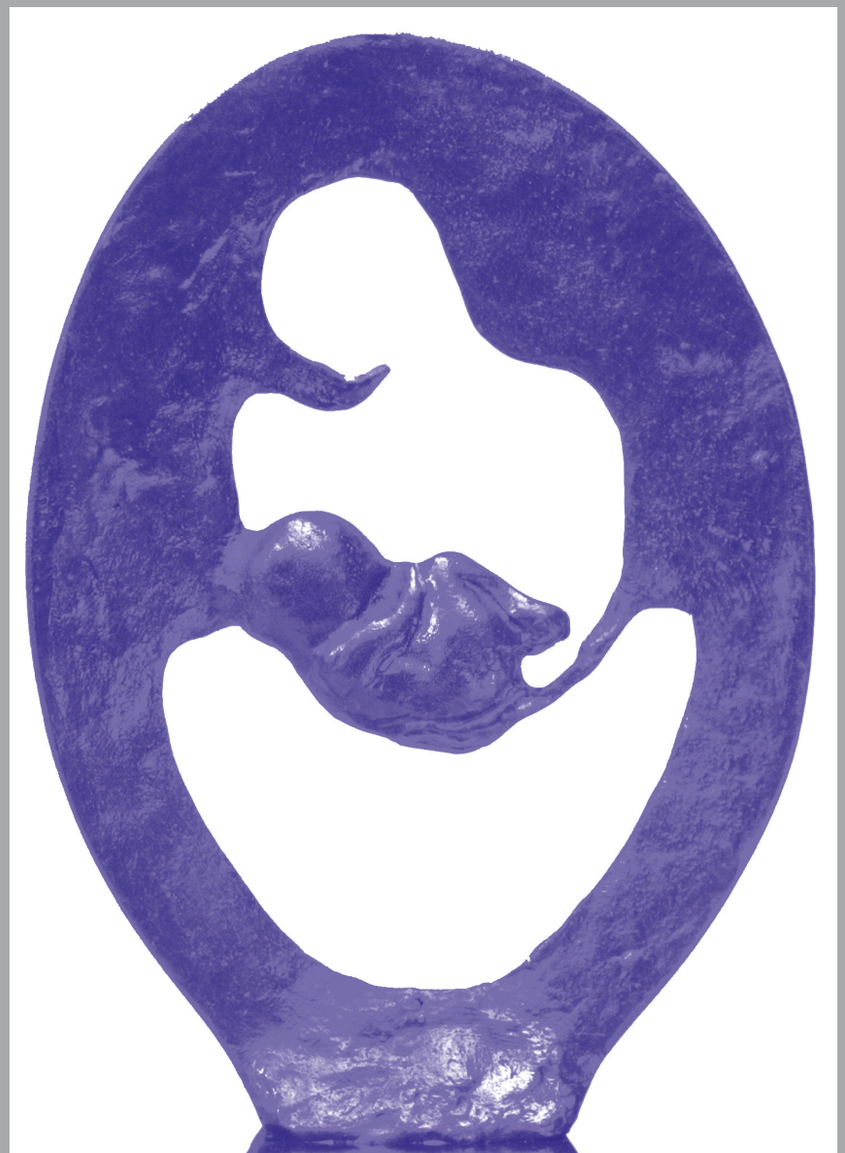
Short Children Born Small for Gestational Age (SGA)

Adrenarche, pubarche, gonadal function, pubertal development, food intake, quality of life and effect of growth hormone treatment



Venje Boonstra

Short Children Born Small for Gestational Age (SGA) Venje Boonstra

Short Children Born Small for Gestational Age (SGA)

Adrenarche, pubarche, gonadal function, pubertal development, food intake, quality of life

and

effects of growth hormone treatment

Venje Boonstra

Sculpture: Sigrid van Rheden
Lay-out: Legatron Electronic Publishing, Rotterdam
Printer: PrintPartners IPSKAMP, Enschede

ISBN 9056773365

Short Children Born Small for Gestational Age (SGA)

Adrenarche, pubarche, gonadal function, pubertal development, food intake,

quality of life

and

effects of growth hormone treatment

Klein geboren kinderen (SGA) met een persisterende kleine lengte

Adrenarche, pubarche, gonadale functie, puberteitsontwikkeling, voedingsinname,
kwaliteit van leven
en
effecten van groeihormoon behandeling

Proefschrift
ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor promoties.

De openbare verdediging zal plaatsvinden op 25 januari 2006 om 13.45 uur

door

Venje Henke Boonstra

geboren te Mqanduli (Zuid Afrika)

Promotiecommissie:

Promotor: Prof.dr. A.C.S. Hokken-Koelega

Overige leden: Prof.dr. F.H. de Jong, Prof.dr. J.M. Wit, Prof.dr. J.B. van Goudoever

The printing of this thesis and the studies described were financially supported by Novo Nordisk Farma B.V., The Netherlands and Novo Nordisk A/S, Denmark.

Govert, Femke, Meintje en mijn ouders

Contents

Chapter 1

Introduction

Introduction

This chapter describes definitions of small for gestational age (SGA), prevalence and etiology of SGA, factors involving fetal growth and long term effects of being born SGA. Also the effects of growth hormone (GH) treatment in these children is discussed. Finally, the aims of the study, the outline of the thesis, the study design and in- and exclusion criteria of the Dutch multicenter GH-trials are described.

1 Small for gestational age (SGA)

Definitions of SGA

In literature, the term SGA has been applied to newborns having a birth weight and/or a birth length below the third or tenth percentile (or below −1.88 or −1.29 SDS) for gestational age. Recently consensus has been reached among investigators regarding the definition of SGA.[1] Although the definition is somewhat arbitrary, the definition of SGA has been delineated as a birth length and/or birth weight < −2 SD for gestational age. It has been agreed to use the reference data of Usher and McLean.[2] From the start in 1991 all Dutch multicenter studies in short SGA children have defined SGA as a birth length below the −2 SDS using the curves of Usher and Mclean.[2]

The term intra-uterine growth retardation (IUGR) has also been used to describe infants with a small size at birth. IUGR, however, refers to fetal growth retardation and can be observed as a deviation of the intra-uterine growth chart. Therefore, IUGR can only be diagnosed when documented by two intrauterine growth assessments by ultrasound measurements. SGA does not refer to fetal growth but refers to body size at birth. Not all SGA infants have suffered from intrauterine growth retardations as an SGA infant may have been small from the beginning of fetal life. Also, growth retardation late in gestation does not need to result in an SGA infant (Figure 1).

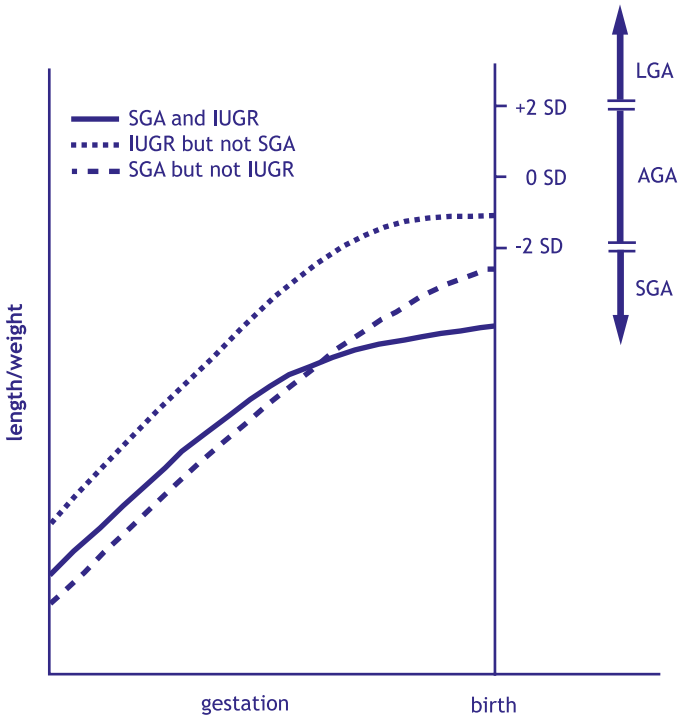


Figure 1: Growth chart showing difference between infants born SGA or IUGR. Definition of SGA is birth length/weight of 2 or more standard deviations (SD) below the mean for gestational age and sex.

According to a frequently used classification, SGA infants can be divided into 'symmetric' versus 'asymmetric' SGA.[3] The term 'symmetric' is applied to SGA neonates having a low birth weight, a small birth length and a small birth head circumference. 'Asymmetric' SGA neonates have a relatively large head since birth weight and birth length are low whereas head circumference at birth is normal. Generally, growth retardation early in fetal life results in symmetrically small neonates whereas growth retardation later in fetal life results in asymmetrical SGA neonates. Another classification distinguishes 'proportionate' from 'disproportionate' SGA.[4] This subdivision uses birth weight and birth length only. Those SGA neonates having both a low birth weight and a small birth length are referred to as 'proportionate' SGA neonates whereas those only having a low birth weight are called 'disproportionate' SGA neonates. Infants can also be classified into 4 categories according to their birth weight (W) and birth length (L).[5] SGA neonates who are both light and short are classified as SGA_{L+W}, short neonates as SGA_L and light neonates as SGA_W (Figure 2). It is important to describe these classifications since different SGA subsets may have different underlying mechanism for SGA and may respond differently to therapy. In this thesis we have described short children born SGA_{L+W} or SGA_L.

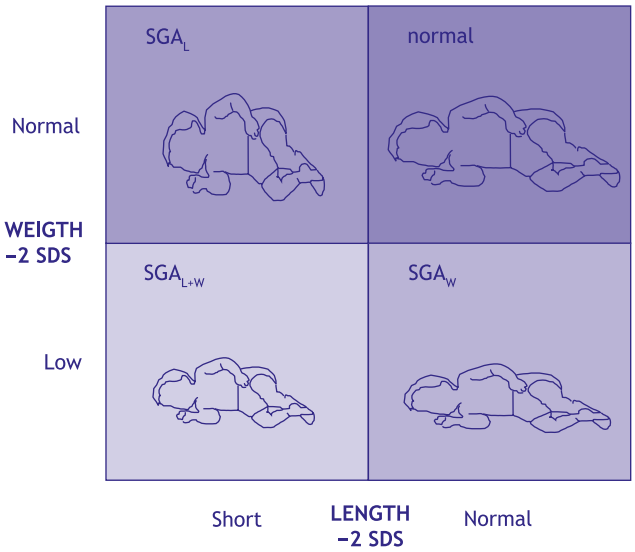


Figure 2. Different classification of SGA infants. The left boxes represent the children described in this thesis. Adapted from Albertsson-Wikland K, Karlberg J Paediatric Research. 1995;733-739.

Prevalence and etiology of SGA

Approximately 4% of all live born neonates are born SGA when SGA is defined as birth length or birth weight below 2 SDS. The cause of SGA should be identified whenever possible as underlying mechanisms are diverse and may influence prognosis and treatment effects. Impaired fetal growth can be caused by a number of fetal, maternal, placental, and demographic factors (Figure 3). Thus SGA infants are not a homogeneous group but consist of several subgroups with distinct etiologies. Growth restriction remains unidentified in up to 40% of cases.[6]

Fetal factors include genetic abnormalities, congenital defects, metabolic diseases and multiple gestations. Specialized genetic tests may be helpful to detect the presently known genetic defects. Fifteen to 20% of SGA cases may be a result of fetal factors. Chromosomal aberrations make up 5 to 7% of all SGA births, with trisomy 21, trisomy 18, and Turner syndrome being common among the abnormalities.[6,7] In fact, approximately 38% of infants with chromosomal abnormalities are born with a weight and length below −2 SDS for gestational age.[3,8]

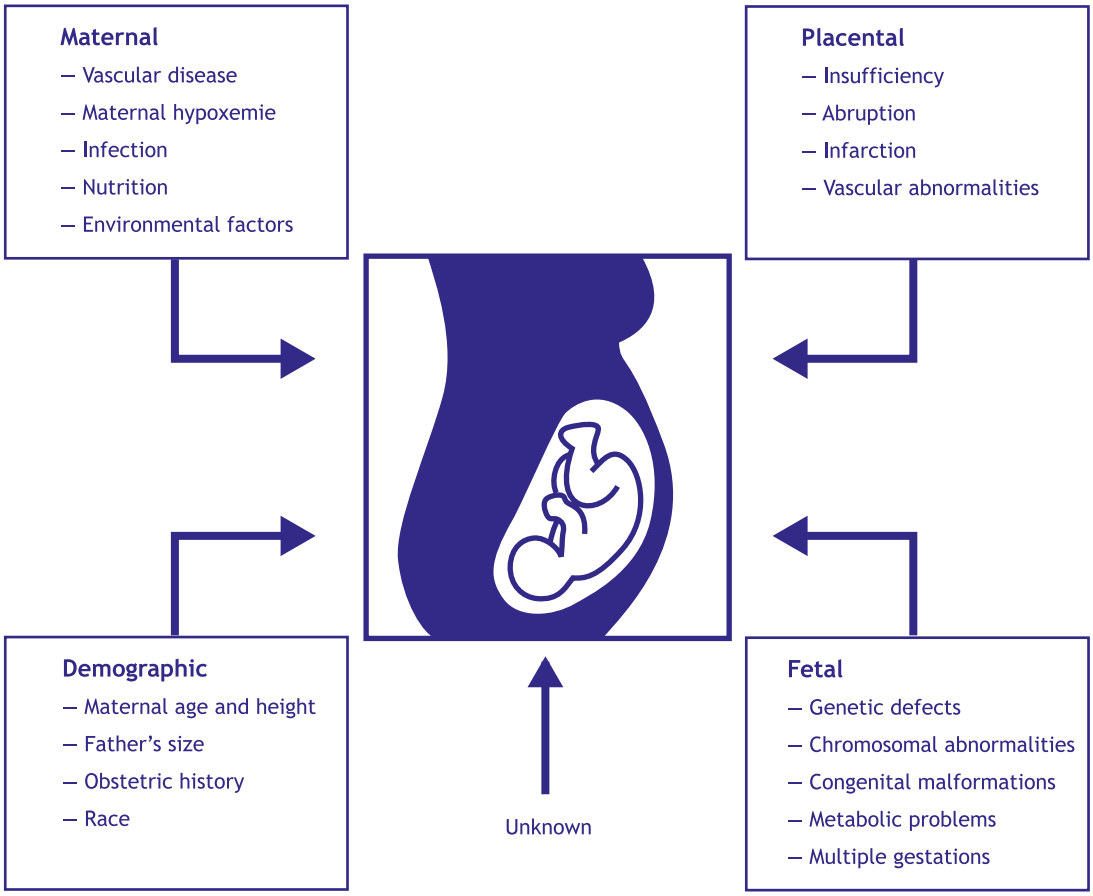


Figure 3. Causes of SGA.

Maternal factors can be divided into medical complications and environmental factors. Medical complications include: chronic vascular diseases (secondary to conditions such as hypertension, diabetes mellitus, systemic lupus erythematosus, renal disorders, and collagen vascular diseases), conditions associated with maternal hypoxemia (such as asthma, sickle cell anemia), infections (particularly toxoplasmosis, rubella, cytomegalovirus and herpes virus), malnutrition. Environmental factors include: use of therapeutic drugs (antimetabolites, anticonvulsants and anticoagulants), illicit drug use (heroin, methadone and cocaine), alcohol abuse and cigarette smoking. Environmental factors seem to directly restrict fetal growth. Cigarette smoking is one of the most common environmental causes of SGA birth. Cigarette smoking is associated with reduced uterine blood flow and impaired fetal oxygenation. Studies show that women who smoke during pregnancy have more than twice the relative risk of delivering an SGA

child compared with their non-smoking counterparts. Up to 5–10% of SGA births may be caused by maternal infection.[6,8,9]

Placental factors involve problems in placental perfusion. As the placenta is essential for nutrient and oxygen supply from mother to fetus, it is clear that any placental dysfunction could result in fetal growth retardation. Placental insufficiency is one of the most common contributors to fetal growth retardation. Examples of placental insufficiency are under or over invasion of fetal trophoblast cells into the maternal deciduas or abruption, infarction or other placental vascular abnormalities. Examination of the placenta by a pathologist might discover specific causes.

Demographic factors include maternal race, obstetric history, age of the mother, height of the mother and father and multiple gestation particularly in case of shared fetal circulation. Older maternal age has been linked to fetal growth restriction, while the influence of very young maternal age is uncertain. Although the incidence of SGA neonates is higher among teenage mothers, it is unclear whether age alone or other socio-economic factors are the cause. Maternal race can also influence fetal growth. For example, studies suggest that black American women are more apt to bear SGA infants than are white American women. Asian and Indian neonates, however, may be "constitutionally small" due to a genetic predisposition rather than fetal growth restriction. In many situations, it has proven difficult to separate genetic, environmental and cultural factors.[8]

Silver-Russel Syndrome

A subgroup of children born SGA consists of children with Silver-Russell Syndrome (SRS) (Figure 4). Typical features of children with SRS are a severely reduced birth weight and birth length, short stature during childhood and adulthood, typical craniofacial abnormalities with a relatively large, prominent forehead (frontal bossing), a small, triangular face, asymmetry of head and limbs, clinodactyly of the fifth finger and some other minor abnormalities.[10] A mean final height of 151.2 (cm) for boys and 139.7 (cm) for girls has been reported in patients with SRS. Chromosome abnormalities have been reported in only a minority of children.[10] The reported genetic abnormalities comprise a heterogeneous group. Maternal uniparental disomy of chromosome 7 is the most frequently observed abnormality and has been described in 7–10% of SRS children.[10] Other less frequently observed abnormalities include a ring chromosome 15, deletion of distal q15, translocation of the distal part of chromosome 17q, trisomy 18 mosaicism and deletion of the short arm of chromosome 8q11–13.[11] However many children with genetically proven SRS do not present all characteristics features of SRS whereas on the other hand some children have classical features without proven chromosomal abnormalities thus far. This means that the diagnosis of SRS is primarily based on clinical

features, only in few children supported by a chromosomal abnormality. A large variety exists in the presence of Silver-Russell symptoms. Some children born SGA show only minor features of SRS (*e.g.* only clinodactyly) which makes it sometimes difficult to diagnose SRS. It seems that a certain range exists varying from Silver-Russell like (only one or two minor symptoms) to the classical SRS (all major symptoms). Since growth patterns of children with SRS do not differ from short children born SGA,[10] children with SRS were included in most trials investigating growth aspect of short children born SGA.

Figure 4. An 11 years old girl with SRS, having striking features such as; frontal bossing, triangular face and facial asymmetry.

2. Fetal growth

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

Growth hormone (GH)

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

However, it was reported that birth length in these infants was on average 1 standard deviation (SD) lower compared to healthy neonates suggesting GH does have an effect on linear growth in utero.[12] GH receptors are already present in fetal tissues although the numbers are lower compared to postnatal tissues.[13,14] It has been assumed that a reduced number of GH receptors may play a role in the moderate effect of GH on fetal growth.

