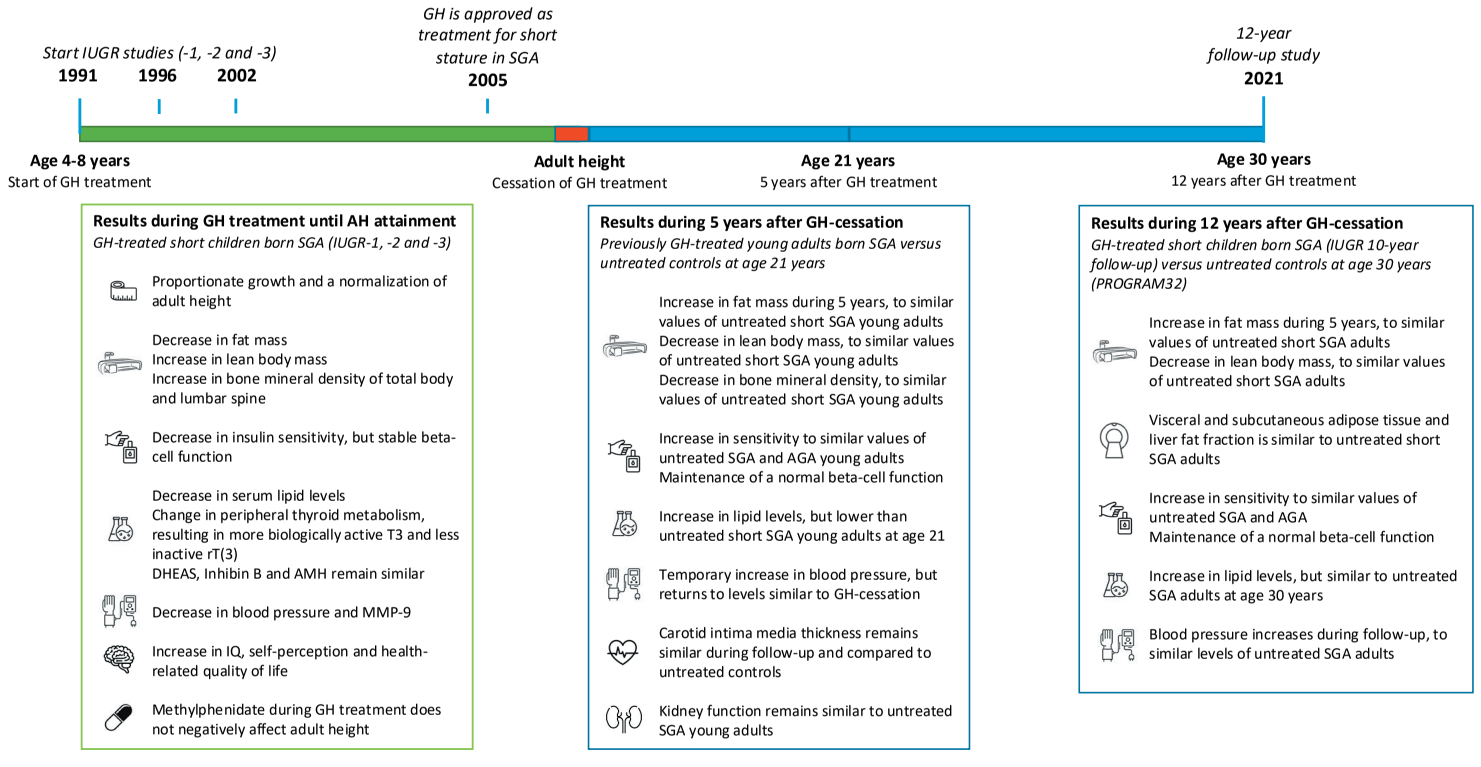


**Figure 1. Overview of the IUGR-1, IUGR-2, IUGR-3 and follow-up studies.**

## References


1. Dahlgren J, Wikland KA, Swedish Study Group for Growth Hormone T. Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res.* 2005;57(2):216-222.
2. Renes JS, Willemsen RH, Mulder JC, et al. New insights into factors influencing adult height in short SGA children: Results of a large multicenter growth hormone trial. *Clin Endocrinol (Oxf).* 2014.
3. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab.* 2003;88(8):3584-3590.
4. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet.* 1993;341(8850):938-941.
5. van der Steen M, Kerkhof GF, Smeets CCJ, Hokken-Koelega ACS. Cardiovascular risk factors and carotid intima media thickness in young adults born small for gestational age after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(12):975-985.
6. van der Steen M, Smeets CC, Kerkhof GF, Hokken-Koelega AC. Metabolic health of young adults who were born small for gestational age and treated with growth hormone, after cessation of growth hormone treatment: a 5-year longitudinal study. *Lancet Diabetes Endocrinol.* 2017;5(2):106-116.
7. Smeets CC, Renes JS, van der Steen M, Hokken-Koelega AC. Metabolic Health and Long-Term Safety of Growth Hormone Treatment in Silver-Russell Syndrome. *J Clin Endocrinol Metab.* 2017;102(3):983-991.
8. Smeets CC, Zandwijken GR, Renes JS, Hokken-Koelega AC. Long-Term Results of GH Treatment in Silver-Russell Syndrome (SRS): Do They Benefit the Same as Non-SRS Short-SGA? *J Clin Endocrinol Metab.* 2016;101(5):2105-2112.
9. Lem AJ, van der Kaay DC, de Ridder MA, et al. Adult height in short children born SGA treated with growth hormone and gonadotropin releasing hormone analog: results of a randomized, dose-response GH trial. *J Clin Endocrinol Metab.* 2012;97(11):4096-4105.
10. Abraham MB, Carpenter K, Baynam GS, Mackay DJ, Price G, Choong CS. Report and review of described associations of Mayer-Rokitansky-Kuster-Hauser syndrome and Silver-Russell syndrome. *J Paediatr Child Health.* 2014.
11. Bellver-Pradas J, Cervera-Sanchez J, Boldo-Roda A, et al. Silver-Russell syndrome associated to Mayer-Rokitansky-Kuster-Hauser syndrome, diabetes and hirsutism. *Arch Gynecol Obstet.* 2001;265(3):155-157.
12. Bruce S, Hannula-Jouppi K, Peltonen J, Kere J, Lipsanen-Nyman M. Clinically distinct epigenetic subgroups in Silver-Russell syndrome: the degree of H19 hypomethylation associates with phenotype severity and genital and skeletal anomalies. *J Clin Endocrinol Metab.* 2009;94(2):579-587.

