Lin Smeets

Silver-Russell Syndrome & Small for Gestational Age

Long-term health perspectives

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Long-term health perspectives

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Lange termijn groei en gezondheid

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Chapter 1

General introduction

INTRODUCTION

For over 25 years, our research group and others have been investigating children born small for gestational age (SGA) with persistent short stature, and the efficacy and safety of biosynthetic growth hormone (GH) treatment in these children. The children that started treatment back then, are now young adults, and the knowledge about the long-term effects of GH treatment has markedly increased.

One of the causes of short SGA is the Silver-Russell syndrome (SRS). Although overlap exists between the medical care for children born SGA and those with SRS, there are management issues that are specific to SRS, and the management of SRS requires a multidisciplinary approach. In the past years, the knowledge about SRS has improved, thanks to, among others, the discovery of new (epi)genetic alterations that cause SRS and a scoring system to facilitate clinicians in diagnosing SRS (1, 2). However, many knowledge gaps remained, some of which are addressed in this doctoral thesis.

Because being born with a lower birth weight leads to an increased risk for age-associated diseases, health in later life has been a concern for those born SGA and/or preterm. One of the goals of this thesis was to investigate parameters associated with health in later life, such as telomere length and bone mineral density, and the effects of GH treatment on these parameters.

SMALL FOR GESTATIONAL AGE

SGA refers to the size of an infant at birth, and is defined as a birth weight and/or birth length of at least two standard deviation scores (SDS) below the mean for gestational age (3, 4). SGA infants may be born either full-term or preterm. By definition, 2.3% of all infants are born SGA.

To determine whether a child is born SGA, it is important to have accurate information on gestational age, birth weight and birth length, as well as an appropriate reference population. The term intrauterine growth retardation (IUGR) is often used synonymously with the term SGA. However, 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).

The causes of SGA birth are heterogeneous: it can be caused by maternal, fetal or demographic factors (Table 1) (5). In a significant proportion of cases, the reason for SGA birth remains unclear.

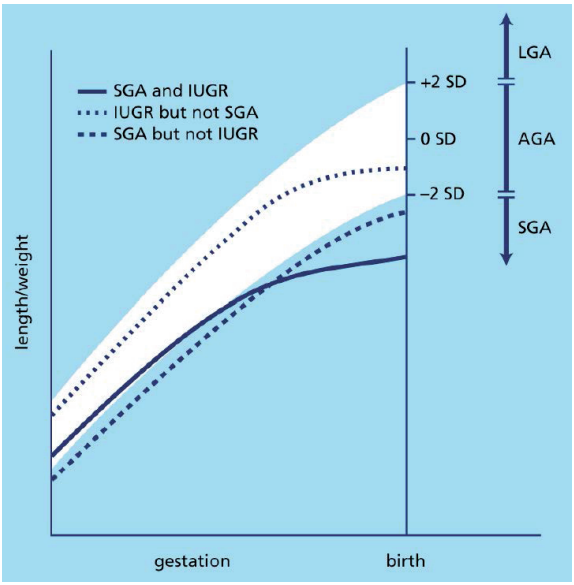

Figure 1. Fetal growth chart showing different intrauterine growth patterns

POSTNATAL GROWTH FAILURE AND SHORT STATURE

Most children born SGA show catch-up growth in the first two years of life, but 10-15% of SGA children fail to show sufficient catch-up growth and remain short, with a height <-2 SDS (6, 7). Short stature is one of the most common medical concerns in childhood and SGA birth is a major risk factor for short stature, accounting for approximately 20% of all cases (8).

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 growth hormone/insulin-like growth factor (IGF)-axis (Figure 2) have also been postulated to play an important role in the insufficient catch-up growth after SGA birth (9-11).

Despite adult height (AH) being one of the most heritable human traits (12), the (epi) genetic nature of short stature in SGA children is still largely unknown. Genome wide association studies have identified genes that contribute to the variation in height, but variations in these genes only have a small effect in the general population (13). Short stature can also be caused by mutations in one gene. Examples of this are IGF1-receptor mutations, 3M syndrome and SHOX deficiency. Also epigenetic changes (i.e. aberrations in regions that control the imprinting of genes) can lead to short stature, which is the case in SRS. Unravelling the (epi)genetic basis of short children born SGA is important for health prognosis, genetic counselling and treatment options.

Table 1. Factors associated with reduced fetal growth (5)

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

SILVER-RUSSELL SYNDROME

SRS is a rare disorder, with an estimated incidence of 1 in 30,000 to 1 in 100,000 life-born infants per year (15). Children with SRS are almost always born SGA, remain short and show various dysmorphic features, such as relative macrocephaly, a triangular shaped head with frontal bossing, clinodactyly, and asymmetry of face and/or body (Figure 3) (16-18). Severe feeding difficulties can be present, especially during infancy and early childhood. Untreated, mean AH is around -4 SDS (i.e. 155 cm for men and 145 cm for women) causing a significant handicap in adulthood (18). Growth charts of a girl with SRS during the first year of life are depicted in Figure 4.

SRS is primarily a clinical diagnosis. Children with SRS can be distinguished from non-syndromic SGA children, based on the presence of characteristic features. Over the years, several clinical scoring systems have been proposed to adequately diagnose SRS.

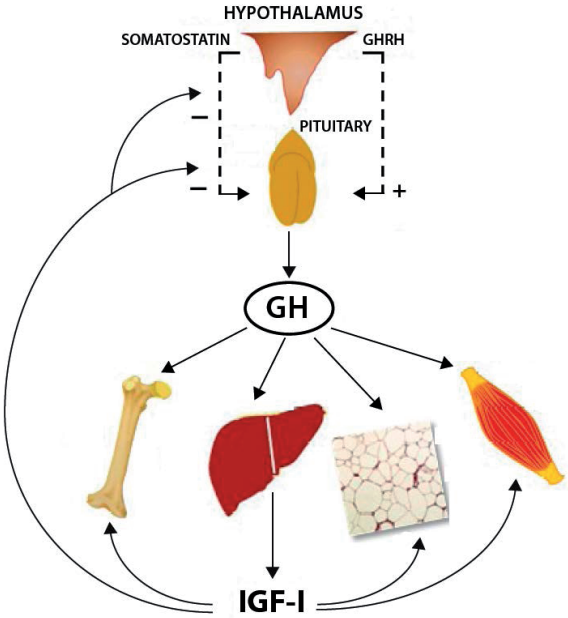

Figure 2. Physiology of the GH-IGF-I axis (adapted from Kumar et al. (14))
GHRH = GH-releasing hormone; GH = Growth hormone; IGF-I = insulin-like growth factor

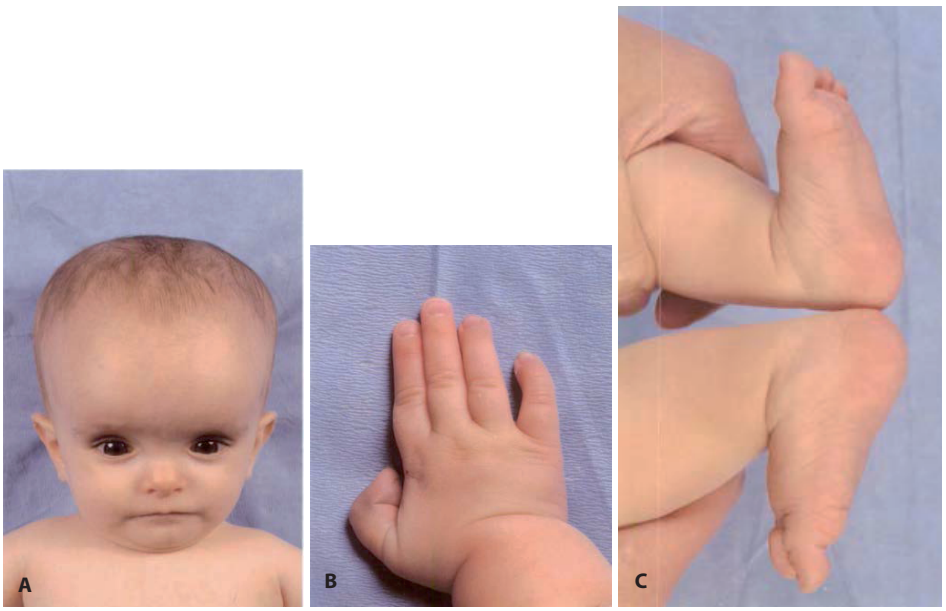

Figure 3. Dysmorphic features in SRS. A) Frontal bossing and triangular shaped head; B) Clinodactyly; C) Asymmetry

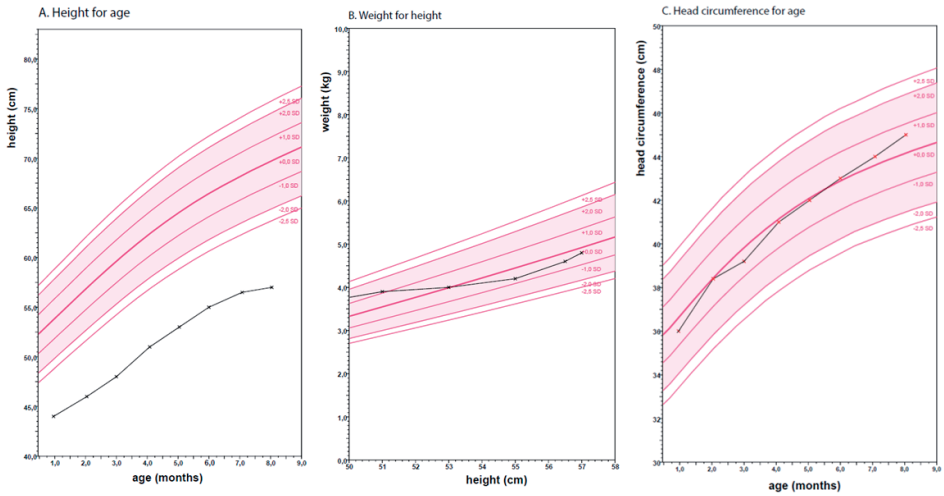

Figure 4. Growth charts of a girl with SRS during the first year of life

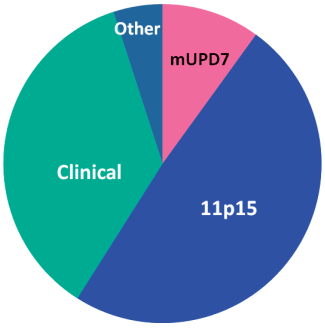

Figure 5. Epigenetic subtypes of SRS

The clinical scoring system with the highest sensitivity (98%) and negative predictive value (89%) is the Netchine-Harbison clinical scoring system (Table 2) (19). If at least four of the criteria of this scoring system are present, the clinical diagnosis SRS can be made. However, since the severity of symptoms varies significantly, and symptoms tend to get less severe as patients get older, the clinical diagnosis can be difficult. Therefore, in case of ≥3 symptoms, molecular testing is indicated (20).

The epigenetic aetiology of SRS is heterogeneous (Figure 5). Approximately 50% of the cases are caused by a loss of methylation in the 11p15 region (11p15 LOM) (1, 2, 21). The 11p15 region contains two imprinting control regions and is important for pre- and postnatal growth (Figure 6). An additional 5-10% of the SRS cases are caused by a maternal uniparental disomy of chromosome 7 (mUPD7) (22). In approximately 40%, the genetic cause remains unknown, which is referred to as idiopathic or clinical SRS. Patients with 11p15 LOM show, on average, a more 'classic' SRS phenotype. Those with

Table 2. Netchine-Harbison clinical scoring system (19)

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

mUPD7 have a milder phenotype, but this subtype is associated with a higher incidence of behavioural problems (23).

GH TREATMENT IN SHORT CHILDREN BORN SGA AND IN SRS

Recombinant GH has been used since 1986 and has replaced GH extracted from human pituitaries. The indications for GH treatment have extended from replacement therapy in children with GH deficiency to a number of conditions. Since 2005, GH treatment is licensed for short SGA children. The aim of GH treatment is achieving an AH within the normal range and/or target height range of the child. Various clinical trials have shown that GH treatment with a dose of 1 mg/m^2/day improves growth rate, and leads to a significant improvement of AH in children born SGA (24-26).

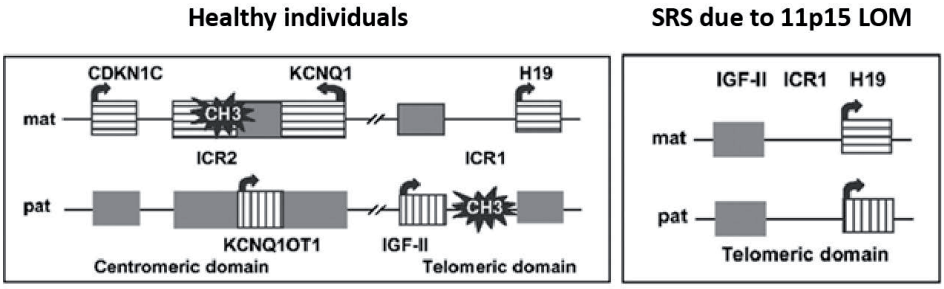

Figure 6. The 11p15 region in healthy individuals (left) and in SRS patients with 11p15 LOM (right). The telomeric imprinting control region (ICR) 1 domain regulates the expression of IGF-II and H19. IGF-II is a growth promotor and expressed exclusively from the methylated paternal allele. H19 is a growth inhibitor and expressed exclusively from the unmethylated maternal allele. Around 50% of the SRS patients have a loss of methylation at ICR1, causing reduction in IGF-II expression and biallelic expression of H19 (Adapted from Netchine et al. (2))

GH in SRS

Because children with SRS are born SGA and remain short, GH treatment is also licensed for children with SRS. SRS was the only syndrome allowed in the clinical trials of GH in short children born SGA that led to indication of GH treatment for these children (20, 27). However, it had never been investigated whether SRS patients benefited equally from GH treatment as non-SRS subjects born SGA. Moreover, it was unknown whether there were differences in GH response among the different genetic subtypes of SRS.

Besides the positive effects on linear growth, GH treatment has also several metabolic effects in children born SGA. An increase in lean body mass, a decline in fat mass, a decrease in blood pressure, and a more favourable lipid profile, but also a lower insulin sensitivity have been reported (28-30). We previously showed that GH treatment in short SGA children is safe on the long-term, and until five years after discontinuation of GH treatment (28, 29, 31). However, the long-term safety of GH treatment and metabolic effects after discontinuation of treatment after AH attainment, had never been investigated in SRS patients.

PUBERTAL DEVELOPMENT AND GONADAL FUNCTION

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 behavioural changes (32). Pubertal changes are regulated by the hypothalamic-pituitary-gonadal axis (Figure 7). Due to an increase in frequency and amplitude of gonadotropin-releasing hormone (GnRH) pulses in the hypothalamus, the secretion of gonadotropins (i.e. luteinizing hormone (LH) and follicle stimulating hormone (FSH)) by the pituitary rises. In the Dutch population, median age of pubertal onset is 10.7 years for girls and 11.5 years for boys (33).

Data on puberty in children born SGA are limited and difficult to compare due to the various definitions of SGA and the various definitions used for the milestones of puberty. Most studies report that puberty in short SGA children starts at a normal age but relatively early for their short stature (34, 35). Moreover, subjects born SGA have a shorter duration of pubertal growth, resulting in a smaller pubertal growth spurt than appropriate for gestational age (AGA) born subjects.

Male gonadal function

Inhibin B is produced by the Sertoli cells of the testis after stimulation of FSH and is a marker of spermatogenesis. Under the stimulation of LH, the Leydig cells of the testis produce testosterone, which is necessary for the development of male characteristics and to continue the process of spermatogenesis.

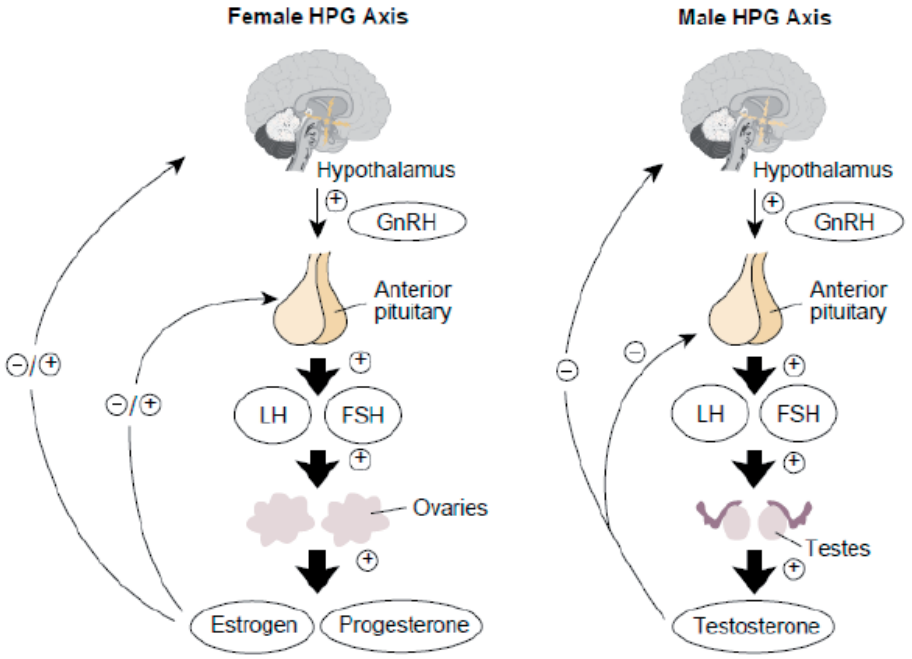

Figure 7. Male and female hypothalamic-pituitary-gonadal axis (adapted from Kong et al. (36))

Previous studies have shown that short SGA boys have a normal number of Sertoli cells and that serum inhibin B levels are the same in GH-treated SGA boys and age-matched AGA boys (37). SGA birth does not affect gonadal function in young men (38).

Female gonadal function

The ovaries fulfil two main functions: the synthesis and secretion of sex hormones and the development and release of the mature oocyte. During fetal life and childhood, follicles develop through primordial and primary stages to preantral and small antral follicles (Figure 8) (39). The loss of primordial follicles already begins prior to birth and continues into childhood. At the time of menarche, approximately 500,000 follicles remain.

From onset of puberty, certain small antral follicles develop into antral follicles. When they reach the antral stage, most follicles undergo atresia, and only a few develop into a Graafian follicle and reach the (pre-)ovulatory stage (40).

Anti-Müllerian hormone (AMH) is produced by the preantral and small antral follicles. This way, serum AMH levels reflect the number of preantral and small antral follicles. Ovarian function can be difficult to evaluate in women because menstrual cycles do not always indicate ovulation. Since AMH is exclusively produced by the ovaries, indepen-

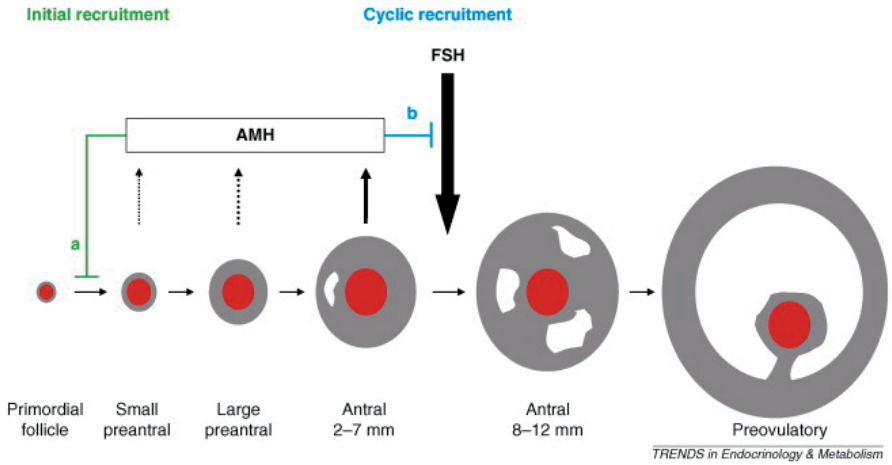

Figure 8. The normal ovarian follicle development (Adapted from Broekmans et al. (45))

dent of the gonadotropic status and menstrual cycle, AMH is an excellent marker of the ovarian follicle pool (41, 42).

Previous studies have shown that there are no adverse effects of SGA birth on AMH levels (43, 44). AMH levels in adolescent girls born SGA are comparable to those of AGA girls, indicating that SGA girls do not have a reduced size of the ovarian follicle pool (44).

Puberty and gonadal function in SRS

Since the majority of individuals with SRS are not routinely followed up, 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 (22, 46). 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 (47, 48). However, pubertal development and gonadal function had never been evaluated in SRS patients.

HEALTH LATER IN LIFE IN CHILDREN BORN SGA AND/OR PRETERM

Several epidemiological studies have shown an association between small size at birth and subsequent catch-up in weight, and the risk for CVD and diabetes mellitus type 2 (DM2) in later life (49-53). 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 largely unknown. One of the hypotheses is the Fetal origin hypothesis by Barker *et al.*, which is based on the postulation that events during pregnancy

leading to fetal malnutrition could result in permanent metabolic changes in the foetus (i.e. reprogramming) (54). During fetal life, this is beneficial, but in the long-term, 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 an unfavourable body composition, an adverse lipid profile, higher blood pressure and lower insulin sensitivity (55-58).

The risk for adult-onset disorders had not been investigated in SRS. There were only two case reports addressing metabolic health in SRS. The first described three SRS patients (all with an 11p15 aberration, two received GH treatment for several years during childhood) who developed adult diseases such as obesity, hypertension, and DM2 in their early twenties (59). The second described the oldest SRS patient known so far, who has DM2, osteopenia, and hypercholesterolemia at the age of 69 years (60). Overall, adult follow-up data in SRS are lacking, which was also emphasized by the recently published consensus statement on diagnosis and management of SRS (20).

LEUKOCYTE TELOMERE LENGTH: A BIOMARKER FOR BIOLOGICAL AGE

Telomeres are noncoding repetitive DNA sequences at the end of each chromosome. Their primary function is to maintain genomic stability (61, 62). Due to the inability of DNA polymerase to fully replicate the end of the chromosome, telomeres shorten with each cell division. When telomeres are reduced to a critical length, the cell goes in senescence (Figure 9) (63, 64).

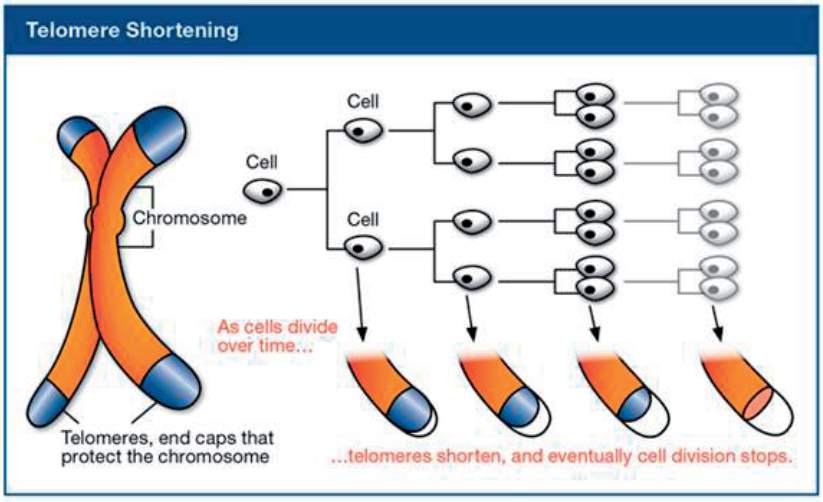

Figure 9. Telomeres shorten with each cell division until a critical length (adapted from www.wholehealthinsider.com (71))

Population studies have shown that telomere length declines with increasing age, which makes it a usable index for biological age, rather than chronological age. Shorter telomere length is also associated with age-associated diseases (65, 66). The telomere hypothesis postulates that at least part of the inter-individual difference in susceptibility to CVD reflects variation in biological ageing. Telomere length can be measured in leukocytes using a PCR-based technique (67) and acts as a valuable marker of this process (68).

Previous studies have highlighted that, next to genetic factors, gender and chronological age, telomere length is also influenced by oxidative stress/chronic inflammation and replicative stress (Figure 10) (69, 70). Since pregnancies resulting in preterm and/or SGA birth are often accompanied by increased stress exposure, and postnatal catch-up growth might induce replicative stress, we postulated that reduced telomere length might be one of the underlying mechanisms of small size at birth and an increased risk for CVD at a relatively young age. However, data on whether preterm and SGA birth influence telomere length were scarce and contradictive. Furthermore, the effects of postnatal growth patterns and possible effects of GH treatment on telomere length were unknown.

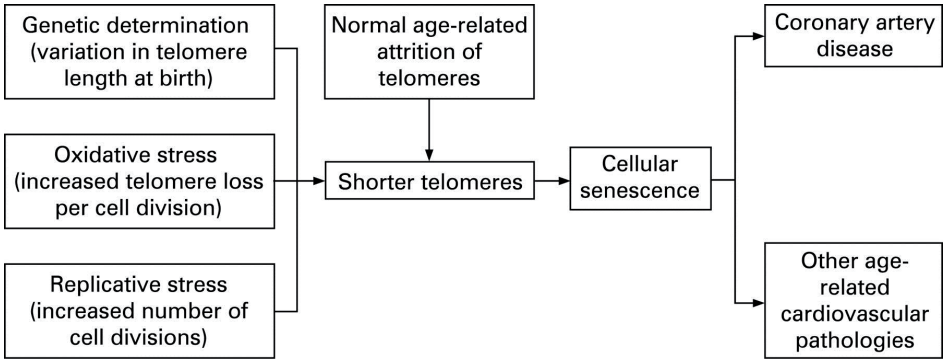

Figure 10. Factors affecting telomere length and how these could explain variation in risk of CVD in different individuals (adapted from Samani et al. (68))

BONE MINERAL DENSITY IN YOUNG ADULTS AFTER CESSATION OF GH

Osteoporosis is a systemic skeletal disorder, characterized by low bone mass and deterioration of bone tissue, leading to bone fragility and susceptibility to fractures (72). Osteoporosis is a world-wide problem with high morbidity and increased mortality (73).

Bone mineral density of the total body (BMD_{TB}) and bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) are important determinants for fracture risk and osteoporosis in later life (74, 75). Each standard deviation decrease in BMD has been shown to be associated with a doubling of fracture risk, in adults as well as in children (74, 75). During childhood, BMD_{TB} and $BMAD_{LS}$ increase, until peak bone mass is attained between the age of 18-20 years in girls and 18-23 years in boys (Figure 11) (76). Attained peak bone

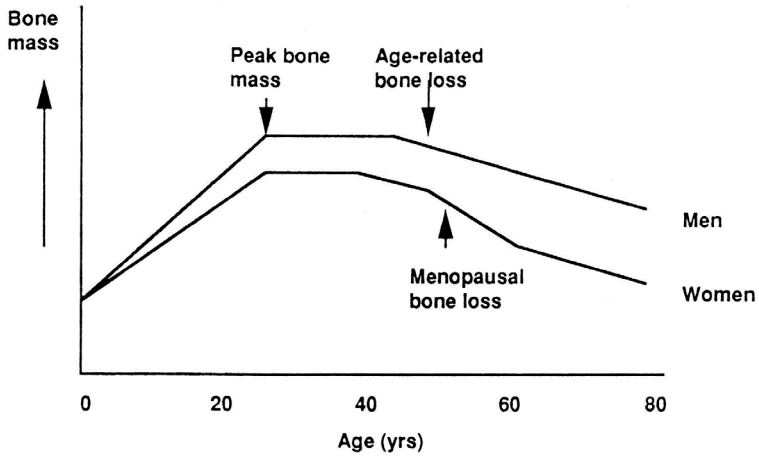

Figure 11. BMD and peak bone mass (adapted from Compston et al. (87))

mass is an important factor determining later life bone health. Thus, maximizing peak bone mass in childhood is relevant to optimizing bone health in later life (77).

BMD_{TB} and $BMAD_{LS}$ are influenced by many factors, such as gender, age, body weight, body composition, calcium intake and physical activity (78-81). The GH-IGF-I axis is also an important factor in the regulation of BMD_{TB} and $BMAD_{LS}$ (82, 83).

BMD_{TB} and $BMAD_{LS}$ can be measured by Dual energy X-ray Absorptiometry (DXA) (Appendix C). DXA is the most widely used clinical tool for the assessment of bone status in children. Since DXA has low radiation dose, great precision and accuracy, it is a suitable technique to use in children and in research (84).

Short SGA children have a lower than average BMD, even after correction for their short stature (85). We have previously shown that GH treatment improves BMD on the short term (85, 86), but long-term studies were lacking, and the effects of discontinuation of GH treatment on BMD_{TB} and $BMAD_{LS}$ were unknown.

STUDY DESIGN

Silver-Russell syndrome

In the past decades, our research group initiated three large GH trials (IUGR studies) to obtain more knowledge about the long-term effects of GH treatment in children born SGA with persistent short stature. The design and in- and exclusion criteria of these trials are described in Appendix A. SRS was the only syndrome allowed in these clinical trials, and there proved to be over 30 SRS patients included in our studies. Since the discovery of 11p15 LOM, we were able to confirm this diagnosis in a large part of the SRS patients. The IUGR trials therefore provided an excellent basis to study long-term effects of GH treatment in SRS patients, and to investigate whether there are differences between SRS patients and their non-syndromic SGA born counterparts regarding long-term response to GH treatment, metabolic health, pubertal development and gonadal function.

Telomere length and bone mineral density

Evaluating the long-term effects of small size at birth and catch-up growth on health is challenging, since it is difficult to obtain large cohorts with enough variance in size at birth and childhood growth patterns. The PROgramming factors for Growth and Metabolism (PROGRAM) and Prematurity and Small for Gestational Age (PREMS) study cohorts consist of a large group of healthy young adults in which subjects with extreme variants of normal growth were oversampled. The design, in- and exclusion criteria are described in Appendix B. These cohorts made it possible to study the long-term effects of preterm birth, size at birth and early life growth patterns on LTL.

One of the problems with studying long-term effects of GH treatment is the lack of an adequate control group. Since most short SGA children are nowadays treated with GH, it is difficult to obtain a control group of untreated short SGA subjects. The PROGRAM study cohort can provide this control group, since subgroups were made by dividing the population into clinically relevant subgroups, consisting of subjects born SGA with and without catch-up growth and a subgroup of young adults born appropriate for gestational age.

AIMS OF THE STUDY

Silver-Russell syndrome

Response to GH treatment in Silver-Russell syndrome

Children with SRS are treated with GH to improve adult height, but there were limited data on response to GH treatment in SRS children and on differences in response among the (epi)genetic SRS subtypes. We therefore compared growth and adult height between GH-treated SGA children with and without SRS, and analyzed differences in GH response among SRS subtypes.

Metabolic health and long-term safety of GH treatment in Silver-Russell syndrome

Data on metabolic health and long-term safety of GH treatment in SRS were lacking. We therefore longitudinally investigated metabolic health in SRS patients during GH treatment and until two years after cessation of GH treatment due to adult height attainment.

Pubertal development and gonadal function in Silver-Russell syndrome

Very little information existed on pubertal development and gonadal function in patients with SRS. We therefore assessed pubertal progression and gonadal function in children, adolescents and young adults with SRS.