Insulin-like growth factors (IGFs)

Fetal IGF-I and IGF–II are very important determinants of fetal growth. Their relative influence seems to be dependent upon gestational age. IGF-II is the dominant IGF in earlier fetal development, whereas after organogenesis and postnatally, IGF-I becomes more important.[8]

The serum levels of IGF-I are regulated by both metabolic and genetic factors. In fetuses and neonates born SGA, low circulating IGF-I levels have been observed suggesting that lower IGF-I levels play a role in fetal growth retardation.[15-20] Gene deletion studies in mice clearly demonstrated the role of IGF-I, as knock-out mice had a birth weight which was about 60% of normal.[21] Postnatal growth in these mice decreased even further resulting in adult weights of about 30% of normal mice.[22] However, these IGF-I knock-out mice behaved normally and appeared proportionate in size. In humans, one homozygous partial deletion and one missense mutation of the IGF-I gene have been described[23,24] These patients were born SGA and showed severe pre- and postnatal growth failure, small head circumference and mental retardation.

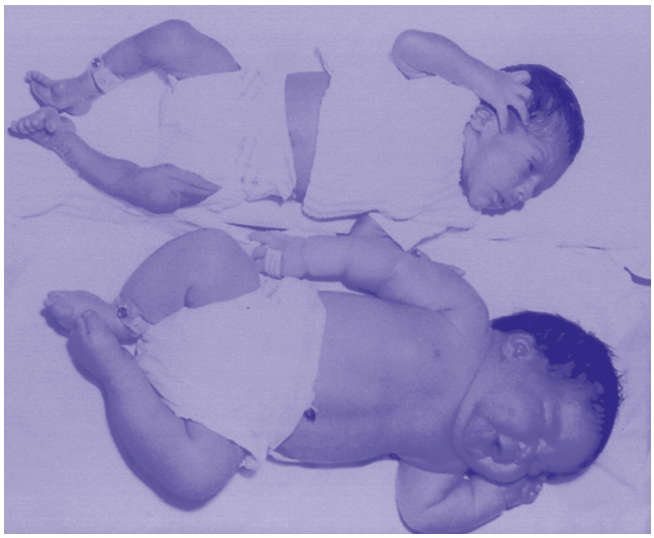
IGF-II predominantly plays a role in prenatal growth. Knockout studies in mice clearly indicate the role of IGF-II.[21] It is also clear from these studies that the growth promoting effect of IGF-II is exerted through the IGF-I receptor. IGF-II was also able to bind to another yet unidentified receptor.

The activity of the peptides IGF-I and -II is modulated by 6 IGF-binding proteins (IGFBPs) that help to transport IGFs to particular tissues during differentiation. Fetal growth restriction had also been associated with reduced serum levels of IGFBP-3 and elevated IGFBP-1 and IGFBP-2 levels.[8]

Insulin

Initially insulin was thought to be the major growth promoting hormone in fetal life. More recently it is assumed that insulin acts directly via stimulation of cellular nutrient (glucose) uptake and indirectly by stimulation of IGF-I production.[25] Glucose availability and the subsequent increase in fetal insulin are the main regulators of fetal IGF-I production during fetal life (postnatal growth, however, is mainly regulated by GH and

IGF-I via the GH-IGF-I axis). Fetal pancreatectomy in sheep resulted in low fetal IGF-I levels and caused severe intra-uterine growth retardation.[26] Intrafetal infusions of either insulin or glucose increased fetal IGF-I levels. Insulin has also lipogenic effects which are well studied in fetuses from mothers with diabetes gravidarum. Fetal hyperinsulinemia and hyperglycemia due to maternal diabetes result in stimulated fetal growth (Figure 5). The increase in birth weight of these infants mainly consists of fat mass due to the lipogenic effects of insulin. Birth length and lean body mass are only slightly increased which is in turn the effect of IGF-I mediated through the high insulin levels.[27]



Figuur 5. The lower baby has macrosomia due to increased insulin production.

3. Long-term effects of being born SGA

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

Several studies have been performed to evaluate the effects of impaired fetal growth on postnatal growth, diseases and disorders. It is generally thought that when the fetus undergoes growth retardation it will adapt to the intrauterine environment and will survive by altering metabolic and endocrine set points, which appear to remain altered postnatally.[28] Children born SGA are at higher risk of a number of chronic diseases and growth retardation in later life.[29,30] However, which diseases and disorders may occur in later life and the exact mechanism of growth retardation are not known at this moment. Two main hypotheses exist. The first one, the ‘fetal origins hypothesis’

postulates that these diseases are the result of malnutrition during a critical period in fetal life (Figure 6).[31] Depending on the type and time of poor fetal and early infant growth, a variety of long-term changes in organ function may develop.[32-34] Malnutrition early in gestation will result in fetal growth retardation as well as postnatal growth retardation and permanent changes in physiology of several major organs including the fetal pancreas. One of the consequences would be that the number of ß-cells will be reduced resulting in low fetal insulin levels. Since insulin is an important fetal growth factor, low fetal insulin levels will result in a low birth weight and/or birth length.

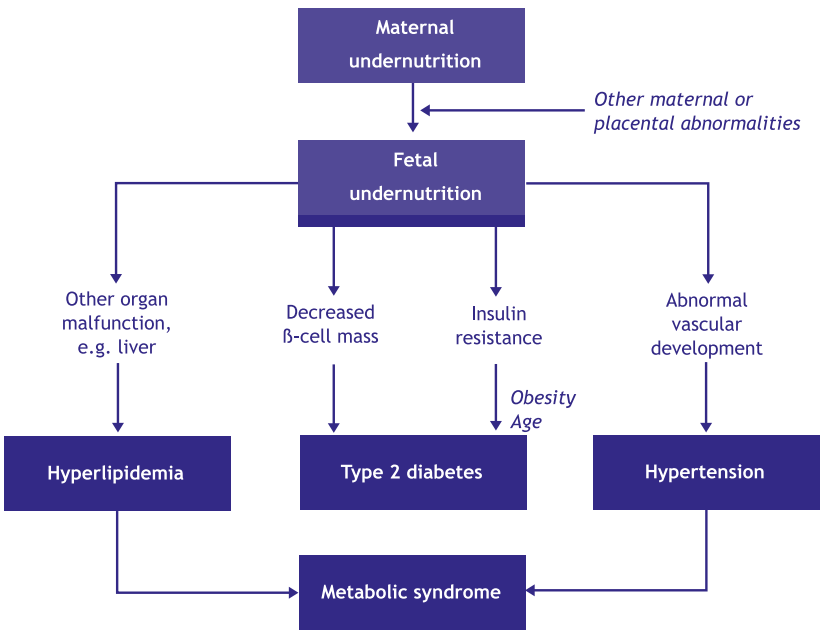


Figure 6. Representation of the fetal origins of syndrome X.
Adapted from Barker DJP *et al*. Diabetologia 1993;36:62. Barker DJP. BMJ.

The second hypothesis is proposed by Hattersley and is called the 'fetal insulin hypothesis' (Figure 7).[35] The fetal insulin hypothesis postulates that genes involving insulin resistance could affect both birth weight and disease in later life.[35-37] In practice, however, the altered endocrine set points and diseases in later life might not solely be the result of environmental factors but rather the result of both genetic and fetal environment factors.

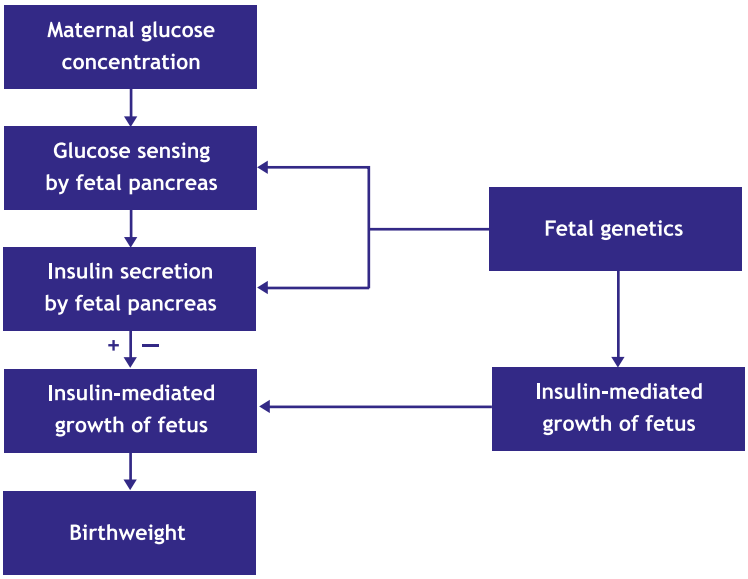


Figure 7. Simplified representation of fetal insulin hypothesis. Insulin related growth reflects not only maternal glycemia but also fetal genetic factors that regulate insulin secretion by the fetal pancreas and the sensitivity of fetal tissues to the effects of insulin (insulin resistance). Adapted from Hattersley *et al*. Lancet 1999;353:1789. 1995;311:171.

Catch-up growth and puberty

Most children born SGA show catch-up growth during the first 2 to 3 years of life. If children have not shown catch-up growth during the first years of life they have a greater risk of remaining short later in life. In a Swedish cohort of 123 infants born SGA, which was defined as a birth weight below −2 SDS, 9% still had a height below −2 SDS at the age of 4 years.[38] Hokken-Koelega *et al* found that 15% of a group of 724 term and preterm SGA infants, which was defined as a birth length below −1.88 SDS, still had a height below −1.88 SDS at the age of two years.[39] Term SGA infants showed a more rapid increase in catch-up growth compared to preterm infants. However at the age of two years the percentage SGA infants without catch-up growth was the same for term and preterm infants (Figure 8).

Longitudinal studies from birth to final height have shown that infants born SGA have an increased risk for short stature in adult life. Chaussain *et al* reported that SGA children born with a birth length < −2 SD for gestational age who remained short during childhood, reached an adult height of 161.9 ± 8.0 cm (boys) and 147.6 ± 7.2 cm (girls).[40] These adult heights were significantly lower than the target heights of these patients. Also, Karlberg *et al* found a 7-fold increased risk for short stature at the age of 18 years in those children who had been born SGA in length and a 5 fold increased risk in those

who had been born SGA in weight.[41] In the normal population important determinants of final height are the height and age at onset of puberty and the magnitude and duration of the pubertal growth.[42-44] Data on puberty in children born SGA are very limited. Some studies evaluated height and age at onset of puberty and not the duration and progression of puberty. Moreover, these study results are difficult to compare due to the various definitions of SGA and the various definitions used for the milestones of puberty.[45,46]

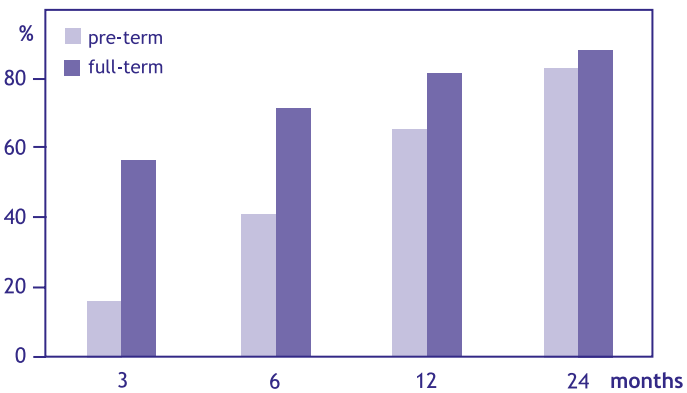


Figure 8. Percentage of SGA infants with postnatal catch-up growth to a height > −1.88 SDS. Data are given for premature (light purpel bars) and full-term infants (purpel bars). Adapted from Hokken-Koelega *et al.* Pediatr Res 1995;38:267.

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

The mechanism underlying persistent short stature in children born SGA is still not fully understood. Disturbances in the GH/IGF-I axis (Figure 9) may play a role. Sixty percent of SGA children with insufficient catch-up growth, defined as a persistent height below −2 SDS, showed a reduction in physiological 24-hour GH secretion whereas 25% showed low GH peaks during GH provocation tests.[47,48] Also, serum IGF-I levels were significantly lower in short children born SGA compared to normal children.[47,48] Arends *et al* showed an association between a polymorphism of the IGF-I gene and low serum IGF-I levels in a group of children born SGA without a catch-up growth who were proportionally small at birth.[49] Genetically determined low serum IGF-I levels may therefore lead not only to a reduction in birth length, weight and head circumference but also to persistent short stature and small head circumference during childhood and adulthood.

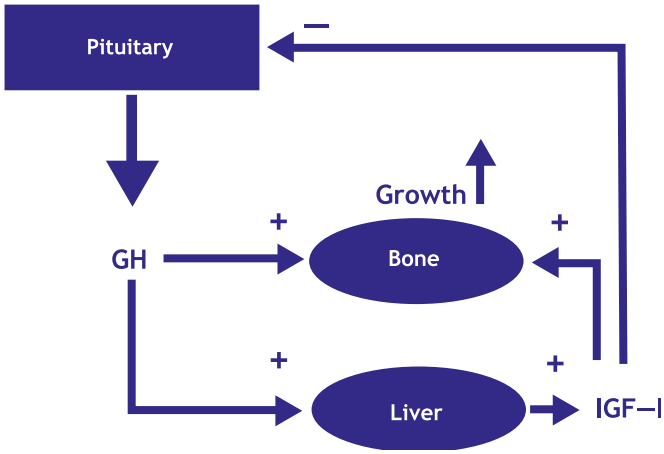


Figure 9. Postnatal growth is mainly regulated by GH via the GH-IGF-I axis.

Body composition

Short children born SGA have a lean appearance. BMI in short SGA children is significantly lower compared to healthy children with the same age and sex. Recently, body composition in short SGA children was measured by Dual Energy X-ray Absorptiometry (DXA), being the most precise method to investigate total body composition.[50] This study showed that in short children born SGA, leanness is mainly the result of a lower lean body mass and to a lesser extent due to a reduction in total body fat.

Parents of short children born SGA often report that their child has a serious lack of appetite and a low dietary intake. Since children with a low dietary intake have low serum IGF-I levels and 60% of the short SGA children do show low serum IGF-I levels it might be that low dietary intake contributes to the failure of complete catch-up growth in these children.

Spontaneous bone maturation

Data on bone maturation in short SGA children are very scarce. A French study reported delayed bone maturation until the age of 8 years in a group of short children born SGA.[51] However, after the age of 8 years bone age accelerated without a concomitant increase in height. This resulted in adult heights which were significantly lower than the predicted adult heights at the age of 8 years. In 1975, Tanner *et al* described in a group of short prepubertal children with Silver-Russel syndrome a similar pattern of acceleration of bone maturation from the age of 5 years.[52] This suggests that SGA children might experience different bone maturation over the years compared to their

healthy peers. For that reason the Advisory Board on SGA concluded that prediction of adult height based on estimates of bone age is unreliable in these children.[1]

Carbohydrate metabolism, blood pressure and cardiovascular system

Epidemiologic studies have shown that type 2 diabetes mellitus (DM), hypertension and cardiovascular diseases occur more frequently among individuals who were born with a low birth weight.[34,53] Also the combination of type 2 DM, hypertension, dyslipidemia and a high body mass index (BMI), called the Metabolic Syndrome, has been associated with a low birth weight.[34,54] Several studies found a relation between low birth weight, hypertension and dyslipidemia.[33,55] The mechanisms underlying these increased risks are still unknown. Insulin resistance and hyperinsulinism are thought to play a key role in the pathogenesis of both type 2 diabetes mellitus and cardiovascular abnormalities.[54, 56,57] In this respect two main hypotheses exist: the "fetal origins hypothesis" and the "fetal insulin hypothesis", as discussed at the beginning of this paragraph.

Adrenarche and pubarche

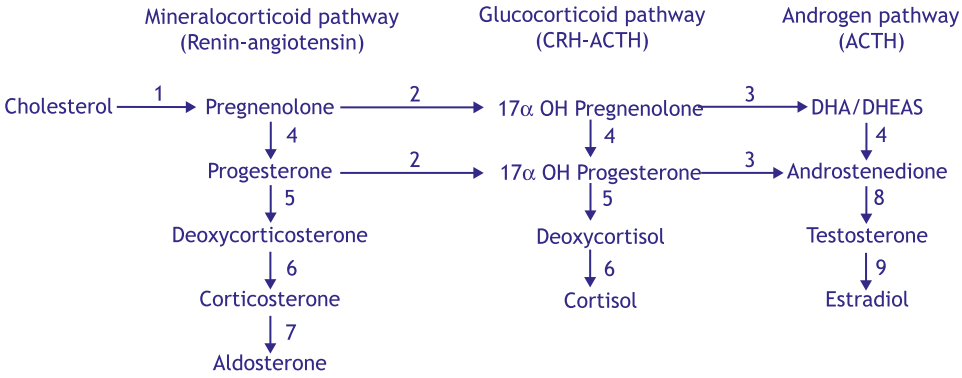
Impaired fetal growth has been associated with hypoplasia of the fetal adrenal zone and with lower fetal serum concentrations of DHEAS.[58-60] However, some studies have reported higher DHEAS levels and an increased incidence of premature pubarche in children born SGA,[59] but other studies did not confirm these results.[61] A possible explanation for these discrepancies could be that various definitions for low birth weight, SGA and catch-up growth were used.

Premature pubarche might be caused by relatively high serum DHEAS levels at a young age. Studies in adolescent girls indicated associations between low birth weight and the occurrence of premature adrenarche, pubarche, hyperandrogenism, polycystic ovary syndrome (PCOS) and hyperinsulinism.[62] These findings might have serious consequences for later life.

Adrenal development

Before birth the fetal adrenal cortex consists of two morphologically distinct zones: the fetal zone and the definitive zone. The fetal adrenal cortex produces steroid hormones which regulate intrauterine homeostasis and the maturation of fetal organ systems necessary for postnatal life.[63] The fetal zone occupies 80% of the enlarged fetal adrenal cortex, exists only during fetal life and undergoes atrophy in the first 6 weeks after birth. During fetal life the fetal zone produces cortisol and dehydroepiandrosterone-sulphate (DHEAS). Fetal DHEAS is secreted by the fetus to the placenta where it is the principal precusor for placental estrone and estradiol synthesis.[58]

The adult adrenal cortex is composed of three zones: the zona glomerulosa, the zona fasciculata and the zona reticularis. The three zones each have their own function and are dependent on different hormones and present of certain enzymes (Figure 10). The zona glomerulosa, the outer zone, comprises 10% of the cortical thickness. It secretes mineralocorticoids, specifically aldosterone, under control of the renin-angiotensin system. The role of aldosterone is the fine-tuning of sodium and potassium exchange mainly in the renal distal tubule but also in the gut and sweat glands. The zona fasciculata accounts for 75% of the thickness of the cortex. It secretes glucocorticoids, primarily cortisol, in the human under the control of pituitary adrenocorticotrophic hormone (ACTH). Glucocorticoids play a role in normal metabolism, resistance to stress and inhibition of inflammation.



Enzymes

1. P450 cholesterol side chain cleavage
2. P450 17α-hydroxylase
3. 17,20-lyase (from same gene as 2)
4. 3β-hydroxysteroid dehydrogenase
5. 21-hydroxylase
6. 11β-hydroxylase
7. Aldosterone synthetase
8. 17β-hydroxysteroid dehydrogenas
9. Aromatase

Figure 10. Pathways of adrenal steroid synthesis.