13. Wakeling EL, Abu Amero S, Alders M, et al. Epigenotype-phenotype correlations in Silver-Russell syndrome. *J Med Genet.* 2010;47(11):760-768.
14. Price SM, Stanhope R, Garrett C, Preece MA, Trembath RC. The spectrum of Silver-Russell syndrome: a clinical and molecular genetic study and new diagnostic criteria. *J Med Genet.* 1999;36(11):837-842.
15. Adachi M, Fukami M, Kagami M, et al. Severe in utero under-virilization in a 46,XY patient with Silver-Russell syndrome with 11p15 loss of methylation. *J Pediatr Endocrinol Metab.* 2019;32(2):191-196.
16. Sujansky E, Riccardi VM. Ambiguous genitalia in the Russell-Silver syndrome. *Am J Dis Child.* 1978;132(2):214.
17. Marks LJ, Bergeson PS. The Silver-Russel syndrome: a case with sexual ambiguity, and a review of literature. *Am J Dis Child.* 1977;131(4):447-451.
18. Yamoto K, Saitsu H, Nakagawa N, et al. De novo IGF2 mutation on the paternal allele in a patient with Silver-Russell syndrome and ectrodactyly. *Hum Mutat.* 2017;38(8):953-958.
19. Masunaga Y, Inoue T, Yamoto K, et al. IGF2 Mutations: Report of Five Cases, Review of the Literature, and Comparison with H19/IGF2:IG-DMR Epimutations. *The Journal of Clinical Endocrinology & Metabolism.* 2019;105(1):116-125.
20. Xu R, Zuo L. Low birthweight and chronic kidney disease. *Nephrology (Carlton).* 2010;15 Suppl 2:18-22.
21. Hughson M, Farris AB, 3rd, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int.* 2003;63(6):2113-2122.
22. Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. *American journal of kidney diseases.* 1994;23(2):171-175.
23. Hallan S, Euser AM, Irgens LM, Finken MJ, Holmen J, Dekker FW. Effect of intrauterine growth restriction on kidney function at young adult age: the Nord Trondelag Health (HUNT 2) Study. *Am J Kidney Dis.* 2008;51(1):10-20.
24. Vikse BE, Irgens LM, Leivestad T, Hallan S, Iversen BM. Low birth weight increases risk for end-stage renal disease. *J Am Soc Nephrol.* 2008;19(1):151-157.
25. Rodriguez-Soriano J, Aguirre M, Oliveros R, Vallo A. Long-term renal follow-up of extremely low birth weight infants. *Pediatr Nephrol.* 2005;20(5):579-584.
26. Böger RH, Skamira C, Bode-Böger SM, Brabant G, von zur Muhlen A, Frolich JC. Nitric oxide may mediate the hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. *Journal of Clinical Investigation.* 1996;98(12):2706-2713.
27. Bello AK, Hemmelgarn B, Lloyd A, et al. Associations among estimated glomerular filtration rate, proteinuria, and adverse cardiovascular outcomes. *Clin J Am Soc Nephrol.* 2011;6(6):1418-1426.
28. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet.* 2010;375(9731):2073-2081.

29. Matsushita K, Coresh J, Sang Y, et al. Estimated glomerular filtration rate and albuminuria for prediction of cardiovascular outcomes: a collaborative meta-analysis of individual participant data. *Lancet Diabetes Endocrinol.* 2015;3(7):514-525.
30. Nichols GA, Deruaz-Luyet A, Hauske SJ, Brodovicz KG. The association between estimated glomerular filtration rate, albuminuria, and risk of cardiovascular hospitalizations and all-cause mortality among patients with type 2 diabetes. *J Diabetes Complications.* 2018;32(3):291-297.
31. Keijzer-Veen MG, Kleinveld HA, Lequin MH, et al. Renal function and size at young adult age after intrauterine growth restriction and very premature birth. *Am J Kidney Dis.* 2007;50(4):542-551.
32. Rodriguez MM, Gomez AH, Abitbol CL, Chandar JJ, Duara S, Zilleruelo GE. Histomorphometric analysis of postnatal glomerulogenesis in extremely preterm infants. *Pediatr Dev Pathol.* 2004;7(1):17-25.
33. Mul D, Versluis-den Bieman HJ, Slijper FM, Oostdijk W, Waelkens JJ, Drop SL. Psychological assessments before and after treatment of early puberty in adopted children. *Acta Paediatr.* 2001;90(9):965-971.
34. Wojniusz S, Callens N, Sutterlin S, et al. Cognitive, Emotional, and Psychosocial Functioning of Girls Treated with Pharmacological Puberty Blockage for Idiopathic Central Precocious Puberty. *Front Psychol.* 2016;7:1053.
35. Lee PA, Houk CP. Cognitive and psychosocial development concerns in children born small for gestational age. *Pediatr Endocrinol Rev.* 2012;10(2):209-216.
36. Puga B, Gil P, de Arriba A, et al. Neurocognitive development of children born small for gestational age (SGA). An update. *Pediatr Endocrinol Rev.* 2012;9(4):716-726.
37. Stephen MD, Varni JW, Limbers CA, et al. Health-related quality of life and cognitive functioning in pediatric short stature: comparison of growth-hormone-naive, growth-hormone-treated, and healthy samples. *Eur J Pediatr.* 2011;170(3):351-358.
38. Downie AB, Mulligan J, Stratford RJ, Betts PR, Voss LD. Are short normal children at a disadvantage? The Wessex growth study. *Bmj.* 1997;314(7074):97-100.
39. Frokjaer VG, Pinborg A, Holst KK, et al. Role of Serotonin Transporter Changes in Depressive Responses to Sex-Steroid Hormone Manipulation: A Positron Emission Tomography Study. *Biol Psychiatry.* 2015;78(8):534-543.
40. Frokjaer VG. Pharmacological sex hormone manipulation as a risk model for depression. *J Neurosci Res.* 2020;98(7):1283-1292.
41. Visser-van Balen H, Geenen R, Moerbeek M, et al. Psychosocial functioning of adolescents with idiopathic short stature or persistent short stature born small for gestational age during three years of combined growth hormone and gonadotropin-releasing hormone agonist treatment. *Horm Res.* 2005;64(2):77-87.
42. Mul D, Oostdijk W, Waelkens JJ, Schulpen TW, Drop SL. Gonadotrophin releasing hormone agonist treatment with or without recombinant human GH in adopted children with early puberty. *Clin Endocrinol (Oxf).* 2001;55(1):121-129.