Telomere length

Telomere length in young adults born preterm

In this study, we investigated whether accelerated biological ageing might be one of the underlying mechanisms of the increased risk for CVD in those born preterm. We used LTL as an ageing biomarker to test this hypothesis, in a large cohort of young adults born either preterm or at term. We also assessed whether LTL was associated with putative cardiovascular risk factors at young adult age.

Effects of size at birth, childhood growth patterns and GH treatment on telomere length

In this study, we investigated whether LTL is influenced by size at birth, childhood growth and long-term GH treatment in a large cohort of young adults with differences in size at birth and childhood growth patterns.

Bone mineral density in early adulthood

Bone mineral density in GH treated young adults born SGA

We investigated BMD in young adults born SGA who were treated with GH during childhood, and had attained an age of 21 years. We also compared BMD at five years after cessation of treatment to age-matched untreated short young adults born SGA, and to AGA born controls with a normal stature.

OUTLINE OF THIS THESIS

Chapter 1 gives an introduction on the topics described in this thesis.
Chapter 2 describes the response to GH treatment in children with SRS.
Chapter 3 shows metabolic health and long-term safety of GH treatment in SRS.
Chapter 4 demonstrates pubertal development and gonadal function in SRS.
Chapter 5 describes the effect of preterm birth on telomere length.
Chapter 6 demonstrates the effects of size at birth, childhood growth patterns and GH treatment on telomere length.
Chapter 7 shows bone mineral density in young adults born SGA, who were treated with GH during childhood.
Chapter 8 provides a general discussion of the results of the studies presented in this thesis.
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, acknowledgments and curriculum vitae.

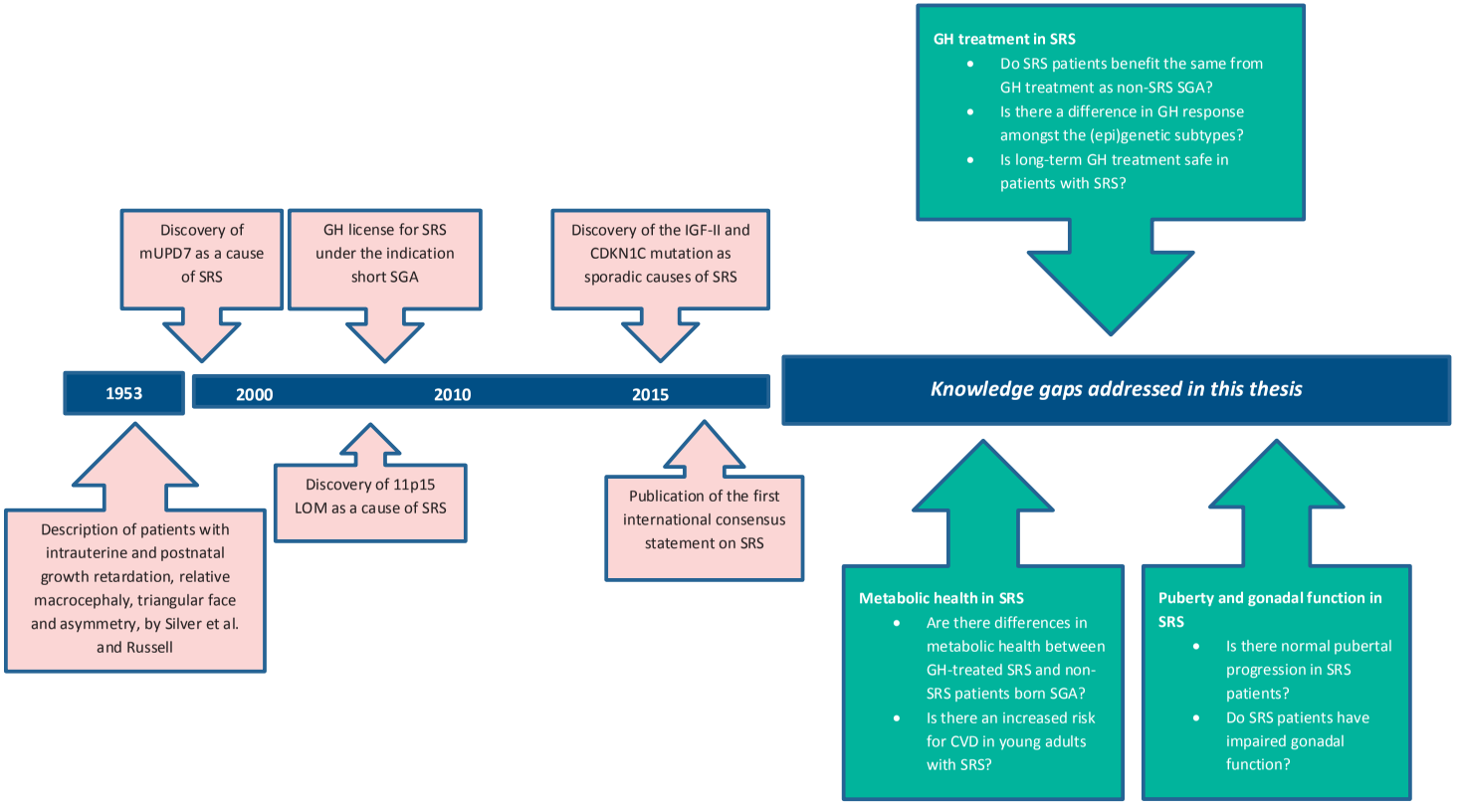

Figure 12. Silver-Russell syndrome – Knowledge gaps addressed in this thesis

APPENDIX A

IUGR-2 and IUGR-3 studies

The second and third Dutch GH trials (IUGR-2 and -3 studies (88, 89)) included children born SGA with persistent short stature.

Design

The IUGR-2 study started in 1996 and the IUGR-3 study in 2003. Both studies are open-labelled, multicentre 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 and/or birth weight SDS for gestational age <-2 SDS (90);
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 (33);
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, or inborn errors of metabolism);
2) Celiac disease or other chronic diseases of the major organs;
3) Chromosomal abnormalities or dysmorphic features suggestive of a syndrome, except Silver-Russell syndrome;
4) Chondrodysplasia;
5) Serious suspicion of psychosocial dwarfism (emotional deprivation);
6) Active malignancy or increased risk of leukaemia;
7) Use of medication that might interfere with growth, such as corticosteroids and sex steroids;
8) Expected non-compliance.

APPENDIX B

PROGRAM and PREMS studies

The PROgramming factors for Growth and Metabolism (PROGRAM) study cohort and Prematurity and Small for Gestational Age (PREMS) study cohort consist of healthy young adults (38, 57). The difference between the two studies is that the subjects in the PROGRAM study were born term, whereas the subjects in the PREMS were born preterm (gestational age <36 weeks). In these participants, several parameters for metabolic health and CVD were determined.

Design

To be able to investigate the influence of different growth patterns during childhood on determinants of adult diseases, subjects with extreme variants of normal growth, such as subjects born SGA with and without catch-up growth, were oversampled. This design created greater contrast in the study population, which contributed to more statistical power.

Inclusion criteria

1) Chronological age at inclusion 18-24 years;
2) 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, broncho-pulmonary dysplasia or other chronic lung disease;
3) Well-documented growth data;
4) Caucasian;
5) Born singleton;
6) Signed informed consent;
7) PROGRAM study: gestational age ≥36 weeks;
8) PREMS study: 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, growth hormone deficiency, malignancies, severe chronic disease);
3) Treatment that could have interfered with growth (such as radiotherapy or growth hormone treatment);
4) Serious suspicion of psychosocial dwarfism (emotional deprivation) during childhood.

APPENDIX C

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

DXA can be used to measure bone mineral density and body composition (i.e. fat mass and lean body mass) (84). The participant lies 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 (approximately 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 (91, 92). 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.

These indicators of glucose regulation were determined by the Bergman's minimal model, calculating paired glucose and insulin data obtained by frequent measurements during an FSIGT test with Tolbutamide. This way, early changes in glucose metabolism can be assessed, many years before the first symptoms of DM2.

Adult Treatment Panel III (ATP-III) score

The 10-year risk for coronary heart disease and DM2 can be assessed using the ATP-III score (93). This score includes the following factors: 1) waist circumference in men >102 cm, and in women >88 cm; 2) triglyceride >1.7 mmol/L; 3) high-density lipoprotein in men <1.03, in women <1.3 mmol/L; 4) blood pressure ≥130/≥85 mmHg; 5) fasting glucose >5.6 mmol/L. Metabolic syndrome is defined as having 3 or more of these risk factors.

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Chapter 2

Long-Term Results of GH Treatment in Silver-Russell Syndrome (SRS): Do They Benefit the Same as Non-SRS Short-SGA?

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ABSTRACT

Context: Silver-Russell syndrome (SRS) is a genetically heterogeneous syndrome characterized by low birth weight, severe short stature and variable dysmorphic features. Growth hormone (GH) treatment is a registered growth-promoting therapy for short children born small for gestational age, including SRS, but there are limited data on the GH response in SRS children and on differences in response among the (epi)genetic SRS subtypes (11p15 aberrations, maternal uniparental disomy of chromosome 7 (mUPD7) and idiopathic SRS).

Objectives: To compare growth and adult height between GH-treated small for gestational age children with and without SRS (non-SRS) and to analyze the difference in GH response among SRS genotypes.

Design and setting: A longitudinal study.

Participants: Sixty-two SRS and 227 non-SRS subjects.

Intervention: All subjects received GH treatment (1 mg/m^2/day).

Main outcome measures: Adult height and total height gain.

Results: The SRS group consisted of 31 children with 11p15 aberrations, 11 children with mUPD7 and 20 children with idiopathic SRS. At the start of GH treatment, mean (SD) height standard deviation score (SDS) was significantly lower in SRS (-3.67 (1.0)) than in non-SRS (-2.92 (0.6), $p<0.001$). Adult height SDS was lower in SRS (-2.17 (0.8)) than in non-SRS (-1.65 (0.8), $p=0.002$), but the total height gain SDS was similar. There was a trend toward a greater height gain in mUPD7 than in 11p15 ($p=0.12$).

Conclusion: Children with SRS have a similar height gain during GH treatment as non-SRS subjects. All (epi)genetic SRS subtypes benefit from GH treatment, with a trend toward mUPD7 and idiopathic SRS having the greatest height gain.

INTRODUCTION

Silver-Russell syndrome (SRS) is characterized by intrauterine growth retardation leading to small size at birth, feeding difficulties during infancy and early childhood, severe short stature, body asymmetry, triangular face with prominent forehead and several other minor anomalies (1-3). Incidence is estimated as 1 in 30000 to 100000 life-born infants per year (4). Without treatment, mean adult height (AH) is around -4.2 standard deviation score (SDS), causing a significant handicap in adulthood (3).

The (epi)genetic etiology of SRS is heterogeneous. In most cases, the 11p15 region, important for controlling growth, is involved (5). The 11p15 region contains two imprinting control regions, ICR1 and ICR2. More than 50% of SRS cases are caused by a hypomethylation of the paternal allele of the ICR1 (6). Maternal duplications of the ICR2 region have also been described (7,8). Recently, mutations in the CDKN1C and IGF-2 genes, also located in the 11p15 region, were discovered as a cause of SRS (9,10). An additional 5-10% of the SRS cases are caused by a maternal uniparental disomy of chromosome 7 (mUPD7) (11). Furthermore, it has been suggested that uniparental disomy events outside chromosome 7 and widespread DNA methylation changes are present in many SRS patients (12). In approximately 40%, the genetic cause remains unknown, which is referred to as idiopathic SRS.

GH treatment is a registered growth-promoting therapy for short children born small for gestational age (SGA), including children with SRS (13). However, there are few data on the growth response to and safety of GH in SRS patients, especially on the long-term. Toumba *et al.* (14) described AH after GH treatment in a small group (n=26) of SRS patients. None of these patients were tested for aberrations in the 11p15 region and the clinical scoring system used to diagnose SRS has been updated since then (15). Binder *et al.* (16) described AH after GH treatment in a group of 37 SRS patients, but most patients measured AH themselves, which might give less reliable results.

Data on genotype-phenotype correlations (ie, differences in phenotype among the different (epi)genetic alterations seen in SRS) are scarce. It has been stated that the 11p15 alterations lead to a more severe SRS phenotype (17), but it is unknown whether there are differences in GH response among the different (epi)genetic aberrations seen in SRS.

In the present study, we compared response to GH treatment between 62 SRS subjects and 227 non-SRS subjects born SGA. Based on previous literature and our clinical experience, we hypothesized that SRS patients have a similar height gain from the start of treatment until AH as non-SRS subjects born SGA. We also compared growth data of subjects with different (epi)genetic causes of SRS, hypothesizing that SRS subjects with mUPD7 or idiopathic SRS attain a larger AH compared to subjects with aberrations in the 11p15 region, since the 11p15 alterations cause the most severe SRS phenotype.

SUBJECTS AND METHODS

Subjects

For the present study, we included 62 SRS and 227 non-SRS subjects. All subjects were born SGA (birth length and/or birth weight SDS below -2.0 for gestational age) (18) and received GH treatment because of short stature (height below -2.5 SDS) (19). Excluded were subjects with chromosomal abnormalities or signs of a syndrome except SRS, subjects who received less than three years of GH treatment and non-SRS subjects who were also treated with a GnRH analog (GnRHa).

Subjects were diagnosed with clinical SRS based on the Netchine-Harbison clinical scoring system (15). This recently developed scoring system includes the following six factors: 1) prenatal growth retardation (birth weight and/or length ≤ -2 SDS for gestational age); 2) postnatal growth retardation (height SDS for calendar age below -2.0 according to national reference (19); 3) relative macrocephaly at birth (head circumference at birth at least 1.5 SDS above birth weight and/or length SDS according to Usher and McLean (18); 4) prominent forehead (defined as forehead that projects beyond the facial plane on a side view as a toddler); 5) body asymmetry (defined as leg length discrepancy of ≥ 0.5 cm or arm asymmetry or leg length discrepancy <0.5 cm with at least two other asymmetrical body parts (with one being a non-face part)); 6) feeding difficulties during early childhood. Patients were classified as clinical SRS if at least four of these six factors were present. All clinical SRS subjects were tested for methylation aberrations in the 11p15 region (ICR1 and ICR2) and for methylation of chromosome 7. Furthermore, 14 out of the 20 clinical SRS patients who tested negative for 11p15 aberrations and mUPD7 were also tested for CDKN1C and IGF-2 mutations. Six patients could not be tested for CKDN1C and IGF-2 mutations because there was no DNA available anymore, and they did not want to revisit the hospital after attainment of AH. Patients with clinical SRS without a genetic alteration were classified as idiopathic SRS.

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

Design

In this prospective study, all participants were treated with biosynthetic GH at a dose of 1 mg/m^2/d (0.035 mg/kg/d). GH was administered sc once daily at bedtime. Height, weight and Tanner stage were determined three-monthly, and GH dose adjusted to the calculated body surface area.

GH treatment was discontinued at AH. AH was defined as the condition when height velocity dropped below 0.5 cm during the previous 6 months and a bone age ≥ 15 years for girls and ≥ 16.5 years for boys.

Measurements

Birth data were obtained from records of hospitals and primary health care centres. Height was measured to the nearest 0.1 cm (Harpenden stadiometer), weight to the nearest 0.1 kg (Servo Balance KA-20-150S). Anthropometric measurements were performed twice according to standardised methods, after which the mean was calculated. Target height (TH) was calculated as TH=[(maternal height + paternal height + 13) / 2 + 4.5] for boys and TH= [(maternal height + paternal height - 13) / 2 + 4.5] for girls (20). Onset of puberty was defined as breast stage 2 according to Tanner for girls and testicular volume ≥ 4 ml for boys (21). Bone age was determined once a year according to Tanner and Whitehouse RUS (22). Bone age delay was calculated as calendar age minus bone age in years.

DNA analysis

DNA analysis for 11p15 methylation aberrations, CDKN1C and IGF-2 mutations, and mUPD7 was performed in clinical SRS patients. Genomic DNA was extracted from peripheral blood leucocytes. DNA methylation testing of the 11p15 region (ICR1 (H19) and ICR2 (KCNQ1OT1)) was performed using Methylation-specific multiplex ligation-mediated Probe amplification (MS-MLPA). The SALSA MS-MLPA kit ME-030 (MRC Holland) was used according to manufacturer's instructions. Testing for mUPD7 was also performed by MS-MLPA. The SALSA MS-MLPA kit Me-032 (MRC Holland) was used according to manufacturer's instructions. To identify CDKN1C mutations (c.836G>T (pArg279Leu)) (10) or IGF2 mutations (IGF2c.191C>A (p.Ser64Ter)) (9), genomic DNA extracted from peripheral blood leucocytes was diluted to a concentration of 5 ng/µl. Target regions were amplified by PCR with Taq polymerase (Qiagen). The forward primer
IGF2exR3 5'-CTCGGCATTATGACCTGTGT-'3 and reverse primer
IGF2ex3R 5'-AGGCGTGTGATGGGAAAG-'3 were used in order to amplify the target including the IGF-2 mutation. The CDKN1C target region containing the mutation was amplified using primers described previously (10).

Calculations and statistical analysis

SDS values for birth length and birth weight were calculated to correct for gestational age and gender (18), SDS for weight and blood pressure to correct for height and gender and SDS for height, IGF-I and IGF binding protein-3 (IGFBP-3) levels to correct for gender and age (19,23). SDS values for birth length, birth weight, height, and weight were calculated using the Growth Analyzer software (http://www.growthanalyser.org). Total height gain was defined as AH SDS minus height SDS at start of GH. Prepubertal height gain was defined as height SDS at onset of puberty minus height SDS at start of GH, and pubertal height gain as AH SDS minus height SDS at onset of puberty. Distance to TH SDS at AH was determined as TH SDS minus AH SDS.

Differences in characteristics between SRS and non-SRS were determined using an independent-sample *t* test (continuous data) or X^2 test (categorical data). Using one-way ANOVA, we compared characteristics and response to GH treatment among the different genetic causes of SRS.

Because follow-up was not complete until AH (AH data were available for 26 SRS subjects (42%) and 159 non-SRS subjects (70%)), in addition to the uncensored cases analysis, we also performed a linear mixed model analysis to compare longitudinal changes in height between SRS and non-SRS, adjusting for missing values. In this analysis, factors were SRS (1 = SRS; 0 = non-SRS) and time (0 = baseline; 1 = 1 year GH, 2 = onset puberty, 3= AH). An unstructured repeated covariance type was used, correcting for total treatment duration and age at start of treatment.

To evaluate which variables associate with total height gain SDS in the total group and in subjects with SRS, we used multiple regression (MR) analyses. We included TH SDS, age at start of treatment, height at start of treatment, and duration of treatment. To analyze whether SRS negatively influenced total height gain, we included the variable SRS (1 = yes, 0 = no) as a dummy variable in the total group analysis. Consecutively, we added 11p15 alteration (1 = yes, 0 = mUPD7 or idiopathic SRS) to the MR analysis for the SRS subjects to test the influence of 11p15 on total height gain in SRS subjects compared to mUPD7 and idiopathic SRS.

Results were considered statistically significant if the *P* value was <.05. All analyses were performed with SPSS for Windows (version 21.0; SPSS Inc).

RESULTS

Clinical characteristics of the SRS and non-SRS subjects born SGA are listed in Table 1. Of the 62 SRS patients, 31 had a methylation defect in the 11p15 region (n = 24 ICR1 hypomethylation, six duplication of the maternal 11p15 allele, one deletion of the paternal allele) and 11 patients an mUPD7. There were no patients with an IGF-2 or CDKN1C mutation in the 14 out of 20 idiopathic SRS patients who were tested for these mutations. Twenty clinical SRS patients tested negative for the (epi)genetic alterations causing SRS and were thus assigned to the idiopathic SRS group.

The ratio males/females was similar between SRS and non-SRS and among the three SRS groups. SRS patients had a significantly lower birth weight and birth length ($P <$ .005). Those with mUPD7 had the greatest birth length SDS of the three SRS groups (-1.92 SDS, vs -4.37 in 11p15 and -4.55 in idiopathic SRS, $P = .002$), and there was a trend toward the greatest birth weight ($P = .10$). TH was 0.30 SDS higher in SRS than in non-SRS ($P = .01$). There was a trend toward a higher TH in 11p15 and mUPD7 than in idiopathic SRS ($P = .08$).

Table 1. Baseline characteristics

	Total group			SRS			
	SRS	**Non-SRS**	***P* value[a]**	**11p15**	**mUPD7**	**Idiopathic**	***P* value[b]**
n	62	227		31	11	20	
No. of males/females	34/28	139/88	.36	19/12	4/7	11/9	.36
Gestational age, wk	37.6 (2.7)	36.4 (3.5)	**.005**	38.0 (2.4)	38.8 (1.5)	36.4 (3.4)	.06
Birth length SDS	-3.99 (1.7)	-3.04 (1.6)	**.001**	-4.37 (1.4)	-1.92 (1.5)	-4.55 (1.3)	**.002**
Birth weight SDS	-2.79 (1.2)	-2.28 (1.2)	**.004**	-2.98 (0.9)	-2.03 (1.2)	-2.86 (1.4)	.10
Birth head circumference SDS	-0.62 (0.6)	-1.02 (0.9)	.29	-1.08 (0.9)	n.a.	-0.70 (1.5)	.63
TH SDS	-0.20 (0.8)	-0.50 (0.8)	**.01**	-0.11 (0.8)	0.10 (0.8)	-0.52 (0.7)	.08

Abbreviations: 11p15, alteration in 11p15 region; n.a., no data available; TH, target height. Values are expressed as mean (SD) unless otherwise specified. Boldface indicates *P* values below .05.

[a] Represents *P* values of SRS vs non-SRS.

[b] Represents *P* values of 11p15 vs mUPD7 vs idiopathic.

Growth during GH treatment in SRS vs non-SRS

Table 2 and Figure 1A show height SDS in SRS and non-SRS from the start of GH treatment until AH. At the start of GH treatment, mean (SD) age was 4.9 (2.2) years in SRS and 6.7 (2.2) years in non-SRS ($P < .001$). Mean height (SD) at the start of GH treatment was -3.67 (1.0) SDS in SRS vs. -2.92 (0.6) SDS in non-SRS ($P < .001$). Weight for height at start was 1.36 SDS lower in SRS subjects than in non-SRS subjects ($P < .001$). Serum IGF-I and IGFBP-3 SDS at start were comparably low in both groups ($P = .96$ and $P = .31$, respectively).

During the first year of GH treatment, height gain was 0.96 (0.5) SDS in SRS vs 0.84 (0.3) SDS in non-SRS ($P = .08$). Serum IGF-I SDS increased to 1.51 (1.3) SDS in SRS vs 0.63 (1.3) SDS in non-SRS ($P < .001$).

SRS subjects, both girls and boys, were significantly younger at the onset of puberty than non-SRS ($P < .001$). Height SDS at onset of puberty was similar in SRS and non-SRS ($P = .81$).

SRS and non-SRS attained AH at a similar age ($P = .17$). From onset of puberty until AH, height SDS declined in both groups: with 0.75 (0.7) SDS in SRS and with 0.44 (0.7) SDS in non-SRS ($P = .051$), resulting in an mean AH SDS of -2.17 (0.8) in SRS and -1.65 (0.8) in non-SRS ($P = .002$) and a larger distance to TH in SRS subjects ($P < .001$). Total height gain SDS from start of treatment until AH was, however, similar in SRS (1.30 (1.0) SDS) and non-SRS (1.26 (0.8) SDS ($P = .81$)). At near AH, IGF-I SDS was similar in SRS and non-SRS ($P = .47$).

The estimated mean AH from the mixed-model analysis (accounting for missing values) was -2.21 (95% confidence interval (CI) -2.50 to -1.92) SDS in SRS and -1.66 (95% CI -1.78 to -1.54) SDS in non-SRS and thus similar to the AH of the uncensored cases analysis.

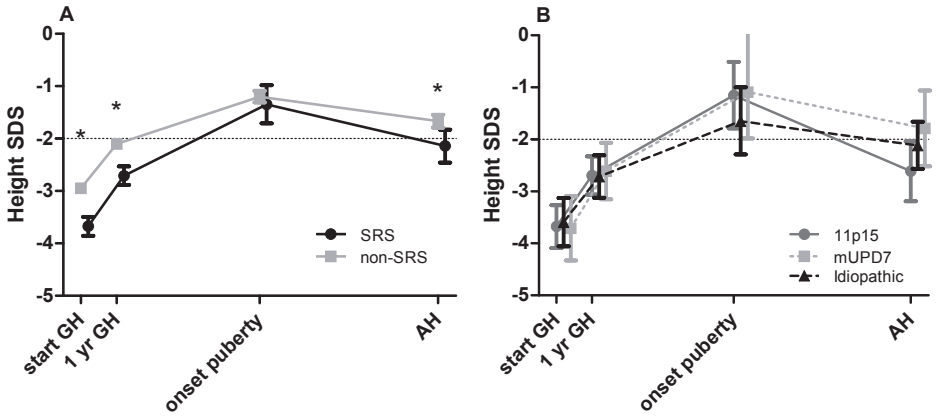

Figure 1. Height SDS during GH treatment in SRS and non-SRS (A) and SRS (B).
Data are expressed as estimated marginal means with 95% CI. *, *P* value <.05 SRS vs non-SRS.

Differences among 11p15, mUPD7, and idiopathic SRS

We evaluated whether the growth response to GH differed among the three SRS groups (Table 2 and Figure 1B). Height SDS and weight for height at start of GH treatment were similar in the three groups. During the first year of GH treatment, height gain was also similar in the three groups (*P* = .57). IGF-I SDS was highest in those with an 11p15 alteration (2.10 (1.0) SDS vs. 0.65 (1.4) in mUPD7 and 0.92 (1.1) in idiopathic SRS; *P* = .001).

Boys with mUPD7 were the youngest at onset of puberty (*P* = .04). There was a trend toward the greatest height SDS at onset of puberty in 11p15 SRS and the lowest in idiopathic SRS (*P* = .12).

The three SRS subgroups attained their AH at a comparable age (*P* = .44). AH SDS was -2.42 (1.0) in 11p15, -2.00 (0.7) in mUPD7 and -1.96 in idiopathic SRS (*P* = .39). There was a trend toward a lower total height gain from the start of GH to AH in 11p15 compared to mUPD7 and idiopathic SRS (*P* = .12). At near AH, IGF-I levels were similar among the three SRS subgroups (*P* = .82).

Factors associated with total height gain

The MR analysis in the total group (SRS and non-SRS; Table 3, left column) showed that TH SDS (*ß* = 0.33, *P* < .001), and total treatment duration (*ß* = 0.14, *P* = .001) positively influenced total height gain. Height SDS at the start of GH treatment (*ß* = -0.49; *P* < .001) was negatively associated with total height gain. SRS patients negatively influenced total height gain of the total group (*ß* = -0.44, *P* = .013). This model explained 35% of the variance in total height gain until AH (*P* < .001).

In the non-SRS group, the variables influencing total height gain were similar to those of the total group (SRS and non-SRS) (Table 3, middle column).

Table 2. First year growth, pubertal growth and AH in SRS vs non-SRS

	Total group			SRS			
	SRS	**Non-SRS**	***P* value[a]**	**11p15**	**mUPD7**	**Idiopathic**	***P* value[b]**
n	62	227		31	11	20	
At start of GH							
Age, y	4.9 (2.2)	6.7 (2.2)	**<.001**	4.6 (2.1)	4.8 (2.2)	5.3 (2.3)	.51
BA delay (age – BA)	1.2 (1.4)	1.2 (1.0)	.41	0.90 (1.6)	1.49 (0.4)	1.58 (1.2)	.84
Height SDS	-3.67 (1.0)	-2.92 (0.6)	**<.001**	-3.69 (1.2)	-3.73 (0.8)	-3.60 (0.9)	.93
Weight/height SDS	-2.64 (1.3)	-1.28 (1.2)	**<.001**	-2.71 (1.4)	-2.33 (1.3)	-2.70 (1.1)	.68
IGF-I SDS	-1.17 (1.7)	-1.16 (1.3)	.96	-0.57 (1.2)	-1.95 (1.4)	-1.36 (2.1)	.61
IGFBP-3 SDS	-1.98 (0.8)	-1.54 (1.3)	.31	-2.54 (0.3)	-2.26 (0.8)	-1.36 (0.8)	.13
After 1 yr GH							
Height SDS	-2.71 (1.0)	-2.08 (0.6)	**<.001**	-2.79 (1.1)	-2.65 (0.6)	-2.62 (0.9)	.80
Height gain SDS	0.96 (0.5)	0.84 (0.3)	.08	0.90 (0.6)	1.08 (0.4)	0.98 (0.3)	.57
Weight/height SDS	-2.15 (1.3)	-0.95 (1.1)	**<.001**	-2.38 (1.5)	-1.61 (0.8)	-2.09 (1.0)	.22
IGF-I SDS	1.51 (1.3)	0.63 (1.3)	**<.001**	2.10 (1.0)	0.65 (1.4)	0.92 (1.1)	**.001**
At onset puberty							
Age, y							
• Total	10.8 (1.3)	11.7 (1.1)	**<.001**	10.9 (1.3)	9.8 (1.0)	11.4 (1.3)	**.02**
• Boys	11.4 (1.1)	12.0 (1.1)	**.02**	11.5 (0.9)	10.3 (1.0)	12.0 (1.0)	**.04**
• Girls	10.2 (1.3)	11.2 (1.0)	**.003**	10.3 (1.4)	9.2 (0.4)	10.7 (1.3)	.16
Duration of GH, y	5.6 (2.5)	5.0 (2.2)	.13	6.1 (2.4)	4.1 (2.3)	5.8 (2.7)	.18
Height SDS	-1.12 (1.1)	-1.17 (0.8)	.81	-0.74 (0.8)	-1.09 (1.3)	-1.64 (1.2)	.12
At AH							
No. that achieved AH	26	159		11	5	10	
Age, y							
• Total	15.6 (1.3)	16.0 (1.1)	.17	15.4 (0.7)	16.3 (1.6)	15.6 (1.7)	.44
• Boys	16.8 (1.5)	16.6 (0.9)	.56	15.8 (0.9)	17.8 (1.2)	17.5 (1.8)	.20
• Girls	15.0 (0.7)	15.3 (0.9)	.34	15.0 (0.5)	15.4 (1.1)	14.8 (0.8)	.51
Duration of GH, y	9.6 (2.5)	8.8 (2.0)	**.02**	9.5 (2.7)	10.7 (2.3)	9.7 (3.0)	.73
Pubertal height gain	-0.75 (0.7)	-0.44 (0.7)	.051	-1.27 (0.4)	-0.52 (0.7)	-0.35 (0.7)	**.01**
AH SDS	-2.17 (0.8)	-1.65 (0.8)	**.002**	-2.42 (1.0)	-2.00 (0.7)	-1.96 (0.2)	.39
Total height gain SDS	1.30 (1.0)	1.26 (0.8)	.81	0.91 (1.0)	2.04 (0.8)	1.37 (1.0)	.12
Distance to TH SDS	2.14 (1.0)	1.11 (0.7)	**<.001**	2.37 (1.4)	2.03 (0.4)	1.97 (0.9)	.69
IGF-I SDS (near AH)	1.31 (1.2)	1.16 (0.7)	.47	1.45 (0.8)	1.38 (1.0)	1.11 (1.6)	.82

Abbreviations: 11p15, alteration in 11p15 region; BA, bone age Greulich & Pyle. Values are expressed as mean (SD), unless specified otherwise. Boldface *P* values are < .05.

[a] Represents *P* values of SRS vs non-SRS.