The zona reticularis, the innermost zone, differentiates during middle childhood and is responsible for the production of adrenal androgens, primarily DHEAS. Serum levels of DHEAS are high in newborns and decline rapidly after birth. At the age of approximately 6–8 years the serum DHEAS levels start to rise, which is called the adrenarche. The highest serum DHEAS levels are seen between the ages of 15–25 years. After the third decade levels decline, which is called the adrenopause (Figure 11).[62,64] In most children, the adrenarche is followed by development of pubic hair, axillary hair, acne and pubertal odour. The appearance of pubic hair growth before the age of 8 years in girls and 9 years in boys is called premature pubarche and is also mostly accompanied by axillary hair, acné and pubertal odour.[62,65] Several hormones such as adrenocorticotrophic hormone (ACTH) and probably Corticotrophic Releasing Hormone (CRH), prolactin, dopamine

and IGF-1 might have an influence on the secretion of DHEAS.[63,66-68] However, current studies indicated that the increase of DHEAS is a result of intra-adrenal changes. The increased level of 17 hydroxylase/17,20 lyase (p450c17), P450 oxidoreductase and cytochrome b5 and the decreased level of 3ß-hydroxysteroid dehydrogenase (3ßHSD) in the reticularis is likely to increase DHEAS production.[62,69,70] Some patients with a premature pubarche showed normal androgen levels, which suggest that these children have increased peripheral sensitivity to these hormones. It is generally thought that the adrenarche is independent of the gonads or gonadotrophins, since children with primary hypogonadism as well as those with an isolated gonadotrophin deficiency show a normal adrenarche.[62,71]

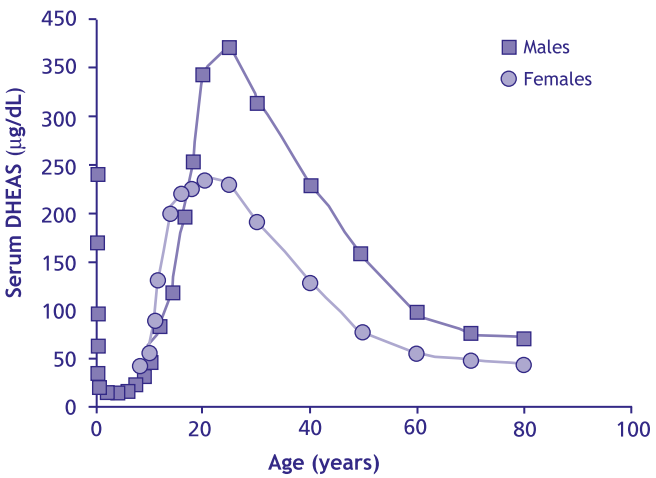


Figure 11. Concentrations of serum DHEAS as a function of age in females and males.

Gonadal development

Some studies have suggested that reduced fetal growth will have an effect on the gonadal development. However, very limited data are yet available. The embryonic gonads play a crucial role in sexual differentiation and reproduction in later life.

Ovarian development

The ovary fulfils two essential functions: the development and release of the mature oocyte and the synthesis and secretion of sex hormones. The oocyte is surrounded by granulosa cells and thecal cells and is called the follicle. The most dynamic phase of ovarian development occurs before birth.[72-74] Human follicle development starts in the twelfth week of intra-uterine life and by the fifth month the maximum size of the

ovarian follicle pool is reached. During fetal life and childhood, follicles develop through primordial and primary stage, to pre-antral and small antral follicles.[72,74] Reduction of the number of primordial follicles, which begins already prior to birth, continues throughout childhood so that at the time of menarche approximately 500,000 follicles remain. From the onset of puberty the small antral follicles will develop to antral follicles. As a result the ovaries will then consist of a mixed population of follicles in different developmental stages. At the antral stage most follicles undergo atresia whereas a few of them, under the cyclic gonadotropin stimulation that starts during puberty, develop into a Graafian follicle and will reach the preovulatory stage.[74]

The granulosa cells of pre-antral and small antral follicles produce the dimeric glycoprotein Anti-Müllerian Hormone (AMH) (Figure 12). AMH, also referred to as Müllerian-inhibiting substance, is a member of the transforming growth factor- ß (TGF ß) super family. During fetal sex differentiation, AMH is produced by Sertoli cells in the male, where it induces degeneration of the Müllerian ducts. In females, AMH is only postnatally produced by the granulosa cells of the ovary and it is involved in the regulation of early folliculogenesis.[74] Serum AMH levels are a good measure of the size of the ovarian follicle pool, since this hormone reflects the number of pre-antral and antral follicles.[75,76]

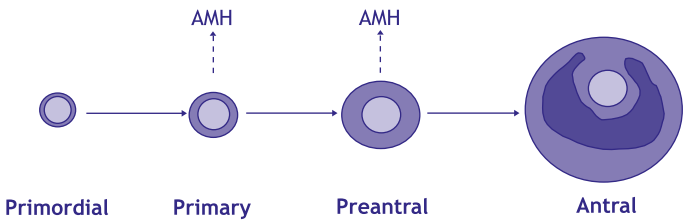


Figure 12. Development of postnatal follicles. AMH is produced by preantral and small antral follicles in the postnatal ovary. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of Anti-Müllerian hormone. Reproduction 2002;124(5):601-9.

At the start of the studies presented in this thesis limited data were available on the ovaries as well as the developmental stage and number of the follicles of short girls born SGA. Some studies suggested that reduced birth size was associated with impaired ovarian development and ovarian hyperandrogenism, reduced ovulation frequency and a reduced size of the uterus and the ovarian follicle pool.[77-79] This would increase the risk for infertility and premature ovarian failure in these girls.

Testicular development

The testis also fulfils two essential functions: the production and maturation of male gametes and the synthesis and secretion of sex hormones. Functionally and anatomically, the testis can be divided into a tubular compartment and an interstitial compartment.

The tubular compartment represents 60–70% of the volume of the human testicular parenchyma. This compartment consists of the seminiferous tubules containing two type of cells: the Sertoli cells and the germ cells in different stages of differentiation from spermatogonium to spermatozoon. Each Sertoli cell can harbour a limited number of germ cells. The number of Sertoli cells is quantitatively determinative for the sperm production. Animal studies have demonstrated that the size of the Sertoli cell population in early fetal life is already important for the testicular size and sperm characteristics in adult life.[80] The interstitial compartment, consisting of Leydig cells which produce testosterone, is situated between the seminiferous tubules.

In prepubertal boys gonadotrophins may be unreliable predictors of testis function because the hypothalamic-pituitary-testis (HPT) axis appears to be quiescent. However, there is a continuous Sertoli cell proliferation and functional activity during the fetal and prepubertal period. As a result these periods are very important for adult testis function.[80-82] Inhibin B and antimullerian hormone (AMH) are produced by the Sertoli cells and for this reason their serum levels are probably important markers of Sertoli cell function in childhood and adulthood.[83,84] From the onset of puberty it is also possible to measure testosterone, LH and FSH as markers of the intactness of the HPT-axis.[85]

Small size at birth, which might reflect fetal growth restriction, might result in an impaired testicular development and a reduced size of the Sertoli cell population. Two retrospective studies concluded that lower birth weight was associated with subfertility and pseudohermaphroditism.[86,87] In both studies size at birth was only judged by low birth weight and not by birth length. At the start of the studies of this thesis, very limited data were available on testis development in SGA boys.

Psychological development and quality of life

Several studies suggest that short children born SGA are psychosocially disadvantaged,[88-90] whereas other studies have demonstrated normal psychosocial functioning of short children.[91] These discrepancies might be due to the use of different study populations or different questionnaires.[91-93] In addition, being born SGA may be associated with an increased risk of subnormal intellectual and psychological development resulting into poor school performance and reduced academic achievements.[94-96] Some studies suggest that in these children there is a relation between low circulating IGF-I, IGF-II and IGFBP-3 and impaired cognitive function.[97,98]

However, most psychological studies regarding short stature concentrate on problems and limitations in general functioning, whereas it is also important to investigate the specific problems and limitations related to short stature. Another problem is that most studies investigated only if there are limitations due to a health problem, also called the health status (HS). It is, however, also important to investigate the emotional

impact that the problem or limitation has on the person's life, called the health related quality of life (HRQOL).[99] At the start of this thesis no data were available about specific problems and limitations in short SGA children and their emotional feelings towards these problems.

4. Growth hormone (GH) treatment in SGA children

In 1991, the first Dutch GH trial was started treating short children born SGA. This was a randomised, double-blind, dose-response multicenter study. These children were treated with either a dose of 1 or 2 mg GH/m^2/day (≈ 0.035 and 0.070 mg/kg/d respectively). All children of the first Dutch GH trial have now reached adult height. From these results could be concluded that GH treatment of short children born SGA leads to a normalization of height during childhood and a normal adult height (AH) for 85% of the short SGA children, even when a GH dose of 1 mg/m^2/day is used (Figure 13).[100,101] In 1996, the second Dutch GH trial was started with a randomised control group for 3 years. After 3 years we found that a GH dose of 1 mg/m^2/day resulted in a normalisation of height.[102] Several other studies also demonstrated that GH treatment is an effective therapy.[103-107]

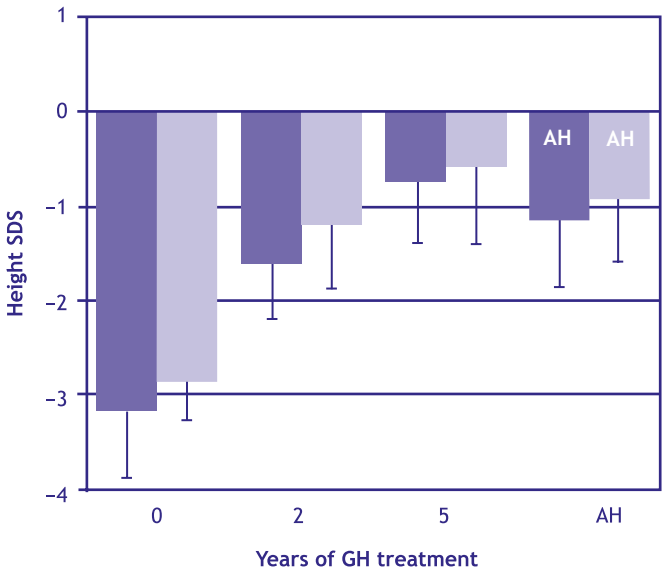


Figure 13. Corrected height SDS (+/− SD) during GH treatment and at Adult Height (AH) for the group treated with 1 mg/m^2/day (dark purpel box) and the group treated with 2 mg/m^2/day (light purpel box). From: Van Pareren *et al.* Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. J Clin Endocrinol Metab 2003;88(8):3584-90.

Safety

The national cooperative growth study (NCGS) published adverse events which have been reported, from 1984 until 1995, during GH treatment in children with various diagnoses. These uncommon events were idiopathic intracranial hypertension, edema and lymphedema, carpal tunnel syndrome, slipped capital femoral epiphysis, diabetes mellitus and carbohydrate intolerance.[108] The authors concluded that adverse events in relation to GH treatment are very rare. They also concluded that in many cases it was difficult to assess the relationship between GH treatment, pre-existing patient factors and an adverse event, because it is limited by incomplete reporting and the lack of reliable data on the occurrence of the event in the general population or in subpopulations of children with other complex medical problems.

Several GH studies in short SGA children have shown that major side-effects were very uncommon.[101,103,109,110] Some studies investigated the effect of GH treatment on insulin sensitivity. It is known that GH treatment decreases insulin sensitivity in normal adults.[111,112] Because of the known association between a low birth weight and a higher risk of developing type 2 diabetes mellitus in adult life, evaluation of glucose intolerance was recommended during GH treatment in short children born SGA.[1] However, limited data are available about possible side-effects of GH treatment on insulin sensitivity in short SGA children. The first Dutch multicenter trial showed that 8% of the short prepubertal SGA children already had an impaired glucose tolerance before the onset of GH treatment.[113] During GH treatment serum glucose levels remained constant while fasting serum insulin levels increased. After 6 years of GH treatment impaired glucose tolerance was found in 4% of the children suggesting that 6 years of continuous GH treatment has no adverse effects on glucose levels in short SGA children.

GH therapy induced a significant and sustained rise in mean IGF-I levels, which in most children is between +1 and +2 SDS during treatment with 1 mg/m^2/day and above +2 SDS during treatment with 2 mg/m^2/day. As the long term effects of persistently high IGF-I levels > 2 SDS are not yet known and continuous therapy with the lower dose appears to be as efficient as the higher GH dose, it seems to be safer to use the lower GH dose of 1 mg/m^2/day.[101,114]

Since epidemiological studies also found a relation between low birth weight and hypertension and dyslipidemia, blood pressure and serum lipids were monitored during GH treatment of short SGA children. Before GH treatment short SGA children had a significantly higher systolic blood pressure than their peers but it decreased significantly during 6 years of GH treatment. The Dutch GH trials have shown that GH treatment had a positive effect on blood pressure and serum lipids.[115] Although these results are reassuring, blood pressure and serum lipids need to be checked regularly during follow up of GH treatment.

5. Aims of the study

Adrenarche and pubarche

To investigate adrenarche by measuring serum DHEAS levels in short SGA children. In addition, we investigated the incidence of premature pubarche in these children and whether there was an association between serum DHEAS levels and bone maturation in SGA children. Finally the effect of GH treatment on serum DHEAS levels was evaluated.

Ovarian development

To assess whether SGA girls have a reduced size of their ovarian follicle pool compared to age-matched AGA girls by measuring serum AMH levels in prepubertal and adolescent girls. Since nowadays many short children born SGA receive growth hormone (GH) treatment, we also investigated the effect of 2 years of GH treatment on serum AMH levels.

Testicular development

To investigate whether prepubertal SGA boys have a reduced size of their Sertoli cell population compared to age-matched prepubertal AGA boys by measuring serum inhibin B and AMH levels in these boys, and to determine the effect of 2 years of GH treatment on serum inhibin B and AMH levels. We also compared the testis function of adolescent GH-treated SGA boys with age-matched AGA boys by measuring serum inhibin B, AMH, testosterone LH and FSH levels.

Pubertal development

To assess the effects of GH treatment in SGA children on pubertal development compared with normal statured children born AGA. The first Dutch GH trial treating short prepubertal children born SGA showed a significant increase in height during 5 years of GH treatment with either a dose of 33 µg/kg/day or 66 µg/kg/day. In addition, important determinants of final height are the height and age at onset of puberty and the magnitude and duration of the pubertal growth.

Food intake

To investigate the food intake and body composition of short SGA children before and after 1 year of GH treatment. Short children born SGA have a serious lack of appetite and a low food intake, according to the parents. They also have a reduced BMI compared to the normal population. Since children with a low food intake have low serum IGF-I levels and since leptin, a protein produced by adipose tissue, is involved in the regulation of appetite and body weight, we also measured IGF-I and leptin in combination with food intake.

Quality of life

To study the effect of GH treatment on quality of life (QOL) in short SGA children, by using two different questionnaires: the TACQOL, a generic QOL questionnaire developed for children with various disorders and diseases, and the TACQOL-Short, a specific QOL questionnaire for children with short stature. These questionnaires investigate the health status (HS) and the emotional feelings towards HS, called the health related quality of life (HRQOL). We hypothesized that GH treatment in short SGA children would lead to a higher quality of life (HS and HRQOL).

6. Outline of the thesis

This doctoral thesis gives a detailed description of various studies in short SGA children. The SGA children were enrolled between 1991-2001 in the first and second Dutch GH trials. Both trials used the same inclusion and exclusion criteria. For a detailed description of both studies the reader is referred to Appendices A and B. The first results of these two studies were reported in the theses of W. de Waal, T. Sas and N. Arends.[116-118]

Chapter 2 describes the adrenarche in short children born SGA and whether they have an increased risk for premature pubarche. In addition, the effect of GH treatment on serum levels of dehydroepiandrosterone-sulphate was investigated. Chapter 3 describes the effect of reduced fetal growth on the number of ovarian follicles in SGA girls compared to normal statured AGA girls. Chapter 4 describes the effect of reduced fetal growth on the testiscular development in short SGA boys compared to normal statured AGA boys. Both chapters 3 and 4 also report on the effect of GH treatment on serum hormone levels secreted by the gonads. Chapter 5 describes the onset and progression of puberty during treatment with different doses of GH. In this study only SGA children of the first Dutch GH-trial were included since all these children have gone through puberty. Chapter 6 describes the food intake before and during one year of GH

treatment in short SGA children in combination with body composition and serum IGF-I, IGFBP3 and leptin levels. In this study children from the second Dutch GH trial were included. Furthermore, Chapter 7 gives the quality of life in short SGA children both at baseline and after 3 years of GH treatment, in comparison with results of untreated short SGA children. In this study the children of the second Dutch GH trial were included.

In chapter 8, the general discussion, our data are discussed in relationship with the present literature. Chapter 9 and 10 present an English and Dutch summary.

Appendix A

Description of the first and second Dutch GH trials (IUGR-1 / IUGR-2)

Patients

Inclusion criteria:
1) birth length standard deviation score (SDS) below −1.88 (that is below 3rd percentile) for gestational age;[2]
2) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia;
3) chronological age (CA) between 3.00 and 7.99 years at start of the study;
4) height SDS for age below −1.88 according to Dutch standards;[119]
5) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth;[56]
6) prepubertal, defined as Tanner stage 1 or a testicular volume < 4 ml;[120]
7) normal liver, kidney and thyroid functions.

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

IUGR-1 study design

Before entering the study, the children underwent a 24 hour plasma GH profile. To stratify for the spontaneous GH secretion during 24 hour GH profile, the total group of 79 children was divided into three groups: “normal profile”, “GH insufficient profile” (area under the curve < 90 µg/L/24h and mean GH < 2.0 µg/L) and “no profile performed”. After stratification for spontaneous GH secretion during 24 hour GH profile and CA all children were randomly and blindly assigned to either one of two GH dosage groups:

group A, 1 mg/m^2 body surface area/day (≈ 33 µg/kg/day), or group B, 2 mg/m^2 body surface area/day (≈ 66 µg/kg/day). Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area. The study was kept double blind by using an equal volume of a reconstituted preparation.

IUGR-2 study design

The study design was an open-labelled multicenter study with a randomized control group. Before entering the study the GH status was evaluated in all children using GH stimulation tests (arginine and/or clonidine). Children with GH deficiency (GHD) which was defined as a GH peak < 10 µg/l during two GH stimulation tests, were not randomized but started GH treatment at dose of 1 mg/m^2 body surface area/day (≈33 µg/kg/day) (GHD-group). The non-GHD children were stratified according to age (3.00–5.50 versus 5.50–7.99) and height of the parents (height of both parents above −1.88 SDS versus height of at least one parent below −1.88 SDS). After stratification the patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group started immediately with GH treatment at a dose of 1 mg/m^2 body surface area/day (≈33 µg/kg/day). The control group remained untreated for 3 years and received subsequently the same GH treatment as the GH-group. Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. Three-monthly, the GH dose was adjusted to the calculated body surface area.