43. Boot AM, De Muinck Keizer-Schrama S, Pols HA, Krenning EP, Drop SL. Bone mineral density and body composition before and during treatment with gonadotropin-releasing hormone agonist in children with central precocious and early puberty. *J Clin Endocrinol Metab.* 1998;83(2):370-373.
44. van der Sluis IM, Boot AM, Krenning EP, Drop SL, de Muinck Keizer-Schrama SM. Longitudinal follow-up of bone density and body composition in children with precocious or early puberty before, during and after cessation of GnRH agonist therapy. *J Clin Endocrinol Metab.* 2002;87(2):506-512.
45. Saggese G, Bertelloni S, Baroncelli GI, Battini R, Franchi G. Reduction of bone density: an effect of gonadotropin releasing hormone analogue treatment in central precocious puberty. *Eur J Pediatr.* 1993;152(9):717-720.
46. Antoniazzi F, Bertoldo F, Zamboni G, et al. Bone mineral metabolism in girls with precocious puberty during gonadotrophin-releasing hormone agonist treatment. *Eur J Endocrinol.* 1995;133(4):412-417.
47. Magiakou MA, Manousaki D, Papadaki M, et al. The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. *J Clin Endocrinol Metab.* 2010;95(1):109-117.
48. Lazar L, Lebenthal Y, Yackobovitch-Gavan M, et al. Treated and untreated women with idiopathic precocious puberty: BMI evolution, metabolic outcome, and general health between third and fifth decades. *J Clin Endocrinol Metab.* 2015;100(4):1445-1451.
49. Aguiar AL, Couto-Silva AC, Vicente EJ, Freitas IC, Cruz T, Adan L. Weight evolution in girls treated for idiopathic central precocious puberty with GnRH analogues. *J Pediatr Endocrinol Metab.* 2006;19(11):1327-1334.
50. Park HK, Lee HS, Ko JH, Hwang IT, Lim JS, Hwang JS. The effect of gonadotrophin-releasing hormone agonist treatment over 3 years on bone mineral density and body composition in girls with central precocious puberty. *Clin Endocrinol (Oxf).* 2012;77(5):743-748.
51. Chiavaroli V, Liberati M, D'Antonio F, et al. GNRH analog therapy in girls with early puberty is associated with the achievement of predicted final height but also with increased risk of polycystic ovary syndrome. *Eur J Endocrinol.*163(1):55-62.
52. Sorensen K, Mouritsen A, Mogensen SS, Aksglaede L, Juul A. Insulin sensitivity and lipid profiles in girls with central precocious puberty before and during gonadal suppression. *J Clin Endocrinol Metab.* 2010;95(8):3736-3744.
53. Tascilar ME, Bilir P, Akinci A, et al. The effect of gonadotropin-releasing hormone analog treatment (leuprolide) on body fat distribution in idiopathic central precocious puberty. *Turk J Pediatr.* 2011;53(1):27-33.
54. Faienza MF, Brunetti G, Acquafredda A, et al. Metabolic Outcomes, Bone Health, and Risk of Polycystic Ovary Syndrome in Girls with Idiopathic Central Precocious Puberty Treated with Gonadotropin-Releasing Hormone Analogues. *Horm Res Paediatr.* 2017;87(3):162-169.
55. Leunissen RW, Kerkhof GF, Stijnen T, Hokken-Koelega A. Timing and tempo of first-year rapid growth in relation to cardiovascular and metabolic risk profile in early adulthood. *JAMA.* 2009;301(21):2234-2242.

56. Leunissen RW, Stijnen T, Hokken-Koelega AC. Influence of birth size on body composition in early adulthood: the programming factors for growth and metabolism (PROGRAM)-study. *Clin Endocrinol (Oxf).* 2009;70(2):245-251.
57. Leunissen RW, Oosterbeek P, Hol LK, Hellingman AA, Stijnen T, Hokken-Koelega AC. Fat mass accumulation during childhood determines insulin sensitivity in early adulthood. *J Clin Endocrinol Metab.* 2008;93(2):445-451.
58. Kerkhof GF, Hokken-Koelega AC. Rate of neonatal weight gain and effects on adult metabolic health. *Nat Rev Endocrinol.* 2012;8(11):689-692.
59. Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. *Obes Rev.* 2005;6(2):143-154.
60. Ekelund U, Ong K, Linne Y, et al. Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: the Stockholm Weight Development Study (SWEDES). *Am J Clin Nutr.* 2006;83(2):324-330.
61. Meas T, Deghmoun S, Alberti C, et al. Independent effects of weight gain and fetal programming on metabolic complications in adults born small for gestational age. *Diabetologia.* 2010;53(5):907-913.
62. Balasuriya CND, Stunes AK, Mosti MP, et al. Metabolic Outcomes in Adults Born Preterm With Very Low Birthweight or Small for Gestational Age at Term: A Cohort Study. *J Clin Endocrinol Metab.* 2018;103(12):4437-4446.
63. Hunt KJ, Resendez RG, Williams K, Haffner SM, Stern MP, San Antonio Heart S. National Cholesterol Education Program versus World Health Organization metabolic syndrome in relation to all-cause and cardiovascular mortality in the San Antonio Heart Study. *Circulation.* 2004;110(10):1251-1257.
64. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev.* 2000;21(6):697-738.
65. Lorbeer R, Rospleszcz S, Schlett CL, et al. Correlation of MRI-derived adipose tissue measurements and anthropometric markers with prevalent hypertension in the community. *Journal of Hypertension.* 2018;36(7):1555-1562.
66. Hiuge-Shimizu A, Kishida K, Funahashi T, et al. Absolute value of visceral fat area measured on computed tomography scans and obesity-related cardiovascular risk factors in large-scale Japanese general population (the VACATION-J study). *Ann Med.* 2012;44(1):82-92.
67. Maiorana A, Cianfarani S. Impact of growth hormone therapy on adult height of children born small for gestational age. *Pediatrics.* 2009;124(3):e519-531.
68. Carel JC, Ecosse E, Landier F, et al. Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J Clin Endocrinol Metab.* 2012;97(2):416-425.
69. Savendahl L, Cooke R, Tidblad A, et al. Long-term mortality after childhood growth hormone treatment: the SAGhE cohort study. *Lancet Diabetes Endocrinol.* 2020;8(8):683-692.
70. Savendahl L, Maes M, Albertsson-Wikland K, et al. Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during

childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. *J Clin Endocrinol Metab.* 2012;97(2):E213-217.

71. Tidblad A, Bottai M, Kieler H, Albertsson-Wikland K, Sävendahl L. Association of Childhood Growth Hormone Treatment With Long-term Cardiovascular Morbidity. *JAMA Pediatr.* 2021;175(2):e205199.
72. Sas T, Mulder P, Hokken-Koelega A. Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. *J Clin Endocrinol Metab.* 2000;85(10):3786-3792.
73. Bratusch-Marrain PR, Smith D, DeFronzo RA. The effect of growth hormone on glucose metabolism and insulin secretion in man. *The Journal of clinical endocrinology and metabolism.* 1982;55(5):973-982.
74. Heptulla RA, Boulware SD, Caprio S, Silver D, Sherwin RS, Tamborlane WV. Decreased insulin sensitivity and compensatory hyperinsulinemia after hormone treatment in children with short stature. *The Journal of clinical endocrinology and metabolism.* 1997;82(10):3234-3238.
75. Cutfield WS, Wilton P, Bennmarker H, et al. Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet.* 2000;355(9204):610-613.
76. Albertsson-Wikland K, Mårtensson A, Sävendahl L, et al. Mortality Is Not Increased in Recombinant Human Growth Hormone-treated Patients When Adjusting for Birth Characteristics. *J Clin Endocrinol Metab.* 2016;101(5):2149-2159.
77. Sas T, de Waal W, Mulder P, et al. Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab.* 1999;84(9):3064-3070.

8

# Chapter 9

## Summary
## Samenvatting

# Summary

**Chapter 1**

This chapter provides an overview of the definition, prevalence and causes of small for gestational age (SGA) birth, and the effects of SGA birth on puberty, adult stature and metabolic health. It describes the effects of growth hormone (GH) and gonadotropin-releasing hormone agonist (GnRHa) treatment during childhood in subjects born SGA and children with Silver-Russell syndrome (SRS), a rare genetic variant causing SGA birth and persistent short stature in most cases. Furthermore, we provide an introduction on parameters addressed in studies in this thesis, namely metabolic health, gonadal function and bone mineral density (BMD). Finally, the aims and outline of this thesis are presented.