[b] Represents *P* values of 11p15 vs mUPD7 vs idiopathic.

Subsequently, we analyzed which variables influenced total height gain in the SRS subjects (Table 3, right column). TH SDS (*P* = .84) and total duration of GH (*P* = .58) did not significantly influence total height gain in this model. Height SDS at start (*ß* = -0.67, *P* = .02) negatively influenced total height gain. There was a trend toward a negative influence of subjects with an 11p15 aberration on total height gain of the SRS subjects (*ß* = -0.52, *P* = .13). This model explained 45% of the variance in total height gain in SRS subjects (*P* = .007).

Table 3. Multiple regression analysis with independent variable total height gain

Total height gain SDS						
	Total group		**Non-SRS**		**SRS**	
Variables	***ß***	***P* value**	***ß***	***P* value**	***ß***	***P* value**
TH SDS	0.33	**<.001**	0.36	**0.001**	0.05	.84
Age at start GH	0.03	.48	0.02	.62	-0.06	.69
Height SDS at start GH	-0.49	**<.001**	-0.43	**0.001**	-0.67	**.02**
Total duration of GH, y	0.14	**.001**	0.11	**.008**	0.09	.58
SRS	-0.44	**.013**	NA	NA	NA	NA
11p15	NA	NA	NA	NA	-0.52	.13
Overall *P* value	**<.001**		**<.001**		**.007**	
R^2 adjusted	0.35		0.32		0.45	

Abbreviations: NA, not applicable; SRS, subjects with Silver-Russell syndrome (non-SRS subjects are the reference group). 11p15 indicates SRS subjects with an alteration of the 11p15 region (mUPD7 and idiopathic SRS are the reference group). Total group columns represent SRS and non-SRS subjects. SRS columns represent SRS subjects only. Boldface *P* values are <.05.

GnRHa treatment in SRS

In 17 SRS subjects (five 11p15; five mUPD7 and seven idiopathic SRS), puberty was postponed for two years due to a low predicted AH using GnRHa (leuprolide acetate depot, 3.75 mg sc every 4 weeks) in addition to GH treatment. Five patients were male and 12 were female.

Mean (SD) age at start of puberty was significantly lower (9.4 (0.9) years) in the SRS girls treated with additional GnRHa than in those without (11.0 (1.2) years; *P* = .004). Height at the onset of puberty was 132.1 (8.8) cm in SRS girls with GnRHa vs 139.4 (9.9) cm in SRS girls without (*P* = .15). Height gain from the onset of puberty until AH was greater in SRS girls with additional GnRHa (26.3 (6.1) cm) than in SRS girls without GnRHa (15.5 (5.5) cm) (*P* = .004), leading to a similar AH (155.0 (4.6) cm with GnRHa and 156.9 (4.9) cm without GnRHa) (*P* = .41).

SRS boys treated with additional GnRHa were also younger at start of puberty than those without (10.7 (1.1) years vs 11.7 (0.9) years, respectively, *P* = .052). Height at the onset of puberty was 142.5 (6.9) cm in SRS boys with GnRHa and 145.2 (6.2) cm in those

without (P = .44). Height gain from onset of puberty until AH was greater in SRS boys with GnRHa than in those without (33.1 (2.8) cm vs. 22.3 (1.9) cm, respectively, P = .03), leading to a similar AH (P = .55).

Safety

GH treatment was well tolerated in SRS and non-SRS. No adverse events considered to be drug-related were observed. After 1 year of treatment, 38.6% of the SRS patients had IGF-I levels above +2 SDS vs. 11.1% of the non-SRS subjects (P < .001). At near AH, 24.0% of the SRS patients and 9.1% of the non-SRS subjects had IGF-I levels above +2 SDS (P = .15).

Two female SRS patients with an ICR1 hypomethylation were diagnosed with a slipped capital femoral epiphysis (SCFE) during GH treatment at 11 and 10 years of age. Both girls underwent surgical fixation of the hip joint. Because these events were not considered related to GH by the treating physicians, after fixation, GH treatment was continued.

DISCUSSION

In this study, the growth response to GH treatment was compared between 62 SRS patients and 227 short, non-syndromic subjects born SGA. Mean total height gain was similar in SRS and non-SRS subjects born SGA. Although SRS subjects did not attain the same AH, GH treatment is similarly effective in SRS as in non-SRS SGA subjects. All SRS subtypes benefit from GH treatment, with a trend toward mUPD7 and idiopathic SRS having the greatest height gain.

Our study represents a large study describing growth during GH treatment in a cohort of SRS subjects treated for a long time. We found that SRS patients attained a mean AH of -2.17 SDS and that total height gain was 1.30 SDS, compared to a mean AH of -1.65 SDS and a total height gain of 1.26 SDS in non-SRS patients. The effectiveness of GH treatment in short children born SGA had been reported (24-26), but long-term effects of GH in SRS patients were very scarce. Rakover *et al.* (27) described 33 SRS patients treated with GH. There was a significant improvement in growth during three years of GH therapy but AH data were missing. To our knowledge, there are only two studies describing AH after GH therapy in SRS patients (14,16). Binder *et al.* (16) described 37 patients with a mean height at start of -3.34 SDS. Overall height gain was 1.18 SDS in males and 1.47 SDS in females, which is comparable with the height gain in our cohort. Unfortunately, in that study a large number of patients measured their height themselves, which might lead to unreliable results. Also, only data at start of treatment and at AH were analyzed, and height data around puberty were lacking. In the study of Toumba *et al.* (14), 26 SRS patients with a median height at start of -2.7 SDS were described. In that study, boys attained a higher AH (-1.0 SDS) than girls (-2.3 SDS), and median

height gain was 1.4 SDS. However, no patients were tested for methylation aberrations on 11p15 and the scoring system to diagnose clinical SRS has been updated since then. In both studies, a control group of short SGA children without SRS was lacking.

Although total height gain in SRS and non-SRS subjects was similar in our study, SRS patients did not attain the same AH because they were significantly shorter at start of GH. Also, puberty started earlier in SRS patients leading to earlier closure of the growth plates and a lower AH at a younger age. Indeed, there was a steeper decline in height SDS from onset of puberty until AH in SRS. A decline in height SDS during puberty is a known phenomenon in short SGA children who are treated with GH (26). Puberty was delayed with GnRHa in 17 SRS patients. Because pubertal height gain was greater in those treated with GnRHa in addition to GH, adding GnRHa treatment can be beneficial in those who start puberty at a young age with a short height. However, our study was not designed to give a definite answer on this matter.

Our study has two important strengths: the application of strict criteria to diagnose clinical SRS (15) and the performance of additional DNA analysis in all clinical SRS patients, including the recently discovered CDKN1C and IGF-2 mutations in a majority of the patients (9,10). Previous studies describing GH treatment in SRS patients did not perform extensive genetic testing (14,27). In our cohort, we did not detect IGF-2 or CDKN1C mutations in 14 out of 20 idiopathic SRS patients who were tested for these mutations. Our findings are in line with those of Muller *et al.* (28) who did not find IGF-2 mutations in 72 patients with a clinical SRS phenotype. Until now, CDKN1C mutations have been described in just one family (10,30). Our study shows that IGF-2 and CDKN1C mutations are not a major molecular cause of SRS and that only in familial cases with paternal inheritance of the SRS phenotype, IGF-2 mutations need to be considered (9,28,30).

To analyze genotype-phenotype correlations, we compared GH response among patients with an 11p15 methylation defect, mUPD7 and idiopathic SRS. Height SDS was similar at start but there was a trend toward a greater height gain in the patients with mUPD7 and idiopathic SRS compared to patients with an 11p15 methylation defect. This is in line with a previous study reporting that the 11p15 alterations cause a more severe phenotype of SRS (17). Patients with mUPD7 or idiopathic SRS thus respond better to GH treatment than those with 11p15. Because those with an 11p15 alteration also increase their height with almost 1 SDS, we also consider GH treatment beneficial for SRS patients with an 11p15 alteration.

The number of patients in the SRS subgroup analysis for AH was relatively small due to the fact that SRS is a rare disorder and not all patients attained AH yet. Thus, definite conclusions should await findings in a larger cohort. Despite applying the most recent genetic assessments regarding SRS, there was still an idiopathic SRS group. We, therefore, suggest that future studies aim at elucidating the genetic causes of idiopathic SRS.

IGF-I levels were similar at the start of GH treatment in SRS and non-SRS groups. However, after 1 year of GH treatment, IGF-I levels were higher in the SRS group due to high IGF-I levels in the SRS patients with an 11p15 alteration. This finding is in concordance with a previous study, suggesting reduced IGF-I sensitivity in SRS patients with an 11p15 epimutation (31). However, the mechanism behind the reduced IGF-I sensitivity remains to be elucidated. At near AH, IGF-I levels were not significantly different anymore between SRS and non-SRS or among the different SRS subgroups, which is reassuring. Based on the higher IGF-I levels in the SRS group after 1 year of GH treatment, we suggest careful monitoring of IGF-I levels in these children, especially in those with an 11p15 alteration. Our findings highlight the importance of attaining more knowledge about the degree of IGF-I insensitivity in SRS children and whether higher IGF-I levels, even within the normal range, have long-term consequences.

GH treatment was well tolerated in SRS and non-SRS groups. Two SRS patients were diagnosed with SCFE during GH treatment. Whether GH-treated SRS subjects are at increased risk for SCFE cannot be concluded from these two events. Besides this, there were no adverse events observed that were considered to be drug-related over a long period of time. We therefore conclude that, besides a possible increased risk for SCFE, there is no safety concern about GH treatment in children with SRS.

In conclusion, we showed that SRS patients respond well to GH treatment and that total height gain is similar in SRS and non-SRS subjects born SGA. There was a trend toward a better response to GH treatment in SRS patients with mUPD7 and idiopathic SRS compared to patients with an 11p15 methylation defect, but GH treatment is also beneficial for those with 11p15. Since no adverse events occurred over a long treatment period, there is no safety concern about GH in children with SRS. Future studies should aim at elucidating the genetic causes of idiopathic SRS and the long-term consequences of short SGA in general and SRS in particular. Children with clinical signs of SRS should be tested for 11p15 methylation aberrations and mUPD7.

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Chapter 3

Metabolic Health and Long-Term Safety of Growth Hormone Treatment in Silver-Russell Syndrome

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ABSTRACT

Context: Children with Silver-Russell syndrome (SRS) are born small for gestational age (SGA) and remain short. Growth hormone (GH) treatment improves height in short SGA children, including those with SRS. Data on metabolic health and long-term safety of GH treatment in SRS are lacking.

Objective: To investigate metabolic health in SRS patients during and until 2 years after discontinuation of GH treatment.

Design: Metabolic health was assessed longitudinally at GH-start, GH-stop, 6 months and 2 years thereafter.

Patients: Twenty-nine SRS patients vs 171 non-SRS subjects born SGA.

Main outcome measures: Lean body mass (LBM) and fat mass percentage (FM%), insulin sensitivity (Si) and β-cell function, blood pressure and serum lipids.

Results: At GH-start (mean age (standard deviation) 5.4 (2.1) years in SRS and 6.7 (2.0) in non-SRS ($P = 0.003$)), blood pressure, serum lipids, glucose and insulin levels were similar and within normal ranges in SRS and non-SRS. LBM SDS and FM% SDS were lower than average in both groups. During treatment, LBM SDS remained stable while FM% SDS increased in both groups. During the 2 years after GH-stop, LBM decreased and FM% increased, while Si and β-cell function improved. At 2 years after GH-stop (mean age 18 years), all parameters were similar and within normal ranges in SRS and non-SRS. None of the SRS patients developed metabolic syndrome, diabetes mellitus type 2, or adverse events.

Conclusion: GH-treated SRS patients have a similar metabolic health and safety profile as non-SRS subjects born SGA, both during and until 2 years after GH-stop.

INTRODUCTION

Silver-Russell syndrome (SRS) is characterized by small for gestational age (SGA) birth, postnatal growth retardation, feeding difficulties, and several dysmorphic features (1-3). Approximately 60% of cases are caused by an aberration in the imprinting control region of the 11p15 region (4) and 5% to 10% by a maternal uniparental disomy of chromosome 7 (mUPD7) (5). In 30% to 40%, the genetic cause is unknown, which is referred to as clinical SRS (6).

Children born with a low birth weight are at increased risk to develop adult-onset disorders such as diabetes mellitus type 2 (DM2), hypertension, and hyperlipidemia at a relatively young age (7). Epigenetic changes could be one of the underlying mechanisms behind this increased risk (8), but the risk for adult-onset disorders has not been investigated in SRS. Overall, adult follow-up data in SRS are lacking, which was also emphasized by the recently published consensus statement on diagnosis and management of SRS (6).

Growth hormone (GH) treatment is a registered growth promoting therapy for short children born SGA (9), including children with SRS. It has been shown that GH treatment is effective at increasing adult height (AH) in SRS (10, 11). GH treatment has also several metabolic effects in children born SGA, namely an increase in lean body mass (LBM), a decline in fat mass (FM), a decrease in blood pressure (BP), and a more favorable lipid profile, but also a lower insulin sensitivity (Si) (12-14). Data on whether these effects also occur in GH-treated SRS patients are lacking.

In the present study, we assessed longitudinal changes in metabolic health (*i.e.* BP, fasting lipid levels, body composition, Si, and occurrence of DM2 and metabolic syndrome) in patients with SRS, from start of GH treatment until 2 years after discontinuation of GH due to AH attainment. We compared these data with GH-treated non-SRS subjects born SGA, hypothesizing that SRS patients have a less favorable metabolic health profile due to their epigenetic changes, but that the metabolic changes during and after GH treatment are similar in SRS and non-SRS subjects.

METHODS

Subjects

The study group comprised 29 SRS and 171 non-SRS subjects who participated in a large, multicenter GH trial (13-15) and had attained AH. All subjects were born SGA (birth length (BL) and/or birth weight (BW) ≤ -2 standard deviation score (SDS) for gestational age (16)) and received biosynthetic GH at a dose of 1 mg/m^2/d (~0.035 mg/kg/d) because of short stature (height ≤ -2.5 SDS (17)), until they attained AH (*i.e.* height velocity <0.5 cm in 6 months and bone age ≥15 years for girls and ≥16.5 years for boys). Excluded were subjects with chromosomal abnormalities or signs of a syndrome except SRS.

From start of GH until 2 years after discontinuation of treatment, parameters of vascular and metabolic health were investigated at 4 time points: 1) at GH-start; 2) when subjects reached AH (*i.e.* GH-stop); 3) at 6 months after GH-stop; and 4) at 2 years after GH-stop. Owing to the time-consuming aspect of the study and the fact that not all patients had already discontinued GH for 2 years, data were available of 18 SRS patients and 165 non-SRS subjects at 6 months after GH-stop and 13 SRS patients and 109 non-SRS subjects at 2 years after GH-stop.

Subjects were diagnosed with SRS based on the Netchine-Harbison clinical scoring system (18), which includes the following 6 factors: 1) prenatal growth retardation (BL and/or BW ≤ -2 SDS for gestational age); 2) postnatal growth retardation (height SDS < -2.0 according to national reference (17); 3) relative macrocephaly at birth (head circumference at birth ≥1.5 SDS above BL and/or BW SDS according to Usher and McLean (16); 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 4 factors were present. SRS patients were tested for methylation aberrations of the 11p15 region and mUPD7, and when negative, also for CDKN1C and insulin-like growth factor (IGF)2 mutations. Patients with SRS based on 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/or their parents.

Measurements

Birth data were obtained from records of hospitals and primary health care centers. Anthropometric measurements were performed twice according to standardized methods, after which the mean was calculated. Height was measured to the nearest 0.1 cm (Harpenden stadiometer), weight to the nearest 0.1 kg (Servo Balance KA-20-150S). Waist circumference was measured midway between the lower margin of the lowest rib and the upper margin of the iliac crest at the end of a normal expiration.

Diastolic BP (DBP) and systolic BP (SBP) were measured after 10 minutes of rest, in sitting position, using the non-dominant arm, with an automatic device (Accutorr Plus, Datascope, Montvale NJ) at every 5 minutes for 1 hour; the mean value was taken to reflect resting BP.

LBM and FM were measured on a dual-energy X-ray absorptiometry (DXA) machine (Lunar Prodigy; GE Healthcare, Chalfont St. Giles, UK). FM was measured as a percentage of total body weight (FM%). Quality control was performed daily.

Glucose homeostasis was assessed at GH-stop and 6 months and 2 years thereafter, by frequently sampled intravenous glucose tolerance (FSIGT) tests with Tolbutamide

after an overnight fast (19). Si, glucose effectiveness (Sg), acute insulin response (AIRg), and disposition index (DI) were calculated using R.N. Bergman's minimal model software (MINMOD 6.01). Si quantifies the capacity of insulin to promote glucose disposal, and Sg reflects the capacity of glucose to mediate its own disposal. AIRg is an estimate of insulin secretory capacity and was measured as the area under the curve from 0 to 10 minutes corrected for baseline insulin levels. DI equals AIRg times Si (DI = AIRg X Si) and indicates the ß-cell function.

Revised criteria of the National Cholesterol Education Program (adult treatment panel III (ATP-III)) were used to determine components of metabolic syndrome (20). Metabolic syndrome was defined as having ≥3 of the following risk factors: 1) waist circumference in men >102 cm, and in women >88 cm; 2) triglycerides (Tg) >1.7 mmol/L; 3) high-density lipoprotein cholesterol (HDLc) in men <1.03, in women <1.3 mmol/L; 4) BP ≥130/≥85 mm Hg; 5) fasting glucose >5.6 mmol/L.

Behavioral problems were defined as attention deficit hyperactivity disorder, pervasive developmental disorder, or autism spectrum disorder, diagnosed by an experienced psychologist.

Laboratory measurements

After centrifugation, all samples were kept frozen until assayed (-80°C). Fasting levels of total cholesterol (TC), Tg and high-density lipoprotein (HDLc) were measured using the CHOD-PAP and the GPO-PAP reagent kits (Roche Diagnostics, Mannheim, Germany) (TC and Tg), and using a homogeneous enzymatic colorimetric assay (Roche Diagnostics) (HDLc). Low-density lipoprotein cholesterol (LDLc) was calculated using the Friedewald formula: LDLc (mmol/L) = TC – HDLc – 0.45 X level of Tg. Fasting glucose levels were determined on an Architect ci8200 system (Abbott Laboratories, Abbott Park, IL). Fasting insulin levels were measured by immunoradiometric assay (Medgenix, Biosource Europe, Nivelles, Belgium). Calculated glomerular filtration rate (GFR) was calculated using the Schwartz equation: GFR (mL/min/1.73 m^2) = (0.41 X Height (cm))/serum creatinine (mg/dL) (21).

DNA analyses

DNA methylation testing of the 11p15 region (ICR1 (H19) and ICR2 (KCNQ1OT1)) and mUPD7 was performed using methylation-specific multiplex ligation-mediated probe amplification (MS-MLPA), as previously described (11). To identify CDKN1C mutations (c.836G>T (pArg279Leu)) (22) or IGF2 mutations (IGF2c.191C>A (p.Ser64Ter) (23), genomic DNA extracted from peripheral blood leucocytes was diluted to a concentration of 5 ng/µl and target regions of CDKN1C and IGF2 were amplified by PCR using primers. The IGF2 region containing the mutation was amplified using the forward primer IGF2exR3 5'-CTCGGCATTATGACCTGTGT-'3 and reverse primer IGF2ex3R 5'-AGGCGTGTGAT-

GGGAAAG-'3, and the CKDN1C target region containing the mutation using the primers described previously (22).

Calculations and statistics

SD scores for BL and BW were calculated in order to correct for gestational age and sex (16), SD scores for height, serum IGF-I, and IGF binding protein-3 (IGFBP3) to correct for sex and age (17, 24) and SD scores for weight and BP to correct for height and sex (25). SD scores for BL, BW, height, and weight were calculated using the Growth Analyzer software (http://www.growthanalyser.org). FM% SDS was calculated according to age- and sex-matched Dutch reference values (26). Because LBM is strongly related to height, LBM was expressed as SDS for height and sex (26).

Distribution of variables was determined by Shapiro-Wilk tests and normal Q-Q-plots. Because of a skewed distribution, Si, Sg, AIR and DI were log transformed. Differences between SRS and non-SRS were analyzed using independent-sample *t* tests. To analyze differences in longitudinal changes during GH treatment between SRS and non-SRS, linear mixed modelling for repeated measurements was used with SRS and time as factors. An unstructured repeated covariance type was used, adjusting for missing values. A *P* value of <0.05 was considered statistically significant. Analyses were performed with SPSS version 21.0.

RESULTS

Clinical characteristics

Clinical characteristics of the SRS and non-SRS subjects are listed in Table 1. Fourteen SRS patients had an 11p15 aberration and 6 patients an mUPD7. There were no patients with an IGF2 or CDKN1C mutation. Nine SRS patients tested negative for all known aberrations causing SRS and were assigned to the clinical SRS group. SRS patients had a lower BL and BW SDS than the non-SRS subjects ($P = 0.005$ and $P = 0.04$, respectively). Head circumference SDS was similar in SRS and non-SRS ($P = 0.56$), but the discrepancy between head circumference and BL was larger in SRS ($P = 0.009$).

At GH-start, SRS patients were significantly younger than non-SRS subjects (mean age (SD) 5.4 (2.1) years vs 6.7 (2.0) years, respectively, $P = 0.003$), and had a lower height SDS ($P < 0.001$) and weight for height SDS ($P < 0.001$). SRS patients attained AH at a younger age (15.7 (1.5) years vs 16.4 (1.3) years, respectively; $P = 0.01$). Mean AH SDS (SD) was -1.63 (0.8) in SRS and -1.43 (0.8) in non-SRS ($P = 0.26$). SRS patients had a lower weight for height SDS at AH ($P < 0.001$). At 2 years after GH-stop, age was similar in the 2 groups ($P = 0.72$).

BP and fasting lipid levels

At GH-start, SRS patients had a lower mean SBP SDS than did non-SRS subjects ($P = 0.04$), whereas DBP SDS was similar in both groups (Table 2). At the end of treatment,

Table 1. Clinical characteristics

	SRS (n=29)	non-SRS (n=171)	*P*
Male/Female	13/16	82/89	0.76
11p15/mUPD7/clinical	14/6/9	N/A	N/A
Gestational age, wk	37.6 (2.8)	35.7 (3.9)	**0.003**
Birth length SDS	-4.26 (1.6)	-3.02 (1.5)	**0.005**
Birth weight SDS	-2.76 (1.4)	-2.21 (1.2)	**0.04**
Birth head circumference SDS	-1.73 (1.5)	-2.02 (1.1)	0.56
Target height SDS	-0.09 (0.7)	-0.48 (0.8)	**0.02**
At GH-start			
Age, y	5.4 (2.1)	6.7 (2.0)	**0.003**
Height SDS	-3.60 (0.8)	-2.96 (0.5)	**<0.001**
Weight/height SDS	-2.76 (1.1)	-1.26 (1.2)	**<0.001**
Head circumference SDS	-0.64 (1.1)	-1.23 (0.9)	**0.003**
IGF-I SDS	-0.33 (1.4)	-0.55 (1.2)	0.49
IGFBP3 SDS	-1.51 (1.2)	-1.38 (1.2)	0.69
At AH (GH-stop)			
Age, y	15.7 (1.5)	16.4 (1.3)	**0.01**
Height SDS	-1.63 (0.8)	-1.43 (0.8)	0.26
Weight/height SDS	-0.30 (1.1)	0.48 (1.0)	**<0.001**
Head circumference SDS	-0.47 (1.0)	-0.82 (0.9)	0.24
IGF-I SDS	1.27 (0.9)	1.25 (0.8)	0.95
IGFBP3 SDS	-0.12 (0.5)	-0.32 (0.6)	0.35
At 2yrs after GH-stop			
Age, y	18.3 (1.6)	18.4 (1.3)	0.72

Values expressed as mean (SD). Boldface *P* values are <0.05.
Abbreviation: N/A, not applicable.

SBP and DBP SDS had remained similar in SRS whereas they had decreased in non-SRS ($P < 0.001$). At GH-stop, SRS patients had a similar SBP and DBP SDS as did non-SRS ($P = 0.44$ and $P = 0.07$, respectively). In the 2 years after GH-stop, SBP and DBP SDS remained stable in SRS, whereas DBP and SBP SDS increased in the 6 months after GH-stop in non-SRS and decreased again in the 18 months thereafter. At 2 years after GH-stop, SBP and DBP SDS were similar and within normal ranges in SRS and non-SRS.

At GH-start, fasting serum levels of TC, LDLc, HDLc and Tg were similar in SRS and non-SRS (Table 2). During treatment, serum lipids remained similar and within normal ranges in SRS. In non-SRS, there was a significant decrease of TC and LDLc during treatment, followed by an increase in the 2 years after GH-stop, whereas HDLc and Tg remained similar during and after GH-stop.

Table 2. Metabolic parameters in SRS and non-SRS at GH-start, GH-stop, and 6 months and 2 years after GH-stop.

	GH-start			GH-stop			6 months after GH-stop			2 years after GH-stop		
	SRS	**non-SRS**	***P***	**SRS**	**non-SRS**	***P***	**SRS**	**non-SRS**	***P***	**SRS**	**non-SRS**	***P***
BP												
SBP SDS	0.37 (1.1)	0.83 (1.0)	**0.04**	0.19 (1.0)	0.04 (0.9)[a]	0.44	0.21 (0.8)	0.43 (1.0)[a]	0.41	0.11 (0.7)	-0.00 (0.8)[a]	0.65
DBP SDS	0.45 (1.1)	0.28 (1.0)	0.44	0.21 (0.6)	-0.04 (0.5)[a]	0.07	0.44 (0.6)	0.36 (0.7)[a]	0.68	0.10 (0.5)	0.00 (0.5)[a]	0.54
Lipid levels												
TC, mmol/L	4.1 (0.4)	4.2 (0.7)	0.15	4.0 (0.9)	4.0 (0.8)[a]	0.85	4.1 (0.9)	4.0 (0.9)	0.73	4.4 (1.0)[a]	4.3 (0.9)[a]	0.31
LDLc, mmol/L	2.3 (0.6)	2.4 (0.7)	0.56	2.2 (0.7)	2.3 (0.7)[a]	0.71	2.4 (0.6)	2.3 (0.7)	0.61	2.7 (0.8)[a]	2.5 (0.8)[a]	0.22
HDLc, mmol/L	1.3 (0.4)	1.4 (0.4)	0.09	1.4 (0.3)	1.5 (0.4)	0.31	1.4 (0.3)	1.5 (0.4)	0.38	1.4 (0.4)	1.5 (0.4)	0.64
TG, mmol/L	1.1 (0.5)	1.0 (0.5)	0.45	1.3 (0.9)	1.0 (0.5)	0.053	1.1 (0.5)	0.9 (0.6)	0.10	1.1 (0.4)	0.9 (0.4)	0.14
Glucose and insulin												
Fasting glucose, mmol/L	4.0 (0.7)	4.4 (0.7)	**0.047**	4.9 (0.5)[a]	5.0 (0.5)[a]	0.69	4.9 (0.5)	4.7 (0.5)[a]	0.21	5.0 (0.3)	4.7 (0.4)	0.12
Fasting insulin, mU/L	13.8 (14.6)	15.0 (14.0)	0.81	15.0 (6.6)	15.1 (7.0)	0.95	8.0 (4.2)[a]	10.9 (4.6)[a]	0.07	9.4 (4.4)	9.9 (4.0)	0.78
ATP-III score [b]												
0	9	85		13	95		9	92		9	70	
1	8	29	0.14	8	34	0.19	4	45	0.57	4	29	0.67
2	2	7		3	5		2	9		0	5	
3	0	0		0	2		0	0		0	0	
Renal function												
Creatinine, umol/l	28.5 (13.1)	35.6 (12.7)	**0.02**	62.3 (13.0)[a]	68.3 (12.4)[a]	**0.04**	70.1 (11.2)[a]	70.6 (13.1)[a]	0.89	70.9 (14.0)	72.0 (12.6)[a]	0.60
Calculated GFR, mL/min/1.73m^2	148.9 (59.7)	119.0 (44.1)	**0.02**	95.5 (20.1)[a]	86.3 (22.8)[a]	0.14	86.6 (11.4)[a]	84.7 (35.1)	0.84	89.6 (15.1)[a]	84.8 (15.8)	0.34

Values expressed as mean (SD) unless stated otherwise. Boldface *P* values are <0.05.

[a] $P < 0.05$ with respect to previous time point.

[b] Expressed as number of patients.

Body Composition

Figure 1 shows the longitudinal changes in LBM and FM%, during and after GH-stop in SRS and non-SRS. At GH-start, estimated mean (SE) LBM SDS was -1.63 (0.9) in SRS vs. -0.53 (0.3) in non-SRS (P = 0.12). During treatment, LBM remained similar in both groups, and SRS patients had a lower LBM at GH-stop (P = 0.007). In the 6 months after GH-stop, LBM SDS deteriorated in both groups, but remained stable in the 18 months thereafter. At 2 years after GH-stop, there was still a trend toward a lower LBM in SRS than in non-SRS (P = 0.10).

At GH-start, FM% SDS was similar in both groups (estimated mean (SE) -0.51 (0.3) in SRS vs -0.65 (0.2) in non-SRS; P = 0.72). During GH treatment, FM% SDS increased in both groups. During the 6 months after GH-stop, FM% SDS increased further in SRS, but remained stable in the 18 months thereafter. In non-SRS, FM% SDS increased persistently until 2 years after GH-stop. At 2 years after GH-stop, FM% SDS was similar in SRS and non-SRS (P = 0.97).

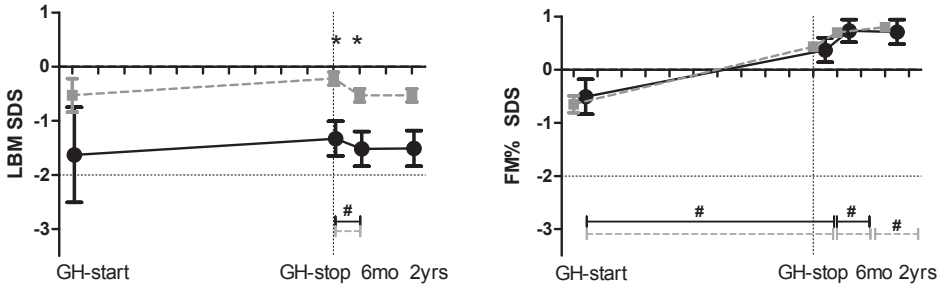

Figure 1. Longitudinal changes in body composition at GH-start, GH-stop and 6 months and 2 years thereafter in SRS (black lines) and non-SRS (gray dotted lines). Data are expressed as estimated marginal means ± standard error of the mean. *P < 0.05 SRS vs non-SRS; # P < 0.05 compared with previous time point (in black for SRS and gray for non-SRS).

Insulin sensitivity and ß-cell function

At GH-start, fasting glucose levels were lower in SRS than in non-SRS (P = 0.047), while fasting insulin levels were similar in the 2 groups (Table 2). During treatment, fasting glucose levels increased, whereas insulin levels remained stable in both groups. After GH-stop, glucose levels remained stable in SRS, whereas they decreased in the 6 months after GH-stop in non-SRS. Insulin levels decreased in the 6 months after GH-stop, and remained stable in the 18 months thereafter in both groups.