References

1. **Lee P, Chernausek S, Hokken-Koelega A, Czernichow P** 2003 International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics*. 2003 111:1253-61
2. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-10.
3. **Pollack RN, Divon MY** 1992 Intrauterine growth retardation: definition, classification, and etiology. *Clin Obstet Gynecol* 35:99-107.
4. **Villar J, Belizan J, Smeriglio V** 1989 Intrauterine growth retardation. In: J.S (ed), Raven Press Ltd., New York 18:261-280
5. **Albertsson-Wikland K, Karlberg J** 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl* 399:64-70
6. **Bernstein PS, Divon MY** 1997 Etiologies of fetal growth restriction. *Clin Obstet Gynecol* 40:723-9.
7. **Wollmann HA** 1998 Intrauterine growth restriction: definition and etiology. *Horm Res* 49:1-6.
8. **Rosenfeld R** 2003 The role of growth hormone therapy in short children born small for gestational age. **www.medscape.com**
9. **Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, Niu T, Wise PH, Bauchner H, Xu X** 2002 Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *Jama* 287:195-202.
10. **Wollmann HA, Kirchner T, Enders H, Preece MA, Ranke MB** 1995 Growth and symptoms in Silver-Russell syndrome: review on the basis of 386 patients. E*uropean Journal of Pediatrics* 154:958-968
11. **Hitchins MP, Stanier P, Preece MA, Moore GE** 2001 Silver-Russell syndrome: a dissection of the genetic aetiology and candidate chromosomal regions. *J Med Genet* 38:810-9.
12. **Gluckman PD, Gunn AJ, Wray A, Cutfield WS, Chatelain PG, Guilbaud O, Ambler GR, Wilton P, Albertsson-Wikland K** 1992 Congenital idiopathic growth hormone deficiency associated with prenatal and early postnatal growth failure. The International Board of the Kabi Pharmacia International Growth Study. *J Pediatr* 121:920-3.
13. **Hill DJ, Riley SC, Bassett NS, Waters MJ** 1992 Localization of the growth hormone receptor, identified by immunocytochemistry, in second trimester human fetal tissues and in placenta throughout gestation. *J Clin Endocrinol Metab* 75:646-50.
14. **Klempt M, Bingham B, Breier BH, Baumbach WR, Gluckman PD** 1993 Tissue distribution and ontogeny of growth hormone receptor messenger ribonucleic acid and ligand binding to hepatic tissue in the midgestation sheep fetus. *Endocrinology* 132:1071-7.
15. **Foley TP, Jr., DePhilip R, Perricelli A, Miller A** 1980 Low somatomedin activity in cord serum from infants with intrauterine growth retardation. *J Pediatr* 96:605-10.
16. **Bennett A, Wilson DM, Liu F, Nagashima R, Rosenfeld RG, Hintz RL** 1983 Levels of insulin-like growth factors I and II in human cord blood. *J Clin Endocrinol Metab* 57:609-12.
17. **Gluckman PD, Johnson-Barrett JJ, Butler JH, Edgar BW, Gunn TR** 1983 Studies of insulin-like growth factor -I and -II by specific radioligand assays in umbilical cord blood. *Clin Endocrinol* (Oxf) 19:405-13.

18. **Lassarre C, Hardouin S, Daffos F, Forestier F, Frankenne F, Binoux M** 1991 Serum insulin-like growth factors and insulin-like growth factor binding proteins in the human fetus. Relationships with growth in normal subjects and in subjects with intrauterine growth retardation. *Pediatr Res* 29:219-25.
19. **Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, Hintz RL, Rosenfeld RG** 1995 Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab* 80:1548-55.
20. **Leger J, Noel M, Limal JM, Czernichow P** 1996 Growth factors and intrauterine growth retardatio.II. serum growth hormone,insulin-like growth factor(IGF) I,and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: Prospective study from birth to two years of age. *Pediatric Resource* 40:101-107
21. **Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A** 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 75:59-72.
22. **Baker J, Liu JP, Robertson EJ, Efstratiadis A** 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75:73-82.
23. **Woods KA, Camacho-Hübner C, Savage MO, Clark AJL** 1996 Intrauterine growth retardation and postnnatal growth failure associated with deletion of the insulin-like growth factor I gene. *Br.Report* 335:1363-1367
24. **Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, Mohan S, Denley A, Forbes B, van Duyvenvoorde HA, van Thiel SW, Sluimers CA, Bax JJ, de Laat JA, Breuning MB, Romijn JA, Wit JM** 2005 Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J Clin Endocrinol Metab* 90:2855-64
25. **Oliver MH, Harding JE, Breier BH, Gluckman PD** 1996 Fetal insulin-like growth factor (IGF)-I and IGF-II are regulated differently by glucose or insulin in the sheep fetus. *Reprod Fertil Dev* 8:167-72.
26. **Gluckman PD, Butler JH, Comline R, Fowden A** 1987 The effects of pancreatectomy on the plasma concentrations of insulin-like growth factors 1 and 2 in the sheep fetus. *J Dev Physiol* 9:79-88.
27. **Milner RD, Hill DJ** 1984 Fetal growth control: the role of insulin and related peptides. *Clin Endocrinol* (Oxf) 21:415-33.
28. **Clark PM** 1998 Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. *Eur J Pediatr* 157 Suppl 1:S7-10.
29. **Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD** 1991 Fetal and infant growth and impaired glucose tolerance at age 64. *Bmj* 303:1019-22.
30. **Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ** 1989 Weight in infancy and death from ischaemic heart disease. *Lancet* 2:577-80.
31. **Barker D** 1994 Mothers, babies and diseases in later life. *British medical journal* Publishing Group, London
32. **Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS** 1993 Fetal nutrition and cardiovascular disease in adult life. *Lancet* 341:938-41.
33. **Barker D** 1997 The fetal origins of coronary heart disease. *Acta Paediatr Suppl* 422:78-82
34. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-7.

35. **Hattersley A, Tooke T** 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 353:1789-1792
36. **Hattersley A, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S** 1998 Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat genet* 19:268-270
37. **Dunger D, Ong K, Huxtable S, Sherriff A, Ring S, Bennett S, Todd J** 1998 Association of the INS VNTR with size at birth. ALSPAC Study team. Avon Longitudinal Study of Pregnancy and Childhood. *Nat genet* 19:98-100
38. **Albertsson-Wikland K, Wennergren G, Wennergren M, Vilbergsson G, Rosberg S** 1993 Longitudinal follow-up of growth in children born small for gestational age. *Acta Paediatrica* 82:438-443
39. **Hokken-Koelega ACS, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: Do they catch up? *Pediatr Res* 38:267-71.
40. **Chaussain JL, Colle M, Ducret JP** 1994 Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. *Acta Paediatr Suppl* 399:72-3.
41. **Karlberg J, Albertsson-Wikland K** 1995 Growth in full-term small-for-gestational-age infants: from birth to final height. *Pediatric Research* 38:733-739
42. **Tanaka T, Suwa S, Yokoya S, Hibi I** 1988 Analysis of linear growth during puberty. *Acta Paediatr Scand Suppl* 347:25-9
43. **Tanaka T, Satoh M, Hibi I** 1996 Combined GH and LHRH analog treatment in short children. *Endocr J* 43 Suppl:S13-7.
44. **Bourguignon JP** 1988 Linear growth as a function of age at onset of puberty and sex steroid dosage: Therapeutic implications. *Endocr Rev* 9:467-88.
45. **Leger J, Levy M, Boch J, Pinet A, Chevenne D, Porquet D, Collin D, Czernichow P** 1997 Reduced final height and indications for insulin resistance in 20 year old born small for gestational age: Regional cohort study. *British medical journal* 315:341-347
46. **Persson I, Ahlsson F, Ewald U, Tuvemo T, Qingyuan M, von Rosen D, Proos L** 1999 Influence of perinatal factors on the onset of puberty in boys and girls: Implications for interpretation of link with risk of long term diseases. *Am J Epidemiol* 150:747-55.
47. **de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL** 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. *Clin Endocrinol* (Oxf) 41:621-30.
48. **Boguszewski M, Rosberg S, Albertsson-Wikland K** 1995 Spontaneous 24-hour growth hormone profiles in prepubertal small for gestational age children. *J Clin Endocrinol Metab* 80:2599-606.
49. **Arends N, Johnston L, Hokken-Koelega A, van Duijn C, de Ridder M, Savage M, Clark A** 2002 Polymorphism in the IGF-I gene: clinical relevance for short children born small for gestational age (SGA). *J Clin Endocrinol Metab* 87:2720.
50. **Arends N, Hokken-Koelega A** 1998 Body composition and daily food intake in children with short stature after intrauterine growth retardation (IUGR). *Horm Res* 50 (suppl. 3):47
51. **Job JC, Rolland A** 1986 Histoire naturelle des retards de croissance a debut intra-uterin. *Archives of french pediatrics* 43:301-306
52. **Tanner J, Lejarraga H, Cameron N** 1975 The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. *Pediatr Res* 9:611-23.

53. **Barker DJ, Bull AR, Osmond C, Simmonds SJ** 1990 Fetal and placental size and risk of hypertension in adult life. *Bmj* 301:259-62.
54. **Reaven G** 1988 Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 37:1595-1607
55. **Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R** 2000 Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. *Pediatr Res* 48:623-8.
56. **Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, Shitrit A, Fuchs Z** 1985 A link between hypertension obesity and glucose tolerance. *J Clin Invest* 75:809-817
57. **Ferrannini E, Haffner S, Mitchell B, Stern M** 1991 Hyperinsulinaemia: the key feature of a cardiovascuar and metabolic syndrome. *Diabetologia* 34:416-422
58. **Turnipseed MR, Bentley K, Reynolds JW** 1976 Serum dehydroepiandrosterone sulfate in premature infants and infants with intrauterine growth retardation. *J Clin Endocrinol Metab* 43:1219-25.
59. **Francois I, de Zegher F** 1997 Adrenarche and fetal growth. *Pediatr Res* 41:440-2.
60. **Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ** 1996 Size at birth and adrenocortical function in childhood. *Clin Endocrinol* (Oxf) 45:721-6.
61. **Jaquet D, Leger J, Chevenne D, Czernichow P, Levy-Marchal C** 1999 Intrauterine growth retardation predisposes to insulin resistance but not to hyperandrogenism in young women. *J Clin Endocrinol Metab* 84:3945-9.
62. **Ibanez L, Dimartino-Nardi J, Potau N, Saenger P** 2000 Premature adrenarche - normal variant or forerunner of adult disease? *Endocr Rev* 21:671-96.
63. **Mesiano S, Jaffe RB** 1997 Developmental and functional biology of the primate fetal adrenal cortex. *Endocr Rev* 18:378-403.
64. **Orentreich N, Brind JL, Rizer RL, Vogelman JH** 1984 Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J Clin Endocrinol Metab* 59:551-5.
65. **Pere A, Perheentupa J, Peter M, Voutilainen R** 1995 Follow up of growth and steroids in premature adrenarche. *Eur J Pediatr* 154:346-52.
66. **Smith R, Mesiano S, Chan EC, Brown S, Jaffe RB** 1998 Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulfate secretion by human fetal adrenal cortical cells. *J Clin Endocrinol Meta*b 83:2916-20.
67. **Parker CR, Jr., Stankovic AM, Goland RS** 1999 Corticotropin-releasing hormone stimulates steroidogenesis in cultured human adrenal cells. *Mol Cell Endocrinol* 155:19-25.
68. **Ibanez L, Potau N, Marcos MV, de Zegher F** 1999 Corticotropin-releasing hormone as adrenal androgen secretagogue. *Pediatr Res* 46:351-3.
69. **Miller WL** 1999 The molecular basis of premature adrenarche: an hypothesis. *Acta Paediatr Suppl* 88:60-6.
70. **Suzuki T, Sasano H, Takeyama J, Kaneko C, Freije WA, Carr BR, Rainey WE** 2000 Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. *Clin Endocrinol* (Oxf) 53:739-47
71. **Sklar CA, Kaplan SL, Grumbach MM** 1980 Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed growth and adolescence. *J Clin Endocrinol Metab* 51:548-56.
72. **Macklon NS, Fauser BC** 1999 Aspects of ovarian follicle development throughout life. *Horm Res* 52:161-70.

73. **de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER** 2001 Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Hum Dev* 60:179-92.
74. **McGee EA, Hsueh AJ** 2000 Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 21:200-14.
75. **de Vet A, Laven JSE, de Jong FH, Themmen APN, Fauser BCJM** 2002 Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 77:357-62.
76. **Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J** 2003 Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 18:323-7.
77. **Ibanez L, Potau N, Enriquez G, de Zegher F** 2000 Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res* 47:575-7.
78. **Ibanez L, Potau N, de Zegher F** 2000 Ovarian hyporesponsiveness to follicle stimulating hormone in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 85:2624-6.
79. **de Bruin JP, Dorland M, Bruinse HW, Spliot W, Nikkels PG, te Velde ER** 1998 Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev* 51:39-46
80. **Orth JM, Gunsalus GL, Lamperti AA** 1988 Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* 122:787-94.
81. **Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE** 1998 Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 83:675-81.
82. **Chemes HE** 2001 Infancy is not a quiescent period of testicular development. *Int J Androl* 24:2-7.
83. **Schmiegelow M, Lassen S, Poulsen HS, Schmiegelow K, Hertz H, Andersson AM, Skakkebaek NE, Muller J** 2001 Gonadal status in male survivors following childhood brain tumors. *J Clin Endocrinol Metab* 86:2446-52.
84. **Kubini K, Zachmann M, Albers N, Hiort O, Bettendorf M, Wolfle J, Bidlingmaier F, Klingmuller D** 2000 Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. *J Clin Endocrinol Metab* 85:134-8.
85. **Pierik FH, Burdorf A, de Jong FH, Weber RF** 2003 Inhibin B: a novel marker of spermatogenesis. *Ann Med* 35:12-20
86. **Francois I, de Zegher F, Spiessens C, D'Hooghe T, Vanderschueren D** 1997 Low birth weight and subsequent male subfertility. *Pediatr Res* 42:899-901.
87. **Francois I, van Helvoirt M, de Zegher F** 1999 Male pseudohermaphroditism related to complications at conception, in early pregnancy or in prenatal growth. *Horm Re*s 51:91-5.
88. **Pryor J, Silva PA, Brooke M** 1995 Growth, development and behaviour in adolescents born small-for-gestational-age. *J Paediatr Child Health* 31:403-7.
89. **van der Reijden-Lakeman I, Slijper FM, van Dongen-Melman JE, de Waal WJ, Verhulst FC** 1996 Self-concept before and after two years of growth hormone treatment in intrauterine growth-retarded children. *Horm Res* 46:88-94.
90. **Low JA, Handley-Derry MH, Burke SO, Peters RD, Pater EA, Killen HL, Derrick EJ** 1992 Association of intrauterine fetal growth retardation and learning deficits at age 9 to 11 years. *Am J Obstet Gynecol* 167:1499-505.

91. **Kranzler JH, Rosenbloom AL, Proctor B, Diamond FB, Jr., Watson M** 2000 Is short stature a handicap? A comparison of the psychosocial functioning of referred and nonreferred children with normal short stature and children with normal stature. *J Pediatr* 136:96-102.
92. **Haverkamp F, Noeker M** 1998 'Short stature in children--a questionnaire for parents': a new instrument for growth disorder-specific psychosocial adaptation in children. *Qual Life Res* 7:447-55.
93. **Theunissen NC, Kamp GA, Koopman HM, Zwinderman KA, Vogels T, Wit JM** 2002 Quality of life and self-esteem in children treated for idiopathic short stature. *J Pediatr* 140:507-15.
94. **Sommerfelt K, Andersson HW, Sonnander K, Ahlsten G, Ellertsen B, Markestad T, Jacobsen G, Hoffman HJ, Bakketeig L** 2000 Cognitive development of term small for gestational age children at five years of age. *Arch Dis Child* 83:25-30.
95. **Paz I, Gale R, Laor A, Danon YL, Stevenson DK, Seidman DS** 1995 The cognitive outcome of full-term small for gestational age infants at late adolescence. *Obstet Gynecol* 85:452-6.
96. **Wake M, Coghlan D, Hesketh K** 2000 Does height influence progression through primary school grades? *Arch Dis Child* 82:297-301.
97. **Wickelgren I** 1998 Tracking insulin to the mind. *Science* 280:517-9.
98. **Singh SP, Ehmann S, Snyder AK** 1996 Ethanol-induced changes in insulin-like growth factors and IGF gene expression in the fetal brain. *Proc Soc Exp Biol Med* 212:349-54.
99. **Vogels T, Verrips GHW, Koopman HM, Theunissen NC, Fekkes M, kamphuis RP** TACQOL Manuel Parent Form and Child Form. Leiden Center for child health and pediatrics LUMC-TNO.
100. **Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 2003 Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab* 88:3584-90.
101. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.
102. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. *Clin Endocrinol* (Oxf) 59:779-87.
103. **Chaussain JL, Colle M, Landier F** 1994 Effects of growth hormone therapy in prepubertal children with short stature secondary to intrauterine growth retardation. *Acta Paediatr Suppl* 399:74-5; discussion 76.
104. **Zegher de F, Albertsson-Wikland K, Wilton P, Chatelain P, Jonsson B, Lofstrom A, Butenandt O, Chaussain J-L** 1996 Growth hormone treatment of short children born small for gestational age: metanalysis of four indepent, randomized, controlled, multicentre studies. *Ac.Paed. suppl* 417:27-31
105. **Dahlgren J, Wikland KA** 2005 Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res* 57:216-22
106. **Ranke MB, Lindberg A** 1996 Growth hormone treatment of short children born small for gestational age or with Silver-Russell syndrome: results from KIGS (Kabi International Growth Study), including the first report on final height. *Acta Paediatr Suppl* 417:18-26.