**Chapter 2**

Children with SRS are mostly born SGA and benefit similarly from GH treatment as those born SGA without SRS. We followed 32 children with SRS until attainment of adult height, investigating the onset and progression of puberty and gonadal function. We compared these data to a control group of subjects born SGA without SRS, and to age-appropriate reference data of healthy Dutch adolescents.



Mean age at onset of puberty was similar in males with SRS (11.5 years) and males without SRS (11.6 years), with similar pubertal duration in both groups. Four of the 14 males with SRS had a postpubertal inhibin-B level below the 5th percentile compared to healthy controls, and two of them an FSH above the 95th percentile, indicating Sertoli cell dysfunction.

Mean age at onset of puberty was similar in females with SRS (10.5 years) and females without SRS (10.7 years), with similar pubertal duration in both groups. Mean age at menarche was similar in females with SRS (13.1 years) and without SRS (13.3 years). All females with SRS had AMH, LH and FSH levels within the reference range. One female with SRS had primary amenorrhea due to Müllerian agenesis.

We also compared pubertal height gain in GH-treated subjects with SRS with and without additional 2 years of GnRHa treatment. This study shows that GH-treated subjects with SRS who additionally received 2 years of GnRHa had significantly better pubertal height gain.

In conclusion, subjects with SRS have a similar age at onset of puberty and a similar pubertal progression as subjects without SRS born SGA. Although gonadal function was on average similar in subjects with SRS or without SRS, disturbances in Sertoli cell function were more common in males with SRS. GH-treated children with SRS with an adult height expectation below -2.5 standard deviation scores (SDS) can benefit from additional GnRHa treatment to increase adult height, similar to children born SGA without SRS.

**Chapter 3**

GH treatment increases glomerular filtration rate (GFR), as serum IGF-I stimulates the renin-angiotensin system. It has been reported that infants born with a low birth weight have a smaller number of nephrons, which causes a lower GFR, a higher blood pressure and a higher albumin-to-creatinine ratio in early adulthood. We followed 261 subjects born SGA who were treated with GH during childhood until 5 years after GH-cessation, investigating kidney function and blood pressure, and compared these data to untreated adults born SGA and appropriate for gestational age (AGA).

Only during the first 6 months after cessation of GH treatment, there was a slight but significant decrease in GFR while remaining well within the normal range, due to the loss of GH properties. From 6 months after cessation of GH treatment until 5 years after GH-cessation, GFR remained stable and within the normal range.

At age 21 years, GH-treated young adults born SGA had similar GFR, blood pressure and urinary albumin-to-creatinine ratio as the healthy untreated controls either born SGA or AGA. We conclude that long-term GH treatment in children born SGA has no unfavourable effects on kidney function in early adulthood.

**Chapter 4**

GH-treated children born SGA with an adult height expectation below -2.5 standard deviation scores (SDS) at onset of puberty can benefit from additional GnRHa treatment for 2 years to increase adult height. We followed 99 subjects born SGA who were treated with GH/GnRHa or GH only during childhood until adult height attainment, investigating the cognition, psychosocial functioning and health-related quality of life at cessation of GH/GnRHa treatment versus only GH treatment in young adults born SGA.

Intelligence quotient scores were similar in the GH/GnRHa and GH-group. Health-related quality of life was similar between both groups, and also compared to reference population. However, perception of cognitive functioning was significantly lower in the GH/GnRHa-group. This result might be explained by higher expectations of their parents, as parents of the GH/GnRHa-treated young adults had higher educational level than of those treated with GH only. Problem behaviour was similar in the GH/GnRHa and GH-group. AH did not correlate with health-related quality of life, self-perception and problem behaviour.

We conclude that additional 2 years of GnRHa treatment has no negative effects on cognitive and psychosocial functioning in previously GH-treated young adults born SGA.

**Chapter 5**

GH-treated children born SGA with an adult height expectation below -2.5 standard deviation scores (SDS) at onset of puberty can benefit from additional GnRHa treatment for 2 years to increase adult height. Studies have reported a decrease in insulin sensitivity and bone mineral density and an increase in weight and fat mass, during GnRHa treatment in children. We followed 363 subjects born SGA who were treated with GH/GnRHa or GH only during childhood until 5 years after cessation of GH treatment, investigating long-term metabolic and cardiovascular safety of additional 2 years of GnRHa treatment during GH treatment in young adults born SGA.

In these young adults born SGA, previously treated with combined GH/GnRHa treatment or GH-only, fat mass increased during 5 years of follow-up after GH-cessation. The changes in insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and bone mineral density were unaffected by the addition of 2 years of GnRHa treatment for postponement of puberty. At 21 years of age, insulin sensitivity, beta-cell function, body composition, blood pressure, serum lipid levels and bone mineral density were similar in GnRHa/GH-treated compared to GH-treated young adults born SGA and untreated young adults born AGA.



In conclusion, our results show that the addition of 2 years of GnRHa treatment to long-term GH treatment of short children born SGA does not have an unfavorable effect on their metabolic health and bone mineralization at the age of 21 years.

**Chapter 6**

At age 21 years, young adults born SGA with higher gain in weight SDS during early childhood had signs of insulin resistance, adverse lipid profile and a significantly higher fat mass than young adults born AGA. We longitudinally followed 287 adults, 170 born SGA, either short (SGA-S) or with catch-up growth (SGA-CU) and 117 born AGA, from age 21 to 32 years, to investigate the development of metabolic health and adiposity into adulthood.

At age 32 years, SGA-CU adults had lower insulin sensitivity than AGA adults, while SGA-S and AGA adults were similar. Fat mass and trunk fat were higher in SGA-CU than AGA adults. SGA-S and AGA adults had similar fat mass parameters, but SGA-S adults had lower lean body mass than SGA-CU and AGA adults. SGA-CU adults had significantly higher levels of adverse lipids than AGA adults. Beta-cell function, visceral fat, liver fat fraction and blood pressure were similar in all groups. SGA birth and catch-up in weight SDS during childhood were associated with a higher risk of metabolic syndrome at age 32 years.

Reassuringly, metabolic health parameters in SGA-CU and SGA-S adults did not worsen compared to AGA adults during 11 years of follow-up.

In conclusion, 32-year-old adults born SGA with spontaneous catch-up in height and weight have a higher risk of a persistently unhealthy metabolic profile. Furthermore, our results show that accelerated weight gain in early childhood is associated with an increased metabolic risk at age 32 years, particularly in children born SGA.

**Chapter 7**

Large population studies, including the SAGhE study, suggested an increase in cardiovascular mortality in GH-treated adults, but these studies lacked an appropriate control group. We, therefore, followed subjects born SGA who were treated with GH during childhood until 12 years after cessation of GH treatment, investigating detailed cardiovascular and metabolic determinants in adulthood in comparison with untreated adults born SGA or AGA at around age 30 years.