Figure 2 shows the longitudinal changes in Si, Sg, AIR, and DI from GH-stop until 2 years thereafter. At GH-stop, Si, Sg, AIR, and DI were similar in SRS and non-SRS. During the 6 months after GH-stop, Si and DI increased significantly in both groups and remained stable in the 18 months thereafter. Sg only increased in non-SRS in the 6 months

after GH-stop, and remained stable in both groups in the 18 months thereafter. At 2 years after GH-stop, SRS patients had a lower AIR than did non-SRS subjects ($P = 0.009$), while Si, Sg and DI were similar in both groups. Until 2 years after GH-stop, none of the SRS and non-SRS patients had developed DM2.

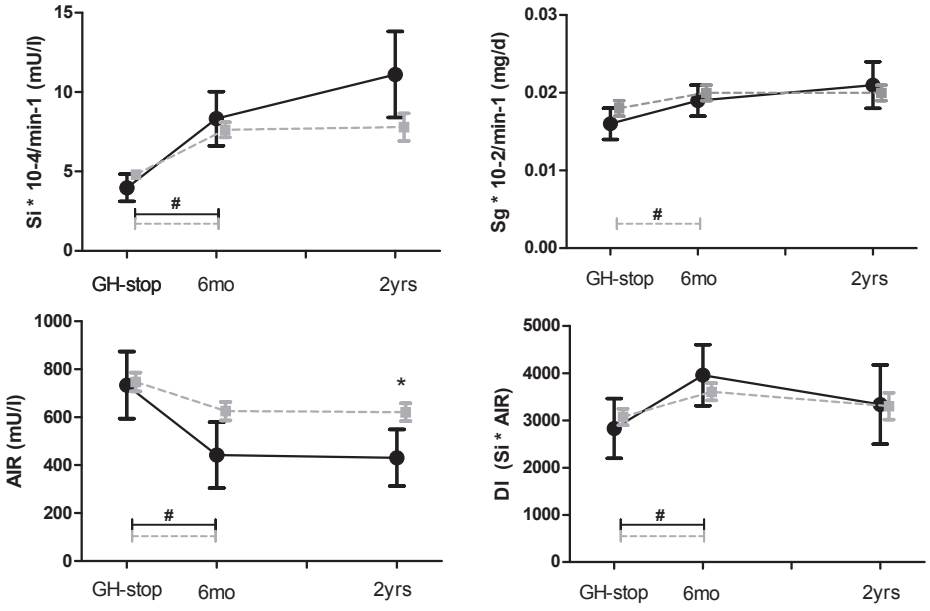

Figure 2. Longitudinal changes in FSIGT-results at GH-stop and 6 months and 2 years thereafter in SRS (black lines) and non-SRS (gray dotted lines).
Data expressed as estimated marginal means ± standard error of the mean. *$P < 0.05$ SRS vs non-SRS; $^{\#}$ $P < 0.05$ compared with previous time point (in black for SRS and gray for non-SRS).

Metabolic syndrome

ATP-III score was similar and overall low in SRS and non-SRS at GH-start, GH-stop and 6 months and 2 years thereafter (Table 2). There were no SRS patients with an ATP-III score ≥3 at any time point. One girl of 15.4 years and one boy of 15.9 years in the non-SRS group had an ATP-III score of 3, and thus met the criteria for metabolic syndrome. Two SRS patients had an ATP-III score of 2 at GH-start due to an adverse lipid profile. In both patients, this improved during treatment, resulting in an ATP-III score of 1 and 0 at GH-stop. Three other SRS patients, all girls, had an ATP-III score of 2 at GH-stop due to adverse lipid levels and high BP. At 2 years after GH-stop, the lipid levels of 1 girl had improved, probably due to dietary changes, resulting in an ATP-III score of 1. Of the other 2 girls, there were no follow-up data available.

Renal function

At GH-start, mean (SD) serum creatinine was significantly lower in SRS than in non-SRS ($P = 0.02$), probably due to the younger age and the trend toward a lower LBM in SRS patients at GH-start (Table 2). Consequently, calculated GFR was higher in SRS than in non-SRS at GH-start. At GH-stop, serum creatinine was lower in SRS ($P = 0.04$), while calculated GFR was similar in the 2 groups. There were no differences in serum creatinine and calculated GFR 6 months and 2 years after GH-stop. Serum creatinine and calculated GFR fell within the normal ranges for age at all time points.

Follow-up of congenital malformations in SRS

Table 3 shows the congenital malformations, anomalies, and developmental problems in the total group of SRS patients and per subgroup based on the underlying epigenetic alteration. Multiple patients had craniofacial and musculoskeletal anomalies such as micrognathia and retrognathia, as well as asymmetry of face or limbs. A congenital heart defect was seen in 1 patient with an 11p15 aberration and in 1 patient with clinical SRS. Genital anomalies were only seen in SRS males, of which 1 patient with an 11p15 aberration had a hypospadias, and 2 patients (1 with an 11p15 aberration and 1 with clinical SRS) had cryptorchidism. Developmental impairments were seen in 33.3% of the mUPD7 patients and in 14.3% of the 11p15 patients, but not in the clinical SRS patients. Behavioral problems were present in half of the mUPD7 patients, vs in <15% of the 11p15 and clinical SRS patients. Besides these known malformations and developmental problems, there were also patients with various other anomalies, such as hearing loss (in 1 patient with mUPD7), strabismus (in 1 patient with an 11p15 aberration) and epilepsy (in 1 patient with clinical SRS).

Safety of GH treatment

At GH-start, SRS and non-SRS subjects had similar mean (SD) serum IGF-I SDS (-0.33 (1.4) in SRS and -0.55 (1.2) in non-SRS; $P = 0.49$), (Table 1). At GH-stop, IGF-I SDS had significantly increased in both groups, but was still similar and within normal ranges in SRS and non-SRS (1.27 (0.9) in SRS and 1.25 (0.8) in non-SRS; $P = 0.95$).

Two female SRS patients with an 11p15 aberration were diagnosed with a slipped capital femoral epiphysis during GH treatment at the ages of 10 and 11 years, after 6 and 7 years, respectively, of GH treatment. Both girls were simultaneously treated with a gonadotropin-releasing hormone analog, for 1 month and 1 year, respectively, when their capital femoral epiphyses slipped. Both girls underwent surgical fixation of the hip joint. After this, GH treatment was continued and they both attained an AH around -1 SDS (-0.95 SDS and -1.04 SDS).

Table 3. Congenital malformations, anomalies and developmental problems in the total group of SRS patients and per subgroup.

	Anomaly	Total SRS (n=29)	11p15 (n=14)	mUPD7 (n=6)	Clinical (n=9)
Craniofacial	Micro/retrognathia	4 (13.8)	2 (14.3)	2 (33.3)	-
	Face asymmetry	3 (10.3)	3 (21.4)	-	-
Musculoskeletal	Limb asymmetry	7 (24.1)	6 (42.9)	1 (16.7)	1 (11.1)
	Scoliosis	2 (6.9)	1 (7.1)	1 (16.7)	-
	Bilateral club feet	1 (3.5)	1 (7.1)	-	-
	Hip dysplasia	1 (3.5)	1 (7.1)	-	-
	Slipped femoral epiphysis	2 (6.9)	2 (14.3)	-	-
	Joint contractures	1 (3.5)	1 (7.1)	-	-
	Inguinal hernia	1 (3.5)	-	-	1 (11.1)
	Exostosis	1 (3.5)	-	-	1 (11.1)
Heart	Atrial septal defect	1 (3.5)	1 (7.1)	-	-
	Ventricular septal defect	1 (3.5)	-	-	1 (11.1)
Genital	Hypospadias	1 (3.5)	1 (7.1)	-	-
	Cryptorchidism	2 (6.9)	1 (7.1)	-	1 (11.1)
Development	Mild impairment	2 (6.9)	-	2 (33.3)	-
	Speech delay	1 (3.5)	1 (7.1)	-	-
	Delayed motor milestones	2 (6.9)	1 (7.1)	1 (16.7)	-
	Behavioral problems	4 (13.8)	1 (7.1)	3 (50.0)	1 (11.1)
Other	Hearing loss	1 (3.3)	-	1 (16.7)	-
	Strabismus	1 (3.5)	1 (7.1)	-	-
	Thrombocytopenia	1 (3.5)	-	1 (16.7)	-
	Lung hypoplasia	1 (3.5)	1 (7.1)	-	-
	Hashimoto thyroiditis	1 (3.5)	-	-	1 (11.1)
	Epilepsy	1 (3.5)	-	-	1 (11.1)

Values are n (%).
-, no anomaly.

DISCUSSION

This study shows long-term data on metabolic health, safety of GH treatment, and phenotype in SRS patients compared to non-SRS short SGA subjects treated with GH. We found that SRS and non-SRS patients have a very similar metabolic health profile at start of treatment, and that, apart from minor variations, the metabolic profile of SRS and non-SRS patients responds similarly to GH treatment. At the age of 18 years, there is no difference in risk for metabolic syndrome between SRS and non-SRS.

This longitudinal study describes extensive metabolic health data in a cohort of GH-treated SRS patients that was followed from childhood into early adulthood. We

used gold standard tests such as dual-energy X-ray absorptiometry to measure body composition, and FSIGT tests with Tolbutamide to assess Si and ß-cell function, making our data unique (27). All major determinants of cardiovascular disease risk were similar in SRS and non-SRS at start of treatment, and SBP and fasting glucose levels were even lower in SRS than in non-SRS. LBM was low in both groups, especially in SRS patients, but this difference did not reach statistical significance. We found some differences between the 2 groups regarding the response to GH treatment. In SRS, BP and lipid levels did not change during treatment, although the lack of difference could be caused by the relatively small number of patients in the SRS group. BP, TC and LDLc significantly decreased in non-SRS, but the actual differences were small, and thus most likely not clinically relevant. Both groups responded similarly to GH with respect to the changes in body composition, but LBM remained lower in SRS patients. During the 2 years after GH-stop, we found several changes related to the loss of pharmacologic effects of GH in SRS patients, such as a decrease in LBM and an increase in FM%, but this was similar as in non-SRS patients. Si and ß-cell function improved after GH-stop. Most importantly, at 2 years after GH-stop, at a mean age of approximately 18 years, there were no significant differences between the groups, and none of the SRS patients had developed DM2 or metabolic syndrome.

To our knowledge, there are only 2 case reports addressing metabolic health in SRS. The first described 3 SRS patients (all with an 11p15 aberration, 2 received GH treatment for several years during childhood) who developed adult diseases such as obesity, hypertension, and DM2 in their early 20s (28). The second described the oldest SRS patient known so far, who has DM2, osteopenia, and hypercholesterolemia at the age of 69 years (29). However, these studies were very small and did not compare the data of SRS patients with those of non-SRS subjects who were similarly treated with GH.

We found multiple malformations, anomalies, and developmental problems in the SRS patients, with differences between the 11p15, mUPD7 and clinical patients. In particular, behavioral problems and mild developmental impairments were very common in the mUPD7 patients. However, most SRS patients went to a normal school and had a similar educational level as their non-SRS peers born SGA. These findings are in contrast with a previous study, that found an impairment of cognitive abilities in half of the SRS patients (30). That study was, however, conducted before genetic testing for SRS was available and patients were compared with healthy controls, instead of short children born SGA. In our cohort of SRS patients, there were 2 patients with a congenital heart defect: one 11p15 patient with an atrial septal defect and 1 clinical SRS patient with a ventricular septal defect. Previous literature showed that the prevalence of congenital heart defects is increased to 5.5% in SRS patients with an 11p15 aberration, compared with 1% in the general population (31). We also found malformations that have not been described in association with SRS, such as hearing loss and epilepsy. Renal anomalies in SRS have

been described (32, 33), but were not present in our study group, and kidney function was similar in SRS and non-SRS. Future studies are needed to decide whether there is an increased risk for these anomalies in SRS. Overall, although we did not perform statistical tests due to the low number of patients in the subgroups, our findings seem in concordance with the study of Wakeling *et al.* (34), who found that 11p15 aberrations are associated with more typical SRS features and congenital defects, and mUPD7 with an increased prevalence of developmental delay.

Considering that SRS is a rare disorder, our study cohort comprises a relatively large group. However, to be able to draw definitive conclusions, larger cohorts are needed. Unfortunately, due to the heterogeneous phenotype and the fact that approximately 40% of the patients remain even nowadays without a genetically confirmed diagnosis, underdiagnosis is still a problem. Therefore, developments in finding new (epi)genetic causes of SRS (4, 22, 23, 35), advanced molecular testing, and guidelines on how to diagnose SRS (6, 18, 36) are valuable to improve awareness and identification of SRS patients.

In conclusion, we showed that there are no metabolic differences between SRS and non-SRS subjects born SGA, before, during and after GH treatment. However, a longer follow-up of SGA born adults, and SRS patients specifically, is needed to see whether this will be maintained over the years when patients reach their 30s and 40s.

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Chapter 4

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

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ABSTRACT

Context: Silver-Russell syndrome (SRS) is characterized by small for gestational age (SGA) birth, postnatal growth failure and several dysmorphic features. Data on puberty and gonadal function in SRS are lacking.

Objective: To evaluate pubertal progression and gonadal function in SRS.

Design: A longitudinal study.

Patients: 31 SRS patients (14 males) and 123 non-SRS patients born SGA (65 males). All received growth hormone and 27.3% received additional gonadotropin-releasing hormone analogue treatment (GnRHa).

Main outcome measures: Pubertal progression, serum levels of inhibin-B, follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, anti-Müllerian hormone (AMH).

Results: Mean age at onset of puberty was 11.5yrs in SRS males vs. 11.6yrs in non-SRS males ($p=0.51$), and 10.5yrs in SRS females vs. 10.7yrs in non-SRS females ($p=0.50$). 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 vs. 13.3yrs in non-SRS ($p=0.62$). One SRS female had primary amenorrhea due to Müllerian agenesis. Four of 14 SRS males had a postpubertal inhibin-B level below the 5th percentile compared to healthy controls, and two 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.

Conclusion: Age at onset of puberty and pubertal progression is similar in SRS and non-SRS subjects born SGA. Sertoli cell dysfunction is more common in SRS males. In SRS females, gonadal function does not seem impaired.

INTRODUCTION

Silver-Russell syndrome (SRS) is characterized by 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 11p15 region (11p15 LOM) (5), and 5-10% by a maternal uniparental disomy of chromosome 7 (mUPD7) (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 AH (7, 8).

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, 9). 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. Another unresolved issue is whether SRS patients have reproductive difficulties. Males with SRS have an increased risk for genital abnormalities such as cryptorchidism and hypospadias (10-12), 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 (13, 14). However, data on gonadal function in SRS are lacking.

In this longitudinal study, we analyzed progression of puberty and gonadal function (i.e. serum levels of inhibin B, follicle stimulating hormone (FSH), luteinizing hormone (LH), anti-Müllerian hormone (AMH) and testosterone) 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 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.

SUBJECTS AND 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 when they had not yet completed puberty, 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 mUPD7, and when negative, also for CDKN1C and IGF2 mutations as previously described (7). Patients with SRS based on 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/or 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 a.m..

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 (17, 18, 24, 25) and with postpubertal results of GH-treated age-matched non-SRS subjects born SGA, collected at similar time points.

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 additionally received gonadotropin-releasing hormone analogue treatment (GnRHa, leuprolide acetate depots, 3.75 mg sc every four weeks) for two years to postpone puberty. A height of less than 140 cm at onset of puberty was used to identify children with an AH expectation of less than -2.5 SDS, based on Dutch reference values (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 velocity dropped below 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 the Endocrine Laboratory, Dept. of Clinical Chemistry, Erasmus UMC. 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 was 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 an 11p15 aberration, seven patients an mUPD7 and no patients had an IGF2 or CDKN1C mutation. In nine SRS patients, no genetic aberration was found, and these subjects were assigned to the clinical SRS group. 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 years in SRS vs. 11.6 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, vs.

Table 1. Clinical characteristics

	SRS (n=31)	Non-SRS (n=123)	p-value
Males (n, %)	14 (45.2)	65 (52.8)	
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 (yrs)	11.5 (0.8)	11.6 (0.8)	0.51
GnRHa (n, %)	4 (28.6)	8 (12.5)	0.13
Females (n, %)	17 (54.8)	58 (47.2)	
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 (yrs)	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

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, vs. in 36.2% of the non-SRS females (p=0.22).

Puberty in SRS vs. non-SRS, treated with GH only

Figure 1 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, vs. 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, vs. 2.9 (0.9) years in non-SRS (p=0.07).

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, vs. 13.3 (1.1) years in non-SRS (p=0.62). There was one SRS female with primary amenorrhea due

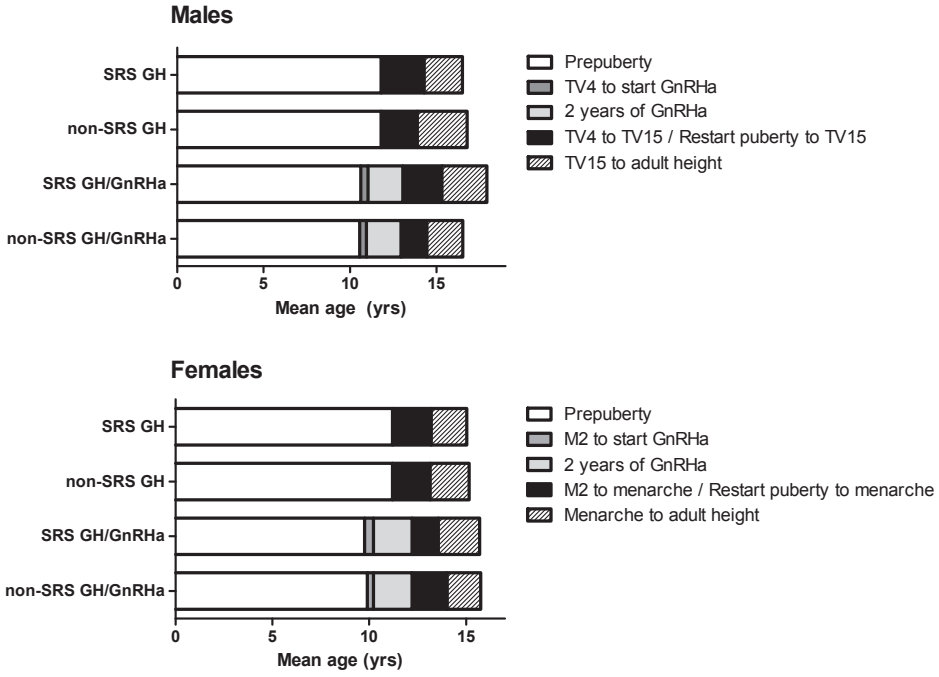

Figure 1. Duration of puberty in SRS and non-SRS.

Abbreviations: GH, growth hormone; GnRHa, gonadotropin-releasing hormone analogue; M2, breast development stage II according to Tanner; TV, testicular volume in ml

to MRKH. The period from menarche to AH lasted 1.8 (1.1) years in SRS vs. 2.0 (0.8) in non-SRS (p=0.61).

Puberty in SRS vs. 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 1). The period from restart of puberty after two years of GnRHa to TV 15 ml lasted 2.3 (0.9) years in SRS vs. 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 vs. 2.1 (0.2) years in non-SRS (p=0.53).

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, vs. 9.9 (1.0) years in non-SRS (p=0.85). There were two 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, vs. 1.5 (0.4) years in non-SRS (p=0.29). Mean age at menarche was 13.2 (1.1) years in SRS, vs. 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, vs. 1.8 (0.6) years in non-SRS (p=0.29).

Pubertal growth in SRS vs. 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 2)). 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.).

Table 2. Pubertal growth in SRS and non-SRS

	Height at onset of 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 includes two years of GnRHa treatment.
Abbreviations: GH, growth hormone; GnRHa, gonadotropin-releasing hormone analogue

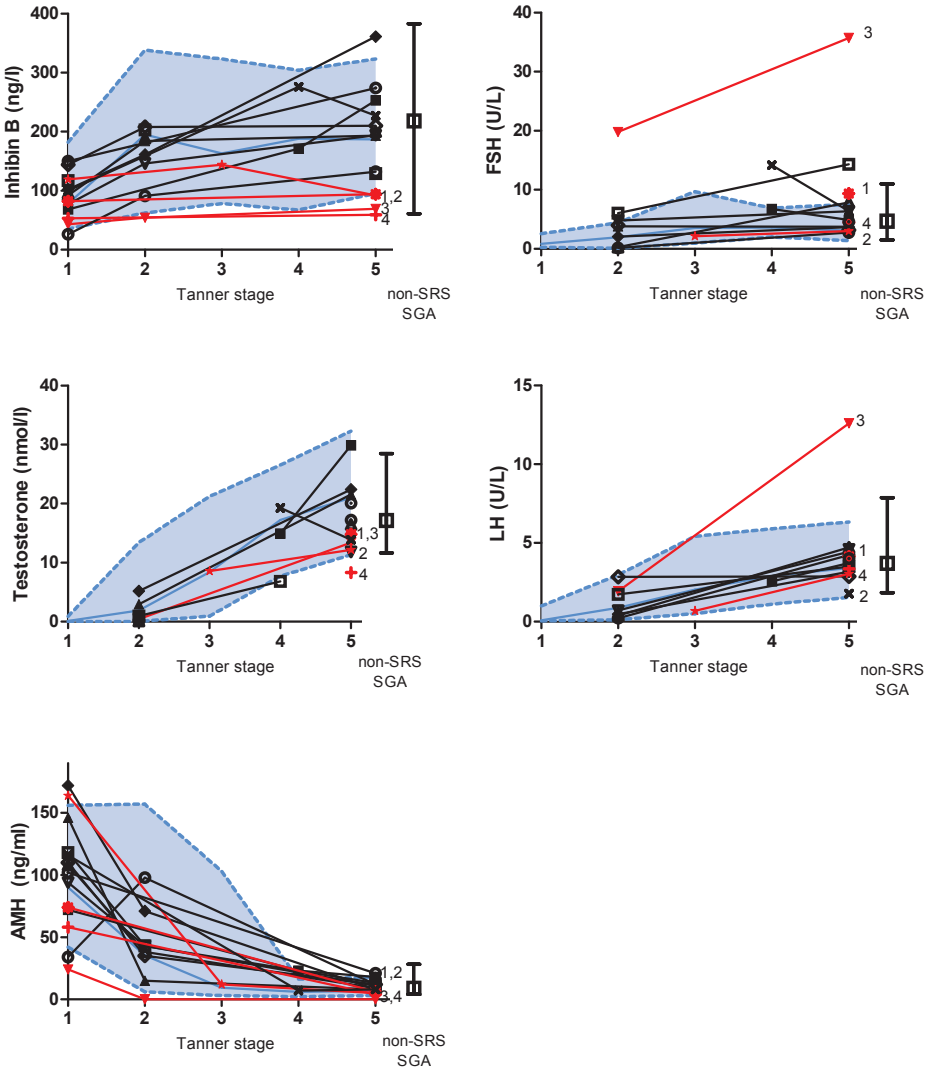

Figure 2. Serum levels of inhibin B, FSH, LH, testosterone and AMH in SRS and non-SRS males
Each black line represents the longitudinal measurements of each SRS male, based on Tanner stage (G1-5) at the moment of the measurement. The shaded area with the blue line indicates the median and 5-95th percentile 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 5-95th percentile of the postpubertal non-SRS subjects born SGA.

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

Longitudinal serum levels of reproductive hormones

Longitudinal serum levels of reproductive hormones in SRS males and females are depicted in Figure 2 and 3, respectively. Postpubertal mean levels were similar in SRS and non-SRS, except for LH levels of females, which were lower in SRS (Table 3). Four of the 14 SRS males (28.6%) had an inhibin B level below the 5th 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 95th 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 5th 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

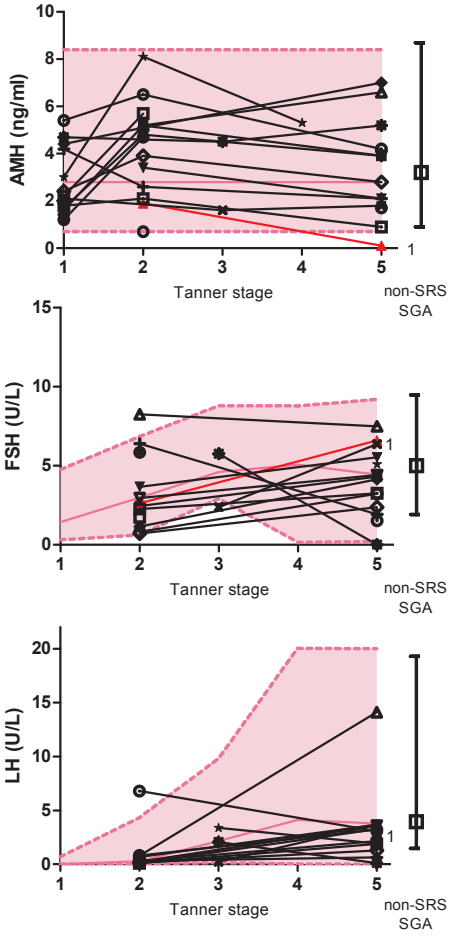

Figure 3. Serum levels of AMH, FSH and LH in SRS and non-SRS females

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 2.5-97.5th percentile per Tanner stage of the healthy population. The red line indicates the patient with AMH levels <2.5th percentile. The vertical bar represents the median and 5-95th percentile of the postpubertal non-SRS subjects born SGA.

Table 3. 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 (yrs)	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.

5th 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 2.5th percentile (patient 1, Figure 3).

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.

The age at onset of puberty was similar in SRS compared to non-SRS subjects born SGA. In the recently published consensus statement, it was mentioned that onset of puberty in SRS is usually within the normal range, although mostly in the younger age of the spectrum (4), which has disadvantages for the eventual AH. In our cohort, 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 (28). 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. To our knowledge, progression of puberty had never been investigated in SRS patients. Our study shows that there seems to be no concern for an abnormal pubertal progression in SRS patients. However, we emphasize that more research is warranted.

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, our study is the first study that assesses gonadal function in SRS patients. More than a quarter of the SRS males had a postpubertal inhibin B level below the 5th percentile for healthy references, and two males also had an FSH level above the 95th 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. Previous literature has shown that both cryptorchidism and orchiopexy are associated with decreased gonadal function (29, 30). In our cohort, four of the 14 SRS males had cryptorchidism, for which two underwent orchiopexy. 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 (10-12). 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 aetiology of Sertoli cell dysfunction in SRS males. 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 case-reports have shown an association between SRS and MRKH (12-14, 31). In three of these case-reports, 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 H19 hypomethylation (12). However, our patient with MRKH had clinical

SRS, similar as the patient in the case-report of Abraham *et al.* (13). More research is thus warranted to establish the aetiology 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 2.5th 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.

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.

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Chapter 5

Leukocyte Telomere Length in Young Adults Born Preterm: Support for Accelerated Biological Ageing

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ABSTRACT

Background: Subjects born preterm have an increased risk for age-associated diseases, such as cardiovascular disease in later life, but the underlying causes are largely unknown. Shorter leukocyte telomere length (LTL), a marker of biological age, is associated with increased risk of cardiovascular disease.

Objectives: To compare LTL between subjects born preterm and at term and to assess if LTL is associated with other putative cardiovascular risk factors at young adult age.

Methods: We measured mean LTL in 470 young adults. LTL was measured using a quantitative PCR assay and expressed as T/S ratio. We analyzed the influence of gestational age on LTL and compared LTL between subjects born preterm (n=186) and at term (n=284). Additionally, we analyzed the correlation between LTL and potential risk factors of cardiovascular disease.

Results: Gestational age was positively associated with LTL (r = 0.11, p=0.02). Subjects born preterm had shorter LTL (mean (SD) T/S ratio = 3.12 (0.44)) than subjects born at term (mean (SD) T/S ratio = 3.25 (0.46)), p=0.003). The difference remained significant after adjustment for gender and size at birth (p=0.001). There was no association of LTL with any one of the putative risk factors analyzed.

Conclusions: Young adults born preterm have shorter LTL than young adults born at term. Although we found no correlation between LTL and risk for CVD at this young adult age, this biological ageing indicator might contribute to CVD and other adult onset diseases at a later age in those born preterm.

INTRODUCTION

Nowadays, 5-13% of all newborns in developed countries are born preterm (i.e. gestational age <37 weeks) (1). Next to respiratory morbidity and neurodevelopmental impairment, the ex-preterm infant phenotype is also characterized by adverse metabolic health in later life (2): Already at a young adult age, ex-preterms have a higher risk for age-associated diseases, such as cardiovascular disease (CVD) (3-7) and have increased cardiovascular mortality (8). The fetal origin hypothesis states that this increased risk is programmed during fetal life and that adverse events during pregnancy lead to reprogramming, causing diseases in later adulthood (9). This way, subjects born preterm, who have a low birth weight and more stress during early life, could be programmed to a different health outcome in later life (10), but the exact underlying mechanism is unknown. Because risk for CVD is age-associated, we hypothesized that accelerated ageing might be one of the mechanisms linking preterm birth and higher risk for CVD.

Telomeres are noncoding repetitive DNA sequences at the end of each chromosome. Their primary function is to maintain genomic stability (11,12). Due to the inability of DNA polymerase to fully replicate the end of the chromosome, telomeres shorten with each cell division. When telomeres are reduced to a critical length, the cell enters a state of arrest (i.e. cell senescence) (13,14). In population studies, telomere length declines with increasing age, which makes leukocyte telomere length (LTL) a usable index for biological ageing (13). Also, several studies found correlations between shorter LTL and age-associated diseases (15-18).

Studies reporting on the relation between telomere length and preterm birth are scarce and contradictive. A small study (n=26) measured LTL in umbilical cords of neonates and found no difference between LTL in preterm and full-term controls (19). Henckel *et al.* (20) found shorter telomere length in ex-preterms with bronchopulmonary dysplasia at the age of 10 years, suggesting a faster telomere attrition rate in these children. Two studies measuring telomere length at an adult age found no differences between ex-preterms and controls (21,22). Because of the large methodologic differences between these studies (i.e. salivary vs. leukocyte telomere length; neonates vs. children vs. adults, TRF vs. PCR-based measurements) many gaps remain in the current literature on the relation between preterm birth and telomere length. This was also emphasized by a recent review on telomere length and preterm birth (2).

We hypothesized that accelerated biological ageing may at least partly explain the increased risk of CVD in subjects born preterm and used LTL as an ageing biomarker to test this hypothesis. We therefore investigated the correlation between gestational age and LTL and analyzed differences in LTL between subjects born preterm and at term. Additionally, we assessed if, at the age of 21 years, putative risk factors for later CVD (i.e.

body composition, blood pressure, lipid levels, insulin sensitivity and the inflammatory biomarker high sensitivity C-reactive protein) correlate with shorter LTL.

MATERIALS AND METHODS

Subjects

The study population consisted of 470 healthy individuals, aged 18-24 years (23,24). Subjects born preterm (gestational age <37 weeks, n=186) had been admitted to the neonatal intensive care unit of the Erasmus University Medical Centre shortly after birth. Subjects born at term were randomly selected from hospitals and schools in The Netherlands. All participants fulfilled the same inclusion criteria: 1) age 18-24 yr; 2) born singleton; 3) Caucasian; 4) uncomplicated neonatal period without signs of severe asphyxia (defined as an Apgar score <3 after 5 min), without sepsis or long-term complications of respiratory ventilation and/or oxygen supply of 2 weeks during the neonatal period. Subjects were excluded if they had been suffering from any serious complication or condition (including necrotizing enterocolitis, intraventricular hemorrhage with a degree of three or more, spastic hemiplegia, or quadriplegia), from any disease or if they had an endocrine or metabolic disorder, chromosomal defect, syndrome, or serious dysmorphic symptoms suggestive for a yet unknown syndrome.