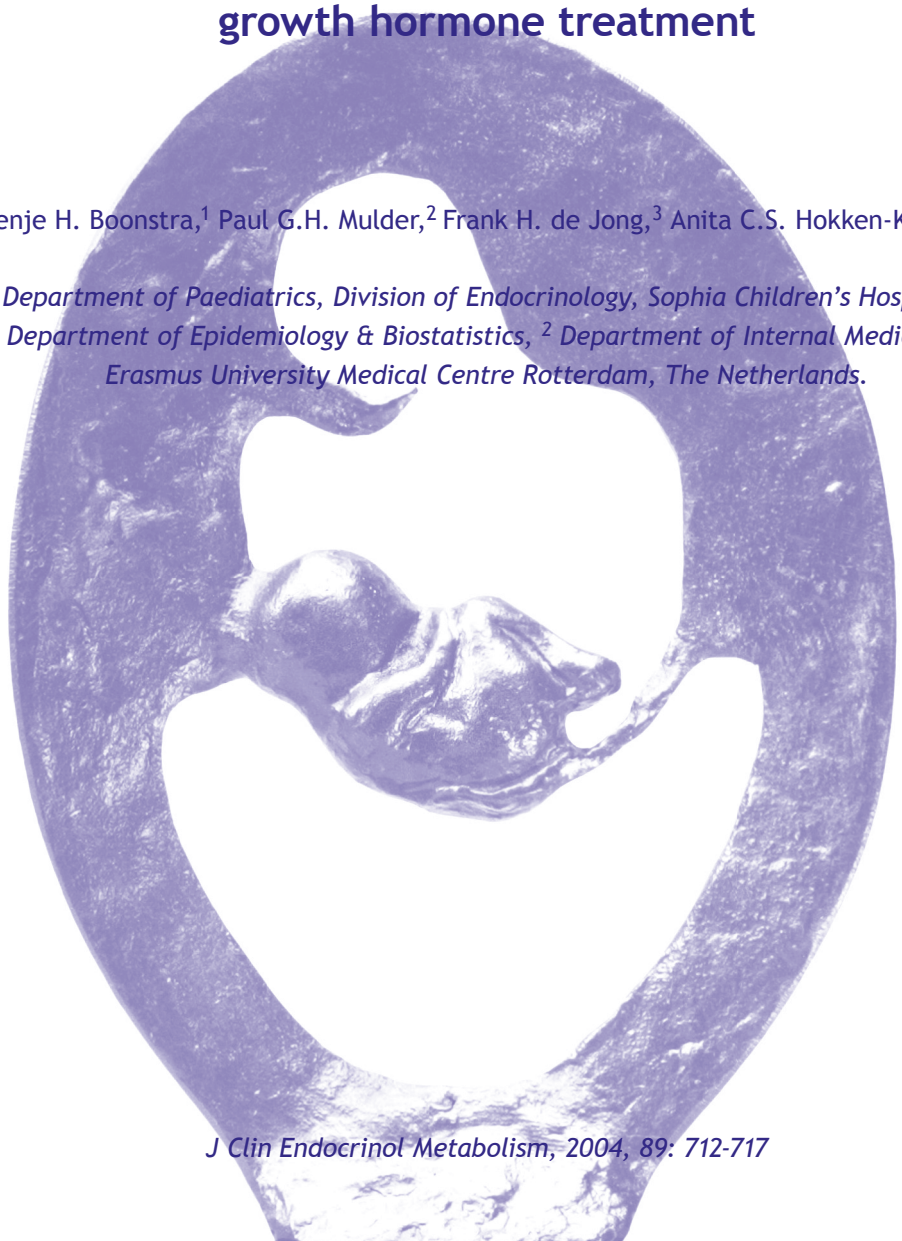
107. **Zegher de F, Francois I, Helvoirt M, Berghe van den G** 1997 Small as fetus and short as child: from endogenous to exogenous growth hormone. *Journal of Clinical Endocrinology and Metabolism* 82:2021-2026
108. **Blethen SL, Allen DB, Graves D, August G, Moshang T, Rosenfeld R** 1996 Safety of recombinant deoxyribonucleic acid-derived growth hormone: the national cooperative growth study experience. *Journal of Clinical Endocrinology and Metabolism* 81:1704-1710
109. **Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P** 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. *Journal of Clinical Endocrinology and Metabolism* 78:904-909
110. **Sas T, de Muinck Keizer-Schrama S, Stijnen T, Drop S** 2000 Carbohydrate (CH) metabolism during long-term growth hormone (GH) treatment and after discontinuation of GH treatment in girls with Turner syndrome participating in a randomized dose-response trial. *J Clin endocrinol metab* 85:769-775
111. **Bratusch-Marrain PR, Smith D, DeFronzo RA** 1982 The effect of growth hormone on glucose metabolism and insulin secretion in man. *J Clin Endocrinol Metab* 55:973-82.
112. **Rizza RA, Mandarino LJ, Gerich JE** 1982 Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. *Diabetes* 31:663-9.
113. **Sas T, Mulder P, Aanstoot H** 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. *Clinical Endocrinology* 54:243-251
114. **Boguszewski M, Jansson C, Rosberg S, Albertsson-Wikland K** 1996 Changes in serum insulin-like growth factor I (IGF-I) and IGF-binding protein-3 levels during growth hormone treatment in prepubertal short children born small for gestational age. *J Clin Endocrinol Metab* 81:3902-8.
115. **Sas T, Mulder P, Hokken-Koelega A** 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. *J Clin Endocrinol Metab* 85:3786-92.
116. **de Waal w** 1996 Influencing the extremes of growth: too tall − too small Erasmus Medical Centre. Erasmus Medical Centre, Rotterdam
117. **Arends N** 2003 Short SGA children; etioloical aspects, metabolic consequences and effects of GH treatment Erasmus Medical Centre. Erasmus Medical Centre, Rotterdam
118. **Sas T** 2000 Long term growth hormone treatment in two growth disorders Erasmus Medical Centre. Erasmus Medical Centre, Rotterdam
119. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-23.
120. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.

Chapter 2

Serum dehydroepiandrosterone sulphate (DHEAS) levels and pubarche in short children born Small for Gestational Age (SGA) before and during growth hormone treatment

Venje H. Boonstra,[1] Paul G.H. Mulder,[2] Frank H. de Jong,[3] Anita C.S. Hokken-Koelega[1]

Department of Paediatrics, Division of Endocrinology, Sophia Children's Hospital,[1] Department of Epidemiology & Biostatistics, [2] Department of Internal Medicine,[3] Erasmus University Medical Centre Rotterdam, The Netherlands.



J Clin Endocrinol Metabolism, 2004, 89: 712-717

Abstract

It has been suggested that the programming of the endocrine axes occurs during critical phases of fetal development and will be affected by intra-uterine growth retardation. As a result children born Small for Gestational Age (SGA) might have several hormonal disturbances. In later life one of the questions is: do short children born SGA have higher serum DHEAS levels than their peers? Therefore, we compared serum DHEAS levels of 181 prepubertal 3–9 years old short children born SGA (birth length (SDS) below −2 for gestational age) with a control group of 170 prepubertal age-matched normal statured children born Appropriate for Gestational Age (AGA) (birth length between −2 and +2 SDS). Since relatively high serum DHEAS levels at a young age might result in a premature pubarche, we investigated the incidence of premature pubarche. We also investigated the association between serum DHEAS levels and bone maturation. In addition, we analysed if one year of GH treatment with 1 and 2 mg/m^2/day (≈ 0.035 and 0.070 mg/kg/d respectively) had an effect on serum DHEAS levels of prepubertal short SGA children.

Serum DHEAS levels of the SGA group were comparable with those of age-matched AGA controls. The incidence of premature pubarche was comparable with that of the normal population. There was a weak negative correlation between serum DHEAS levels and bone maturation after the age of 7 years. After one year of GH treatment the increase of serum DHEAS levels was the same for both GH dosage groups and the untreated group.

In conclusion, this study shows that small size at birth, which might be a feature of fetal growth restriction, has no effect on serum DHEAS levels before the age of 9 years. The incidence of premature pubarche is comparable with the normal population. Finally, one year of GH treatment has no effect on serum DHEAS levels.

Introduction

Epidemiological studies have shown a correlation between low birth weight and hypertension, diabetes mellitus type II, hyperlipidemia and cardiovascular disease at a relatively young age.[1] It has been suggested that the programming of the endocrine axes occurs during critical phases of fetal development which will be affected by intra-uterine growth retardation.[2-4] As a result children born SGA might have several hormonal disturbances during later life.

One of the unresolved questions is whether children born SGA without catch-up growth have a disturbed adrenarche, the prepubertal rise in the secretion of the adrenal steroids dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulphate (DHEAS) and androstenedione, and whether these children are at increased risk for a premature pubarche. Premature pubarche is defined as the appearance of pubic hair growth before the age of 8 years in girls and 9 years in boys, and is mostly accompanied by axillary hair, acné and pubertal odour.[5,6] Some studies have reported higher DHEAS levels in children born SGA,[7,8] but other studies did not confirm these results.[9] A possible explanation for these discrepancies could be that various definitions for low birth weight, SGA and catch-up growth were used. Premature pubarche might be caused by relatively high serum DHEAS levels at a young age. Studies in adolescent girls indicated associations between low birth weight and the occurrence of premature adrenarche, pubarche, hyperandrogenism, polycystic ovary syndrome (PCOS) and hyperinsulinism.[10] These findings might have serious consequences for later life.

DHEAS arises primarily from the adrenal cortex, has a relatively long half-life in the circulation and therefore does not exhibit a circadian rhythm.[6,11-13] For that reason determination of serum DHEAS levels are appropriate for evaluation of adrenarche.

In the first part of our study, we assessed whether short prepubertal 3–9 years old children born SGA had serum DHEAS levels which differ from those in normal statured age-matched children born appropriate for gestational age (AGA). In addition, we investigated the incidence of premature pubarche in short children born SGA and whether there was an association between serum DHEAS levels and bone maturation in SGA children.

Many children born SGA without a catch-up growth are being treated with GH. Therefore, in the second part of this study we evaluated the effect of GH treatment (1 or 2 mg/m^2/day) on serum DHEAS levels in short children born SGA.

Patients and methods

Study groups

The SGA group comprised of 181 prepubertal children (91 boys and 90 girls) with short stature born SGA. They were enrolled between 1991 and 2001 in two Dutch multicenter GH-trials in which short children born SGA were treated with GH. The following inclusion criteria were used: 1) birth length below −2 SD for gestational age according to the standards of Usher & McLean,[14] 2) an uncomplicated neonatal period, 3) height SDS for chronological age (CA) below −2.00 according to Dutch reference,[15] 4) height velocity SDS for CA ≤ zero to exclude children presenting spontaneous catch-up growth,[15,16] 5) normal liver, kidney and thyroid functions, 6) prepubertal stage defined as Tanner breast stage I for girls, and testicular volume less than 4 ml for boys,[17] 7) age between 3 and 9 years. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, chondrodysplasia) or syndromes (with exception of Silver-Russell syndrome (SRS)) and previous or present use of drugs that could interfere with growth and GH treatment. Mean birth length (SDS) was −3.4 ± 1.6, birth weight (SDS) −2.5 ± 1.1, gestational age 36.4 ± 3.7 weeks and BMI (SDS) −1.3 ± 1.2.

The control group comprised of 170 healthy age-matched prepubertal children (94 boys and 76 girls) with a normal stature born AGA who were referred to the hospital for a minor surgical procedure. Blood was obtained before anaestheia was given. Normal stature was defined as a height between −2 and +2 SDS according to Dutch references.[15] All children were between 3 and 9 years. None of the children had a syndrome or chromosomal abnormality, endocrine or metabolic disorder, or any other illness or use of drugs that might have effected DHEAS levels.

The study was approved by the Ethics Committee of each participating centre. Written informed consent was obtained from the parents or custodians of each child.

Study design

In the first part of this study the serum DHEAS levels were compared between the SGA group and the AGA controls. Venous blood samples were obtained for determination of serum DHEAS levels. In the SGA group blood was obtained before the start of GH treatment, the incidence of premature pubarche was assessed and bone maturation determined.

In the second part of this study the 1-year effect of GH treatment (1 or 2 mg GH/m^2/day) on serum DHEAS levels was evaluated in the SGA group in comparison with changes after one year in a randomised untreated SGA group. The SGA group consisted

of participants of two Dutch GH trials who met the same inclusion and exclusion criteria. All patients had a GH stimulation test to exclude GH-deficiency. The first study consisted of 56 short SGA children who were 1:1 randomly and blindly assigned to either a group receiving 1 mg or 2 mg GH/m^2/day (≈ 0.035 and 0.070 mg/kg/d respectively).[18] The second study consisted of 125 short SGA children who were 1:2 randomly assigned to either a group without GH treatment (untreated group) or a group receiving 1 mg GH/m^2/day (≈ 0.035 mg/kg/d). Together this resulted into three study groups: an untreated control group (n = 42), a group who received 1 mg GH/m^2/day (n = 111) and a group treated with 2 mg GH/m^2/day (n = 28). Biosynthetic GH (r-hGH NorditropinR, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime with a pen injection system (Nordiject 24). Every three months the total GH dose was adjusted to the calculated body surface. Venous blood samples were obtained after one year of GH treatment or non-treatment. All samples were frozen (−80°C) until assayed.

Physical examination

Before start of GH treatment and every 3 months after the start of GH treatment SGA children had a physical examination including measurement of standing height, weight and pubertal stage. Height was measured according to Cameron[19] using a Harpenden stadiometer. Height and weight were expressed as standard deviation score (HSDS).[15] Pubertal stages were assessed according to Tanner[17] using an orchidometer in boys. Premature pubarche was defined as the appearance of pubic hair stage 2 or more before the age of 8 years in girls and 9 years in boys.

Hormone assays

DHEAS levels were determined in one central laboratory and measured using a chemiluminescence based competitive immunoassay (Immulite1, Diagnostic Products Corporation, Los Angeles, California). The interassay coefficient was 8%. The limit of detection was 0.2 µmol/l. Values below this limit of detection were considered to be 0.1 µmol/l.

Bone maturation

For the SGA children an X-ray of the left hand was made before start of GH therapy and after one year of GH treatment. All bone ages (BA) were determined by one investigator according to Tanner and Whitehouse radius, ulna and short bones score (BArus).[20]

Statistical analyses

Serum DHEAS levels are presented as median and interquartile range, bone ages as mean ± SD. The SGA children and the AGA controls were divided into various age groups: group I: 3.00–4.99 years, group II: 5.00–6.99 years and group III: 7.00–8.99 years. Differences in serum DHEAS levels between the SGA children and AGA controls were tested per subgroup of age and gender. In case of a non-Gaussian shaped DHEAS distribution the Mann-Whitney test was used. If the DHEAS distribution within a group was Gaussian shaped, the ANCOVA was used with age as covariate. Differences between bone-age and chronological-age were tested using the paired samples t-test. The Spearman rank correlation test was used to test the correlations between serum DHEAS levels and age, BMI and difference between chronological age and bone age. The correlations between DHEAS and birth weight and birth length were tested with the partial correlation test corrected for age at time of study. A p-value < 0.05 was considered significant. All analyses were performed using SPSS version 10.0.

Results

Untreated SGA children versus AGA controls

Table 1 shows the serum DHEAS levels and age in the various age groups for the SGA children compared to the age-matched AGA controls, for boys and girls. For both the SGA children and AGA controls, we found higher serum DHEAS levels with increasing age. There was no significant difference in age between the SGA group and AGA controls. The serum DHEAS levels per age group were not higher for the SGA group compared to AGA controls. SGA girls aged 7.00–8.99 years had even significantly lower serum DHEAS levels compared to age-matched AGA girls. Table 1 also shows the BMI SDS for the SGA children. In all age groups the mean BMI SDS for the SGA children was significantly lower than zero. Only one SGA boy in age group III had a BMI SDS above 2.

For both the SGA group and AGA controls a positive correlation was found between serum DHEAS levels and age ($r = 0.47$, $P < 0.001$ and $r = 0.56$, $P < 0.001$, respectively). A weak but significant negative correlation was found between serum DHEAS levels and birth weight SDS and birth length SDS in children born SGA ($r = -0.30$, $p < 0.01$ and $r = -0.29$, $p < 0.01$ respectively), but after correction for age at time of study the correlations disappeared. No correlation was found between serum DHEAS levels and BMI SDS at time of study.

Of the 90 SGA girls, 2 girls had first signs of pubarche (P2) before the age of 8 years. As pubic hair development stage > 1 was not an exclusion criterion for the GH

trials, these girls were enrolled in the study at age of 8.5 years. At that age serum DHEAS levels were 1.60 µmol/l and 3.00 µmol/l and bone ages were 9.8 years and 9.6 years, respectively. The normal interval range for serum DHEAS for girls aged 7.0 to 8.9 is 1.19 (0.37–2.00) µmol/l. None of the boys had signs of pubarche before the age of 9 years.

Table 2 shows that all age groups of SGA children had a bone age delay (ΔCA−BA), with a significantly lower mean bone age than chronological age (CA) ($p < 0.001$). Until the age of 7 years we did not find a correlation between serum DHEAS levels and degree of bone age delay, but after the age of 7 years a weak negative correlation was found between serum DHEAS levels and degree of bone age delay ($r = -0.34$, $p < 0.001$).

Table 1. Age and serum DHEAS levels for boys and girls per age group in SGA children and AGA controls and BMI SDS for SGA children.

		SGA children				AGA controls		
	Age group	n	Age	DHEAS	BMI SDS*	n	Age	DHEAS
			(years)	(µmol/l)			(years)	(µmol/l)
Boys	I	26	3.9 (3.7–4.6)	0.1 (0.1 – 0.3)	−1.5 (1.2)[1]	30	3.7 (3.3–4.3)	0.1 (0.1 – 0.1)
	II	29	6.0 (5.4–6.7)	0.1 (0.1 – 0.7)	−1.0 (1.0)[1]	30	6.0 (5.4–6.4)	0.3 (0.1 – 0.8)
	III	36	7.7 (7.2–8.3)	1.0 (0.2 – 2.3)	−1.0 (1.1)[1]	34	7.9 (7.4–8.5)	0.5 (0.3 – 0.9)
Girls	I	23	4.0 (3.5–4.5)	0.1 (0.1 – 0.3)	−1.9 (1.6)[1]	26	4.3 (3.6–4.6)	0.1 (0.1 – 0.2)
	II	37	6.2 (5.3–6.6)	0.3 (0.1 – 0.5)	−1.5 (1.2)[1]	30	5.9 (5.4–6.3)	0.3 (0.2 – 0.4)
	III	30	7.9 (7.4–8.6)	0.5 (0.2 – 1.2)	−0.9 (1.0)[1]	20	7.9 (7.5–8.6)	1.2 (0.4 – 2.0)[2]

Data expressed as median (interquartile range). *Data expressed as mean (SD). *1)* $p < 0.01$, *vs* 0 SDS., *2)* $p < 0.05$, AGA *vs* SGA. Age group I: age 3.00 to 4.99 years; II: age 5.00−6.99 years; III: age 7.00−8.99 years.

Table 2. Bone age compared to chronological age in 164 SGA children.

Age group	n	Chronological Age (years)	Bone age (years)#
I (3-4.99)	43	4.0 ± 0.6	3.2 ± 0.9*
II (5-6.99)	59	6.0 ± 0.7	5.0 ± 1.3*
III (7-8.99)	62	7.9 ± 0.6	6.9 ± 1.7*

Data expressed as mean ± SD, # Bone age according to TW−RUS score, *$p < 0.001$.

Effect of 1 year of GH treatment versus no treatment

Table 3 shows that baseline clinical data were comparable for the 3 study groups. The mean age of the children receiving 2mg GH/m^2/day was 11 months older than that of

the untreated group but this age-difference was not significant. Table 4 shows the age, serum DHEAS levels and progression of bone age after one year of GH treatment or no treatment. In the oldest age group the age was significantly different between the three study groups. We therefore used the ANCOVA test adjusted for age. After adjusting for age, the serum DHEAS levels of the 3 study groups per age group were not significantly different between the three SGA study groups. The progression of bone maturation (the increase of bone age divided by the increase of chronological age) in age group II was significantly faster in the group receiving 2 mg GH/m^2/day compared to the groups without GH treatment and the group receiving 1 mg GH/m^2/day .

Table 3. Clinical data at start of GH trial in 181 short SGA children.

	SGA study groups		
	Untreated group	1 mg GH/m^2/d	2 mg GH/m^2/d
Male/Female	19 / 23	57 / 54	15 / 13
Gestational age (wk)	36.3 ± 3.5	36.6 ± 3.7	35.6 ± 4.1
Birthlength SDS	−3.7 ± 2.3	−3.3 ± 1.2	−3.6 ± 1.7
Birthweight SDS	−2.8 ± 1.0	−2.3 ± 1.1	−2.7 ± 1.2
Chronological age (yr)	5.8 ± 1.5	6.1 ± 1.6	6.7 ± 1.9
Height SDS	−3.1 ± 0.6	−3.0 ± 0.7	−3.1 ± 0.7

Data expressed as mean ± SD.