During 12 years of follow-up, previously GH-treated adults born SGA maintained a normal beta-cell function and showed an increase in insulin sensitivity, fat mass, total cholesterol and blood pressure to similar levels as SGA-S adults at age 30 years. Furthermore, SGA-GH adults had similar metabolic and cardiovascular health parameters as age-matched AGA adults, except of lower lean body mass and higher adverse serum lipid levels, which were present in all SGA groups. Abdominal adiposity, liver fat fraction, blood pressure and metabolic syndrome components (MetS) were similar between SGA-GH and control groups at age 30 years.

In conclusion, long-term GH treatment in children born SGA has no adverse effects on metabolic and cardiovascular health up to 12 years after GH-cessation.

**Chapter 8**

In the General Discussion, we discuss the most important findings of our studies in a broader context, and compared to literature data. We emphasize the clinical implications and give directions for future research.

# Samenvatting

**Hoofdstuk 1**
Dit hoofdstuk beschrijft de definities, prevalentie en oorzaken van een kleine lengte en/of een laag gewicht bij de geboorte (SGA, small for gestational age) en de effecten van SGA op puberteit, volwassen lengte en metabole gezondheid. Het geeft achtergrondinformatie over de effecten van groeihormoon (GH) en GnRH-analoga (GnRHa) in SGA-geboren kinderen en in kinderen met het Silver-Russell syndroom (SRS) een zeldzame genetische variant die in de meeste gevallen gepaard gaat met SGA-geboorte en persisterend kleine lengte. Daarnaast wordt een introductie gegeven over de parameters die in deze thesis worden beschreven, parameters voor metabole gezondheid, gonadale functie en botdichtheid. Aan het einde van dit hoofdstuk worden de doelstellingen van de studies en de indeling van dit proefschrift besproken.

**Hoofdstuk 2**
Kinderen met SRS worden meestal ook SGA geboren en ervaren dezelfde voordelen van GH als SGA-geboren kinderen zonder SRS. Wij volgden 32 GH-behandelde kinderen met SRS en evalueerden de start en progressie van de puberteit en de gonadale functie gedurende de GH-behandeling tot de stop tot behalen van de volwassen lengte. Daarnaast vergeleken we deze data met een controlegroep van SGA-geboren kinderen zonder SRS en met referentie data van gezonde Nederlandse adolescenten met eenzelfde leeftijd.



De gemiddelde leeftijd bij start van puberteit van vergelijkbaar in mannen met SRS (11.5 jaar) en zonder SRS (11.6 jaar), met een vergelijkbare duur van de puberteit in beide groepen. Vier van de 14 mannen met SRS had een inhibine B onder het 5$^{e}$ percentiel na de puberteit, vergeleken met gezonde controles. Twee van hen had ook een FSH boven het 95$^{e}$ percentiel, wijzend op een Sertoli cel dysfunctie.

De gemiddelde leeftijd bij start van puberteit van vergelijkbaar in vrouwen met SRS (10.5 jaar) en zonder SRS (10.7 jaar), met een vergelijkbare duur van de puberteit in beide groepen. De gemiddelde leeftijd van de menarche was gelijk in vrouwen met SRS (13.1 jaar) en zonder SRS (13.3 jaar). Alle vrouwelijke deelnemers met SRS hadden AMH, LH en FSH binnen het normale gebied van een gezonde referentiepopulatie, echter had één van hen primaire amenorroe ten gevolge van agenesie van de buizen van Müller.

We hebben ook de lengtegroei tijdens de puberteit vergeleken in GH behandelde kinderen met SRS met en zonder additionele behandeling met 2 jaar GnRHa behandeling. Kinderen met SRS met een additionele behandeling met GnRHa had een significant betere lengtegroei tijdens de puberteit, hetgeen leidde tot een toename van de volwassen lengte.

Kortom, kinderen met SRS hebben een vergelijkbare leeftijd van start van puberteit en een vergelijkbare progressie van de puberteit als SGA-geboren kinderen zonder SRS. Ondanks het feit dat gonadale functie gemiddeld genomen vergelijkbaar was tussen de adolescenten met SRS en zonder SRS, kwam Sertoli cel dysfunctie vaker voor in mannen met SRS. Kinderen met SRS met een volwassen lengte predictie onder -2.5 standaard deviatie scores (SDS) ervaren eenzelfde voordeel van additionele behandeling met GnRHa op het verbeteren van de volwassen lengte als SGA-geboren kinderen zonder SRS.

**Hoofdstuk 3**

Studies hebben laten zien dat GH behandeling zorgt voor een toename in glomerulaire filtratie (GFR, glomerular filtration rate), door een stimulatie van het renine-angiotensine systeem door IGF-I. Kinderen die geboren worden met een laag geboortegewicht hebben een lager aantal nefronen, waardoor ze een lagere GFR hebben, een hogere bloeddruk en een hogere albumine-creatinine ratio op jongvolwassen leeftijd. Wij volgden 261 SGA-geboren jongvolwassenen die behandeld zijn met GH als kind, tot 5 jaar na de stop van GH behandeling en onderzochten de nierfunctie en bloeddruk en vergeleken deze resultaten met onbehandelde SGA-geboren jongvolwassenen en jongvolwassenen met een normaal geboortegewicht (AGA, appropriate for gestational age).

Enkel gedurende de eerste 6 maanden na de stop van GH is er sprake van een milde maar significante daling van GFR, binnen het normale bereik van een gezonde referentiepopulatie, door het verlies van de stimulerende neveneffecten van GH. In de periode tussen 6 maanden en 5 jaar na de stop van GH behandeling blijft GFR stabiel en binnen het normale bereik.

Op de leeftijd van 21 jaar hebben SGA-geboren jongvolwassenen die als kind behandeld zijn met GH een vergelijkbare GFR, bloeddruk en albumine-creatinine ratio als onbehandelde gezonde controles na SGA-geboorte of AGA-geboorte.

**Hoofdstuk 4**

In SGA-geboren kinderen die met GH behandeld worden, met een volwassen lengte predictie onder de -2.5 SDS bij de start van puberteit is een additionele behandeling met GnRHa gedurende 2 jaar effectief op het verbeteren van de volwassen lengte. Wij volgden 99 SGA-geboren deelnemers die behandeld waren met GH/GnRHa of alleen GH als kind en vergeleken bij de stop van GH behandeling bij het behalen van volwassen lengte in deze twee groepen de cognitie, het psychosociaal functioneren en de gezondheids-gerelateerde kwaliteit van leven.

Intelligentie quotiënt scores waren vergelijkbaar in de GH/GnRHa- en GH-groep. Gezondheids-gerelateerde kwaliteit van leven was vergelijkbaar in beide groepen en ook in vergelijking met de referentiepopulatie. De perceptie van cognitief functioneren was significant lager

in de GH/GnRHa-groep. Dit kan verklaard worden door de hogere verwachtingen van ouders, die een hoger opleidingsniveau hadden in de GH/GnRHa-groep in vergelijking met de GH-groep. Probleemgedrag was vergelijkbaar in de GH/GnRHa- en GH-groep. Volwassen lengte correleerde niet met gezondheids-gerelateerde kwaliteit van leven, zelfperceptie en probleemgedrag.