The Medical Ethics Committee of Erasmus Medical Centre approved the study. Written informed consent was obtained from all participants.

Measurements

Participants were invited to visit the Erasmus University Medical Centre. Prior to the visit, participants fasted for at least 12 hours and abstained from smoking and alcohol for at least 16 hours. Birth data regarding gestational age and birth size were obtained from hospital records, primary health care records and general practitioner records. Information regarding socioeconomic status (SES) and smoking of the participants was obtained using questionnaires. Education level of the participants was used as socioeconomic indicator to determine SES (25). Height was measured to the nearest 0.1 cm (Harpenden stadiometer), weight to the nearest 0.1 kg (Servo Balance KA-20-150S). Lean body mass and fat mass were measured on one Dual-energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, England). Systolic and diastolic blood pressure (SBP and DBP) were measured after 10 minutes at rest, in the sitting position, using the non-dominant arm with an automatic device (Accutorr Plus, Datascope Corp, Montvale, New Jersey) (26) every five minutes for one hour and the mean value was taken to reflect the resting blood pressure. To measure insulin sensitivity (Si), which plays an important role in the pathogenesis of Diabetes Mellitus type 2 (DM2), a frequent sampled intravenous glucose tolerance (FSIGT) test with Tolbutamide was performed

(27). Si quantifies the capacity of insulin to promote glucose disposal and was calculated using Bergman's minimal model (MINMOD 6.01, copyright R.N. Bergman).

Laboratory Methods

After centrifugation, all blood samples were kept frozen until assayed (-80°C). For measurement of hsCRP, an important predictor of future atherosclerotic events, an in-house-high-sensitivity ELISA with polyclonal rat CRP antibodies for catching and tagging (DAKO, Denmark) was used. Total cholesterol level was measured using the CHOD-PAP and the GPO-PAP reagent kit (Roche Diagnostics, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol level was measured using a homogeneous enzymatic colorimetric assay (Roche Diagnostics). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula: LDL cholesterol level in mmol/L = total cholesterol – HDL cholesterol level – 0.45 x level of triglycerides.

LTL assessment

Genomic DNA was isolated from peripheral leukocytes using standard procedures. All LTL measurements were made in the same laboratory at the University of Leicester, without knowledge of birth status. Mean LTL was measured by the quantitative PCR-based technique as previously described (28,29). Telomere sequence copy number (T) was compared with a single copy gene number in the genome 36B4 (S) and telomere length expressed as a T/S ratio. All T and S values were calculated relative to a calibrator DNA (genomic DNA from the K562 cell line) that was included on every plate. This allows correction for inter-run variation. For quality control, all samples were checked for concordance between duplicate values. Samples showing a difference of greater than 0.2 cycles in the take-off value or amplifying outside of the linear range of the assay were excluded and re-run. Reproducibility of the assay was tested by re-running samples on separate days. The mean inter-run CV for the T/S ratio was 3.13%.

Statistical analysis

Standard deviation (SD)-scores for birth length and birth weight were calculated in order to correct for gestational age and gender (30). SD-scores for adult height and weight were calculated to correct for gender and age (31). SD-scores for blood pressure, fat mass percentage and lean body mass were calculated to correct for gender and height. SD-scores were calculated using growth analyzer software (http://www.growthanalyser.org).

Baseline characteristics of normally distributed data are presented as mean (SD) and of non-normally distributed data as median (interquartile range (IQR)). ANOVA (continuous data) and Chi square tests (categorical data) were used to determine differences between participants born either preterm or at term. The association between gestational age and LTL was determined using multiple linear regression analysis. After assessing

the linear correlation (Model A), adjustments were made for age, gender, birth length SDS, birth weight SDS and adult height SDS (Model B). The interaction term birth length SDS * adult height SDS was added to the analysis because the study group had been selected on birth length and adult height, in order to ensure that the effect of these variables was modeled correctly. Additionally, we adjusted for smoking and SES (Model C). Difference in LTL between subjects born preterm and at term was analyzed with an independent samples t-test. In an ANCOVA model, we additionally corrected for gender, birth length SDS and birth weight SDS. The association between LTL and risk factors of CVD was assessed with linear regression, with LTL as dependent variable and the differ-

Table 1. Clinical characteristics and risk factors for CVD of the total study population and the subjects born preterm and at term

	Total group (n=470)	Preterm (n=186)	At Term (n=284)	p-value
Male/Female	204/266	90/96	114/170	0.08
Age (yrs)[1]	20.9 (1.7)	20.9 (1.7)	20.9 (1.7)	0.61
Gestational age (wks)[1]	36.7 (3.9)	32.5 (2.4)	39.4 (1.5)	<0.001
Birth weight (SDS)[1]	-0.97 (1.6)	-0.57 (1.9)	-1.22 (1.3)	<0.001
Birth length (SDS)[1]	-1.44 (1.6)	-1.36 (1.9)	-1.49 (1.4)	0.41
Height SDS[1]	-0.85 (1.3)	-0.39 (1.0)	-1.13 (1.3)	<0.001
Weight SDS[1]	-0.48 (1.3)	-0.23 (1.2)	-0.66 (1.4)	0.001
BMI SDS[1]	0.00 (1.2)	0.01 (1.2)	-0.00 (1.1)	0.94
Fat mass % SDS[1]	0.00 (1.0)	0.30 (1.7)	-0.02 (0.9)	0.19
Lean body mass SDS[1]	-0.48 (1.0)	-0.42 (1.1)	-0.49 (1.0)	0.76
SBP SDS[1]	-0.08 (0.7)	-0.03 (0.7)	-0.12 (0.8)	0.24
DBP SDS[1]	0.21 (0.5)	0.12 (0.5)	0.29 (0.5)	0.002
TC (mmol/L)[1]	4.4 (0.9)	4.3 (0.8)	4.5 (1.0)	0.005
HDLc (mmol/L)[1]	1.4 (0.4)	1.4 (0.3)	1.4 (0.4)	0.49
LDLc (mmol/L)[1]	2.6 (0.8)	2.5 (0.7)	2.7 (0.9)	0.12
Tg (mmol/L)[1]	1.0 (0.5)	1.0 (0.5)	1.0 (0.5)	0.47
hsCRP (mg/l)[2]	1.12 (3.1)	1.24 (3.7)	0.75 (1.6)	0.78
Si * 10^{-4}/min (μU/ml)[2]	6.32 (6.2)	6.41 (6.4)	5.59 (6.7)	0.13
Smoking (%)	26.8	26.7	27.9	0.53
SES (%) 1	12.4	14.1	11.5	
2	26.6	30.2	24.5	0.70
3	60.9	55.7	64.0	

[1]Values are given as mean (sd).

[2]Values are given as median (IQR).

p-values below 0.05 are shown in bold type. Preterm = gestational age <37 wks, at term = gestational age >37 wks. BMI = Body mass index; DBP = diastolic blood pressure; HDLc = high-density lipoprotein cholesterol; hsCRP = C-reactive protein; LDLc =low-density lipoprotein cholesterol; SBP = systolic blood pressure; SES = Socioeconomic status; Si = Insulin sensitivity; TC = Total cholesterol; Tg = triglycerides.

ent risk factors as independent variables. Finally, we assessed if there were differences in these risk factors between subjects in the bottom quartile for LTL and subjects in the upper quartile for LTL, using independent samples t-tests. Results were considered statistically significant if the p-value was <0.05. Statistical package SPSS version 21.0 (SPSS, Inc., Chicago, IL) was used for all analyses.

RESULTS

Characteristics of the study population are shown in Table 1. The total population consisted of 470 subjects with a mean (SD) T/S of 3.20 (0.46). Gestational age varied between 27 and 43 weeks, with 186 subjects born preterm (gestational age <37 weeks) and 284 subjects born at term (gestational age >37 weeks). Birth weight SDS, adult height SDS and weight SDS were significantly higher in subjects born preterm. Diastolic blood pressure SDS and total cholesterol were significantly lower in subjects born preterm.

Variables at birth and at young adult age influencing telomere length

We evaluated the relative contribution of several variables at birth and at young adult age to LTL in a multiple regression analysis (Table 2). There was a significant positive correlation between gestational age and LTL (Model A, $R^2 = 0.01$, p=0.02). Adding age, gender, height SDS, birth weight SDS, birth length and the interaction term birth length SDS * adult height SDS to the model (Model B, $R^2 = 0.04$), improved the model and the

Table 2. Multiple regression analysis for variables influencing telomere length in the total study population

	Model A		Model B		Model C	
Variables	**ß**	**p**	**ß**	**p**	**ß**	**p**
GA (wks)	0.01	**0.02**	0.02	**0.03**	0.02	**0.04**
Age (yrs)			0.02	0.30	0.01	0.46
Female gender			0.16	**0.002**	0.16	**0.003**
Adult height SDS			-0.02	0.48	-0.03	0.27
Birth weight SDS			0.01	0.64	0.00	0.95
Birth length SDS			0.01	0.81	0.02	0.50
BL*AH (SDS)			0.00	0.86	0.01	0.56
Smoking					-0.07	0.26
SES					-0.01	0.83
Overall p-value	0.02		0.006		0.008	
R^2 adjusted	0.01		0.04		0.05	

p-values below 0.05 are shown in bold type. GA = Gestational age; BL*AH = Interaction term birth length * adult height. SES = Socioeconomic status (Low and middle socioeconomic status are used as reference for SES analyses).

correlation between gestational age and LTL remained significant (p=0.03). Also, a positive association was found between female gender and LTL (p=0.002). Gestational age and gender both remained significant after adding smoking and socioeconomic status (Model C, R^2 = 0.05) to the analysis (p=0.04 and p=0.003, respectively).

Telomere length in subjects born preterm versus at term

Figure 1 shows the difference in LTL between subjects born preterm and at term. Unadjusted for possible confounders, subjects born preterm had significantly shorter LTL than subjects born at term (mean (SD) T/S of 3.12 (0.44) and 3.25 (0.46), respectively, p=0.003). This difference remained significant after correction for gender, birth length SDS and birth weight SDS (Table 3, p=0.001).

Relationship between telomere length and CVD and DM2 risk factors

We assessed the relation between LTL and several putative CVD risk factors, including insulin sensitivity (Si), at 21 years of age (Table 3). There were no significant associations between LTL and these variables. Moreover, these risk factors did not significantly differ between subjects in the bottom and upper quartile for LTL (S1 Table).

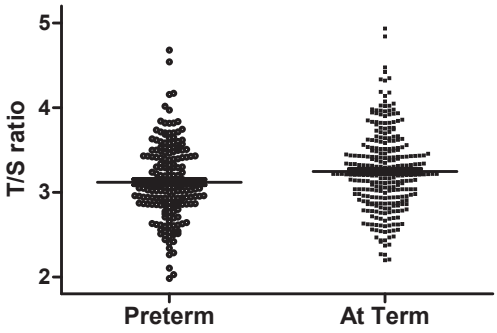

Figure 1. Distributions of mean telomere lengths in subjects born preterm and at term.
T/S ratio = Telomere to single-gene copy ratio; Preterm = gestational age < 37 wks; The horizontal bars represent the mean values.

DISCUSSION

In this study, we analyzed the association between gestational age and LTL in 470 young adults and compared LTL between subjects born preterm and at term. We found that gestational age is positively associated with LTL and that subjects born preterm have shorter LTL than subjects born at term. This difference remained significant after correction for birth length and birth weight, indicating an independent effect of gestational age on LTL, not confounded by birth size. We also found that females had longer telomeres than males, which is in concordance with earlier studies (29). At this young age, no relation between LTL and other putative risk factors for CVD was found.

Table 3. Relation of telomere length with preterm birth and cardiovascular risk factors

	Telomere length	
	ß	**p**
Preterm	-0.13	**0.003**
Preterm adjusted[1]	-0.15	**0.001**
SBP SDS	-0.01	0.79
DBP SDS	-0.00	0.99
Fat mass % SDS	0.02	0.58
Lean body mass SDS	-0.04	0.13
TC (mmol/l)	0.04	0.09
LDLc (mmol/l)	0.04	0.26
HDLc (mmol/l)	-0.01	0.90
Tg (mmol/l)	0.05	0.31
hsCRP (mg/l)	0.00	0.95
Si * 10^{-4}/min (µU/ml)	0.00	0.54

p-values below 0.05 are shown in bold type. ß coefficients equate to the difference in telomere length per unit change in the variable. DBP = diastolic blood pressure; HDLc =high-density lipoprotein cholesterol; hsCRP = C-reactive protein; LDLc = low-density lipoprotein cholesterol; SBP = systolic blood pressure; Si = Insulin sensitivity; TC = Total cholesterol; Tg = triglycerides. The variable preterm equates to the difference between subjects born preterm and the reference group subjects born at term. [1] = Adjusted for gender, birth weight SDS and birth length SDS.

S1 Table. Difference in cardiovascular risk factors between highest and lowest quartile telomere length

	Lowest quartile LTL	**Highest quartile LTL**	**p-value**
SBP SDS	-0.02 (0.7)	-0.08 (0.8)	0.58
DBP SDS	0.20 (0.5)	0.21 (0.6)	0.93
Fat mass % SDS	0.10 (1.1)	0.19 (1.1)	0.63
Lean body mass SDS	-0.61 (1.0)	-0.82 (0.9)	0.17
TC (mmol/l)	4.38 (0.9)	4.57 (0.9)	0.12
LDLc (mmol/l)	2.55 (0.7)	2.64 (0.7)	0.37
HDLc (mmol/l)	1.38 (0.4)	1.38 (0.4)	0.96
Tg (mmol/l)	0.99 (0.6)	1.06 (0.5)	0.33
hsCRP (mg/l)	5.11 (7.4)	4.43 (7.2)	0.53
Si * 10^{-4}/min (µU/ml)	6.72 (3.8)	7.72 (6.1)	0.28

Values are given as mean (sd). DBP = diastolic blood pressure; HDLc = high-density lipoprotein cholesterol; hsCRP = C-reactive protein; LDLc = low-density lipoprotein cholesterol; SBP = systolic blood pressure; Si = Insulin sensitivity; TC = Total cholesterol; Tg = triglycerides.

Thus far, there is limited literature on the association between preterm birth and LTL and the results are contradictive. Friedrich *et al.* (19) measured telomere length in umbilical cords of neonates and found no relation between preterm birth and telomere

length. Although this study provides valuable insights on telomere length at birth, it lacks data on telomere length at a later age and had a very small sample size (n=26). Henckel *et al.* (20) measured telomere length in children at the age of 10 and found that ex-preterm children with bronchopulmonary dysplasia had shorter telomeres than at term children with asthma, suggesting a faster telomere attrition in preterm infants, already present at the age of 10 years old. However, these results are difficult to compare to ours as children with asthma or bronchopulmonary dysplasia comprise a very different study population. A recent study of Hadchouel *et al.* (21) found a correlation between telomere length and abnormal airflow in adolescents born extremely preterm. However, there was no association found between telomere length and gestational age or perinatal events, suggesting that preterm birth *per se* is not a risk factor for shortening of telomeres. In contrast to our study, telomere length was measured in saliva in that study, which questions the comparability of their results to our findings. Kajantie *et al.* (22) described the relation between several birth factors and adult LTL. In contrast to our study, no correlation was found between preterm birth and LTL. One of the reasons for the different results could be that the percentage of preterms was very low compared to those born at term (5.9%).

Previous studies have highlighted oxidative stress as an important determinant of LTL (32,33) and showed that intrauterine stress causes shorter LTL (34-36). Since pregnancies resulting in preterm birth are often accompanied by increased stress exposure (37) and preterm born infants are frequently exposed to stressful events, we think it is plausible that oxidative stress is one of the explanations for the difference in LTL between those born preterm and at term. Other determinants of LTL are replicative stress and genetic factors (38). Most preterm born infants go through a phase of slow postnatal growth due to feeding problems, followed by a phase of accelerated growth (i.e. catch-up growth) mostly from term age onwards. Since catch-up growth can induce replicative stress, preterms could be exposed to increased replicative stress, causing shorter telomeres. To analyze this, we added both birth length SDS and adult height SDS to the multiple regression analysis, indicating the change in height SDS during childhood. If catch-up growth influences LTL, we would have expected a significant association between the change in height SDS during childhood and LTL. Because we did not find this, we think that replicative stress does not explain the findings in our study. This is in concordance with previous studies (22,39). To our knowledge, there is no reason to believe that parents of preterm infants have shorter LTL than those of term infants and we thus do not consider genetic factors to be the cause for the difference in LTL between preterms and terms. A future study that measures LTL and oxidative stress biomarkers during fetal and early postnatal life and, subsequently at a later age, would be a good way to evaluate if increased perinatal oxidative stress is indeed the mechanism behind shorter LTL in those

born preterm. Ideally, LTL would be measured in parents too, to analyze the influence of genetic factors.

To provide a more meaningful context in terms of kilobases for the observed difference in T/S ratio between those born preterm and those born at term, we used data from a previous study from the same laboratory where a comparison had been made between LTL measured by PCR and in kilobases by Southern blotting (40,41). On this basis, a difference in T/S ratio of 0.13 equates to approximately 180 base pairs. Since age-related decline in LTL has been reported to be between 15 and 35 base pairs per year (15,18,41,42) the difference of 180 base pairs equates to approximately 5 to 12 years. This might suggest that young adults born preterm are 5-12 biological years older than young adults born at term with a comparable calendar age. However, since this conversion is based on data of a previous study, we have to be cautious with drawing definitive conclusions from this calculation. Because longitudinal telomere length measurements were lacking and all participants had the same age, we were unable to calculate the mean telomere attrition rate per year in our cohort. Therefore, we cannot take inter-individual telomere attrition rates into account. Previous studies showed that telomere attrition rates vary at different ages, with the most rapid loss early in life, followed by a plateau between age 3-4 and young adulthood, and gradual attrition later in life (43-45). Since the participants of our study had a comparable calendar age, the conversion from base pairs to years was not influenced by this.

Previously, we have shown that several putative risk factors for CVD are already increased at a young age in subjects born preterm (3,4). We therefore investigated whether there was an association between LTL and these risk factors. However, in these young adults, correlations between LTL and risk factors for CVD were not found. This is in concordance with earlier studies, indicating that the association between LTL and CVD is independent of risk factors for CVD, including markers of inflammation (15,40,41). Although we found no correlation between LTL and risk for CVD at this young adult age, we think that this biological ageing indicator may contribute to CVD and other adult onset diseases at a later age in those born preterm. It would therefore be very interesting to analyze how LTL and CVD progress over time when these young adults reach their 30s and 40s.

In conclusion, our data show that gestational age is positively correlated with LTL and that young adults born preterm have shorter LTL than young adults born at term. This could reflect pre- and postnatal oxidative stress and in turn could partly explain the association between preterm birth and later life risk of CVD. Since the prevalence of preterm birth and survival is rapidly increasing, our results are of clinical relevance for a large and increasing number of subjects worldwide.

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Chapter 6

Effects of Size at Birth, Childhood Growth Patterns and Growth Hormone Treatment on Leukocyte Telomere Length

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ABSTRACT

Background: Small size at birth and rapid growth in early life are associated with increased risk of cardiovascular disease in later life. Short children born small for gestational age (SGA) are treated with growth hormone (GH), inducing catch-up in length. Leukocyte telomere length (LTL) is a marker of biological age and shorter LTL is associated with increased risk of cardiovascular disease.

Objectives: To investigate whether LTL is influenced by birth size, childhood growth and long-term GH treatment.

Methods: We analyzed LTL in 545 young adults with differences in birth size and childhood growth patterns. Previously GH-treated young adults born SGA (SGA-GH) were compared to untreated short SGA (SGA-S), SGA with spontaneous catch-up to a normal body size (SGA-CU), and appropriate for gestational age with a normal body size (AGA-NS). LTL was measured using a quantitative PCR assay.

Results: We found a positive association between birth length and LTL ($p=0.04$), and a trend toward a positive association between birth weight and LTL ($p=0.08$), after adjustments for gender, age, gestational age and adult body size. Weight gain during infancy and childhood and fat mass percentage were not associated with LTL. Female gender and gestational age were positively associated with LTL, and smoking negatively. After adjustments for gender, age and gestational age, SGA-GH had a similar LTL as SGA-S ($p=0.11$), SGA-CU ($p=0.80$), and AGA-NS ($p=0.30$).

Conclusions: Larger size at birth is positively associated with LTL in young adulthood. Growth patterns during infancy and childhood are not associated with LTL. Previously GH-treated young adults born SGA have similar LTL as untreated short SGA, SGA with spontaneous catch-up and AGA born controls, indicating no adverse effects of GH-induced catch-up in height on LTL.

INTRODUCTION

Small size at birth and catch-up in weight for length in early life are associated with an increased risk for cardiovascular disease (CVD) in later life (1-3). The mechanisms underlying these associations are not fully understood, but it appears that early life growth trajectories have programming effects on later health outcomes (4,5). Ten percent of all children born small for gestational age (SGA) show insufficient catch-up growth and remain short (6). These children can nowadays be treated with growth hormone (GH) from the age of four years, resulting in a significant catch-up in length (7). GH treatment has several positive effects on metabolic health, but the long-term effects on later life health are less known (8). Previous studies have suggested that shorter, smaller bodies have advantages in terms of health and longevity (9,10).

Telomeres are noncoding repeating DNA sequences at the end of each chromosome. Their primary function is to maintain genomic stability (11,12). Telomeres shorten with each cell division due to the inability of DNA polymerase to fully replicate the end of the chromosome. When telomeres are reduced to a critical length, the cell enters a state of arrest (13). Since leukocyte telomere length (LTL) declines with increasing age, it can serve as an index for biological ageing. LTL is influenced by oxidative and replicative stress, and shorter LTL is associated with increased risk for CVD (14,15).

In this study, we investigated whether size at birth, growth patterns during infancy and childhood, and GH treatment influence LTL of young adults. We hypothesized that small size at birth and accelerated weight gain during infancy lead to shorter LTL. We also hypothesized that the gradual catch-up in length caused by GH treatment does not lead to increased attrition of telomeres and thus does not influence LTL. To address the fact that those born SGA have an already increased risk for CVD, we compared the data of previously GH-treated young adults born SGA with untreated short young adults born SGA. To study whether GH-induced catch-up growth has a similar effect on LTL as spontaneous catch-up after SGA birth, we also compared the GH-treated group to a group of young adults born SGA with spontaneous catch-up (SGA-CU).

METHODS

Subjects

The total population consisted of 545 participants: 470 healthy participants from the PROGRAM and PREMS study cohorts (2,16), and 75 age-matched participants who had participated in a GH trial (8,17). The 470 healthy participants fulfilled the same inclusion criteria: 1) age 17-24 yr; 2) born singleton; 3) Caucasian; 4) uncomplicated neonatal period without severe asphyxia (defined as an Apgar score below three after five minutes), sepsis, or long-term complications of respiratory ventilation and/or oxygen supply.

Participants were randomly selected from hospitals in the Netherlands, where they had been registered because of their small size at birth (birth length <-2 standard deviation score (SDS)), short stature (adult height <-2 SDS) or being born preterm (gestational age <36 weeks). Young adults born appropriate for gestational age (AGA) were asked to participate via advertisements at schools with different educational levels. For the last analysis, we additionally included the 75 GH-treated subjects. All subjects were born SGA (birth weight and/or birth length <-2 SDS) and received GH treatment during childhood because of their short stature, for ≥7 years. All participants received biosynthetic GH at a dose of 1 mg/m^2/day (0.035 mg/kg/d), sc at bedtime. Every three months, GH dose was adjusted to the calculated body surface area. The data of the GH group were compared to those of three subgroups based on their size at birth and their adult stature: untreated young adults born SGA (birth length <-2 SDS) with persistent short stature (adult height <-2 SDS) (SGA-S, n=48); young adults born SGA (birth length <-2 SDS) with spontaneous catch-up growth resulting in a normal adult height (>-1 SDS) (SGA-CU, n=89); and young adults born appropriate for gestational age with a normal adult height (>-1 SDS) (AGA-NS, n=135). In order to increase the statistical power for subgroup comparison, 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 study was conducted according to the Helsinki Declaration. The Medical Ethics Committee of Erasmus Medical Centre approved the study. Written informed consent was obtained from all participants and/or their parents.

Measurements

Birth data were obtained from hospital records, primary health care records and general practitioner records. Height was measured to the nearest 0.1 cm by a Harpenden stadiometer, weight to the nearest 0.1 kg by a scale (Servo Balance KA-20-150S). All anthropometric measurements were performed by a trained investigator, according to standardized methods. The measurements were performed twice, the mean value was used for analyses. Fat mass, fat mass percentage and lean body mass were measured on one Dual-energy X-ray Absorptiometry (DXA) machine (Lunar Prodigy, GE Healthcare, Chalfont St Giles, England) (18). Quality control was performed daily. Information regarding socioeconomic status (SES) based on education level, and smoking of the participants was obtained using questionnaires (19).

LTL assessment

Genomic DNA was isolated from peripheral leukocytes using standard procedures. All LTL measurements were performed in the laboratory of the University of Leicester, using the quantitative PCR-based technique as previously described (20). Telomere sequence copy number (T) was compared with a single copy gene number in the genome 36B4 (S)

and telomere length expressed as a T/S ratio. All T and S values were calculated relative to a calibrator DNA (genomic DNA from the K562 cell line) that was included on every plate, minimizing the potential for inter-run variation. All samples were checked for concordance between duplicate values. Samples showing a difference of greater than 0.2 cycles in the take-off value or amplifying outside of the linear range of the assay were excluded and re-run. Reproducibility of the assay was tested by re-running samples on separate days. The mean inter-run coefficient of variation for the T/S ratio was 3.33%.

Calculations and statistical analysis

Standard deviation scores (SDS) for birth length and birth weight were calculated in order to correct for gender and gestational age, and SDS for adult height and weight were calculated to correct for gender and age (21), all using the growth analyzer software (http://www.growthanalyser.org). Fat mass percentage SDS was calculated according to age- and sex-matched Dutch reference values (22). Because lean body mass is strongly related to height, lean body mass was expressed as SDS for height and sex (22).

Means and SD were used to describe the distribution of continuous variables. Multiple linear regression analyses were performed to determine whether size at birth (i.e. birth length and birth weight) and childhood growth patterns (i.e. the degree of catch-up in length and weight from birth to adulthood) were significant predictors of LTL. Because of collinearity between birth weight and birth length, these variables were analyzed in separate models. Adjustments were made for age and gender, and additionally for gestational age, body composition, smoking and SES. Because the study group had been selected on birth length and adult height, the interaction term birth length SDS*adult height SDS was added to the analysis, in order to ensure that the effect of these variables was modeled correctly.

Quartiles of weight gain during the first 12 months of life were determined in the total group, except for the GH-treated subjects and for men and women separately. ANCOVA was used to determine differences in LTL between the lowest and highest quartile, corrected for age and gestational age. Lastly, we analyzed whether there were differences in LTL between the SGA-GH subgroup and the SGA-S, SGA-CU and AGA subgroups. In this analysis, we additionally adjusted for age, gender and gestational age. Results were considered statistically significant if the p-value was <0.05. Statistical package SPSS version 21.0 (SPSS, Inc., Chicago, IL) was used for all analyses.

RESULTS

Clinical characteristics of the total study population (n=470), and for men and women separately are shown in Table 1. Mean (SD) age of the total population was 20.9 (1.7) years. Mean (SD) lean body mass SDS was higher in men than in women (-0.12 (1.1)

versus -0.62 (1.3), resp.; p<0.001). The other clinical characteristics were similar in men and women. Mean (SD) LTL was shorter in men than in women (p=0.02).

Table 1. Clinical characteristics

	Total group (n=470)	**Men** (n=204)	**Women** (n=266)	**p-value**
Age (yrs)	20.9 (1.7)	21.0 (1.7)	20.8 (1.7)	0.29
Gestational age (wks)	36.7 (3.9)	36.3 (4.0)	37.0 (3.7)	0.06
Birth weight SDS	-0.97 (1.6)	-0.83 (1.7)	-1.07 (1.5)	0.10
Birth length SDS	-1.43 (1.6)	-1.33 (1.6)	-1.52 (1.6)	0.22
Adult height SDS	-0.85 (1.3)	-0.86 (1.2)	-0.85 (1.3)	0.94
Adult weight SDS	-0.52 (1.4)	-0.57 (1.3)	-0.49 (1.4)	0.55
BMI	22.5 (3.7)	22.3 (3.2)	22.6 (4.0)	0.36
Fat mass % SDS	0.62 (0.9)	0.63 (0.9)	0.62 (0.9)	0.89
Lean body mass SDS	-0.41 (1.2)	-0.12 (1.1)	-0.62 (1.3)	**<0.001**
Smoking (%)	27.5	29.5	25.9	0.39
SES (%) 1	12.4	13.5	11.7	
2	26.6	27.5	26.0	0.78
3	60.9	59.1	62.3	
LTL	3.20 (0.5)	3.14 (0.4)	3.24 (0.5)	**0.02**

Values are given as means (SD). P-values <0.05 are shown in bold. BMI = body mass index; LTL = Leukocyte Telomere Length (in T/S ratio); SES = socioeconomic status

Factors associated with LTL in the total group

We analyzed the effects of size at birth and postnatal growth on LTL in a multiple regression analysis. First, birth length was analyzed (Table 2). As expected, female gender was positively associated with LTL (p=0.004). Age was not a significant confounder of LTL in our analyses, probably because of the fact that most subjects in our study population had approximately the same age. In Model A, birth length SDS did not predict LTL (p=0.36). In Model B, we added gestational age to the model, which proved to be a significant predictor of LTL (β=0.02, p=0.002). In Model C, we added adult height and the interaction term birth length SDS*adult height SDS to the model. In this model, there was a trend toward a positive relation between birth length and LTL (β=0.03, p=0.06). Adult height was not associated with LTL, and since birth length was included in the model, this shows us that gain in height from birth to adulthood does not predict LTL. Then, parameters of body composition were added to the model (Model D), showing a trend toward a positive relation between LBM SDS and LTL (β=0.04, p=0.06). The relation between birth length and LTL was still close to significant in this model (p=0.08). Finally, we included the possible confounders smoking and SES in the model (Model E), show-

Table 2. Multiple regression analysis for variables associated with leukocyte telomere length at 21 years - Analysis including birth length

	Model A		Model B		Model C		Model D		Model E	
Variables	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**
Female gender	0.13	**0.004**	0.12	**0.009**	0.12	**0.006**	0.16	**0.001**	0.15	**0.002**
Age	0.00	0.85	0.00	0.77	0.00	0.80	0.00	0.79	-0.00	0.97
Birth length SDS	0.01	0.53	0.01	0.36	0.03	0.06	0.03	0.08	0.04	**0.04**
Gestational age			0.02	**0.002**	0.02	**0.02**	0.01	**0.048**	0.02	**0.02**
Adult height SDS					-0.02	0.30	-0.02	0.49	-0.03	0.26
Fat mass % SDS							-0.02	0.49	-0.01	0.68
Lean body mass SDS							0.04	0.06	0.03	0.22
SES									0.00	0.99
Smoking									-0.12	**0.03**
Overall p-value	**0.03**		**0.001**		**<0.001**		**<0.001**		**<0.001**	
R^2 adjusted	0.01		0.03		0.04		0.05		0.07	

ß = regression coefficient. A positive value indicates that the dependent variable LTL will increase with that amount for every unit increase of the independent variable. All analyses where adult height was included were additionally adjusted for the interaction term birth length*adult height SDS. P-values <0.05 are shown in bold.
SDS = standard deviation score; SES = Socioeconomic status (Lowest socioeconomic status is used as reference for SES analyses).

ing an inverse association between smoking and LTL (ß -0.12, p=0.03). In this last model, gender, gestational age and birth length SDS were all positive predictors of LTL.