Discussion

We investigated the serum DHEAS levels in a large group of 181 short prepubertal, 3–9 years old children born SGA in comparison with 171 age-matched AGA controls. Our data show that short prepubertal children born SGA have normal serum DHEAS levels and that one year of GH treatment had no influence on the serum DHEAS levels. Premature pubarche was found in 2.2% of the girls and in none of the boys. Age was positively correlated with serum DHEAS levels. After the age of 7 years a weak negative correlation was found between serum DHEAS levels and bone age delay. No correlation was found between serum DHEAS levels and BMI SDS.

Normal serum DHEAS levels were found in short prepubertal SGA children compared to age-matched AGA controls. SGA girls aged 7.00–8.99 years had even significantly lower serum DHEAS levels compared to their AGA controls. Previously, other studies reported higher serum DHEAS levels in individuals born SGA.[2,7,8,21,22] However, most of these studies are much smaller and used different definitions. For example some studies included individuals with low birth weight not corrected for gestational age or evaluated the effect of birth weight or length in a group of children born AGA. In most studies serum DHEAS levels were studied in individuals during or after puberty or in SGA

children who underwent a spontaneous catch-up growth after birth. Our results are in agreement with those of a French study of normal statured adult women born SGA in whom also no differences were found in serum DHEAS levels between women born SGA and an AGA control group.[9] Dahlgren *et al* also did not find significantly different serum DHEAS levels in 33 short SGA children (defined as a weight or a length at birth below −2SDS) compared to 35 normal statured AGA children. However, this study included both prepubertal and pubertal children with an age between 2.8 and 15.5 years.[23] To our knowledge our study is the first one investigating serum DHEAS levels in a large group of prepubertal short SGA children.

Serum DHEAS levels were positively correlated with age. However, we did not find a correlation between birth weight (SDS), birth length (SDS) and serum DHEAS levels in SGA children after correction for age at time of study. Dahlgren *et al* found a negative correlation between serum DHEAS levels and birth weight in SGA and AGA children together, but this correlation disappeared after the age of 9 years. A possible explanation for this discrepancy is that these authors did not correct for age at investigation below the age of 9 years, while they also found a significant correlation between serum DHEAS levels and age.[23]

Ibanez *et al* reported a significantly lower birth weight in girls with ovarian hyperandrogenism who also had a premature pubarche.[24] It was concluded that girls with a premature pubarche born SGA are at higher risk getting Polycystic Ovarian Syndrome (PCOS).[10] However, these studies have been performed in a relatively small patient group from a specific part of Spain. Their patients presented with abnormalities and the association with low birth weight was found retrospectively. We feel that selection on low birth weight is more appropriate for studying the consequences of a small size at birth on serum DHEAS levels at varying prepubertal ages. In the Dutch GH trials pubic hair development stage > 1 was not an exclusion criterion.[17] Serum DHEAS levels in our SGA group were normal, it is not surprising that only 2.2% of the girls and none of the boys had a premature pubarche. This is comparable with the incidence of premature pubarche in the normal population, in which the incidence in white girls younger than 8 years is 2.8%.[25] One of the two SGA girls with a premature pubarche had DHEAS levels above the normal range.

Some studies reported a positive correlation between weight and serum DHEAS levels. Particularly marked weight gain and obesity were associated with high serum DHEAS levels.[26] In contrast, we did not find a correlation between BMI SDS and serum DHEAS levels in our prepubertal SGA group. One of the explanations might be that our SGA children were lean with a mean BMI SDS significantly lower than zero. Only one boy had a BMI SDS above 2. In addition there was only a narrow variation in the BMI SDS of our prepubertal SGA children.

The SGA children had a one-year delay in bone age which was similar for all three age groups. Mean bone maturation was not advanced, at least not until a mean (SD) age of 7.9 (0.6) years, which does not exclude that acceleration of bone maturation might occur at a later age as has previously been reported. Tanner found an acceleration of bone age from the age of 8 years in short children with Silver Rusell syndrome born SGA.[27] Before the age of 7 years we did not find a correlation between serum DHEAS levels and bone age delay. But after the age of 7 years we found a weak but significant negative correlation between serum DHEAS levels and bone age delay, suggesting that DHEAS might be one of the factors responsible for the acceleration of bone maturation in SGA children after the age of 7 years. In addition the two SGA girls who had a premature pubarche showed high serum DHEAS levels and an advanced bone age.

In several studies short SGA children are being treated with biosynthetic growth hormone (GH). It is known that GH increases IGF-1 levels and that IGF-1 plays an important role in the biosynthesis of adrenal steroids.[6,18] For this reason we investigated if GH treatment might have an influence on the adrenarche of SGA children. However, we showed that one year of GH treatment has no influence on serum DHEAS levels in SGA children regardless of the GH dose of 1 or 2 mg GH/m^2/day. The age in age group III was significantly different between the three GH treatment groups. For this reason the DHEAS levels were different after one year of GH treatment but after correction for age there was no significantly difference anymore between serum DHEAS levels in the three groups. It has also been reported that the administration of GH in children with idiopathic GH deficiency did not modify the adrenal androgen plasma levels.[28] These data support our data indicating that GH treatment does not induce higher serum DHEAS levels. After one year the progression of bone maturation was only significantly higher in age group II who received 2 mg GH/m^2/day. However, van Pareren *et al* showed in the same group that there was no GH-dose effect on bone maturation after 5 years of GH treatment.[29]

In conclusion, this study shows that small size at birth, which might be a feature of fetal growth restriction, has no effect on serum DHEAS levels before the age of 9 years. The incidence of premature pubarche is comparable with the normal population. In addition one year of GH treatment has no effect on serum DHEAS levels.

Table 4. Age, serum DHEAS levels and ΔBA/ΔCA after 1 year of GH-treatment in SGA children.

GH treatment groups									
Age group	0 mg/m^2/d (n=42)			1 mg/m^2/d (n=111)			2 mg/m^2/d (n=28)		
	Age (yr)	DHEAS (μmol/l)	ΔBA/ΔCA	Age (yr)	DHEAS (μmol/l)	ΔBA/ΔCA	Age (yr)	DHEAS (μmol/l)	ΔBA/ΔCA
I	4.9 (4.4 – 5.0)	0.2 (0.1–0.7)	0.9 (0.6 – 1.4)	5.1 (4.7 – 5.6)	0.3 (0.1–0.5)	1.3 (0.9 – 1.8)	4.8 (4.3 – 5.6)	0.2 (0.1–0.5)	1.0 (0.9 – 1.9)
II	7.0 (6.3 – 7.4)	0.6 (0.4–1.4)	0.9 (0.6 – 1.4)	6.9 (6.3 – 7.7)	0.4 (0.2–1.3)	1.4 (0.9 – 2.2)	7.5 (6.6 – 7.8)	1.2 (0.4–2.9)	2.8 (1.8 – 3.1)[4,5]
III	8.2 (8.1 – 8.7)	0.8 (0.3–1.1)	1.1 (0.4 – 1.5)	8.8 (8.4 – 9.3)[1]	1.3 (0.6–1.8)	1.5 (0.7 – 1.8)	9.6 (9.0 – 9.7)[2,3]	2.0 (1.4–2.5)	1.2 (0.1 – 2.5)

Data expressed as median (interquartile range). *1)* p < 0.05, 0mg *vs* 1mg., *2)* p < 0.05, 1mg *vs* 2mg., *3)* p < 0.001, 0mg *vs* 2mg., *4)* p < 0.05, 1mg *vs* 2mg., *5)* p<0.05, 0mg vs 2mg.

References

1. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM.** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-67.
2. **Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ.** 1996 Size at birth and adrenocortical function in childhood. *Clin Endocrinol* (Oxf) 45:721-726.
3. **Chatelain PG, Nicolino M, Claris O, Salle B, Chaussain J.** 1998 Multiple hormone resistance in short children born with intrauterine growth retardation? Horm Res 49:20-22.
4. **Clark PM.** 1998 Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. *Eur J Pediatr* 157 Suppl 1:S7-10.
5. **Pere A, Perheentupa J, Peter M, Voutilainen R.** 1995 Follow up of growth and steroids in premature adrenarche. *Eur J Pediatr* 154:346-352.
6. **Ibanez L, Dimartino-Nardi J, Potau N, Saenger P.** 2000 Premature adrenarche - normal variant or forerunner of adult disease? *Endocr Rev* 21:671-696.
7. **Francois I, de Zegher F.** 1997 Adrenarche and fetal growth. *Pediatr Res* 41:440-442.
8. **Szathmari M, Vasarhelyi B, Tulassay T.** 2001 Effect of low birth weight on adrenal steroids and carbohydrate metabolism in early adulthood. *Horm Res* 55:172-178
9. **Jaquet D, Leger J, Chevenne D, Czernichow P, Levy-Marchal C.** 1999 Intrauterine growth retardation predisposes to insulin resistance but not to hyperandrogenism in young women. *J Clin Endocrinol Metab* 84:3945-3949.
10. **Ibanez L, Potau N, Marcos MV, De Zegher F.** 2000 Adrenal hyperandrogenism in adolescent girls with a history of low birthweight and precocious pubarche. *Clin Endocrinol* (Oxf) 53:523-527.
11. **Thomas G, Frenoy N, Legrain S, Sebag-Lanoe R, Baulieu EE, Debuire B.** 1994 Serum dehydroepiandrosterone sulfate levels as an individual marker. *J Clin Endocrinol Metab* 79:1273-1276.
12. **Orentreich N, Brind JL, Rizer RL, Vogelman JH.** 1984 Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. J *Clin Endocrinol Metab* 59:551-555.
13. **Wierman ME, Beardsworth DE, Crawford JD, Crigler JF, Jr., Mansfield MJ, Bode HH, Boepple PA, Kushner DC, Crowley WF, Jr.** 1986 Adrenarche and skeletal maturation during luteinizing hormone releasing hormone analogue suppression of gonadarche. *J Clin Invest* 77:121-126.
14. **Usher R, McLean F.** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-910.
15. **van Wieringen JC, Roede MJ, Wit JM.** 1985 [Growth diagrams for patient care]. *Tijdschr Kindergeneeskd* 53:147-152.
16. **Rikken B, Wit JM.** 1992 Prepubertal height velocity references over a wide age range. *Arch Dis Child* 67:1277-1280.
17. **Tanner JM, Whitehouse RH.** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-179.
18. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A.** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-3070.

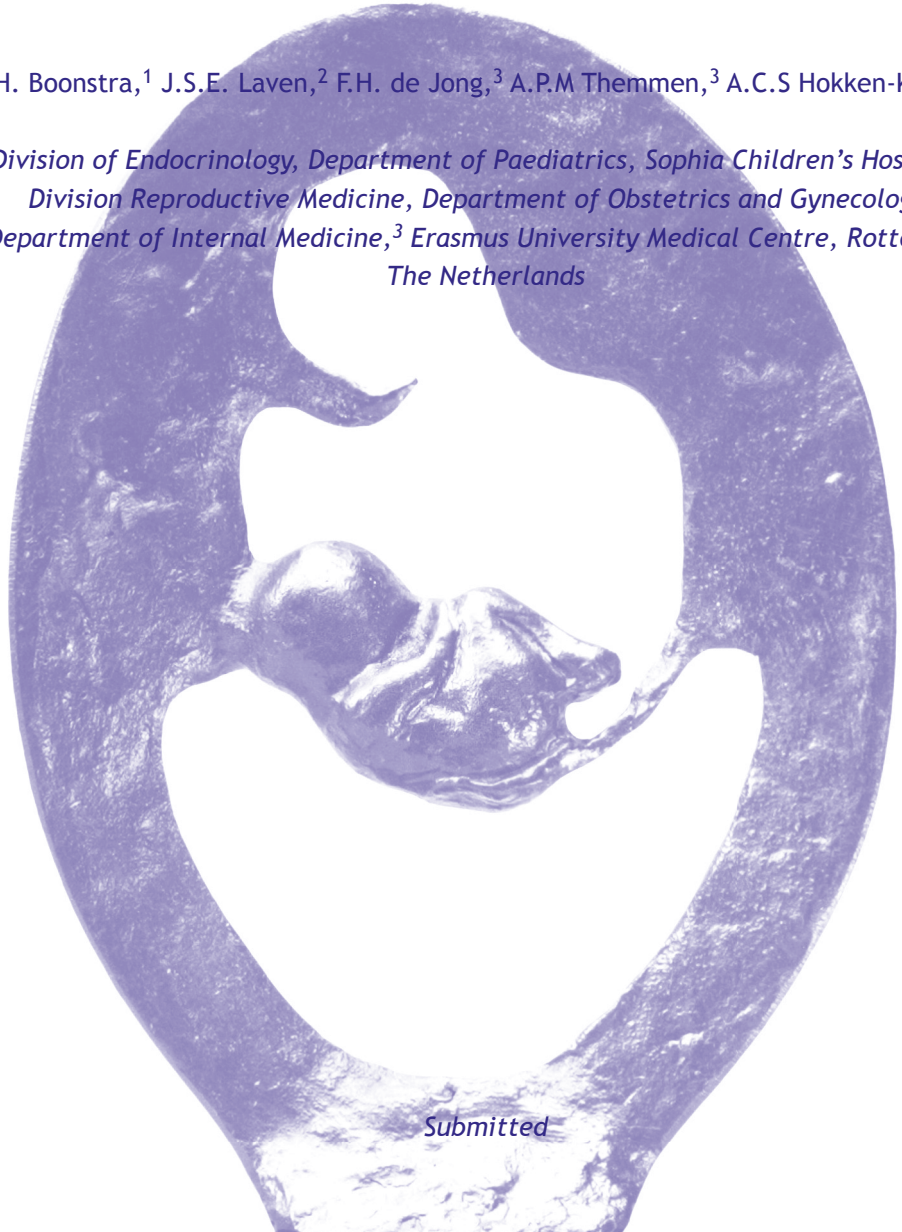
19. **Cameron N** 1978 The methods of auxological anthropometry. In: Falkner F, Tanner J (eds) Human growth, postnatal growth 2 ed. Tindall, london
20. **Tanner J, Whitehouse R, Cameron N, Marchall W, Healy M, Goldstein H** 1983 Assessment of skeletal maturity and prediction of adult height (TW2-method) 2 ed. academic press, london
21. **Ibanez L, Potau N, Marcos MV, de Zegher F.** 1999 Exaggerated adrenarche and hyperinsulinism in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 84:4739-4741.
22. **Ghirri P, Bernardini M, Vuerich M, Cuttano A, Coccoli L, Merusi I, Ciulli C, D'Accavio L, Bottone U, Boldrini A.** 2001 Adrenarche, pubertal development, age at menarche and final height of full-term, born small for gestational age (SGA) girls. *Gynecological Endocrinology* 15:91-97
23. **Dahlgren J, Boguszewski M, Rosberg S, Albertsson-Wikland K.** 1998 Adrenal steroid hormones in short children born small for gestational age. *Clin Endocrinol* (Oxf) 49:353-361.
24. **Ibanez L, Potau N, Francois I, de Zegher F.** 1998 Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab* 83:3558-3562.
25. **Herman-Giddens ME, Slora EJ, Wasserman RC, Bourdony CJ, Bhapkar MV, Koch GG, Hasemeier CM.** 1997 Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Ped* 99:505-512.
26. **Remer T, Manz F.** 1999 Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab* 84:3936-3944.
27. **Tanner J, Lejarraga H, Cameron N.** 1975 The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. *Pediatr Res* 9:611-623.
28. **Rossi E, Merola B, Longobardi S, Esposito V, Tommaselli AP, Colao A, Lombardi G.** 1995 Acute and chronic effects of human recombinant GH (hrGH) on adrenal steroidogenesis in children affected with isolated GH deficiency (IGHD). *J Clin Endocrinol Metab* 80:2251-2254
29. **Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A.** 2003 Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab* 88:3584-3590.

Chapter 3

Normal serum Anti-Müllerian Hormone (AMH) levels in short girls born Small for Gestational AGA (SGA)

V.H. Boonstra,[1] J.S.E. Laven,[2] F.H. de Jong,[3] A.P.M Themmen,[3] A.C.S Hokken-Koelega[1]

Division of Endocrinology, Department of Paediatrics, Sophia Children's Hospital,[1] Division Reproductive Medicine, Department of Obstetrics and Gynecology,[2] Department of Internal Medicine,[3] Erasmus University Medical Centre, Rotterdam, The Netherlands



Submitted

Abstract

It has been suggested that impaired fetal growth might lead to permanent changes in organ structure and physiology. As a consequence short girls born SGA might have a reduced size of the ovarian primordial follicle pool, which might result in earlier follicle pool depletion. This would increase the risk of infertility and premature ovarian failure for these girls. Since serum Antimullerian Hormone (AMH) levels correlate well with the size of the ovarian follicle pool, we investigated whether short girls born SGA have a reduced ovarian follicle pool compared to age-matched girls born Appropriate for Gestational Age (AGA), by measuring serum AMH levels.

We compared serum AMH levels of 35 prepubertal short girls born SGA (birth length SD and height < −2SD) with 16 age-matched prepubertal normal statured girls born AGA (birth length and height SD between −2 and +2). We also compared serum AMH levels of growth hormone (GH)-treated prepubertal SGA girls (n = 30) with age-matched untreated SGA girls (n = 35) and normal AGA girls (n = 16). In addition, we compared serum AMH levels of post-menarchal SGA girls (n = 31) with age-matched AGA girls (n = 27).

Median (interquartile) serum AMH levels of prepubertal untreated short SGA girls, GH-treated SGA girls and AGA controls were 1.8 (1.1–3.3), 1.6 (0.7–2.9) and 1.8 (1.0–2.8) µg/l, respectively. No significant differences were found between these levels. The median serum AMH levels of the post-menarchal SGA and AGA girls, being 1.9 (0.9–3.7) and 2.0 (1.7–3.5), respectively, were also not significantly different.

In conclusion, our study indicates that small size at birth, which might reflect fetal growth restriction, has no effect on the serum AMH levels in short SGA girls. Therefore, our results strongly suggest that the primordial follicle pool in short SGA girls is comparable to that of age-matched AGA girls.

Introduction

Since the first reports demonstrating an association between low birth weight (LBW) and high blood pressure,[1] a large number of studies have elaborated on the consequences of LBW in relation to adult diseases. Impaired fetal growth may lead to permanent changes in organ structure and physiology as suggested by the fetal programming hypothesis.[2, 3] Consequently short girls born small for gestational age (SGA) might have impaired ovarian development and a reduced ovarian follicle size, since the most dynamic phase of ovarian development occurs before birth.[4,5] This might result into earlier follicle pool depletion and would increase the risk for infertility and premature ovarian failure for these girls.

Human follicle development starts in the twelfth week of intra-uterine life and by the fifth month the maximum size of the ovarian follicle pool is reached. During fetal life and childhood, follicles develop through primordial, pre-antral stages, into small antral follicles.[4,6] As a result, the ovaries consist of a mixed population of follicles in different developmental stages.