Wij concluderen dat een additionele GnRHa behandeling geen negatieve effecten op cognitief en psychosociaal functioneren heeft

**Hoofdstuk 5**
Andere studies beschreven een afname van insuline sensitiviteit en botdichtheid en een toename van gewicht en vetmassa gedurende een GnRHa behandeling. Wij volgden 363 deelnemers gedurende de GH behandeling, met of zonder een additionele GnRHa behandeling van 2 jaar, tot 5 jaar na stop en evalueerden metabole en cardiovasculaire veiligheid van GnRHa gedurende een GH behandeling in SGA-geboren jongvolwassenen.

In 363 SGA-geboren jongvolwassenen, die behandeld zijn met een GH/GnRHa-behandeling of een GH-behandeling, hebben we een toename in vetmasse gezien gedurende 5 jaar na stop van GH. Veranderingen in insulinesensitiviteit, beta-celfunctie, lichaamssamenstelling, bloeddruk, serum lipide en botdichtheid gedurende 5 jaar waren vergelijkbaar in GH/GnRHa- en GH-behandelde jongvolwassenen. Op de leeftijd van 21 jaar was insulinesensitiviteit, beta-celfunctie, lichaamssamenstelling, bloeddruk, serum lipide en botdichtheid vergelijkbaar in beide groepen en ook vergeleken met AGA-geboren jongvolwassenen.



Kortom, onze resultaten laten zien dat een additionele behandeling met GnRHa voor 2 jaar tijdens een GH behandeling geen negatieve effecten heeft op metabole gezondheid en hun botmineralisatie op de leeftijd van 21 jaar.

**Hoofdstuk 6**
Op de leeftijd van 21 jaar hadden SGA-geboren jongvolwassenen met postnatale inhaalgroei tekenen van insulineresistentie, ongunstig lipideprofiel en een significant hogere vetmassa dan AGA-geboren jongvolwassenen. 287 volwassenen (170 SGA-geboren, zonder inhaalgroei (SGA-S, short adults born SGA) of met inhaalgroei (SGA-CU, adults born SGA with postnatal catch-up) en 117 AGA-geboren volwassenen) werden longitudinaal gevolgd van 21 tot 32 jaar. Wij evalueerden de progressie van metabole gezondheid en adipositas gedurende deze periode tot volwassen leeftijd.

Op de leeftijd van 32 jaar hadden SGA-CU volwassenen een lagere insulinesensitiviteit dan AGA volwassenen, dit was gelijk in SGA-S en AGA volwassenen. Vetmassa en rompvet waren hoger in SGA-CU dan in AGA volwassenen. SGA-S en AGA volwassenen hadden

vergelijkbare vetmassaparameters, maar SGA-S had een lagere spiermassa dan SGA-CU en AGA volwassenen. SGA-CU had hogere ongunstige lipides dan AGA volwassenen. Beta-celfunctie, visceraal vet, levervetfractie en bloeddruk waren vergelijkbaar in alle groepen. SGA-geboorte en inhaalgroei in gewicht waren geassocieerd met een hoger risico op het ontwikkelen van metabool syndroom op de leeftijd van 32 jaar.

De parameters van metabole gezondheid in SGA-CU en SGA-S volwassenen verslechterden niet gedurende de periode van 11 jaar ten opzichte van de AGA volwassenen, hetgeen geruststellend was.

Kortom, SGA-geboren volwassenen met postnatale groei in gewicht en lengte SDS hebben een hoger risico op een persisterend ongezond metabool profiel. Onze resultaten laten zien dat versnelde gewichtstoename in vroege kindertijd geassocieerd is met een toename in metabool risico op de leeftijd van 32 jaar, voornamelijk in SGA-geboren kinderen.

**Hoofdstuk 7**
Grote populatiestudies, zoals de SAGhE studie, suggereren een toename in cardiovasculaire mortaliteit in GH-behandelde volwassenen. Deze studies hebben echter geen geschikte controlegroep gebruikt. Om deze reden, hebben wij gedetailleerde cardiovasculaire en metabole determinanten in volwassenen die met GH zijn behandeld als kind onderzocht en vergeleken met onbehandelde volwassenen met een SGA-geboorte of AGA-geboorte op de leeftijd van 30 jaar.

Gedurende 12 jaar na stop van groeihormoon behouden SGA-geboren volwassenen een normale betacelfunctie en hebben een toename in insulinesensitiviteit, vetmassa, totaal cholesterol en bloeddruk, waardoor ze op de leeftijd van 30 jaar vergelijkbaar zijn met onbehandelde SGA-S volwassenen. SGA-GH volwassenen zijn ook vergelijkbaar met AGA volwassenen, behalve de spiermassa die net als in SGA-S volwassenen significant lager is en een ongunstig lipideprofiel dat vergelijkbaar is met onbehandelde SGA-geboren volwassenen op de leeftijd van 30 jaar. Abdominale adipositas, levervetfractie, bloeddruk en metabool syndroom (MetS) was vergelijkbaar in alle groepen op de leeftijd van 30 jaar.

Kortom, lange termijn GH behandeling in SGA-geboren kinderen heeft geen negatieve effecten op de metabole en cardiovasculaire gezondheid tot 12 jaar na stop van de behandeling.

**Hoofdstuk 8**
In dit hoofdstuk bespreken we de belangrijkste resultaten van onze studies in een bredere context, en vergelen met beschikbare literatuurdata, evenals de klinische implicaties en suggesties voor toekomstig onderzoek.

# Chapter 10

List of abbreviations
List of publications
List of co-authors and affiliations
PhD portfolio
Acknowledgements
Curriculum Vitae

# List of abbreviations

| | |
|---|---|
| 11p15 LOM | loss of methylation in the 11p15 region |
| ABCL | Adolescent Behaviour Checklist |
| ACR | albumin-to-creatinine ratio |
| AGA | appropriate for gestational age |
| AH | adult height |
| AIRg | acute insulin response to glucose |
| AMH | anti-Müllerian hormone |
| ATP-III | adult treatment panel III |
| ANCOVA | analysis of covariance |
| BL | birth length |
| $BMAD_{LS}$ | bone mineral apparent density of the lumbar spine |
| BMD | bone mineral density |
| $BMD_{LS}$ | bone mineral density of the lumbar spine |
| $BMD_{TB}$ | bone mineral density of the total body |
| BMI | body mass index |
| BP | blood pressure |
| BW | birth weight |
| CBCL | Child Behaviour Checklist |
| CBSA | Self-perception Profile of Adolescents |
| CI | confidence interval |
| CPP | central precocious puberty |
| CVD | cardiovascular disease |
| DBP | diastolic blood pressure |
| DI | disposition index |
| DM2 | diabetes mellitus type 2 |
| DXA | dual energy X-ray absorptiometry |
| FM | fat mass |
| FSH | follicle stimulating hormone |
| FSIGT-test | frequently sampled intravenous glucose tolerance test |
| GA | gestational age |
| GFR | glomerular filtration rate |
| GH | growth hormone |
| GH-axis | growth hormone axis |
| GnRHa | gonadotropin-releasing hormone analogue |
| HDLc | high-density lipoprotein cholesterol |
| HPG-axis | hypothalamic-pituitary-gonadal axis |
| HRQoL | health-related quality of life |
| ICR | imprinting control region |