The same models were used to analyze the relation between birth weight and LTL (Table 3). Instead of height SDS, we included weight SDS to the model, to analyze the influence of weight gain from birth to adulthood. Regarding the variables gender, age, gestational age, LBM and smoking, this analysis showed similar results, except that LBM was a significant predictor of LTL. There was a (trend toward a) significant positive relation between birth weight and LTL in all models. Weight SDS was not a significant predictor of LTL. Since birth weight was included in this model, this shows us that weight gain from birth to adulthood does not predict LTL. Due to the high collinearity between weight and FM%, weight was excluded in Model D. In the final model, there was still a trend toward a positive relation between birth weight SDS and LTL (ß=0.02, p=0.08).

Effects of weight gain and fat mass accumulation during infancy on LTL

We analyzed the effect of weight gain and fat mass accumulation during the first 12 months of life on LTL. We found no significant correlation between weight gain in the first 12 months of life and LTL (*r*= -0.08, p=0.15). Subsequently, we stratified the population into quartiles based on weight gain in kg and Δweight (kg)/Δlength (cm) during the first 12 months of life. Figure 1 shows LTL of the total group and men and women separately, adjusted for age and gestational age.

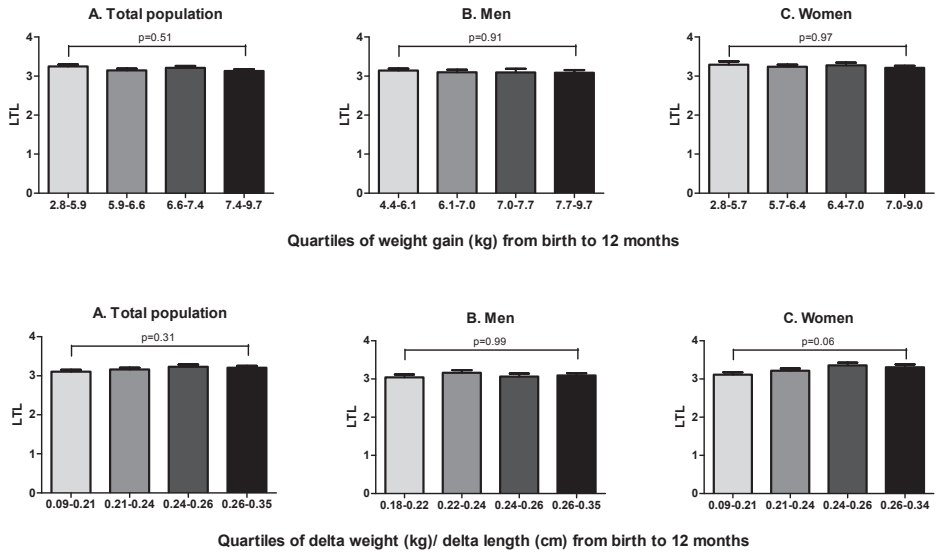

Figure 1. Weight gain and fat mass accumulation during infancy and LTL. Values are given as estimated means ± SEM, adjusted for age and gestational age.

Table 3. Multiple regression analysis for variables associated with leukocyte telomere length at 21 years - Analysis including birth weight

	Model A		Model B		Model C		Model D		Model E	
Variables	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**
Female gender	0.11	**0.01**	0.12	**0.03**	0.10	**0.03**	0.15	**0.001**	0.14	**0.004**
Age	0.00	0.96	0.00	0.92	0.00	0.96	0.00	0.85	-0.01	0.89
Birth weight SDS	0.02	0.11	0.01	**0.03**	0.03	0.07	0.03	0.06	0.02	0.08
Gestational age			0.02	**0.004**	0.02	**0.002**	0.01	**0.02**	0.02	**0.003**
Weight SDS					-0.02	0.70				
Fat mass % SDS							-0.01	0.71	0.06	0.86
Lean body mass SDS							0.04	**0.02**	0.07	**0.048**
SES									-0.02	0.76
Smoking									-0.12	**0.03**
Overall p-value	**0.04**		**0.002**		**<0.001**		**<0.001**		**<0.001**	
R^2 adjusted	0.01		0.03		0.04		0.04		0.05	

ß = regression coefficient. A positive value indicates that the dependent variable LTL will increase with that amount for every unit increase of the independent variable. P-values <0.05 are shown in bold.

SDS = standard deviation score; SES = Socioeconomic status (Lowest socioeconomic status is used as reference for SES analyses).

The weight gain analyses showed no difference in LTL between the lowest and highest quartile of weight gain during the first 12 months of life in the total population (p=0.51), in men (p=0.91) and women (p=0.97). The analyses for Δweight/Δlength during the first 12 months of life neither showed significant differences in LTL between the lowest and highest quartile in the total population (p=0.31) and in men (p=0.99), and there was a trend toward longer LTL in women with the highest Δweight/Δlength (p=0.06), indicating no negative effect of fat mass accumulation during the first 12 months of life on LTL in early adulthood.

Effects of growth hormone treatment on LTL

The effect of long-term GH treatment on LTL was analyzed by comparing LTL between subjects born SGA and treated with GH (SGA-GH) versus age-matched untreated short subjects born SGA (SGA-S), subjects born SGA with spontaneous catch-up during childhood (SGA-CU), and AGA born controls with a normal adult stature (AGA-NS). Clinical

Table 4. Clinical characteristics of the subgroups

	SGA-GH (n=75)	**SGA-S** (n=48)	**SGA-CU** (n=89)	**AGA-NS** (n=135)
Male/female	42/33[1,2]	16/32	35/54	64/71
Age (yrs)	20.2 (2.4)[2]	20.8 (1.8)	20.9 (1.6)	20.8 (1.7)
Gestational age (wks)	36.2 (4.0)[1]	38.2 (3.1)	36.3 (3.2)	36.3 (4.0)
Birth weight SDS	-2.44 (1.2)[3]	-2.07 (0.9)	-2.31 (0.8)	0.29 (1.3)
Birth length SDS	-3.42 (1.5)[2,3]	-3.05 (0.9)	-2.93 (0.8)	0.22 (0.8)
Adult height SDS	-1.42 (0.8)[1,2,3]	-2.55 (0.5)	-0.17 (0.6)	0.18 (0.8)
Adult weight SDS	-1.01 (1.3)[2,3]	-1.44 (1.5)	0.08 (1.2)	0.09 (1.0)
BMI	20.5 (2.7)[1,2,3]	23.3 (4.4)	22.8 (4.3)	22.3 (3.1)
Fat mass % SDS	0.88 (0.9)[1]	1.60 (0.8)	1.19 (0.8)	0.97 (0.8)
Lean body mass SDS	-0.68 (1.3)[1]	0.09 (1.6)	-0.72 (1.1)	-0.63 (1.0)
Smoking (%)	28.6	25.0	29.4	24.1
SES (%) 1	9.1	20.9	16.4	3.4
2	63.6[3]	30.2	31.5	17.8
3	27.3	48.8	52.1	78.8
LTL	3.12 (0.5)	3.30 (0.4)	3.07 (0.4)	3.20 (0.5)

Values are given as means (SD).
[1]= p<0.05 compared to SGA-S.
[2]= p<0.05 compared to SGA-CU.
[3]= p<0.05 compared to AGA-NS.
BMI = body mass index; LTL = Leukocyte Telomere Length (in T/S ratio); SES = socioeconomic status; SGA-GH = birth length <-2 SDS, treated with growth hormone; SGA-S = birth length <-2 SDS, adult height <-2 SDS; SGA-CU = birth length <-2 SDS, with spontaneous catch-up to adult height >-1 SDS; AGA-NS = birth length >-1 SDS, adult height >-1 SDS

characteristics of the subgroups are shown in Table 4. There were significant differences in gender, age, gestational age, birth length SDS, birth weight SDS, adult height SDS, weight SDS, BMI, body composition and distribution of SES between the groups.

Figure 2 shows estimated mean (SE) LTL of the subgroups, adjusted for gender, age and gestational age. The SGA-GH subgroup had a similar LTL as the SGA-S group (p=0.11), the SGA-CU group (p=0.80) and the AGA-NS group (p=0.30).

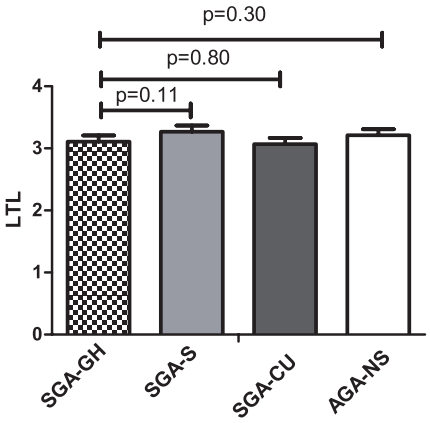

Figure 2. Comparison of LTL in the subgroups. Values are given as estimated means ± SEM, adjusted for gender, age and gestational age. SGA-GH = birth length <-2 SDS, treated with growth hormone; SGA-S = birth length <-2 SDS, adult height <-2 SDS; SGA-CU = birth length <-2 SDS, with spontaneous catch-up to adult height >-1 SDS; AGA-NS = birth length >-1 SDS, adult height >-1 SDS

DISCUSSION

We found a positive association between birth length and LTL, and a trend toward a positive association between birth weight and LTL. No associations were found between gain in weight for length during infancy and childhood and adult body size, and no influence of GH-induced catch-up growth on LTL.

We performed a multiple regression analysis in the total group to analyze the effects of size at birth, adult body size and weight gain during childhood on LTL. Birth length was positively associated with LTL, and there was a trend toward a positive association between birth weight and LTL. These associations were adjusted for possible confounders, such as gender and gestational age, indicating an independent effect of size at birth on LTL. Since previous reports have shown that small size at birth is associated with risk for CVD in later life, it could be that LTL is one of the links between birth size and later life CVD-risk. Our results are in concordance with a recent study of de Zegher *et al.*, showing that telomere lengths are shorter in SGA newborns than in AGA newborns (23). On the other hand, a study of Kajantie *et al.* found no correlation between size at birth and LTL (24). Future studies should aim at exploring the possible underlying mechanisms of the association between size at birth and LTL, such as increased oxidative stress in those born after intra-uterine growth restriction.

We found a positive association between gestational age and LTL. In a previous report we have shown that those born preterm have shorter LTL than those born at term (25), which could be due to increased oxidative stress in those born preterm. Our findings that female gender is positively associated with LTL, and smoking negatively, also correspond to previous studies (26,27), although it is striking that smoking already influences LTL at such a young age.

In our study, extensive data on adult body composition were available: next to weight SDS and BMI, we also measured fat mass and lean body mass using DXA. We found no relation between fat mass percentage and LTL. This result is in contrast with previous studies, showing that obesity is associated with shorter LTL in both children and adults, probably due to the fact that obesity causes increased oxidative stress, which exacerbates telomere attrition (28,29). The main difference with our study is that these studies compared groups with a high BMI to groups with a normal BMI, while we modelled the effect of the continuous variables weight SDS, fat mass and lean body mass in a multiple regression analysis. It could be that the relation between obesity and shorter LTL is subtle, and therefore not present in our group of healthy young adults, with a low percentage of obese participants.

Gain in weight, gain in height and fat mass accumulation from birth to adulthood were not associated with LTL in our study. We previously showed that growth patterns during infancy have programming effects on health in later life (2,30). We, therefore, additionally analyzed whether catch-up in weight and fat mass accumulation during the first year of life were associated with LTL. This analysis showed no difference in LTL between those in the lowest and highest quartile of weight gain and fat mass accumulation during the first year of life. Our results are in contrast with a recent study, that also measured LTL by quantitative PCR, and found an inverse association between weight gain in the first 12 months and LTL at the age of 70 (31). This association was only found in women. Based on these results, the investigators suggested that rapid growth during the perinatal period accelerates cellular ageing in late adulthood. The main differences with our study are that the effects of Δweight/Δlength as a proxy of fat mass accumulation were not tested in that study and the fact that the participants were much older. It would, therefore, be interesting to analyze whether the association between weight gain during infancy and LTL becomes significant at a later age, when age-associated diseases also become more apparent.

To our knowledge, we are the first to evaluate whether GH treatment has an effect on LTL. Young adults born SGA who were treated with GH during childhood had similar LTL as age-matched untreated short subjects born SGA, subjects born SGA with spontaneous catch-up and controls born AGA with a normal stature. Thus, GH-induced catch-up in length does not lead to shorter LTL in young adults born SGA. It seems that a gradual catch-up in length, after the age of four years (when GH therapy is usually started), does

not lead to increased replicative stress. Since data on age-associated diseases, such as CVD, long after cessation of GH treatment are scarce, this result is reassuring (32). Our results support previous data, showing that there are no adverse effects of long-term GH treatment on CVD-risk and that adults who were treated with GH during childhood do not have increased mortality rates (33-35).

Although the multiple regression analyses resulted in significant associations between multiple variables and LTL, it should be noted that the R^2 was small in all analyses, indicating that there are other determinants of LTL, that were not included in our analyses.

Telomere lengths measured in different tissues of the same patient are highly correlated (36,37). Therefore, LTL not only mirrors the ageing process in circulating immune cells, but in other tissues as well. This way, LTL might reflect the vulnerability of our cells to exogenous stress factors in general. The mechanisms underlying telomere shortening are complex. It is known that, next to genetic factors, oxidative and replicative stress are main determinants of LTL. However, the generalizability of shorter telomere length due to replicative stress to other cells is not well studied. For future studies, it would be interesting to measure telomere length in other tissues as well, to see whether low birth weight and subsequent catch-up growth influence telomere length in other tissues that might be more prone to replicative stress (for example bone and muscle tissue).

One of the main strengths of the present study is the large group of young adults that was included, with a great variation in size at birth and childhood growth patterns. Because we oversampled subjects with extreme variants of normal growth, such as subjects born SGA with and without catch-up growth, we created greater contrast in the study population, which contributed to more statistical power.

In conclusion, we found that size at birth, gestational age and female gender are positively associated with LTL and smoking negatively, while adult fat mass and gain in weight and height from birth to adulthood and during infancy were not associated with LTL. Young SGA adults who received GH treatment during childhood have similar LTL as age-matched untreated short SGA, SGA with spontaneous catch-up and controls born AGA, indicating no adverse effects of GH treatment on LTL, which is reassuring.

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Chapter 7

Bone Mineral Density after Cessation of GH Treatment in Young Adults Born SGA: A 5-Year Longitudinal Study

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ABSTRACT

Context: Short children born small for gestational age (SGA) have a bone mineral density (BMD) below average. Growth hormone (GH) treatment improves height and BMD in short SGA children. Longitudinal data on BMD in adults born SGA after cessation of GH treatment are lacking.

Objectives: To determine BMD in young adults born SGA during 5yrs after GH cessation.

Methods: In 173 GH-treated adults born SGA (SGA-GH), BMD of total body (BMD_{TB}) and bone mineral apparent density of lumbar spine ($BMAD_{LS}$) were measured longitudinally at adult height (GH-stop), and 6 months, 2yrs and 5yrs thereafter. At 5yrs after GH-stop (age 21yrs), data were compared with 45 untreated short SGA adults (SGA-S), 59 SGA adults with spontaneous catch-up (SGA-CU), and 81 adults born appropriate for gestational age (AGA).

Results: At GH-stop (mean age 16.4yrs), estimated mean (SE) BMD_{TB} standard deviation score (SDS) was -0.40 (0.1) in males and -0.51 (0.1) in females followed by a trend toward a decrease of BMD_{TB} in males to -0.59 (0.1) at 5yrs after GH-stop ($p=0.06$), while it remained stable in females (-0.57 (0.1), $p=0.33$). At GH-stop, $BMAD_{LS}$ SDS was -0.01 (0.1) in males and -0.29 (0.1) in females, followed by a decrease in males and females to -0.38 and -0.55 at 5yrs after GH-stop, resp. ($p<0.001$). At 5yrs after GH-stop, BMD_{TB} and $BMAD_{LS}$ in SGA-GH were similar compared to SGA-S, SGA-CU and AGA.

Conclusion: After cessation of GH treatment, there is a gradual decline of $BMAD_{LS}$, but at the age of 21yrs, BMD_{TB} and $BMAD_{LS}$ are similar as in untreated short SGA adults.

INTRODUCTION

Short stature persists in approximately 10% of children born small for gestational age (SGA) (1). The mechanisms underlying this lack of catch-up growth are largely unknown, but could be related to disturbances in the growth hormone(GH)-insulin like growth factor-I(IGF-I)-axis, which are present in approximately 60 percent of short SGA children (2).

Osteoporosis is a worldwide problem with high morbidity and increased mortality (3,4). Bone mineral density of the total body (BMD_{TB}) and bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) are important determinants of fracture risk and osteoporosis in later life (5,6). BMD_{TB} and $BMAD_{LS}$ increase during childhood and peak bone mass is normally attained between the age of 18-20 years in girls and 18-23 years in boys (7). Bone strength in later life largely depends on this attained peak bone mass (8).

GH and IGF-I have a well-recognized role not only in bone elongation and skeletal maturation, but are also important factors in the regulation of BMD (9-11). This could be the reason why a low BMD_{TB} and $BMAD_{LS}$ have been found in children with GH deficiency, idiopathic short stature, and Prader-Willi syndrome (12-14). Previous studies have shown that short SGA children have a lower than average BMD_{TB} and $BMAD_{LS}$, even after correction for their short stature (15-17).

Short children born SGA can nowadays be treated with GH during childhood to improve adult height (AH). GH treatment also improves BMD_{TB} and $BMAD_{LS}$ on the short-term. One study reported an increase of BMD_{TB} from -0.9 standard deviation score (SDS) to 0.2 SDS and an increase of $BMAD_{LS}$ from -0.6 SDS to 0.3 SDS during three years of GH treatment (15). A second study reported an increase of $BMAD_{LS}$ with 0.5 SDS during six years of treatment (16). However, long-term studies on BMD_{TB} and $BMAD_{LS}$ in GH-treated short SGA patients are lacking, and the effects after cessation of treatment are unknown.

In this study, we longitudinally investigated BMD_{TB} and $BMAD_{LS}$ in young adults born SGA who were treated with GH during childhood, and had attained an age of 21 years, so that peak bone mass could be evaluated. We hypothesized that BMD_{TB} and $BMAD_{LS}$ had improved during long-term GH treatment and would not be significantly different from 0 SDS at attainment of AH. We also hypothesized that BMD_{TB} and $BMAD_{LS}$ decline in the first months after cessation of GH treatment due to the loss of pharmacologic effects of GH. We compared the data at five years after cessation of GH treatment to young adults born SGA with persistent short stature who were never treated with GH, hypothesizing that BMD_{TB} and $BMAD_{LS}$ of GH-treated SGA adults would return to levels of untreated short young adults born SGA. Additionally, we compared the data at five years after cessation of GH treatment to those of healthy young adults born appropriate for gestational age (AGA).

METHODS

Subjects

The total study group comprised 358 young adults of which 173 young adults born SGA had participated in a GH trial (18-20). These participants started GH when prepubertal, had a birth length and height below -2.5 SDS and had no growth failure caused by other disorders. Subjects with a GH deficiency (defined as a serum GH peak response of <20 mU/l during a GH stimulation test) were excluded. Once daily, 1 mg/m^2 biosynthetic GH (Norditropin; Novo Nordisk A/S, Denmark) was given subcutaneously at bedtime. Every three months, the GH dose was adjusted to the calculated body surface area.

At attainment of AH (i.e. when height velocity dropped below 0.5 cm in six months and bone age was ≥15 years for girls and ≥16.5 years for boys), GH treatment was discontinued. Patients were invited to participate in the current longitudinal study evaluating BMD at four time points: at AH (GH cessation), at six months, two years and five years after GH cessation. Additionally, data at five years after GH cessation were compared with three clinically relevant groups of age-matched healthy young adults (21,22): 45 untreated young adults born SGA (birth weight and/or birth length <-2 SDS) with persistent short stature (AH <-2 SDS) (SGA-short, SGA-S), 59 young adults born SGA (birth weight and/or birth length <-2 SDS) with catch-up growth resulting in a normal AH (>-1 SDS) (SGA-catch-up, SGA-CU) and 81 young adults born appropriate for gestational age (birth length >-1 SDS) with a normal stature (AH >-1 SDS) (AGA).

This study was performed according to the Helsinki declaration. The Medical Ethics Committee of the Erasmus University Medical Centre approved this study. Written informed consent was obtained from all participants and/or their parents.

Measurements

Height was measured to the nearest 0.1 cm (Harpenden stadiometer), weight to the nearest 0.1 kg (Servo Balance KA-20-150S). Anthropometric measurements were performed twice according to standardized methods, after which the mean was calculated. Information regarding oral contraceptive use was obtained using questionnaires.

In all participants, BMD_{TB} and BMD of the lumbar spine (BMD_{LS}), bone mineral content, lean body mass (LBM) and fat mass percentage (FM%) were measured by dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy, GE Healthcare, Chalfont St. Giles, UK). BMD_{TB} measurements included the head. All measurements of both the GH-treated subjects and the control groups were performed on one machine, and quality assurance was performed daily. All measurements were performed between 2003 and 2016, by the same personnel of the same department. The coefficients of variation were 0.64% for BMD_{TB} and 1.04% for BMD_{LS} (23).

Serum IGF-I levels were expressed as SDS adjusted for age and gender, using reference values for healthy children with normal stature, determined in the same laboratory (24).

Calculations

Height and weight were expressed as SDS adjusted for age and gender according to Dutch reference data (25) and calculated using the Growth Analyzer software (http://www.growthanalyser.org).

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 ($BMAD_{LS}$) (26). $BMAD_{LS}$ 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}, $BMAD_{LS}$, and FM% are dependent on age and gender, SDS were calculated based on age- and gender-matched reference values from the Dutch population (27,28). Because LBM is strongly related to height, LBM was expressed as SDS for height and gender (27).

Statistical analysis

Distribution of variables was determined by Shapiro-Wilk tests and normal Q-Q plots. Data were compared to zero using one sample t-tests. Longitudinal changes after cessation of GH treatment were analyzed using repeated measurements analyses with an unstructured covariance type, adjusting for missing data. Multiple linear regression analyses were performed to determine the associations between GH treatment and BMD_{TB} and $BMAD_{LS}$, with corrections for the possible confounders age, gender, birth weight, height, LBM, FM%, and, in girls, oral contraceptive use. An analysis of covariance (ANCOVA) was used to compare the four groups, corrected for the confounders identified in the multiple regression analyses.

Results were considered statistically significant if the p-value was <0.05. All analyses were performed with SPSS version 21.0.

RESULTS

Clinical characteristics

Table 1 shows the clinical characteristics of the 173 GH-treated SGA adults (82 males; 91 females), at start of GH treatment and at AH, when GH treatment was discontinued and the follow-up study started. Mean (SD) age at start of GH treatment was 6.2 (2.0) years and height -3.00 (0.6) SDS. After a mean (SD) treatment duration of 10.2 (2.1) years, subjects attained an AH of -1.69 (0.8) SDS.

Table 1. Clinical characteristics of the GH-treated SGA subjects at start of GH and at GH cessation

	At start of GH			At GH cessation		
	Total	**Males**	**Females**	**Total**	**Males**	**Females**
N	173	82	91	173	82	91
Age (years)	6.2 (2.0)	6.3 (2.1)	6.1 (1.8)	16.4 (1.3)	17.2 (1.1)	15.6 (0.9)
Height SDS	-3.00 (0.6)	-3.08 (0.6)	-2.93 (0.6)	-1.69 (0.8)	-1.66 (0.9)	-1.72 (0.8)
Weight for height SDS	-1.22 (1.2)	-1.22 (1.1)	-1.22 (1.4)	0.58 (0.8)	0.65 (0.6)	0.52 (1.0)
BMI SDS	-1.21 (1.1)	-1.17 (1.0)	-1.24 (1.2)	-0.04 (0.9)	-0.05 (0.8)	-0.04 (1.1)
BMD_{TB} SDS	-1.00 (1.0)[a]	-1.32 (1.2)	-0.73 (1.0)	-0.44 (0.9)	-0.43 (1.1)	-0.45 (0.9)
$BMAD_{LS}$ SDS	-0.48 (0.8)[a]	-0.33 (0.6)	-0.61 (0.9)	-0.14 (1.1)	0.04 (1.2)	-0.30 (0.9)
BMC SDS	-2.43 (0.9)[a]	-2.92 (1.0)	-2.03 (0.4)	-1.23 (0.7)	-1.25 (0.8)	-1.21 (0.7)
IGF-I SDS	-0.53 (1.1)	-0.42 (1.1)	-0.63 (1.3)	1.22 (0.8)	1.28 (0.8)	1.17 (0.8)
GH duration (years)	NA	NA	NA	10.2 (2.1)	10.9 (2.2)	9.5 (1.7)

Data are expressed as mean (SD).

GH = Growth hormone; NA = Not applicable; SDS = standard deviation score

[a] at start of GH treatment only available of 31 participants

Longitudinal changes in BMD_{TB} and $BMAD_{LS}$ after GH cessation

Figure 1 shows the longitudinal changes in BMD_{TB} and $BMAD_{LS}$ SDS in males and females, until five years after GH cessation. At GH cessation, estimated mean (SE) BMD_{TB} SDS was -0.40 (0.1) in males, and -0.51 (0.1) in females. This was both within the normal range but significantly lower than 0 SDS ($p<0.001$). Both males and females had a significantly higher BMD_{TB} at cessation of GH treatment compared to at onset of treatment ($p<0.001$ and $p=0.002$ resp.). In the six months after GH cessation, BMD_{TB} SDS increased significantly in males to -0.30 (0.1) ($p=0.008$), followed by a decrease from then onwards ($p=0.004$). At five years after GH cessation, estimated mean (SE) BMD_{TB} SDS was -0.59 (0.1) in males ($p=0.06$ compared to BMD_{TB} at GH cessation and $p<0.001$ compared to 0 SDS). In females, BMD_{TB} SDS remained stable after cessation of GH-treatment: BMD_{TB} SDS was -0.57 (0.1) at five years after GH cessation ($p=0.33$ compared to BMD_{TB} at GH cessation and $p<0.001$ compared to 0 SDS).

At GH cessation, estimated mean (SE) $BMAD_{LS}$ SDS was -0.01 (0.1) in males ($p=0.76$ compared to 0 SDS), and -0.29 (0.1) in females ($p=0.003$ compared to 0 SDS). Both males and females had a significantly higher $BMAD_{LS}$ at cessation of GH treatment compared to at onset of treatment ($p=0.006$ and $p=0.002$ resp.). In both males and females, $BMAD_{LS}$ SDS did not change in the six months after GH cessation, but started to decrease in the 18 months thereafter in males ($p<0.001$), and between two and five years after GH cessation in females ($p<0.001$). At five years after GH cessation, estimated mean (SE) $BMAD_{LS}$ SDS was -0.38 (0.1) in males ($p<0.001$ compared to $BMAD_{LS}$ at GH cessation and $p=0.02$ compared to 0 SDS), and -0.55 (0.1) in females ($p<0.001$ compared to $BMAD_{LS}$ at GH cessation and 0 SDS).

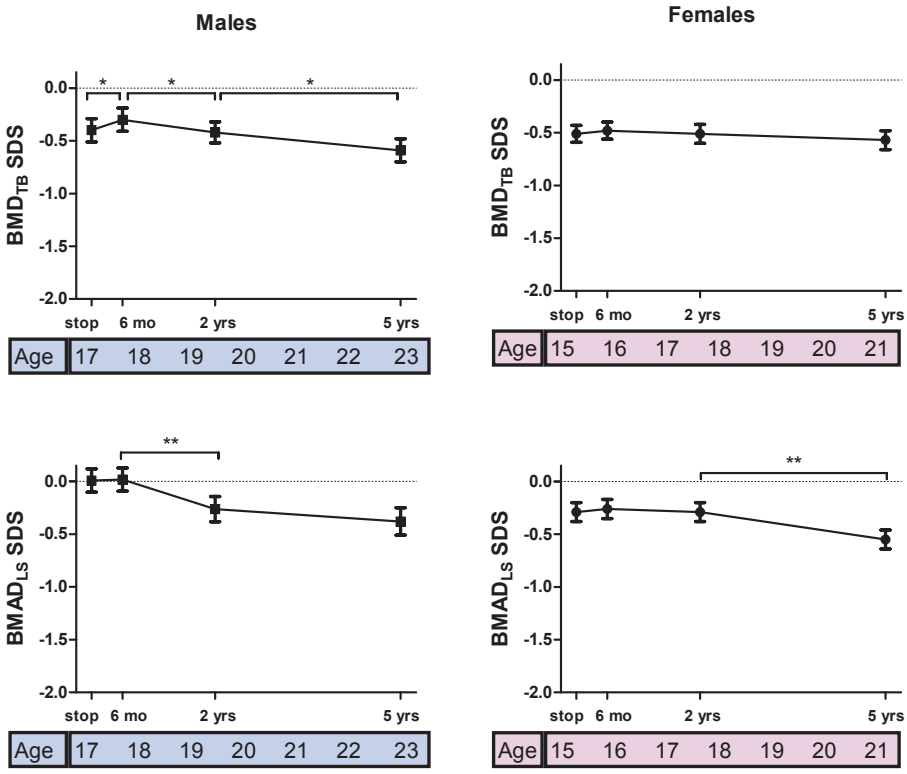

Figure 1. Longitudinal changes in BMD_{TB} and $BMAD_{LS}$ in in GH-treated males and females, until 5 years after GH cessation

Data expressed as estimated marginal means ± SEM. * = p<0.05; ** = p<0.001

In order to compare the individual BMD_{TB} and $BMAD_{LS}$ of the SGA-GH patients to the healthy population, BMD_{TB} and $BMAD_{LS}$ data points at GH cessation and two and five years thereafter were plotted against the reference curve of the healthy Dutch population (Figure 2). At GH cessation, 5.2% of the study population (males and females) had a BMD_{TB} ≤-2 SDS, which was a higher proportion than the expected 2.3% in the reference population (p=0.019). At five years after GH cessation, only 1.3% of the study group had a BMD_{TB} ≤-2 SDS, which was similar to the expected proportion in the reference population (p=0.48). At GH cessation, 3.5% had a $BMAD_{LS}$ ≤-2 SDS (p=0.21 compared to 2.3%). Also at five years after GH cessation, the proportion of patients with a $BMAD_{LS}$ ≤-2 SDS was not significantly different from the expected 2.3% (p=0.66).