It appears that the number of pre-antral and small antral follicles is closely linked to the size of the primordial follicle pool.[6-8] The granulosa cells of pre-antral and small antral follicles produce the dimeric glycoprotein Anti-Műllerian Hormone (AMH). For this reason serum AMH levels reflect the number of pre-antral and antral follicles which in turn is a good measure of the size of the ovarian follicle pool.[7,8] AMH, also referred as Müllerian-inhibiting substance, is a member of the TGF ß superfamily. During fetal sex differentiation, AMH is produced by Sertoli cells in the male, in which it induces degeneration of the Müllerian ducts. In females, AMH is produced only postnatally by the granulosa cells of the ovary and is involved in the regulation of early folliculogenesis. [6,7,9-12]

If it is true that SGA girls have a reduced size of the ovarian follicle pool compared to AGA girls, their serum AMH levels should be lower because of a lower number of pre-antral and small antral follicles.

In this study we assessed whether short prepubertal SGA girls have a reduced size of their ovarian follicle pool compared to age-matched prepubertal AGA girls by measuring serum AMH levels. In addition, since many short children born SGA are now treated with growth hormone (GH), we investigated the effect of 2 years of GH treatment on serum AMH levels. We also investigated whether the number of pre-antral and small antral follicles in GH-treated post-menarchal SGA girls was different compared to age-matched AGA girls by measuring serum AMH levels.

Patients and Methods

Subjects

The SGA group consisted of 35 short prepubertal girls without GH treatment (aged 6.0 to 7.9 years), 30 GH-treated prepubertal girls (aged 6.0 to 7.9 years), and 31 postmenarchal girls who had been treated with GH because of short stature (aged 14.0 to 17.9 years). They were originally enrolled in 2 Dutch multicenter GH trials in which short children born SGA were treated with GH. At start of the GH trials, all children fulfilled the same inclusion criteria: 1) birth length and birth weight below −2 SD for gestational age;[13] 2) height SD score for age below −2 SD score according to Dutch standards;[14] 3) height velocity SD score for age below zero to exclude children with spontaneous catch-up growth; 4) an uncomplicated neonatal period without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes) or long term complications of respiratory ventilation; 5) age between 3 and 9 years; 6) prepubertal stage defined as Tanner breast stage I.[15] Children with emotional deprivation, systemic disorders or syndromes (except the Silver Russell syndrome) were excluded. The SGA girls had a mean (SD) birth length standard deviation score (SDS) of −3.4 (1.5), birth weight SDS of −2.5 (1.1), gestational age of 36.4 (3.8) weeks, height SDS of −3.2 (0.7) and a BMI SDS of −1.3 (1.2).

The first GH trial started in 1991 and consisted of 31 girls. At start of the GH trial they were randomly and blindly assigned (1:1) to either a group receiving 1 mg or 2 mg GH/m^2/day (≈ 0.035 and 0.070 mg/kg/d, respectively).[16] At inclusion in the present study, they all had had their menarche and had reached adult height. These girls were treated with GH for 4.9 (1.8) years. The second study started in 1996 and consisted of 65 short SGA girls who were randomly assigned (1:2) to either a group without GH treatment (untreated group) or a group receiving 1 mg GH/m^2/day (≈ 0.035 mg/kg/d).[17] At inclusion in the present study these girls were still prepubertal.

The AGA control group consisted of 16 AGA prepubertal girls, aged 6.0 to 7.9 years and 27 post-menarchal girls, aged 14.0 to 17.9 years, with normal sature born AGA. All these girls had a birth length between −2 SD and +2 SD. Normal stature was defined as a height between −2 SD and +2 SD according to Dutch standards (14). None of the girls had a systemic disorder or syndrome.

Serum AMH levels of the untreated prepubertal short SGA girls (n = 35) and GH-treated SGA girls (n = 30) were compared with the serum AMH levels of prepubertal age-matched AGA girls (n = 16). Serum AMH levels of the post-menarchal SGA girls (n = 31) were compared with age-matched post-menarchal AGA girls (n = 27). Serum AMH levels of the prepubertal girls were also compared with the levels of post-menarchal girls. This study was approved by the Ethics Committee of each participating centre. Written informed consent was obtained from the parents or custodians of each child.

Assays

Serum levels of AMH were determined in one central laboratory. The serum levels were measured by an ultrasensitive enzyme-linked immunosorbent assay (Immunotech-Coulter, Marseilles, France). The intra- and inter-assay coefficients of variation were below 5% and 8%, respectively.[7]

Statistics

Serum AMH levels are presented as median and interquartile range, since the data were skewed. Differences between the groups were tested using Mann-Whitney tests. The corresponding 95% confidence interval (CI) was used in case of no significance. The non-parametric CI according to Hodges-Lehmann was used based on the difference between the median of the two groups. Correlations between AMH and birth length, birth weight and age were tested with the Spearman correlation test. All analyses were performed using SPSS version 10.0. A p value < 0.05 was considered significant.

Results

The median (interquartile range) ages of the short prepubertal SGA group without GH treatment, with GH treatment and AGA controls were 7.0 (6.6–7.4), 7.1 (6.4–7.4) and 6.9 (6.3–7.6) years, respectively. No significant differences were found between the median ages of the three prepubertal groups. Table 1 shows the serum AMH levels of the prepubertal SGA and AGA girls. The GH-treated SGA girls were treated with 1 mg or 2 mg GH/m^2/day. Since there was no significant difference (p = 0.8) in serum AMH levels between the GH dosages, data of both GH dosage-groups were analysed together.

Table 1. Serum AMH levels (µg/L) in prepubertal and adolescent SGA girls without or with GH treatment *vs* prepubertal and adolescent AGA controls

	SGA				AGA	
	n	No GH treatment	n	GH treatment	n	No GH treatment
Prepubertal (6.0 – 7.9 yr)	35	1.8 (1.1 – 3.3)[1,2]	30	1.6 (0.7 – 2.9)[3]	16	1.8 (1.0 – 2.8)
Adolescent (14.0 – 18.0 yr)			31	1.9 (0.9 – 3.7)[4]	27	2.0 (1.7 – 3.5)

Data expressed as median (interquartile range)

1) CI of the difference between SGA without GH *vs* AGA: 0.1 (–0.6–0.9)
2) CI of the difference between SGA with GH *vs* SGA without GH: –0.2 (-1.0–0.3)
3) CI of the difference between SGA with GH *vs* AGA: –0.3 (-1.0–0.6)
4) CI of the difference between SGA *vs* AGA: –0.4 (-1.2–0.5)

Serum AMH levels were not significantly different between short prepubertal SGA girls and age-matched AGA girls. Similarly, no significant difference in serum AMH levels was found between GH-treated prepubertal SGA girls and untreated short prepubertal SGA girls or AGA controls.

The median (interquartile range) ages of the post-menarchal SGA group and AGA controls were 15.3 (14.3–15.9) and 15.8 (14.5–17.7) years, respectively. There was no significant difference in median age between these girls. The median (interquartile range) age of the menarche in the SGA group was 13.0 (12.4–14.0) years and in the AGA group 13.2 (11.9–14.2), and did not differ significantly. The median serum AMH levels of the post-menarchal SGA girls were similar to those of the post-menarchal AGA girls (Table 1).

Furthermore, we found no significant difference in serum AMH levels between prepubertal short SGA girls and post-menarchal SGA girls or between prepubertal AGA girls and post-menarchal AGA girls. In the prepubertal and post-menarchal SGA group and AGA group we found no correlation between serum AMH levels and age. Taken the prepubertal and post-menarchal groups together we did not find a correlation between serum AMH levels and age. In the SGA group we found no correlations between serum AMH levels and birth length SDS or birth weight SDS ($r = 0.01$, $p = 0.6$; $r = -0.04$,$p = 0.8$, respectively). The scatterplot showed a random pattern between the two variables, indicating no relationship between them (not shown).

Discussion

In this study we investigated whether fetal growth restriction has an effect on the size of the ovarian follicle pool in short SGA girls. Serum AMH levels were used as a measure of the ovarian follicle pool size. The effect of size at birth on the ovarian follicle pool was measured by comparing the serum AMH levels of short prepubertal SGA girls to age-matched AGA girls. In addition, we compared serum AMH levels of short untreated SGA girls to GH-treated SGA girls. Furthermore, serum AMH levels of GH-treated post-menarchal SGA girls were compared to age-matched AGA girls. Our data show that there is neither a significant difference in prepubertal AMH levels between short SGA and AGA girls nor between GH-treated or untreated prepubertal SGA girls. Also during adolescence AMH levels appeared comparable for SGA and AGA girls. Finally, there was no difference in serum AMH levels between prepubertal and post-menarchal girls.

In girls, serum AMH appears to reflect ovarian activity, since production of this hormone is found exclusively in the preantral and antral follicles of the ovary. The number of preantral and small antral follicles correlates well with the size of the ovarian follicle pool. For this reason the serum AMH level can be used as an indirect measurement of

the ovarian follicle pool size.[8,18] This is supported by a study of de Vet *et al*[7] in which serum AMH levels were strongly correlated with the number of preantral and small antral- follicles in the ovary of women, established by transvaginal ultrasonography. Subsequently, they found that AMH levels decrease with an increasing age after the age of 20 years and become undetectable after menopause. This is not surprising since ovarian follicles decrease with age and are depleted after menopause.

We found that serum AMH levels in short prepubertal SGA girls did not differ significantly from those of prepubertal AGA girls, indicating that the size of the ovarian follicle pool of short prepubertal SGA girls is not reduced compared to those of age-matched AGA girls. This is supported by the recent study of Bruin *et al* in which fetal growth restriction was also not associated with a disturbed ovarian development.[5] In this study an autopsy was done in 7 stillborn fetuses, in which the follicles were determined from histological sections of the ovarian cortex of these fetuses. These results stand in contrast to a preliminary study of Bruin *et al* which showed that prenatal growth restriction was associated with a reduced ovarian fraction of primordial follicles in 4 growth-restricted stillborn fetuses.[19] The authors explained the contrasting findings by the larger number of fetuses and an improved methodology in the most recent study.[5] In addition, we did neither find a correlation of serum AMH levels with birth weight SDS nor with birth length SDS, suggesting that the size of the ovarian follicle pool is not related to the fetal size at birth. Ibanez *et al* concluded that SGA girls with a catch-up growth might have an impaired ovarian development.[20,21] However, these studies are not comparable with our data since they studied different indices in a different population.

Many short children born SGA who remain short after birth are nowadays treated with GH. Major adverse effects of GH replacement are uncommon and several studies showed that GH is safe and well tolerated; however, it is important to monitor these children carefully. Our study shows that there is no significant difference between GH-treated SGA girls and untreated SGA girls or AGA girls, which indicates that GH treatment does not change the size of the ovarian follicle pool in SGA girls.

The serum AMH levels of prepubertal girls were comparable with those of post-menarchal girls. No correlation was found between serum AMH levels and age in the age range of 6 to 18 years. De Vet *et al* reported for normo-ovulatory women, aged 20 to 40 years, a negative correlation between serum AMH levels and age.[7] The reason that we did not find such a correlation might be that serum AMH levels start to decline as a function of age after the age of 20 years.[11,22]

All adolescent SGA and AGA girls in this study had had their menarche, at least 2 years before. Venous blood samples were obtained independent of the menstrual cycle. However, this should not have affected the AMH results as very early follicular development to preantral and small antral stage is independent of gonadotropin secretion

and thus cycle.[4] In previous studies from our group it was already shown that AMH levels found in patients as well as in controls were not significantly different between follicular and luteal phase samples, which is consistent with the continuous noncyclic growth of preantral and small antral follicles.[7,23,24]

From the onset of puberty, follicles develop through small antral follicles to antral follicles. At the antral stage most follicles undergo atresia whereas a few of them, under the cyclic gonadotropin stimulation that starts during puberty, reach the preovulatory stage.[6] Our data show that post-menarchal SGA girls without a spontaneous catch-up growth compared to age-matched AGA girls have similar serum AMH levels, indicating that the number of small antral follicles is the same in these girls. These girls have been using GH treatment but this should not have influenced the serum AMH levels as we also did not find a difference in serum AMH levels between untreated and GH-treated prepubertal girls.

In conclusion, our study shows that short prepubertal girls born SGA have normal serum AMH levels. In addition, AMH levels in post-menarchal SGA girls are comparable to those of age-matched AGA girls. These data indicate that short SGA girls do not have a reduced size of the ovarian follicle pool, suggesting that they do not have an increased risk for earlier follicle pool depletion. Finally, this study shows that GH treatment has no effect on serum AMH levels.

References

1. **Wadsworth M, Cripps H, Midwinter R, Colley J** 1985 Blood pressure in a national birth cohort at the age of 36 related to social and familial factors, smoking, and body mass. *British medical journal* (Clin Res Ed) 291:1534-1538
2. **Barker D** 1997 The fetal origins of coronary heart disease. *Acta Paediatr Suppl* 422:78-82
3. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-7.
4. **Macklon NS, Fauser BC** 1999 Aspects of ovarian follicle development throughout life. *Horm Res* 52:161-70.
5. **de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER** 2001 Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Hum Dev* 60:179-92.
6. **McGee EA, Hsueh AJ** 2000 Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 21:200-14.
7. **de Vet A, Laven JSE, de Jong FH, Themmen APN, Fauser BCJM** 2002 Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 77:357-62.
8. **Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J** 2003 Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 18:323-7.
9. **Cook CL, Siow Y, Brenner AG, Fallat ME** 2002 Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 77:141-6.
10. **Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC** 2004 Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *J Clin Endocrinol Metab* 89:318-23.
11. **Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, Hasegawa Y, Noto RA, Schoenfeld D, MacLaughlin DT** 1996 Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. *J Clin Endocrinol Metab* 81:571-6.
12. **Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE** 1999 Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. *J Clin Endocrinol Metab* 84:3836-44.
13. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-10.
14. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-23.
15. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.
16. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.

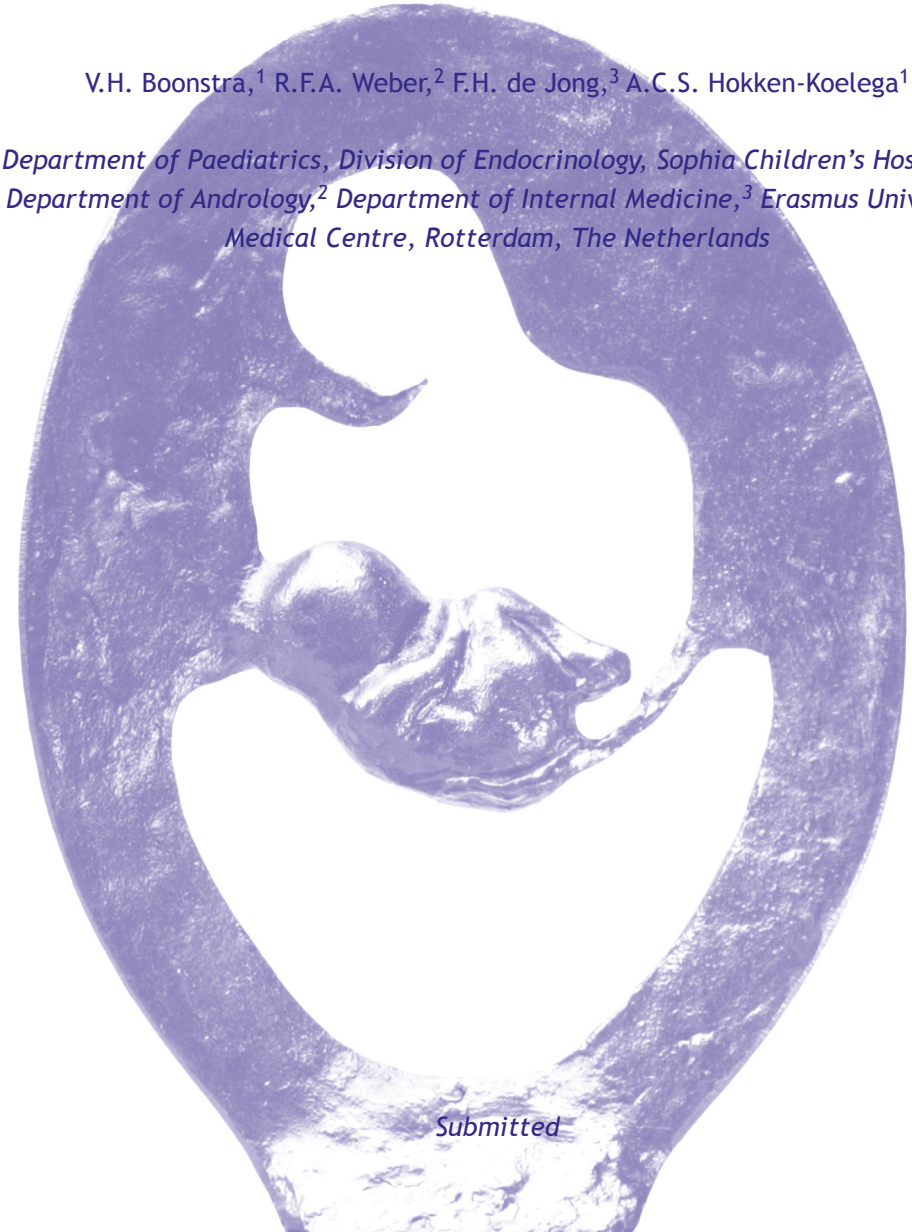
17. **Arends N, Hokken-Koelega A** 1998 Body composition and daily food intake in children with short stature after intrauterine growth retardation (IUGR). *Horm Res* 50 (suppl. 3):47
18. **Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM** 2002 Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril* 77:468-71.
19. **de Bruin JP, Dorland M, Bruinse HW, Spliot W, Nikkels PG, te Velde ER** 1998 Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev* 51:39-46
20. **Ibanez L, Potau N, Enriquez G, de Zegher F** 2000 Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res* 47:575-7.
21. **Ibanez L, Potau N, de Zegher F** 2000 Ovarian hyporesponsiveness to follicle stimulating hormone in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 85:2624-6.
22. **Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM** 1996 Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 379:632-5.
23. **Misra M, MacLaughlin DT, Donahoe PK, Lee MM** 2003 The role of Mullerian inhibiting substance in the evaluation of phenotypic female patients with mild degrees of virilization. *J Clin Endocrinol Metab* 88:787-92.
24. **Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J** 2005 High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod* 20:923-7

Chapter 4

Testis function in prepubertal boys and young men born Small for Gestational Age (SGA)

V.H. Boonstra,[1] R.F.A. Weber,[2] F.H. de Jong,[3] A.C.S. Hokken-Koelega[1]

Department of Paediatrics, Division of Endocrinology, Sophia Children's Hospital,[1] Department of Andrology,[2] Department of Internal Medicine,[3] Erasmus University Medical Centre, Rotterdam, The Netherlands



Submitted

Abstract

It has been suggested that the programming of the endocrine axes occurs during critical phases of fetal development and will be affected by intra-uterine growth retardation. As a result boys born Small for Gestational Age (SGA) might have an impaired gonadal development resulting in reduced testis function later in life.