| | |
|---|---|
| IGF | insulin-like growth factor |
| IGF-I | insulin-like growth factor-I |
| IUGR | intrauterine growth retardation |
| IQR | interquartile range |
| IQ | intelligence quotient |
| LBM | lean body mass |
| LDLc | low-density lipoprotein cholesterol |
| LF | limb fat |
| LH | luteinizing hormone |
| LLD | leg length discrepancy |
| M2 | breast development stage II according to Tanner |
| MetS | metabolic syndrome |
| MRI | magnetic resonance imaging |
| MRKH | Mayer-Rokitansky-Küster-Hauser syndrome |
| NCEP | National Cholesterol Educational Program |
| SAGhE | Safety and Appropriateness of Growth Hormone treatments in Europe |
| SAT | subcutaneous adipose tissue |
| SBP | systolic blood pressure |
| SD | standard deviation |
| SDS | standard deviation score |
| SES | socio-economic status |
| 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 |
| SPSS | Statistical Package for Social Sciences |
| SRS | Silver-Russell syndrome |
| TAAQOL | TNO-AZL Adults Quality of Life |
| TC | total cholesterol |
| TF | trunk fat |
| Tg | triglyceride |
| TH | target height |
| TV | testicular volume |
| UPD(7)mat | maternal uniparental disomy of chromosome 7 |
| VAT | visceral adipose tissue |
| WAIS-III | Wechsler Adult Intellegence Scales-III |
| WISC-III | Wechsler Intellegence Scale for Children-III |

# List of publications

**Goedegebuure W.J. et al.** Long-term Follow-Up After Bilateral Percutaneous Epiphysiodesis Around the Knee to Reduce Excessive Predicted Final Height. *Arch Dis Child 2018* Mar;103(3):219-223.

**Goedegebuure W.J. et al.** Cognition, Health-Related Quality of Life, and Psychosocial Functioning After GH/GnRHa Treatment in Young Adults Born SGA. *J Clin Endocrinol Metab 2018* Nov 1;103(11):3931-3938.

**Goedegebuure W.J. et al.** Gonadal Function and Pubertal Development in Patients with Silver-Russell Syndrome. *Hum Reprod. 2018* Nov 1;33(11):2122-2130.

**Goedegebuure W.J. et al.** Aromatase Inhibitor as Treatment for Severely Advanced Bone Age in Congenital Adrenal Hyperplasia: A Case Report. *Horm Res Paediatr. 2019*;92(3):209-213.

**Goedegebuure W.J. et al.** Glomerular Filtration Rate, Blood Pressure and Microalbuminuria in Adults Born SGA: A 5-year Longitudinal Study After Cessation of GH Treatment. *Clin Endocrinol. 2019* Dec;91(6):892-898.

**Goedegebuure W.J. et al.** Longitudinal Study on Metabolic Health in Adults SGA During 5 Years After GH With or Without 2 Years of GnRHa Treatment. *J Clin Endocrinol Metab. 2020 Aug 1;105(8).*

**Donze S.H. et al.** Evidence for Accelerated Biological Aging in Young Adults with Prader-Willi Syndrome. *J Clin Endocrinol Metab. 2020* Jun 1;105(6):2053-2059.

**De Fluiter K.S. et al.** Longitudinal Body Composition Assessment in Healthy Term-Born Infants Until 2 Years of Age Using ADP and DXA With Vacuum Cushion *Eur J Clin Nutr. 2020* Apr;74(4):642-650.

**Goedegebuure W.J. et al.** Accelerated weight gain during infancy leads to an unfavorable metabolic health profile and increased adiposity in adults born small for gestational age at age 32 years. *Submitted.*

**Goedegebuure W.J. et al.** Childhood growth hormone treatment does not increase metabolic and cardiovascular risk in adults born SGA: A 12-year follow-up study after GH-cessation. *Submitted.*

## List of co-authors and affiliations

A.C.S. Hokken-Koelega, MD, PhD
*Dutch Growth Research Foundation, Rotterdam, the Netherlands. Department of Pediatric Endocrinology, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands.*

M. van der Steen, MD, PhD
*Department of Pediatric Endocrinology, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands.*

C.C.J. Smeets, MD, PhD
*Department of Pediatric Endocrinology, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands.*

G.F. Kerkhof, MD, PhD
*Department of Pediatric Endocrinology, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands.*

J.S. Renes, MD, PhD
*Department of Pediatric Endocrinology, Erasmus University Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands.*

J.L. de With
*Dutch Growth Research Foundation, Rotterdam, the Netherlands.*

Y.B. de Rijke, PhD
*Department of Clinical Chemistry, Erasmus University Medical Centre, Rotterdam, The Netherlands.*

# PhD portfolio

**Name**: Wesley Jim Goedegebuure
**Promotor:** Prof. dr. A.C.S. Hokken-Koelega
**Copromotor:** Dr. M. van der Steen
**Affiliations:** Department of Pediatric Endocrinology, Erasmus University Medical Center – Sophia Children's Hospital, Rotterdam, the Netherlands.
**PhD period:** March 2017 – April 2021

| **Summary of PhD training** | **Year** | **ECTS** |
|---|---|---|
| ***General courses*** | | |
| Good Clinical Practice (BROK), Erasmus MC | 2016 | 1.5 |
| Biostatistical Methods I, NIHES, Erasmus MC | 2017 | 5.7 |
| Research Integrity, Erasmus MC | 2019 | 1.0 |
| ***Specific courses*** | | |
| Basic Introduction to SPSS, Molmed, Erasmus MC | 2016 | 1.0 |
| Basic and translational endocrinology | 2019 | 2.2 |
| Genetics | 2018 | 1.0 |
| R Statistics | 2017 | 1.8 |
| Radiation and MRI Safety | 2017 | 0.6 |
| ***Seminars and workshops*** | | |
| Weekly research meeting, Pediatric Endocrinology, Erasmus MC | 2017-2020 | 4.0 |
| Annual PhD day, Erasmus MC | 2017-2019 | 1.5 |
| Annual Sophia Research Day, Sophia Children's Hospital, Erasmus MC | 2017-2019 | 1.2 |
| ***International and national conferences*** | | |
| 10th International Joint Meeting of Pediatric Endocrinology (IMPE), Washington, USA (poster presentation) | 2017 | 1.0 |
| 57th Meeting of the European Society of Pediatric Endocrinology (ESPE), Athens, Greece (poster presentation) | 2018 | 1.0 |
| 58th Meeting of the European Society of Pediatric Endocrinology (ESPE), Vienna, Austria (poster presentation) | 2019 | 1.0 |
| Silver-Russell Symposium | 2018 | 1.0 |
| ***Other activities*** | | |
| Expert center Rare Growth Disorders / Silver-Russell Syndrome | 2017-2021 | 4.0 |
| Peer review | 2018 | 1.0 |
| Medical Advisor SGA Platform | 2017-2021 | 2.0 |

# Acknowledgements – Dankwoord

Een promotietraject doe je niet alleen. Er zijn een heleboel mensen die mij de afgelopen jaren hebben gesteund, die ik graag zou willen bedanken.