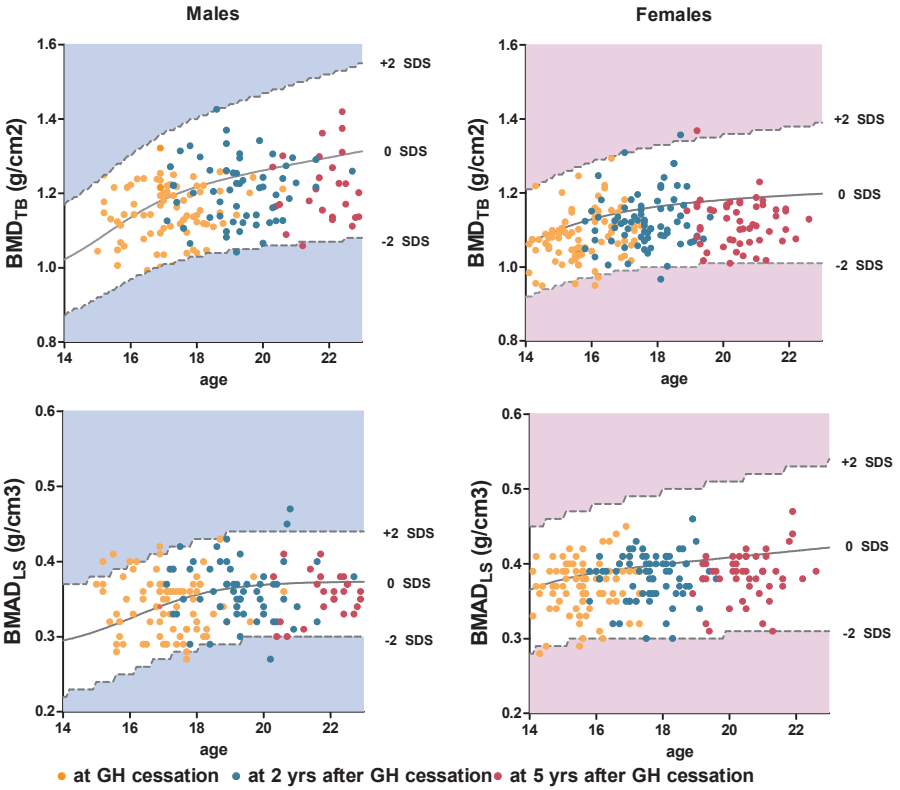

Figure 2. BMD$_{TB}$ and BMAD$_{LS}$ in GH-treated males and females, plotted against the reference curve of the healthy Dutch population

Factors influencing the change in BMD$_{TB}$ and BMAD$_{LS}$ during the 5 years after GH cessation

From GH cessation until five years thereafter, there was a decrease in LBM of 0.39 SDS, and an increase in FM% of 0.56 SDS. We analyzed whether there was a correlation between these changes in body composition and the deterioration in BMD$_{TB}$ and BMAD$_{LS}$ SDS during the five years after GH cessation. There was no correlation between ΔLBM SDS and ΔBMD$_{TB}$ SDS (r=0.17, p=0.24), nor between ΔFM% SDS and ΔBMD$_{TB}$ SDS (r=0.05, p=0.75).There was also no correlation between ΔLBM SDS and ΔBMAD$_{LS}$ SDS (r=0.17, p=0.24), nor between ΔFM% SDS and BMAD$_{LS}$ (r=0.18, p=0.22).

From GH cessation until five years thereafter, serum IGF-I levels decreased with 1.66 SDS. There was no correlation between ΔIGF-I SDS and ΔBMD$_{TB}$ SDS (r=0.40, p=0.20). However, there was a trend toward a significant correlation between the decrease in IGF-I SDS and the decrease in BMAD$_{LS}$ SDS (r=0.57, p=0.05).

BMD_{TB} and $BMAD_{LS}$ at five years after GH cessation, compared to SGA-S, SGA-CU and AGA

Table 2 shows the clinical characteristics of the SGA-GH young adults at five years after GH cessation, compared to the SGA-S, SGA-CU and AGA young adults. The percentage of males/females was similar in the four groups. Mean age was around 21 years in all groups. Due to the selection criteria, there were significant differences in birth length SDS, birth weight SDS, height SDS, weight for age SDS and body composition. The percentage of smokers and of girls who used oral contraceptives was similar in all groups.

We analyzed which variables contributed to BMD_{TB} and $BMAD_{LS}$ in the total group consisting of SGA-GH, SGA-S, SGA-CU and AGA at 21 years, in a multiple regression analysis (Table 3). In the first model with BMD_{TB} as dependent variable, we included the variables gender, age, birth weight SDS, height SDS, LBM SDS (corrected for height and gender) and FM% SDS (corrected for age and gender). Male gender, height SDS, LBM SDS and FM% SDS were all positively associated with BMD_{TB} ($p<0.001$ for gender, height and LBM, and $p=0.01$ for FM%). Age and birth weight SDS were not associated with BMD_{TB}. This model accounted for 38% of the variance in BMD_{TB}. In the second model, we evaluated the effect of oral contraceptive use in girls, which was not a significant

Table 2. Clinical characteristics of the total study group at age 21 years

	SGA-GH	SGA-S	SGA-CU	AGA
N	89	45	59	81
Male/female	38/51	15/30	22/37	30/51
Gestational age (wks)	36.3 (3.6)[1]	39.5 (1.5)[4]	38.3 (1.5)[6]	39.4 (1.7)
Birth length SDS	-3.21 (1.7)[6]	-3.01 (1.1)[6]	-2.90 (0.8)[6]	0.07 (0.7)
Birth weight SDS	-2.39 (1.2)[3,6]	-1.92 (0.9)[5,6]	-2.37 (0.8)[6]	-0.10 (1.3)
Age	21.4 (1.5)[3,7]	20.7 (1.7)	21.1 (1.5)	20.8 (1.7)
Height SDS	-1.56 (0.9)[1]	-2.62 (0.6)[4,6]	-0.14 (0.8)[7]	0.24 (0.9)
BMI SDS	-0.36 (1.7)	0.13 (1.1)	0.11 (1.3)	-0.06 (1.0)
Lean body mass SDS	-0.94 (1.3)[2,7]	0.04 (1.5)[5,7]	-0.67 (1.3)	-0.54 (1.1)
Fat mass % SDS	0.85 (0.9)	0.79 (0.8)	0.90 (0.8)	0.78 (0.8)
OC-use (% of females)	89.5	78.3	80.0	71.1
IGF-I SDS	-0.46 (1.0)	-0.29 (0.8)	-0.26 (0.7)	-0.26 (0.8)

Data are expressed as mean (SD).

[1] P<0.001 compared to the other groups; [2] P<0.001 compared to SGA-S; [3] P<0.05 compared to SGA-S; [4] P<0.001 compared to SGA-CU; [5] P<0.05 compared to SGA-CU; [6] P<0.001 compared to AGA; [7] P<0.05 compared to AGA

SGA-GH = growth hormone treated subjects born SGA, 5 years after GH cessation; SGA-S = untreated subjects born SGA with short stature; SGA-CU = subjects born SGA with spontaneous catch-up growth; AGA = subjects born appropriate for gestational age

Lean body mass was corrected for height and gender; fat mass percentage was corrected for age and gender; OC-use = oral contraceptive use

predictor of BMD_{TB} (p=0.27). In Model 3, we included the subjects who were GH-treated during childhood as a dummy variable, showing that GH treatment was not a significant predictor of BMD_{TB} at five years after GH cessation (p=0.63).

The same variables were then included in the model with $BMAD_{LS}$ as dependent variable. Male gender was negatively associated with $BMAD_{LS}$ (p<0.001). Height SDS was not significantly associated with $BMAD_{LS}$, which was expected since $BMAD_{LS}$ is already corrected for bone size. LBM SDS and FM% SDS were positively associated with $BMAD_{LS}$ (p=0.04 and p=0.001 resp.). This model accounted for 18% of the variance in $BMAD_{LS}$. In the second model, we evaluated the effect of oral contraceptive use in girls. This model showed that oral contraceptive use did not influence $BMAD_{LS}$ (p=0.28). Finally, the dummy GH treatment was included in the model, showing that this was neither a predictor of $BMAD_{LS}$ (p=0.14).

Figure 3 shows the comparisons of BMD_{TB} and $BMAD_{LS}$ between the SGA-GH young adults at five years after GH cessation, and the SGA-S, SGA-CU and AGA young adults. Adjustments were made based on the significant predictors in the multiple regression analysis. BMD_{TB} in SGA-GH, adjusted for gender, height SDS, LBM SDS and FM% SDS, was not different from BMD_{TB} in SGA-S and SGA-CU (p=0.22 and p=0.76, resp.). There was a trend toward a lower BMD_{TB} in SGA-GH than in AGA (p=0.09). SGA-S and SGA-CU had a similar BMD_{TB}, but both lower than AGA (p=0.03). $BMAD_{LS}$ SDS, adjusted for gender, LBM SDS and FM% SDS, was similar in SGA-GH and SGA-S, SGA-CU and AGA (p=0.63, p=0.72 and p=0.26, resp.).

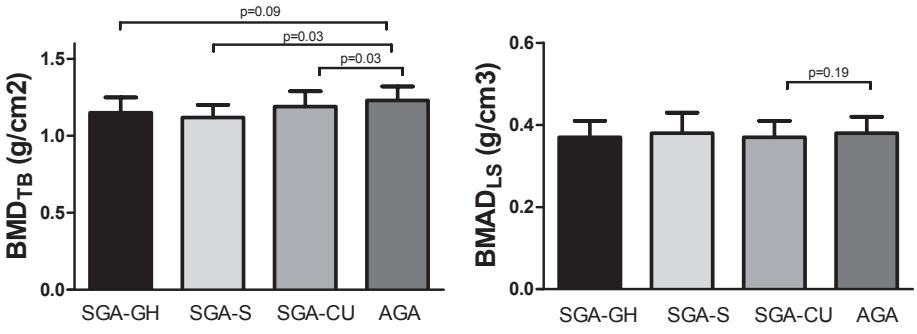

Figure 3. BMD_{TB} and $BMAD_{LS}$ at 5 years after GH cessation, compared to SGA-S, SGA-CU and AGA
Data expressed as estimated means (SEM). BMD_{TB} was additionally adjusted for gender, height SDS, LBM SDS and FM% SDS and $BMAD_{LS}$ for gender, LBM SDS and FM% SDS

Table 3. Multiple regression analysis for BMD_{TB} and $BMAD_{LS}$ in the total group at age 21 years

	BMD_{TB} (g/cm^2)						$BMAD_{LS}$ (g/cm^3)					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	ß	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**	**ß**	**p**
Male gender	0.06	**<0.001**	0.04	**0.04**	0.06	**<0.001**	-0.04	**<0.001**	-0.05	**<0.001**	-0.04	**<0.001**
Age (years)	0.01	0.22	0.01	0.15	0.01	0.22	0.00	0.60	0.00	0.99	0.00	0.58
Birth weight SDS	0.00	0.89	-0.00	0.85	0.00	0.81	-0.00	0.64	-0.00	0.52	-0.00	0.43
Height SDS	0.03	**<0.001**	0.03	**<0.001**	0.03	**<0.001**	0.00	0.91	0.00	0.94	-0.00	0.68
LBM SDS	0.02	**<0.001**	0.02	**<0.001**	0.02	**<0.001**	0.01	**0.04**	0.00	0.16	0.00	0.07
FM% SDS	0.02	**0.01**	0.01	0.10	0.02	**0.01**	0.01	**0.001**	0.01	0.06	0.01	**0.001**
OC-use			-0.02	0.27					-0.01	0.28		
GH treatment					0.01	0.63					-0.00	0.14
Overall p-value	**<0.001**		**<0.001**		**<0.001**		**<0.001**		**0.001**		**<0.001**	
R^2 adjusted	0.38		0.34		0.37		0.18		0.12		0.19	

ß = unstandardized regression coefficient. p=p-value (<0.05 in bold).

LBM SDS = Lean body mass, corrected for height and gender; FM% SDS = Fat mass percentage, corrected for age and gender; OC-use = Oral contraceptive use; GH = Growth hormone

DISCUSSION

This study presents five-year longitudinal data on BMD_{TB} and $BMAD_{LS}$ after cessation of GH treatment in young adults born SGA. We show that cessation of GH treatment is accompanied with a trend toward a decline of BMD_{TB} in males and a gradual decline of $BMAD_{LS}$ in males and females. However, at five years after cessation of treatment, previously GH-treated SGA young adults had a similar BMD_{TB} and $BMAD_{LS}$ as young adults who remained untreated and as controls born AGA with a normal stature.

To our knowledge, this is the first study describing BMD_{TB} and $BMAD_{LS}$ after cessation of GH treatment in a large group of young adults born SGA. Until now, there were limited data available. Our research group reported on BMD_{TB} and $BMAD_{LS}$ in children born SGA during GH treatment (15,16). These studies were performed during respectively three and six years of treatment, and reported a BMD_{TB} SDS of -0.9 and a $BMAD_{LS}$ SDS of -0.6 at onset of treatment. Although we found that BMD_{TB} was lower than average at cessation of GH treatment, both BMD_{TB} and $BMAD_{LS}$ were higher than the baseline BMDs reported in previous studies. The same studies showed that during the first years of GH treatment there was an increase of BMD_{TB} and $BMAD_{LS}$, which was maintained at the same level up to six years after the start of treatment. We can now conclude that these positive effects are maintained until adult height attainment.

During the five years after cessation of GH treatment, there was a gradual decline of $BMAD_{LS}$, and a trend toward a decline of BMD_{TB} in males, probably due to the loss of pharmacological effects of GH treatment. The correlation between the decline in IGF-I SDS levels and the deterioration in $BMAD_{LS}$ in the five years after GH cessation provides support for this explanation. In contrast to other metabolic changes after GH cessation (29), the decrease in BMD_{TB} and $BMAD_{LS}$ did not commence directly after cessation. In males, there was even an increase of BMD_{TB} in the first six months after GH cessation. It could be that GH treatment has relatively longer-lasting effects on bone metabolism than on body mass and fat mass, and that, therefore, there is not an immediate effect of GH cessation on BMD. Despite the deteriorations in the years after GH cessation, average BMD_{TB} and $BMAD_{LS}$ at five years after GH cessation were above -1 SDS, and only a very small percentage of all subjects had a BMD_{TB} or $BMAD_{LS}$ below -2 SDS, which is reassuring. Most of the skeletal mass in the total body and lumbar spine is attained in the first years of the third decade (28,30,31). This attained peak bone mass is an important determinant of osteoporosis in later life (8). The higher BMD_{TB} and $BMAD_{LS}$ at young adult age, the lower the risk of osteoporosis in later life. Thus, although BMD_{TB} and $BMAD_{LS}$ in formerly GH-treated subjects were within the normal range, it is unfortunate that the beneficial effects of GH treatment disappear after cessation of treatment. Future studies should aim at investigating how BMD_{TB} and $BMAD_{LS}$ progress when GH-treated subjects born SGA get older, and what the long-term clinical implications will be.

Our multiple regression analysis could explain a relatively large part of the total variance in BMD_{TB} and $BMAD_{LS}$. Gender, height, LBM and FM% were significant determinants of BMD_{TB}, and gender, LBM and FM% of $BMAD_{LS}$. The combination of LBM and FM% can also be seen as a measure of physical fitness, which is an important determinant of BMD_{TB} and $BMAD_{LS}$ (32,33). Although GH-treated subjects had BMD_{TB} and $BMAD_{LS}$ below average at five years after GH cessation, this did not significantly influence BMD_{TB} and $BMAD_{LS}$ of the total group at the age of 21 years. We found no effect of oral contraceptive use in females on BMD_{TB} and $BMAD_{LS}$, although the fact that there were relatively few females who did not use oral contraceptives makes it difficult to draw definite conclusions. Another factor influencing BMD_{TB} and $BMAD_{LS}$ in adolescence is pubertal stage, but since all participants were postpubertal in our cohort, this was already accounted for. Although the most important determinants of BMD_{TB} and $BMAD_{LS}$ in a healthy young population were assessed in this study, there are of course other determining factors, such as calcium and vitamin D status. Unfortunately, there were no data on these factors available.

At five years after cessation of GH treatment, we additionally compared the data of the GH-treated subjects to age-matched clinically relevant subgroups of young adults born SGA with persistent short stature who were never treated with GH, young adults born SGA who showed spontaneous catch-up growth, and AGA born controls with a normal stature. After correction for the significant determinants of BMD_{TB} and $BMAD_{LS}$, we found no differences between the GH-treated and untreated young adults born SGA who remained short. There was a trend toward a lower BMD_{TB} in the GH-treated subjects than in the AGA born controls. Interestingly, subjects born SGA with spontaneous catch-up growth had also a lower BMD_{TB} than the AGA born subjects, suggesting that BMD_{TB} in subjects born SGA is not only reduced due to short stature, but that there are other determining factors of BMD_{TB} that are disturbed in subjects born SGA. $BMAD_{LS}$ was, however, similar in all groups.

In conclusion, this study shows that GH treatment improves BMD_{TB} and $BMAD_{LS}$ in subjects born SGA. However, after cessation of GH, $BMAD_{LS}$ gradually deteriorates after GH cessation and there is a trend toward a deterioration of BMD_{TB} in males. At the age of 21 years, BMD_{TB} and $BMAD_{LS}$ are, however, similar in GH-treated and untreated subjects born SGA. Although this study describes the largest and longest follow-up thus far on BMD_{TB} and $BMAD_{LS}$ measured by DXA in young adults born SGA who were treated with GH during their childhood, for definite conclusions a longer follow-up is required to see how these parameters develop as subjects progress further into adulthood.

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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). Since then, several clinical trials have proved that GH treatment is safe and effective to improve adult height (AH) in these children (1-3). In the studies presented in this thesis, we focused on important knowledge gaps that remained in the research about short children born SGA.

In the first part of this thesis we present studies about Silver-Russell syndrome (SRS), which is one of the causes of short SGA. Issues that we addressed were long-term effects of GH treatment on growth and metabolic health (Chapter 2 and 3) and pubertal development and gonadal function (Chapter 4). In the second part of this thesis, we explored a possible underlying mechanism behind the increased risk for cardiovascular diseases (CVD) in those born with a low birth weight. To do so, we used the ageing parameter telomere length, which we measured in young adults who were born preterm (Chapter 5), and in young adults with differences in size at birth and postnatal growth patterns, including GH-induced catch-up growth (Chapter 6). Finally, we longitudinally assessed bone mineral density (BMD) and peak bone mass in young adults born SGA, during five years after cessation of GH treatment due to AH attainment (Chapter 7).

This chapter offers a general discussion of the results from the studies presented in this thesis. The clinical implications of these data are addressed, as well as future directions for the field.

SILVER-RUSSELL SYNDROME

SRS is one of the causes of short SGA and children with SRS can be treated with GH under the indication short SGA. However, whether children with SRS benefit the same from GH treatment as non-SRS subjects born SGA had never been investigated. Since the majority of individuals with SRS are not routinely followed up, there was very little information in the literature regarding the long-term natural history of SRS. It is well known that children born SGA have an increased risk for metabolic health issues in adulthood (4-6), but this had never been investigated in SRS patients. Moreover, while it had been shown that GH treatment has several metabolic effects in those born SGA (7-10), the long-term effects of GH treatment on metabolic health in SRS were unknown. Another issue that had not been evaluated in SRS patients was their pubertal progression and gonadal function.

GH treatment in Silver-Russell syndrome

In Chapter 2, we evaluated whether children with SRS benefit the same from GH treatment as non-syndromic subjects born SGA, and compared growth response from start

of GH treatment until AH attainment among the SRS subtypes. We demonstrated that SRS patients are significantly shorter than non-SRS patients at start of GH treatment, but gain more height in the first years of treatment, resulting in a similar height SDS at onset of puberty in SRS and non-SRS. However, there was a steeper decline in height SDS from onset of puberty until AH attainment in SRS compared to non-SRS, resulting in an AH of -2.17 SDS in SRS, versus -1.65 SDS in non-SRS. Although SRS subjects did not attain the same AH, total height gain was similar in SRS and non-SRS subjects born SGA and we therefore conclude that GH treatment is similarly effective in SRS and non-SRS subjects born SGA. Studies examining the long-term effects of GH treatment on growth in SRS patients are sparse, with only two studies describing AH after GH therapy (11, 12). In both studies, total height gain was similar as in our cohort.

The finding that SRS subjects did not attain the same AH as non-SRS subjects born SGA could be due to the fact that patients were shorter at start of GH treatment and the fact that puberty started earlier in SRS patients, leading to earlier closure of the growth plates. This implies that SRS patients have a shorter time window in which height gain can be achieved. Expanding this window might be beneficial for SRS patients. This was also suggested by Binder *et al.*, since their study group found that duration of GH treatment was strongly positively correlated with both AH and overall height gain (11). We therefore suggest that GH treatment should be started at a young age in SRS patients, and SRS patients who start puberty at a young age could be additionally treated with a gonadotropin-releasing hormone analogue (GnRHa) to postpone puberty.

We additionally compared growth data from onset of GH treatment until AH attainment among the different SRS subtypes and found a trend toward a better response to GH treatment in SRS patients with mUPD7 and clinical SRS, compared to patients with 11p15 LOM. A previous study on genotype-phenotype correlations in SRS concluded that patients with 11p15 LOM show the more 'classic' SRS phenotype (13). Based on our results, we conclude that this is also the case for their growth and response to GH treatment. Since those with 11p15 LOM still gained almost 1 SDS in height during treatment, we do consider GH treatment beneficial for SRS patients with 11p15 LOM.

Due to the rarity of SRS, it remains difficult to conduct studies on the differences among the SRS subtypes with sufficient statistical power. Expertise centres that provide optimal care for SRS patients and initiating international collaborations with other study groups, could aid in obtaining larger cohorts of SRS patients.

Metabolic health in Silver-Russell syndrome

In addition to positive effects on linear growth, GH treatment has also several metabolic effects in children born SGA, namely an increase in lean body mass, a decline in fat mass, a decrease in blood pressure, and a more favourable lipid profile, but also a lower insulin sensitivity (Si) (7, 8, 10). After cessation of GH treatment, some of these effects are lost,

due to the loss of pharmacologic effects of GH (9). The long-term safety of GH treatment and metabolic effects after cessation of treatment after AH attainment had never been investigated in SRS patients. Up until now, there were only case reports of adults with SRS, in which an adverse metabolic health profile was described (14, 15). In Chapter 3, we therefore assessed longitudinal changes in metabolic health (i.e. blood pressure, fasting lipid levels, body composition, Si and occurrence of diabetes mellitus type 2 (DM2) and metabolic syndrome) in 29 SRS patients, from start of treatment until two years after cessation of GH due to AH attainment. We compared these data to those of 171 GH-treated non-SRS subjects born SGA.

We found that SRS and non-SRS patients have a similar metabolic health profile at start of treatment and that, apart from minor variations, SRS and non-SRS patients respond similarly to GH treatment regarding their metabolic health profile. In the months after cessation of GH, we found several changes related to the loss of pharmacologic effects of GH in SRS patients, such as a decrease in lean body mass and an increase in fat mass percentage, but this was similar as in non-SRS patients. Si and ß-cell function improved after cessation of GH. Most importantly, at two years after cessation of GH, at a mean age of approximately 18 years, there were no significant differences between the groups, and none of the SRS patients had developed DM2 or metabolic syndrome.

In general practice, the majority of the SRS patients are not routinely followed up after AH attainment, which is the reason why there is very little information on the long-term history of SRS. There are only two case reports addressing metabolic health in SRS (14, 15). The first described three SRS patients (all with 11p15 LOM, two having received GH treatment for several years during childhood) who developed adult diseases such as obesity, hypertension, and DM2 in their early twenties. The second described the oldest SRS patient reported so far, who has DM2, osteopenia, and hypercholesterolemia at the age of 69 years. These studies did, however, not compare the data of SRS patients to those of non-SRS subjects born SGA. Our study is the first study describing metabolic health data in a cohort of GH-treated SRS patients that was followed from childhood into early adulthood. We used gold standard tests such as dual-energy x-ray absorptiometry (DXA) to measure body composition, and frequently sampled intravenous glucose tolerance test with Tolbutamide to assess Si and ß-cell function, which makes our data unique.

Although our results are reassuring, larger cohorts are needed to be able to draw definite conclusions. Due to the rarity of the syndrome, this remains a challenging issue. Moreover, patients in our study were young adults, and although we used appropriate tests with which we were able to establish the subtlest changes in metabolic health, it could be that they develop metabolic health related issues at an older age. Thus, a longer follow-up of SRS patients is needed to establish whether the fact that there are no metabolic differences between SRS and non-SRS subjects born SGA before, during and after GH treatment will be maintained over the years when patients progress further

into their adulthood. It is therefore important that SRS patients are not lost to follow-up once they reach AH and GH treatment is discontinued.

Pubertal development and gonadal function in Silver-Russell syndrome

There was very little information in the literature regarding pubertal development and possible reproductive issues in SRS patients. It is known that boys with SRS have an increased risk for genital abnormalities such as cryptorchidism and hypospadias (13, 16, 17). In girls with SRS, an association has been described with Mayer-Rokitansky-Kuster-Hauser syndrome, a disorder characterized by hypoplasia of the uterus and upper part of the vagina (18, 19). In Chapter 4, we longitudinally assessed pubertal development and gonadal function in 31 SRS patients. We compared these data to those of a control group of 123 GH-treated subjects born SGA without SRS and healthy references.

We found that age at onset of puberty was similar in SRS and non-SRS subjects born SGA. Puberty progressed similarly in SRS and non-SRS, and all SRS patients attained an adult Tanner stage. Thus, our study shows that there is neither concern for a precocious nor for a delayed puberty in SRS patients.

In the consensus statement it was stated that onset of puberty in SRS is usually within the normal range, although mostly in the younger age of the spectrum (20), which has disadvantages for eventual AH. We found that SRS patients have the same benefit from two years of additional GnRHa treatment as the non-SRS patients when they are still short at onset of puberty, improving their growth from onset of puberty until AH (21). We, therefore, suggest considering two years of GnRHa treatment in addition to GH treatment in SRS patients with an expected AH below -2.5 SDS at onset of puberty.

We longitudinally assessed gonadal function in the SRS patients. Since all females with SRS had LH, FSH and AMH levels within the reference range, the follicle pool and gonadal function do not seem to be impaired in SRS females, although there was one female with clinical SRS with a normal pubertal progression but primary amenorrhea due to Müllerian agenesis. Previous case-reports have shown an association between SRS and Mayer-Rokitansky-Küster-Hauser syndrome (18, 19), but the etiology of this association and whether it is more common in certain SRS subtypes remains to be elucidated. We found that more than a quarter of the SRS males had a postpubertal inhibin B level below the 5th percentile for healthy references, and two males also had an FSH level above the 95th percentile. These results imply that Sertoli cell dysfunction is more common in SRS patients. This might at least be partly caused by the increased risk for cryptorchidism in SRS males. Larger cohorts with a longer follow-up period are needed to confirm these findings and to study the etiology of possible reproductive issues in SRS males. Evaluating Sertoli cell function at an early age might lead to earlier identification of a dysfunction and better opportunities for successful conception in later life.

We therefore recommend assessing gonadal function in SRS males, also in those with normal pubertal progression.

Methodological considerations in studies regarding SRS

In the studies presented in this thesis, we adhered to strict guidelines to diagnose SRS and performed extensive genetic testing to confirm the molecular diagnosis. Nevertheless, approximately 40% of the SRS patients remain without a molecular diagnosis, and there is no adequate way to establish whether these patients are not falsely diagnosed with SRS while another syndrome underlies their symptoms. Testing for mUPD7 has been possible for a long time, but this aberration is only present in 5-10% of all SRS patients. More recently, it was discovered that over half of the SRS patients have an aberration in the 11p15 region (22, 23). All studies on SRS that were conducted before 2005 thus describe cohorts of SRS patients that are only based on a clinical diagnosis or mUPD7. Due to the highly variable phenotype of SRS, and the fact that symptoms tend to become less severe with age, it can be very difficult to diagnose SRS based on clinical findings. The recently developed Netchine-Harbison clinical scoring system is a practical scoring system for clinicians, since four of the six criteria are objective (23). It has a sensitivity of 98% and negative predictive value of 89%, which gives a high degree of confidence that patients who have less than four of the six clinical criteria for diagnosis are truly unaffected by SRS. On the other hand, the specificity of this scoring system is low, which could result in false positive results when the diagnosis is only based on clinical findings. Lately, a lot of research has focused on unravelling the mechanism(s) underlying clinical SRS. Although new mechanisms have been discovered, such as the CDKN1C and IGF2 mutation (24-26), these mutations do not seem to explain a large part of the SRS patients (27, 28). Thus, future studies are needed to elucidate the underlying mechanisms of clinical SRS. Not only would this be an important step for the patients who now remain without an established diagnosis, but it will also help in attaining SRS cohorts that are not biased by other syndromes, which might improve the results of future studies and their applicability for clinical practice.

Conclusions and clinical implications regarding Silver-Russell syndrome

From our studies, we conclude that GH treatment is safe and effective in patients with SRS. This is true for each SRS subtype, although those with 11p15 LOM might attain a lower AH. Starting GH treatment at a young age and postponing puberty by means of two years of GnRHa treatment might enlarge the window in which height gain can be achieved. Future research should aim at elucidating this. Until the age of 18 years, there is no difference in metabolic health between those with SRS and those born SGA without SRS. Although this is reassuring, a longer follow-up is needed to see whether this is maintained over the years as subjects reach their thirties and forties. Since Sertoli

cell dysfunction is more common in SRS, we recommend assessing gonadal function in SRS patients, particularly in males. Finally, we emphasize the need for expertise centres that provide the most experienced care for SRS patients and will in the future be able to obtain larger cohorts of SRS patients.

TELOMERE LENGTH IN YOUNG ADULTS BORN PRETERM AND/OR SGA

Low birth weight, whether due to preterm birth or SGA, followed by accelerated weight gain during infancy is associated with an increased risk for adult diseases such as CVD and DM2 in later life (4, 5, 29). The mechanisms underlying these associations are largely unknown, but it appears that growth trajectories in early life have programming effects on later health outcomes. Rapid weight gain during infancy is also associated with an advanced bone age and early menarche in girls (30, 31), suggesting that a faster early growth trajectory represents a more rapid development, and might also predict earlier cellular ageing (32).

Telomere length is a marker of biological age and a shorter telomere length is associated with increased CVD risk (33-35). Telomere length can be measured in leukocytes (i.e. leukocyte telomere length (LTL)) using a quantitative PCR assay (36). Next to biological age, attrition of LTL is influenced by genetic factors and exogenous stressors (37, 38).

In Chapter 5 and 6, we postulated that accelerated biological ageing might be one of the underlying mechanisms behind the increased risk for CVD in those born with a low birth weight due to premature or SGA birth. We used LTL as a marker of biological age to test this hypothesis. Since both preterm birth and poor fetal growth can lead to small size at birth, we studied these effects separately. Moreover, we assessed LTL in young adults with different infant growth trajectories, to assess whether rapid weight gain influences LTL. Finally, we measured LTL in a group of GH-treated young adults born SGA to investigate whether GH-induced catch-up growth influences LTL.

Telomere length in young adults born preterm

In Chapter 5, we assessed LTL in 470 young adults, of which 186 were born preterm. We found a positive association between gestational age and LTL. Young adults born preterm had shorter LTL than young adults born at term. This difference remained significant after correction for size at birth, indicating an independent effect of gestational age on LTL.

Previous studies have highlighted oxidative stress as an important determinant of LTL (37, 38) and have shown that intrauterine stress causes shorter LTL (39-41). Since pregnancies resulting in preterm birth are often accompanied by increased stress exposure (42) and preterm infants are more frequently exposed to stressful events, we think it is plausible that oxidative stress is one of the explanations for the difference in

LTL between young adults born preterm and those born at term. A future study that longitudinally measures LTL and oxidative stress biomarkers in early postnatal life and late adulthood, would be a good way to evaluate if increased perinatal oxidative stress is indeed the mechanism behind shorter LTL in those born preterm.

We found no correlations between LTL and putative risk factors for CVD in these young adults. Future studies should aim to explore whether LTL may contribute to CVD and other adult onset diseases at a later age in those born preterm.

Effects of size at birth, childhood growth patterns and GH treatment on telomere length

In Chapter 6, we assessed LTL in 470 participants with different sizes at birth and infant growth trajectories. Additionally, we measured LTL in 75 young adults born SGA who were treated with GH during childhood. We found a positive association between birth length and LTL, and a trend toward a positive association between birth weight and LTL, indicating shorter LTL in young adults who were born with a low birth weight and/or length. These associations were found after adjustments for possible confounders, such as gender and gestational age, indicating an independent effect of size at birth on LTL. Our results imply that LTL could play a role in the association between small size at birth and later life CVD risk. The underlying mechanism for this association could be increased oxidative stress, similarly as in those born preterm. Future studies should aim at further exploring these mechanisms.

We found no association between weight gain during early infancy and LTL. Our results are in contrast to those of a previous study, that found an inverse association between weight gain in the first 12 months and LTL at the age of 70 (32). That association was, however, only found in women. The main difference with our study is the fact that the participants in that study were much older. For future studies, it would be interesting to analyze whether the association between weight gain during infancy and LTL becomes significant at a later age, when age-associated diseases become more apparent.

Our study was the first study to evaluate whether GH treatment is associated with LTL. Young adults born SGA who were treated with GH during childhood had similar LTL as age-matched untreated short subjects born SGA, subjects born SGA with spontaneous catch-up and controls born appropriate for gestational age (AGA) with a normal stature. We thus concluded that GH-induced catch-up in length does not lead to shorter LTL in young adults born SGA.

Conclusions and clinical implications regarding LTL in subjects born preterm and/or SGA

Our findings indicate that preterm birth and small size at birth have adverse effects on telomere length in later life, at least in early adulthood. Since telomere length is a marker

of biological age, we conclude that young adults born preterm and/or with a low birth weight, have an older biological age compared to young adults born at term with a normal birth weight. The fact that this association was already present at a young adult age, when manifestations of age-associated diseases are not present yet, is particularly intriguing. We postulate that pregnancies leading to preterm or SGA birth are associated with increased oxidative stress, which causes faster attrition of telomere length. However, before statements on clinical implications can be made, more (longitudinal) research is required. Since the prevalence and survival rate of preterm birth and low birth weight for gestational age are increasing (43, 44), our results are relevant for an increasing number of subjects.

BONE MINERAL DENSITY IN YOUNG ADULTS BORN SGA AFTER GH CESSATION

Short SGA children have a lower than average BMD, even after correction for their short stature (10, 45). A lower BMD and subsequent lower peak bone mass are risk factors for osteoporosis and bone fractures in later life (46, 47). BMD can be assessed by measuring BMD of the total body (BMD_{TB}), and of the lumbar spine by means of DXA. Since BMD of the lumbar spine in short individuals is underestimated by the areal presentation, it should be corrected for bone size by calculating the bone mineral apparent density ($BMAD_{LS}$). Previous studies in short SGA patients have shown that GH treatment improves BMD_{TB} and $BMAD_{LS}$ on the short-term (10, 45), but long-term studies were lacking. Moreover, the effects of cessation of GH treatment after AH attainment on BMD_{TB} and $BMAD_{LS}$ were unknown. In Chapter 7, we investigated BMD_{TB} and $BMAD_{LS}$ longitudinally during five years after GH cessation in young adults born SGA who were treated with GH during childhood until AH, and who had attained an age of 21 years. In addition, we compared BMD_{TB} and $BMAD_{LS}$ at five years after cessation of GH treatment to young adults born SGA with persistent short stature who were never treated with GH, and to AGA born controls with a normal stature.

We demonstrate that both BMD_{TB} and $BMAD_{LS}$ at AH are higher than the baseline BMDs reported in previous studies (10, 45). We can thus conclude that the positive effects of GH treatment are maintained until AH attainment. In the five years after GH cessation, we observed a trend toward a decline of BMD_{TB} in males, and a gradual decline of $BMAD_{LS}$ in males and females, probably due to the loss of pharmacological effects of GH treatment. The higher BMD_{TB} and $BMAD_{LS}$ at young adult age, the lower the risk of osteoporosis in later life. Thus, although BMD_{TB} and $BMAD_{LS}$ in formerly GH-treated subjects were within the normal range, it is unfortunate that the beneficial effects of GH treatment are partly lost after cessation of treatment.

Despite the deteriorations in the years after GH cessation, average BMD_{TB} and $BMAD_{LS}$ at five years after GH cessation were above -1 SDS, and only a very small percentage of

all subjects had a BMD_{TB} or $BMAD_{LS}$ below -2 SDS. Moreover, at five years after cessation of treatment, previously GH-treated young SGA adults had a similar BMD_{TB} and $BMAD_{LS}$ as young SGA adults who remained untreated and as controls born AGA with a normal stature, which is reassuring.

Conclusions and clinical implications regarding BMD in young adults born SGA

We demonstrated that GH treatment improves BMD_{TB} and $BMAD_{LS}$ in subjects born SGA. After cessation of GH, $BMAD_{LS}$ gradually deteriorates and there is a trend toward a deterioration of BMD_{TB} in males. However, BMD_{TB} and $BMAD_{LS}$ at five years after GH cessation were above -1 SDS, and only a very small percentage of all subjects had a BMD_{TB} or $BMAD_{LS}$ below -2 SDS. At the age of 21 years, BMD_{TB} and $BMAD_{LS}$ are similar in GH-treated and untreated subjects born SGA. Future studies should aim at investigating how BMD_{TB} and $BMAD_{LS}$ progress when formerly GH-treated adults born SGA get older, and what the long-term clinical implications will be.

GENERAL CONCLUSIONS

SRS is one of the causes of short SGA and children with SRS can be treated with GH under the indication short SGA. However, whether SRS patients benefit the same from GH treatment as non-SRS patients born SGA was unknown. Moreover, metabolic health in SRS patients had never been evaluated before, and there were no data on the long-term natural history of SRS regarding pubertal development and gonadal function. These issues are addressed in this thesis. From our studies we conclude that GH treatment is safe and effective in patients with SRS. This is true for each SRS subtype, although those with 11p15 LOM might attain a lower AH. Furthermore, we conclude that at least until the age of 18 years, there is no difference in metabolic health between SRS patients and non-SRS patients born SGA. Finally, we conclude that puberty progresses similarly in SRS and non-SRS patients born SGA, but that Sertoli cell dysfunction is more common in SRS males. In females with SRS, gonadal function does not seem to be impaired, but there seems to be an increased risk of Müllerian agenesis.

In the second part of this thesis, we measured the ageing biomarker LTL in young adults who were born preterm and in young adults with differences in size at birth and postnatal growth patterns, including GH-induced catch-up growth. Our findings indicate that preterm birth and small size at birth have adverse effects on telomere length in early adulthood. GH-induced catch-up in length does not lead to shorter LTL in young adults born SGA. To draw definite conclusions on the clinical implications of these results, more longitudinal research is needed.

Finally, we longitudinally assessed BMD_{TB} and $BMAD_{LS}$ in young adults born SGA, during five years after GH cessation due to AH attainment. We found that BMD_{TB} and $BMAD_{LS}$

improve during GH treatment, but that there is a gradual deterioration of $BMAD_{LS}$ and a trend toward a deterioration of BMD_{TB} after cessation of GH in males. At five years after GH cessation, at the age of 21 years, BMD_{TB} and $BMAD_{LS}$ are similar in GH-treated and untreated subjects born SGA.

CLINICAL IMPLICATIONS OF THIS THESIS

- SRS patients benefit from GH treatment and should start GH therapy at a young age, to enlarge the window during which height gain can be achieved.
- SRS patients with an expected AH below -2.5 SDS at onset of puberty benefit from the addition of two years of GnRHa treatment to postpone puberty.
- Although metabolic health is similar in SRS and non-SRS SGA at the age of 18 years, it is important that SRS patients are not lost to follow-up once they have attained AH and GH treatment is discontinued, so that the long-term natural history of SRS can be monitored.
- It is recommended to assess gonadal function in SRS patients once they attain an adult pubertal stage. This is particularly the case for SRS males, since Sertoli cell dysfunction is common. Although gonadal function does not seem to be impaired in females with SRS, it is important to be aware of the increased risk for Müllerian agenesis, so that a timely diagnosis can be made.
- There is a need for expertise centres which combine research with optimal care for SRS patients.
- Subjects born preterm and/or SGA have shorter telomere length, but more longitudinal research is required before statements on clinical implications can be made.

DIRECTIONS FOR FUTURE RESEARCH

- Elucidating the underlying (epi)genetic mechanisms of clinical SRS is important to improve the care for clinical SRS patients that now remain with an unknown genetic diagnosis.
- It is important to participate in international collaborations with other study groups, to obtain larger cohorts of SRS patients.
- Setting up studies with a long follow-up to assess metabolic health in adults with SRS, beyond the age of 18 years, will help in learning more about the long-term natural history of SRS.
- Longitudinally measuring oxidative stress biomarkers in early postnatal life, childhood and late adulthood, will help to elucidate whether increased perinatal oxidative stress is one of the mechanisms underlying the association between small size at birth, shorter LTL and adult onset diseases.

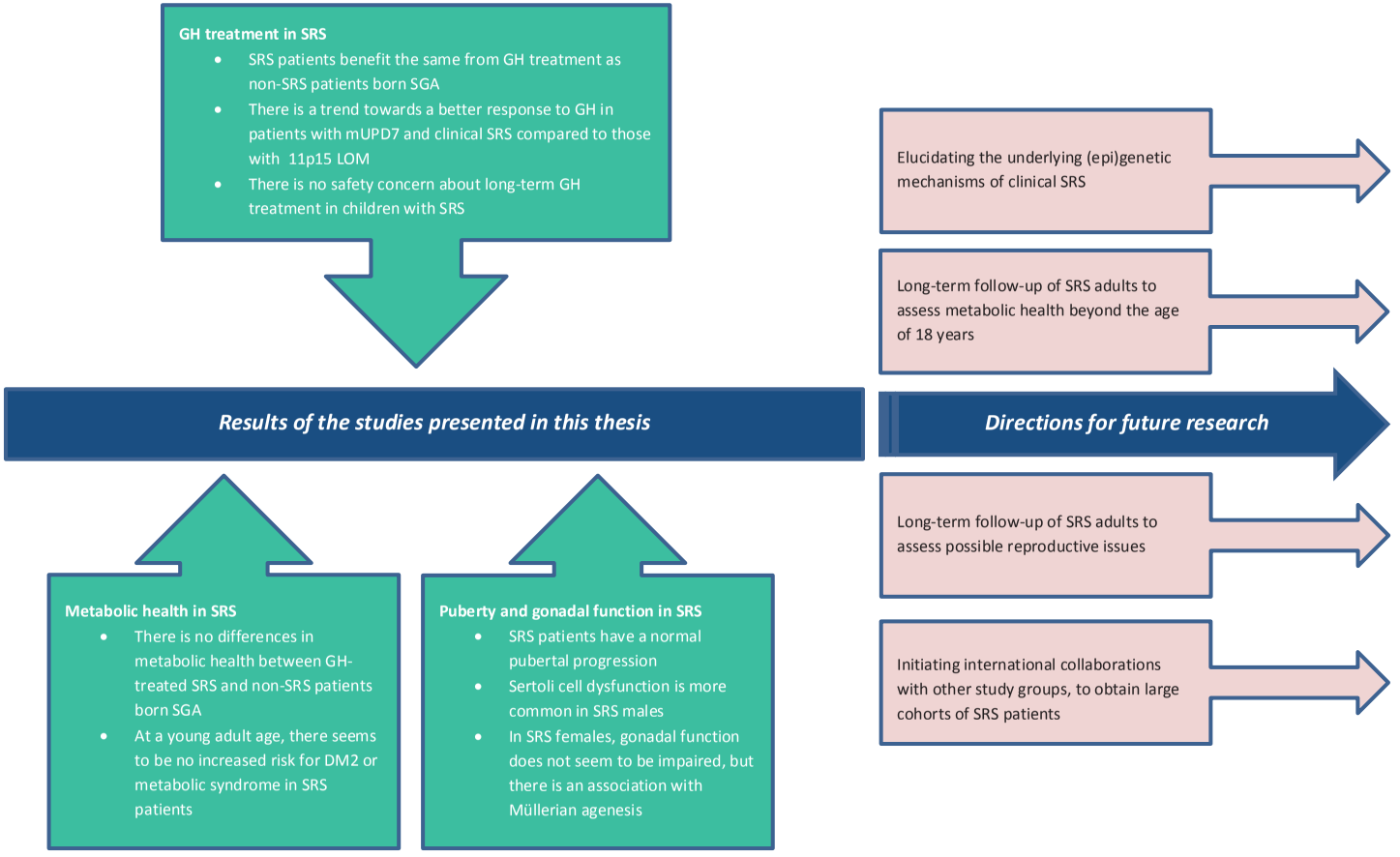

Figure 1. Silver-Russell syndrome – Results of the studies presented in this thesis and directions for future research

- Since previous studies found an association between rapid weight gain during infancy and CVD risk later in life, it is important to analyze whether there is an association between accelerated weight gain during infancy, shorter LTL and adult onset diseases.
- Since we found a deterioration of $BMAD_{LS}$ after cessation of GH treatment in subjects born SGA, it is important to investigate how BMD_{TB} and $BMAD_{LS}$ progress on the very long-term after cessation of GH treatment in subjects born SGA.

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Chapter 9

Summary

Samenvatting

SUMMARY

Chapter 1

In Chapter 1 we provide an overview of the definition, prevalence and causes of small for gestational age (SGA) birth. We describe the etiology and clinical aspects of Silver-Russell syndrome (SRS) and give an update about the effects of growth hormone (GH) treatment in children with and without SRS who were born SGA. We also provide an introduction on parameters addressed in this thesis, namely gonadal function, leukocyte telomere length and bone mineral density (BMD). Finally, we present the aims and outline of this thesis.

Chapter 2

In Chapter 2, we compared response to GH treatment between 62 SRS patients and 227 short, non-syndromic subjects born SGA (non-SRS). We show that SRS patients respond well to GH treatment and that total height gain is similar in SRS and non-SRS subjects born SGA. There was a trend toward a better response to GH treatment in SRS patients with mUPD7 and idiopathic SRS compared to patients with 11p15 LOM, but GH treatment proved also beneficial for those with 11p15 LOM. Since no adverse events occurred over a long treatment period, there is no safety concern about GH in SRS patients.

Chapter 3

In Chapter 3, we compared long-term data on metabolic health and safety of GH treatment between SRS and non-SRS patients. We found that SRS and non-SRS patients have a very similar metabolic health profile at start of treatment, and that, apart from minor variations, the metabolic health profile of SRS and non-SRS patients responds similarly to GH treatment. Two years after cessation of GH treatment, at the age of 18 years, there was no difference in risk for metabolic syndrome between SRS and non-SRS, and none of the patients had developed diabetes mellitus type 2.

Chapter 4

In Chapter 4, we assessed onset and progression of puberty and gonadal function in 32 SRS patients. We compared these data to a control group of subjects born SGA without SRS, and to age-appropriate reference data of healthy Dutch adolescents. We found that SRS patients have a similar age at onset of puberty and a similar pubertal progression as non-SRS subjects born SGA. Although gonadal function was on average similar in SRS and non-SRS subjects born SGA, disturbances in Sertoli cell function were more common in SRS males. Gonadal function does not seem to be impaired in SRS females with a normal pubertal progression.

Chapter 5

In Chapter 5, we analyzed the association between gestational age and leukocyte telomere length (LTL) in 470 young adults born preterm and at term. We found that gestational age is positively associated with LTL and that young adults born preterm have shorter LTL than age-matched subjects born at term. This difference remained significant after correction for birth length and birth weight, indicating an independent positive effect of gestational age on LTL, not confounded by birth size. These results could reflect pre- and postnatal oxidative stress and could partly explain the association between preterm birth and risk for cardiovascular disease (CVD) in later life. At this young age, we found no relation between LTL and other putative risk factors for CVD.

Chapter 6

In Chapter 6, we investigated whether size at birth, childhood growth patterns and adult body size influence LTL. We also analyzed the effect of GH treatment on LTL by comparing LTL between previously GH-treated young adults born SGA with age-matched untreated short subjects born SGA, SGA born subjects with spontaneous catch-up to a normal height and controls born appropriate for gestational age (AGA) with a normal stature. We found that size at birth, gestational age and female gender were positively associated with LTL and smoking negatively, while adult fat mass and gain in weight and height during infancy and from birth to adulthood were not associated with LTL. Since the subjects who received GH treatment during childhood had similar LTL as age-matched untreated short SGA subjects, subjects born SGA with spontaneous catch-up and controls born AGA, we concluded that there are no adverse effects of GH treatment on LTL.

Chapter 7

In Chapter 7, we present longitudinal data on BMD of the total body (BMD_{TB}) and bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) in young adults born SGA, during the five years after cessation of GH treatment due to adult height attainment. We found that GH treatment improves BMD_{TB} and $BMAD_{LS}$ in subjects born SGA. After cessation of GH, $BMAD_{LS}$ gradually deteriorates and there is a trend toward a deterioration of BMD_{TB} in males, although BMD remained within the normal range and almost all participants had a BMD above -2 SDS. At the age of 21 years, BMD_{TB} and $BMAD_{LS}$ were similar in GH-treated and untreated subjects born SGA.

Chapter 8

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

SAMENVATTING

Hoofdstuk 1

In hoofdstuk 1 beschrijven we de definitie, prevalentie, en oorzaken van een kleine lengte en/of laag geboortegewicht voor de zwangerschapsduur (small for gestational age (SGA)). Ook bespreken we de etiologie en de klinische aspecten van Silver-Russell syndroom (SRS), evenals de effecten van groeihormoon (GH) behandeling bij SGA geboren kinderen met en zonder SRS. We geven een introductie over parameters die we beschrijven in dit proefschrift, zoals telomeerlengte (leukocyte telomere length (LTL)) en botdichtheid. Ten slotte bespreken we de doelstellingen van de studies.

Hoofdstuk 2

In Hoofdstuk 2 onderzochten we de effecten van GH behandeling op de groei bij 62 patiënten met SRS. We vergeleken deze data met die van 227 kleine, niet-syndromale kinderen die SGA geboren waren (non-SRS). We laten zien dat SRS patiënten goed reageren op GH en dat de totale lengtewinst vergelijkbaar is tussen SRS en non-SRS. Er was een trend naar een betere respons bij SRS patiënten met mUPD7 of idiopathische SRS in vergelijking met patiënten met 11p15 LOM, maar GH behandeling heeft ook gunstige effecten bij de patiënten met 11p15 LOM. Aangezien er geen bijwerkingen werden gevonden gedurende een lange behandelduur, zijn er geen zorgen over de veiligheid van GH behandeling bij SRS patiënten.

Hoofdstuk 3

In Hoofdstuk 3 laten we lange-termijn data zien betreffende het gezondheidsprofiel en de veiligheid van GH behandeling bij SRS patiënten in vergelijking met non-SRS. We laten zien dat SRS en non-SRS patiënten een zeer vergelijkbaar gezondheidsprofiel hebben bij start van de behandeling. Los van kleine verschillen reageerden SRS en non-SRS patiënten vergelijkbaar op GH behandeling. Op de leeftijd van 18 jaar was er geen verschil in risico op metabool syndroom tussen SRS en non-SRS, en waren er geen patiënten met diabetes type 2.

Hoofdstuk 4

In Hoofdstuk 4 onderzochten we het verloop van de puberteit en gonadale functie bij SRS patiënten. We vergeleken deze data met een groep van SGA geboren jongvolwassenen en met een groep gezonde jongvolwassenen. SRS patiënten hadden een vergelijkbare leeftijd bij start van de puberteit, en een vergelijkbare progressie van puberteitsstadia als non-SRS patiënten. Hoewel de gonadale functie over het algemeen vergelijkbaar was tussen SRS en non-SRS, kwam dysfunctie van de Sertoli cellen meer voor bij mannen met SRS. Bij vrouwen met SRS lijkt de gonadale functie niet verstoord.

Hoofdstuk 5

In Hoofdstuk 5 analyseerden we de associatie tussen zwangerschapsduur en LTL bij 470 jongvolwassenen. We vonden dat zwangerschapsduur positief geassocieerd is met LTL en dat prematuur geboren jongvolwassenen kortere telomeren hebben dan a term geboren jongvolwassenen. Dit verschil bleef significant na correctie voor geboortelengte en geboortegewicht, wat indiceert dat er een onafhankelijk effect is van zwangerschapsduur op LTL. Dit zou het gevolg kunnen zijn van perinatale oxidatieve stress en zou één van de verklaringen kunnen zijn van de associatie tussen prematuriteit en het risico op hart- en vaatziekten op latere leeftijd. We vonden op deze jonge leeftijd geen relatie tussen LTL en determinanten van hart- en vaatziekten.

Hoofdstuk 6

In Hoofdstuk 6 onderzochten we de invloed van geboortelengte en –gewicht, groei in het eerste levensjaar, en volwassen lichaamssamenstelling op LTL. Ook onderzochten we het effect van GH behandeling op LTL bij SGA geborenen. Geboortegewicht en –lengte, zwangerschapsduur en vrouwelijk geslacht waren positief geassocieerd met LTL, en roken negatief. Toename in gewicht en lengte gedurende het eerste levensjaar en van geboorte tot aan de jongvolwassen leeftijd waren niet geassocieerd met LTL. GH behandelde SGA geboren jongvolwassenen hadden een vergelijkbare LTL als onbehandelde SGA geboren jongvolwassenen, SGA geboren jongvolwassenen met spontane inhaalgroei en controles met een normaal geboortegewicht en –lengte.

Hoofdstuk 7

In Hoofdstuk 7 presenteren we longitudinale data over botdichtheid van het totale lichaam en de lumbale wervelkolom tot vijf jaar na het staken GH behandeling bij SGA geboren jongvolwassenen. We lieten zien dat GH behandeling de botdichtheid verbetert. Na het staken van GH neemt de botdichtheid van de lumbale wervelkolom af en er is een trend naar een afname van de botdichtheid van het totale lichaam bij mannen, hoewel deze binnen de normale range blijft en vrijwel alle deelnemers een botdichtheid boven de -2 SDS hadden. Op de leeftijd van 21 jaar hadden GH behandelde en onbehandelde SGA geboren jongvolwassenen een vergelijkbare botdichtheid.

Hoofdstuk 8

In dit hoofdstuk bespreken we de belangrijkste resultaten van onze studies in een bredere context, evenals de klinische implicaties en suggesties voor toekomstig onderzoek.

Chapter 10

List of abbreviations

List of co-authors and affiliations

List of publications

PhD portfolio

Acknowledgments

About the author

LIST OF ABBREVIATIONS

11p15	LOM loss of methylation in the 11p15 region
AGA	appropriate for gestational age
AH	adult height
AIR	acute insulin response
AIRg	acute insulin response to glucose
AMH	anti-Müllerian hormone
ANCOVA	analysis of covariance
ATP-III	adult treatment panel III
BL	birth length
BMAD	bone mineral apparent density
$BMAD_{LS}$	bone mineral apparent density of the lumbar spine
BMD	bone mineral density
BMD_{TB}	bone mineral density of the total body
BMI	body mass index
BP	blood pressure
BW	birth weight
CI	confidence interval
CVD	cardiovascular disease
DBP	diastolic blood pressure
DI	disposition index
DM2	diabetes mellitus type 2
DXA	dual energy X-ray absorptiometry
FM	fat mass
FM%	fat mass percentage
FSH	follicle stimulating hormone
FSIGT-test	frequently sampled intravenous glucose tolerance test
GA	gestational age
GFR	glomerular filtration rate
GH	growth hormone
GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone analogue
HDLc	high-density lipoprotein cholesterol
hsCRP	high sensitivity C-reactive protein
ICR	imprinting control region
IGF-I	insulin-like growth factor-I
IGFBP-3	insulin-like growth factor binding protein-3
IUGR	intrauterine growth retardation

IQR	interquartile range
LBM	lean body mass
LDLc	low-density lipoprotein cholesterol
LH	luteinizing hormone
LTL	leukocyte telomere length
M2	breast development stage II according to Tanner
MR	multiple regression
MRKH	Mayer-Rokitansky-Küster-Hauser syndrome
MS-MLPA	methylation-specific multiplex ligation-mediated probe amplification
mUPD7	maternal uniparental disomy of chromosome 7
OC	oral contraceptive
PCR	polymerase chain reaction
SBP	systolic blood pressure
SCFE	slipped capital femoral epiphysis
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
SRS	Silver-Russell syndrome
T/S	ratio telomere to single-gene copy ratio
TC	total cholesterol
Tg	triglyceride
TH	target height
TV	testicular volume

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LIST OF PUBLICATIONS

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 Mar; 102(3):983-991.*

Smeets CC, Codd V, Samani N, Hokken-Koelega AC. Effects of size at birth, childhood growth patterns and growth hormone treatment on leukocyte telomere length. *PLoS One. 2017 Feb; 12(2):e0171825.*

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 Feb; 5(2):106-116.*

Smeets CC, Zandwijken G, Renes JS, Hokken-Koelega AC. Long-term results of growth hormone treatment in children with Silver-Russell syndrome (SRS): Do They Benefit the Same as Non-SRS Short-SGA? *J Clin Endocrinol Metab. 2016 May; 101(5):2105-12.*

Smeets CC, Codd V, Samani N, Hokken-Koelega AC. Leukocyte telomere length in young adults born Preterm: support for accelerated biological Ageing. *PLoS One 2015 Nov; 10(11):e0143951.*

Smeets CC, Goedegebuure WJ, Renes JC, de Rijke YB, Hokken-Koelega AC. Pubertal development and gonadal function in patients with Silver-Russell syndrome. *Submitted.*

Smeets CC, van der Steen M, Renes JS, Hokken-Koelega AC. Bone mineral density after cessation of GH treatment in young adult born SGA: *A 5-year longitudinal study. J Clin Endocrinol Metab 2017; In press.*

van der Steen M, Kerkhof GF, **Smeets CC**, Hokken-Koelega AC. A 5-year longitudinal study on cardiovascular risk factors and cIMT in young adults born small for gestational age after cessation of GH treatment. *Submitted.*

PHD PORTFOLIO

Summary of PhD training and teaching activities

Erasmus MC Department	Pediatrics, Subdivision of Endocrinology
PhD period	July 2013 – September 2017
Promotor	Prof. dr. A.C.S. Hokken-Koelega
Copromotor	Dr. J.S. Renes
Research School	Molecular Medicine Postgraduate School (MolMed)

General courses	**Year**	**ECTS**
Presenting skills for scientists (Molmed)	2016	1.0
Research Integrity	2015	0.3
English Biomedical writing and communication (Molmed)	2015	3.0
Presenting skills (Molmed)	2014	1.0
Biostatistical Methods I (NIHES)	2014	5.7
Patient oriented research (CPO)	2014	0.3
Good Clinical Practice	2013	1.0
Specific courses		
Safety Training MRI (Radiology department)	2016	0.3
Access (Molmed)	2016	0.3
Excel (Molmed)	2016	0.3
Photoshop and Illustrator (Molmed)	2016	0.3
GraphPad Prism (Molmed)	2015	0.3
Basic Human Genetics course (Molmed)	2015	0.5
Endnote (Medical Library)	2013	-
Seminars and workshops		
TULIPS Child Health Symposium	2016	0.6
NWO Women in Science day	2015	0.6
Young investigators day, TULIPS/NVK	2015	0.6
Weekly Pediatric Endocrinology meetings, Erasmus MC	2013 – 2017	4.0
Annual PhD day	2013 – 2017	1.8
Annual Pediatric research day, Erasmus MC	2013 – 2017	1.2
Conferences		
International		
10th IMPE, Washington *oral + poster presentation*	2017	2.0
4th International Conference Nutrition&Growth, Amsterdam *invited speaker*	2017	2.0
55th Annual Meeting of ESPE, Paris *oral + poster presentation*	2016	2.0
54th Annual Meeting of ESPE, Barcelona *poster presentation*	2015	1.0
53th Annual Meeting of ESPE Dublin *poster presentation*	2014	1.0

National		
Dutch society for pediatrics (NVK) *slam session*	2017	1.0
Dutch Endocrine Meeting *poster pitch + presentation*	2017	1.0
Dutch society for pediatrics (NVK) *slam session*	2015	1.0
Lecturing		
Dutch Advisory Board Growth Hormone, Pediatric Endocrinology	2016	1.0
SGA symposium	2016	1.0
Laboratory of Genetics, Amsterdam Medical Centre	2016	1.0
Annual SGA Day (SGA platform)	2013-2017	1.0
IMC Weekendschool "Growth&Development"	2013	1.0
Contribution ESPE e-learning	2013	2.0
Supervising		
Coach of bachelor students, Faculty of Medicine Rotterdam	2016-2017	2.0
Advising		
Medical advisor SGA platform	2017	0.5
Research Proposals		
Follow-up study of subjects who participated in the IUGR-1, IUGR-2 and PROGRAM/ PREMS studies during childhood and early adulthood.	2015	5.0
Long term effects of growth hormone many years after discontinuation.		
Other activities		
Peer review of articles for international scientific journals	2016-2017	0.5
Writing Guideline "GH treatment in SRS patients" (Dutch Advisory Board Growth Hormone)	2016-2017	1.0
Contribution to application Expert Centre "Rare Growth disorders"	2016-2017	2.0
Organizing committee "SOV education committee"	2014-2015	2.0
Organizing committee Sophia Research day	2015-2016	2.0

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ABOUT THE AUTHOR

Lin Smeets was born on April 15th 1987 in Nijmegen, the Netherlands. She completed her high school at the 'Nijmeegse Scholengemeenschap Groenewoud' in 2005 after which she moved to Utrecht to start her medical training at the Faculty of Medicine of Utrecht University.

Lin conducted her internship Gynecology and Obstetrics in Léon, Nicaragua. During the final year of her medical training, she combined her interest for pediatrics and research at the Wilhelmina Children's Hospital in Utrecht, where she investigated pulmonary complications in children after stem cell transplantation. She obtained her medical degree in 2012, upon which she started working as a pediatric resident at the Amphia hospital in Breda.

In July 2013, Lin started working on a clinical research project at the department of Pediatric Endocrinology of Erasmus MC – Sophia Children's Hospital. The results of this PhD project are presented in this thesis. During this period, Lin went to Kenya with a medical team of medical checks for children, where she screened over a 1000 children for diseases such as malaria, malnutrition and HIV. Furthermore, Lin was a member of the Sophia Education Committee, was a coach of bachelor students, organized the Sophia Research Day of 2016 and is medical advisor of the SGA platform.

For over 25 years, our research group and others have been investigating children born small for gestational age (SGA) with persistent short stature, and the efficacy and safety of biosynthetic growth hormone treatment (GH) in these children.

One of the causes of short SGA is Silver-Russell syndrome (SRS). This doctoral thesis presents the response to, and safety of GH treatment, and describes pubertal progression and metabolic health in patients with SRS.

Because being born with a lower birth weight leads to an increased risk for age-associated diseases, health in later life has been a concern in patients born SGA. In the second part of this thesis, we present data on parameters associated with health in later life, such as telomere length and bone mineral density, and the effects of GH treatment on these parameters.