Allereerst wil ik natuurlijk de deelnemers en hun ouders bedanken voor de inzet gedurende al die jaren van de studie. Voor mij is het altijd heel motiverend geweest om de connectie te behouden met de kliniek, om zo te zien hoe relevant ons onderzoek is. Daarnaast vind ik het fantastisch om te zien hoe toegewijd jullie zijn ten opzichte van onze studie. Ik wens jullie allen veel geluk toe voor de toekomst.

In het bijzonder wil ik mijn promotor bedanken, prof. Anita Hokken-Koelega. Beste Anita, voor mij zullen onze overleggen en poli's me altijd bijblijven, de ongelimiteerde toewijding voor het onderzoek en de zorg zijn bewonderenswaardig. U was voor mij gedurende 4 jaar een bron van motivatie, inspiratie en zo ontzettend veel kennis. Ik hoop dat u de komende jaren nog vaak stil kunt staan bij alle mooie dingen die dankzij u zijn bereikt.

Manouk van der Steen, mijn co-promotor, vanaf afstand toch zo betrokken bij mijn promotie. Ik heb onze samenwerking altijd erg gewaardeerd en hoop dat we deze vaak zullen gebruiken in de toekomst.

Prof. A. van der Lelij, dank voor uw bereidwilligheid om de rol van secretaris binnen de kleine commissie te vervullen. Prof. F. Chiarelli and Prof. S. Cianfarani, thank you for your willingness to participate in the PhD review committee.
Prof. dr. J. Dahlgren, prof. dr. W. Kiess and dr. M. Houdijk, dank voor het plaatsnemen in de grote commissie, thank you for taking place in the committee.
Alle kinderartsen die hebben meegewerkt aan het onderzoek, dank voor het meedenken en jullie gastvrijheid tijdens de buitenpoli's.
Dr. Hackeng, bedankt voor al die duizenden FSIGT's die u met zoveel inzet heeft verricht.

Roel Odink, dank voor de introductie in de wereld van de kinderendocrinologie. U bent een inspiratie geweest om dit pad te kiezen.

Mijn collega's zijn de mensen die mijn tijd in het Sophia Kinderziekenhuis hebben gevormd en ervoor hebben gezorgd dat ik me daar thuis heb gevoeld. Ik heb er altijd ontzettend van genoten om deel uit te mogen maken van zo'n leuk team. Jullie waren er altijd en ik kon terugvallen op jullie rust, humor en nuchtere visie. Zonder jullie was het nooit gelukt.
Christel Bruinings, Esther Lems, José Bontenbal en Naomi Khieroe, ik heb genoten van de nuchtere kijk, de gezelligheid, jullie enthousiasme en gesprekken. Ik heb zoveel geluk gehad om met jullie te mogen samenwerken en mag alleen dromen dat ik in de toekomst collega's

krijg zoals jullie. Karaokeavond, eten in de mooiste tuin van Krimpen en de talloze koffies zal ik altijd blijven onthouden. Lin Smeets, Stephany Donze en Layla Damen, ik wil jullie bedanken voor het goede voorbeeld, de samenwerking en de leuke congressen die we samen hebben gehad.

Collega's van de Pluto-studie, Prader-Willi studie en de Stichting Kind en Groei, ondanks dat we elkaar sporadisch zagen was het heel leuk om deel uit te maken van een groter geheel. Ik wil jullie graag bedanken voor de gezelligheid op de poli en de gastvrijheid op de Westzeedijk!

Het MRI-studententeam, Inez, Laura, Myrea en Thijs, wil ik graag bedanken voor de vele uren dat we samen naar een scherm hebben gestaard en de flexibiliteit die jullie hebben gehad tijdens onze samenwerking.

Ik heb heel wat meters afgelegd tijdens mijn promotie, ben daarin altijd gesteund door de fijne mensen om me heen. Velen hielden me letterlijk uit de wind, gingen mee of initieerden (te) wilde plannen en waren de gezellige thuishaven gedurende deze periode. Speciale dank voor de reizen naar de bergen, de mooie tijd in Utrecht en de vriendengroep uit Sittard. Samen met jullie is geen berg te hoog, geen stukje Nederland te vlak en kan ik genieten van iedere dag, waarvoor duizendmaal dank.



Mijn paranimfen, Kirsten de Fluiter en Harry Hendrix. Kirsten, ik heb ontzettend genoten van jou als collega. Hoewel we aan volledig andere projecten werkten, was je altijd betrokken bij mijn studie en projecten. Ik wil je bedanken voor alle appjes, telefoontjes, koffietjes en de vele langdurige gesprekken. Heel veel geluk bij de revalidatiegeneeskunde, ze mogen in Amsterdam blij zijn met zo'n gedreven en betrokken collega. Harry, ik ben blij dat je voor vandaag mijn paranimf wilde zijn. We hebben samen een mooie band gecreëerd tijdens de studietijd, ik kijk ernaar uit getuige te zijn op je bruiloft.

Mijn familie, dankzij jullie voel ik me nooit alleen. Mama, jouw steun en vertrouwen heb ik altijd bij me en dat maakt me gelukkig, ik ben blij te zien dat je zo gelukkig bent met Wilfried. Janine, je bent zo'n fijne zus. Dank voor het maken van de tekening van de voorkant, de avonden in Maastricht en het voorbeeld dat je voor me bent. Kim, van jou kan ik zoveel leren. Ik bewonder je prachtige gezin en hoe je iedereen in zijn/haar waarde laat. Ik ben trots oom te mogen zijn van Marvin, Mara & Casper.
Robert, Hans en Ingrid, dank voor alle support tijdens mijn promotie. Ik ben blij dat we na al die jaren weer een leuke band hebben opgebouwd.

Lieve Tess, jij maakt me gelukkig. Ik ben trots hoe we de afgelopen jaren onze ambities hebben kunnen combineren met elkaar, ondanks de afstand. Dat onze volgende uitdagingen ook zo mooi afgesloten kunnen worden. Ik kijk uit naar de komende jaren!

# Curriculum Vitae

Wesley Jim Goedegebuure was born on September 28, 1991 in Berkel en Rodenrijs, the Netherlands. After graduation from secondary school at Trevianum Scholengroep in Sittard, he moved to Maastricht to study Medicine at Maastricht University. In 2016, he finished his Medicine study. In March 2017, he started as a PhD candidate at Erasmus Medical Center – Sophia Children's Hospital in Rotterdam. He investigated the long-term effects of being born small for gestational age and growth hormone treatment to increase adult height, which resulted in this thesis. During his PhD, he worked under supervision of prof. dr. Hokken-Koelega in the expertise center for rare growth disorders and Silver-Russell Syndrome. Following this PhD period, he will continue his pursuit of becoming a pediatrician in the Catharina Hospital Eindhoven.