Before puberty the best marker to evaluate testis development is the assessment of the number of Sertoli cells, by estimation of inhibin B and antimullerian hormone (AMH). From the onset of puberty levels of testosterone, LH, FSH and inhibin B can serve as markers of the intactness of the hypothalamic-pituitary-testis (HPT) axis.

In this study we assessed if SGA boys have a reduced size of their Sertoli cell population by comparing serum inhibin B and AMH levels of 73 prepubertal 3–9 years old short children born SGA (birth length (SDS) below −2 for gestational age, height < −2 SDS) with levels of 72 age-matched children born Appropriate for Gestational Age (AGA) (birth length between −2 and +2 SDS, height > −2 SDS). Since many short children born SGA are treated with GH, we also analysed if two years of GH treatment had an effect on serum inhibin B and AMH levels of prepubertal short SGA children. In addition, we compared serum inhibin B, AMH, testosterone, LH and FSH levels between young adults: 21 short SGA men, 15 normal statured SGA men and 25 normal statured AGA men.

This study shows that serum inhibin B and AMH levels of prepubertal short SGA boys were not significantly lower compared to levels of age-matched boys born AGA. Serum AMH levels were even significantly higher in SGA boys. In 25 GH-treated SGA boys, no difference was found between serum inhibin B and AMH levels before and after 2 years of GH treatment. Also, serum inhibin B and AMH levels in the GH-treated SGA boys were not significantly different compared to levels in AGA boys. In young men, serum inhibin B, testosterone, LH and FSH were not significantly different between the three groups. Serum AMH levels were significantly higher in the young SGA adults.

In conclusion, small size at birth, which might reflect fetal growth restriction, does not reduce the number of Sertoli cells. In addition, serum inhibin B and AMH levels are the same in GH-treated SGA boys and age-matched AGA boys. Finally, the HPT-axis is functioning normal in young men born SGA.

Introduction

Since the first reports demonstrating an association between low birth weight (LBW) and high blood pressure,[1] a large number of studies have elaborated on the consequences of LBW in relation to diseases in adulthood. The fetal programming hypothesis suggests that reduced fetal growth might lead to permanent changes in organ structure and physiology.[2,3] Consequently, boys born small for gestational age (SGA) might have an impaired testicular development. Some studies suggested a relation between being born small for gestational age and a small testicular size and a reduced number of Sertoli cells in adults.[4,5]

In the testes, Sertoli cells play an important role in the paracrine control of spermatogenesis. Each Sertoli cell can harbour only a limited number of germ cells in different stages of spermatogenesis.[6,7] As a result the size of the Sertoli cell population determines the number of germ cells in adults. Animal studies have demonstrated that the size of the Sertoli cell population in early life is important for the testicular size and sperm characteristics in adult life.[8,9] Inhibin B and antimullerian hormone (AMH) are produced by the Sertoli cells and for this reason their serum levels are probably important markers of Sertoli cell number and function in childhood in adulthood. Several studies have indicated that prepubertal serum inhibin B level is a good marker of the number of Sertoli cells.[9-11] Before puberty, basal levels of gonadotropins and testosterone are low. During this period assessment of Sertoli cell number, by measuring serum inhibin B and AMH levels is particularly useful.[6,12-14] From the onset of puberty it is also possible to measure testosterone, LH and FSH as markers of the intactness of the hypothalamic-pituitary-testis (HPT) axis.[6]

In this study we assessed if short prepubertal SGA boys have a reduced size of their Sertoli cell population compared to age-matched normal statured prepubertal AGA boys by measuring serum AMH and inhibin B levels. Since many short children born SGA are treated with growth hormone (GH) we also investigated the effect of 2 years of GH treatment on serum AMH and inhibin B levels. Finally, testis function of GH-treated young SGA men was compared to that of age-matched men with a normal stature born either SGA or AGA, by measuring serum AMH, inhibin B, testosterone, FSH and LH levels.

Patients and methods

Study groups

The SGA groups consisted of: 73 prepubertal boys aged 3.0–9.0 years with short stature, 21 young men born SGA treated with GH because of short stature and 15 young men born SGA with a normal stature. The following criteria were used: Birth length below –2 SD for gestational age according to the standards of Usher & McLean,[15] and an uncomplicated neonatal period. Short stature was defined as a height below –2 SD according to Dutch references.[16] We defined normal stature as a height above –2 SD according to Dutch references.[16] The 73 short prepubertal children were analysed before the start of GH treatment and 25 of them were analysed again after 2 years of GH treatment. These children were between 5.00–6.99 years at the moment of analysis. The 21 SGA men had reached adult height and had been treated with GH before puberty until they had reached adult height. The 15 young SGA men met the same inclusion criteria except for height. They all had shown a spontaneous catch-up growth, reaching a height between –2 SD and +2 SD. They also had reached adult height.

The control groups consisted of 72 age-matched prepubertal boys and 26 young men with a normal stature, all born appropriate for gestational age (AGA). AGA was defined as a birth length and birth weight between –2 and +2 SDS according to the standards of Usher & McLean.[15] Normal stature was defined as a height between –2 and +2 SDS according to Dutch references.[16]

None of the SGA or AGA boys and men had a genital disorder, syndrome or chromosomal abnormality, endocrine or metabolic disorder and none of them underwent orchidopexy or had any other illness or use of medication that might have affected gonadal function. In the SGA and AGA group prepubertal stage was defined as testicular volume less than 4 ml.[17]

The study was approved by the Ethics Committee of each participating centre. Written informed consent was obtained from the parents or custodians of each child.

Study design

In this study the serum inhibin B and AMH levels were compared between the prepubertal SGA boys and the age-matched AGA controls. Venous blood samples were obtained for determination of serum inhibin B and AMH levels.

In addition, we measured the 2-years effect of GH treatment on serum inhibin B and AMH levels in 25 of the 73 prepubertal SGA boys and compared these levels with levels before the start of GH treatment and with levels in age-matched AGA boys. Before the start of GH treatment all boys had a GH stimulation test to exclude GH-

deficiency.[18,19] Biosynthetic GH (r-hGH Norditropin^{R} , Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime with a pen injection system (Nordiject 24). Every three months the total GH dose was adjusted to the calculated body surface.
We also compared serum AMH, inhibin B, testosterone, FSH and LH levels in 21 young men born SGA who had been treated with GH with levels in 15 normal statured young men born SGA and in 26 normal statured young men born AGA.

Hormone assays

All blood samples were drawn between 0800-1300 h and centrifuged. All samples were kept frozen (−80°C) until assayed. All hormone levels were determined in one laboratory. Serum inhibin B levels were measured using kits purchased from Serotec limited, Oxford, Uk. The intra- and inter-assay coefficients of variance were below 9 and 15%, respectively. Serum levels of AMH were measured using an ultrasensitive immuno-enzymometric assay (Immunotech-Coulter, Marseilles, France). The intra- and inter-assay coefficients of variance were below 5 and 8%, respectively. Serum FSH and LH were determined using an automated luminescence-based immunoassay (immulite 2000. diagnostic Products Corporation, Los Angelse, CA). The intra- and inter-assay coefficients of variance were below 5 and 12% for LH and below 3% and 8% for FSH. Total serum testosterone was determined by RIA (Diagnostic Products Corporation). The intra- and inter-assay coefficients of variance were below 6 and 9%. Per patient, all hormone concentrations were analysed in the same blood sample.

Statistical analyses

The serum inhibin B and AMH levels of prepubertal boys are presented as mean and standard deviation. Differences between groups were tested using independent Student's t-test. We show boxplots of serum AMH and inhibin B levels in prepubertal SGA and AGA boys, divided in 3 age groups: group I, 3.0−4.9 yr; group II, 5.0−6.9 yr; group III, 7.0−8.9 yr. Differences between the SGA and AGA groups per age-group were tested with the Mann-Whitney U test.

Serum inhibin B and AMH levels during GH treatment are shown as median and interquartile range since these data were skewed. Before testing the differences between the groups the data were logarithmically transformed to obtain approximate normal distributions. Differences between groups were tested using independent Student's t-tests, except for the difference between SGA boys without GH treatment and AGA boys. For these groups the ANCOVA was used with age as covariate since age was significantly different between these two groups. A repeated measurement analysis of variance was used to test the difference of serum inhibin B and AMH levels before the

start of GH treatment and after 2 years of GH treatment in the SGA group. The Pearson's correlation test was used to test the correlation between inhibin B, AMH and age.

Data of young adult men are shown as median and interquartile range, since parts of these data were skewed. The differences in serum inhibin B, AMH, testosterone, LH and FSH levels were tested using independent t-tests. LH and FSH levels were logarithmically transformed in the analyses.

A p-value < 0.05 was considered significant. All analyses were performed using SPSS version 10.0.

Results

Table 1 shows the serum inhibin B and AMH levels of the prepubertal SGA boys compared to prepubertal age-matched AGA boys. Boys born SGA had serum inhibin B levels comparable to AGA boys. The serum AMH levels of boys born SGA were significantly higher than those of AGA boys. Figure 1 shows the serum inhibin B levels of prepubertal SGA and AGA boys divided in age groups of 2 years. The median inhibin B levels in the group with age between 3 and 5 years were significantly higher in the SGA group (p = 0.03). In the groups with ages between 5 and 7 and between 7 and 9 years the median levels were not significantly different. Figure 2 shows the serum AMH levels of prepubertal SGA and AGA boys divided in the same age groups. No significant differences were found in these groups. Before the age of 9 years, we found a very weak correlation between inhibin B and age (r = −0.3, p < 0.001) and between AMH and age (r = −0.2, p = 0.03).

Table 1. Serum inhibin B and AMH levels in prepubertal SGA boys with short stature and AGA boys with normal stature, aged 3.0−8.9 years.

	SGA (n = 73)	AGA (n = 72)
Inhibin B (ng/ml)	87.3 (33.6)	78.2 (34.5)
AMH (µg/L)	75.6 (24.0)*	63.6 (23.0)

Data expressed as mean (standard deviation), * p < 0.05.

Table 2 shows the serum inhibin B and AMH levels in prepubertal untreated and GH-treated SGA boys compared to prepubertal AGA boys, all aged between 5.0−7.9 years. Serum inhibin B levels were not significantly different between GH-treated and untreated SGA boys. Serum AMH levels were significantly lower in GH-treated SGA boys than in untreated SGA boys (p = 0.04). However, in the group of GH-treated SGA boys we found no significant change in the serum inhibin B and AMH levels from baseline until two years of GH treatment, after adjustment for time. Serum inhibin B and AMH levels were not

significantly different between the GH-treated SGA boys and AGA boys. Again, we found no significant difference between untreated SGA boys and AGA boys.

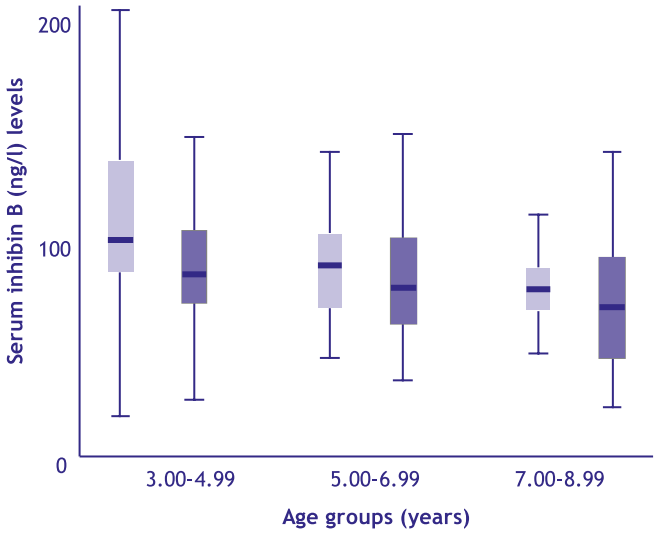


Figure 1. Serum inhibin B levels in prepubertal boys divided in two years age group. Values are given as the median and the first (boxes) and second quartiles below and above the median. SGA = light purpel boxes, AGA = dark purpel boxes.

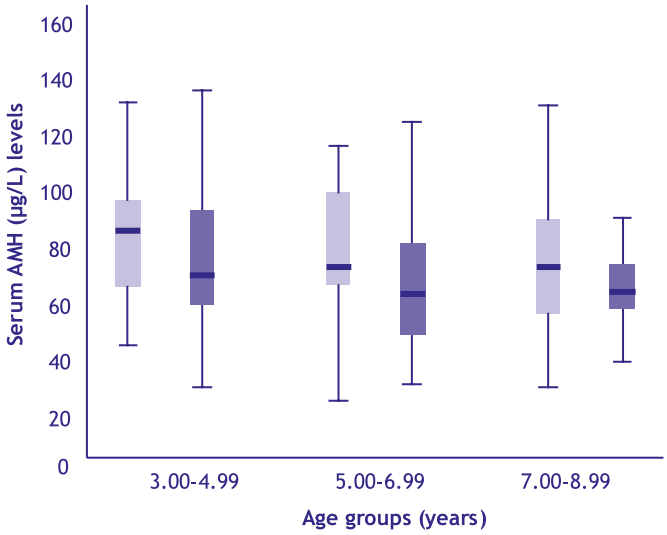


Figure 2. Serum AMH levels in pubertal boys divided in 2 years groups. Values are given as the median and the first (boxes) and second quartiles below and above the median. SGA = light purpel boxes, AGA = dark purpel boxes.

Table 2. Serum inhibin B and AMH levels in untreated SGA boys, GH treated SGA boys and normal statured AGA boys, aged 5.0–7.9 yrs.

	SGA		AGA
	without GH (n = 42)	with GH (n = 25)	(n = 42)
Age (yr)	7.1 (6.4 – 7.5)*	6.6 (5.8 – 7.4)	6.3 (5.6 – 7.3)
Inhibin B (ng/ml)	75.0 (62.5 – 91.0)	66.0 (44.5 – 84.0)	73.0 (53.4 – 105.0)
AMH (µg/L)	63.3 (50.0 – 70.7)**	56.6 (42.0 – 68.1)	59.9 (46.6 – 76.3)

Data expressed as median (interquartile range)
* p < 0.01 SGA without GH *vs* AGA
** p = 0.04 SGA without GH *vs* SGA with GH

Serum inhibin B, AMH, testosterone, FSH and LH levels in young men born SGA with and without short stature compared to normal statured men born AGA are shown in Table 3. In all groups serum inhibin B levels were significantly higher and serum AMH levels were significantly lower compared with levels before puberty. Serum AMH levels were significantly higher in GH-treated SGA men and normal statured SGA men compared to normal statured AGA men. No differences were found in serum inhibin B, testosterone, FSH and LH between all groups.

Table 3. Serum inhibin B, AMH, testosterone, FSH and LH levels in SGA males without spontaneous catch-up growth, SGA males with catch-up growth and normal statured AGA males.

	SGA (n = 21)	SGA (n = 15)	AGA (n = 25)
	Without catch-up growth	With catch-up growth	
Age (yrs)	18.0 (17.4 – 20.6)*	21.4 (19.9 – 22.2)**	19.5 (18.4 – 21.2)
Inhibin B (ng/ml)	186 (111 – 226)	154 (114 – 196)	146 (112 – 203)
AMH (µg/L)	8.2 (6.5 – 12.1)***	9.4 (6.7 – 11.6)****	7.3 (5.5 – 8.5)
Testosterone (nmol/l)	20.2 (17.0 – 23.0)	18.8 (12.8 – 22.9)	18.1 (15.4 – 21.2)
FSH (U/L))	4.5 (2.6 – 6.9)	5.0 (3.2 – 6.2)	4.2 (3.1 – 5.5)
LH (U/L)	4.3 (2.6 – 6.0)	4.2 (2.6 – 9.0)	4.8 (3.7 – 5.5)

* p < 0.01 SGA without catch-up growth *vs* SGA with catch-up growth
** p = 0.03 SGA with catch-up growth *vs* AGA
*** p = 0.03 SGA without catch-up growth *vs* AGA
**** p = 0.03 SGA with catch-up growth *vs* AGA

Discussion

In this study we investigated whether fetal growth restriction has an effect on the endocrine function of the testes before and after puberty. No significant difference was found in serum inhibin B levels between short prepubertal SGA boys and age-matched AGA controls. Short prepubertal SGA boys had significantly higher serum AMH levels compared to age-matched AGA controls. No differences in serum inhibin B and AMH levels were found before and after GH treatment. Serum levels of inhibin B, testosterone, FSH and LH appeared comparable for SGA men, either with or without a spontaneous catch-up growth, and AGA controls. Serum AMH levels were higher in young men born SGA compared to young men born AGA.

In prepubertal boys gonadotrophins may be unreliable predictors of testis function because the HPT-axis seems to be quiescent. However, there is a continuous Sertoli cell proliferation and functional activity during the fetal and prepubertal period. As a result these periods are very important for adult testis function.[6,8,20,21] The total number of Sertoli cells increases rapidly during the fetal and neonatal period and this increase slows down during infancy.[22,23] Around puberty there is a high Sertoli cell proliferation due to an activated HPT-axis. As the number of Sertoli cells is believed to be a determinant of spermatogenic function, potential adverse effects on Sertoli cell proliferation may be expected to result in impaired sperm output in adulthood.[20] This is supported by animal studies demonstrating that perinatal reduction of the number of Sertoli cells results in lower serum inhibin B levels before puberty, and a reduced sperm output in adulthood.[6]

Since inhibin B and AMH are produced exclusively by the Sertoli cells, they may be used as markers of Sertoli cell function and number, thereby predicting future male fertility.[6, 7,10,12,14,20,24] In our study, we found that serum inhibin B and AMH levels in short prepubertal SGA boys were not lower compared to age matched prepubertal AGA boys. Serum AMH levels were even significantly higher in SGA boys. This indicates that the number of Sertoli cells in short prepubertal SGA boys is not reduced compared to that of age-matched AGA boys.

To our knowledge the present study is the first prospective evaluation of testes function by measuring serum inhibin B and AMH levels in prepubertal SGA boys aged 3 to 9 years. One study compared serum FSH and inhibin B levels in 13 SGA boys at the age of 4 months with levels of 7 age-matched AGA boys and found significantly higher serum FSH levels and normal inhibin B levels in SGA boys.[5] This age is characterized by a high level of activity of the HPT-axis. The authors hypothesized that the testes of SGA infants need an augmented FSH drive to fulfil the inhibin B requirements because poor fetal growth conditions had resulted in a reduced number of Sertoli cells within the gonads. Their data are not comparable with ours because they studied a much younger group, including infants with and without postnatal catch-up growth.

The inverse correlation between serum Inhibin B levels and age before puberty was supported by other studies, showing that Inhibin B decreases to a nadir between 6–10 years, followed by a rapid increase in early adolescence.[10,25,26] We only found a very weak inverse correlation between serum AMH levels and age. Data on serum AMH levels before puberty are limited. One study reported that serum AMH levels peak within 3–6 months after birth, are maintained throughout infancy and childhood and then decrease just before puberty.[27]

Many children born SGA with persistent short stature are nowadays treated with GH. We evaluated whether GH treatment had an effect on serum inhibin B and AMH levels. There is limited knowledge about the long-term effects of GH therapy on testicular development and function. Some studies demonstrate that GH/IGF-I is important for pubertal development and affects gonads and gonadotrophins. This indicates that GH treatment might have a stimulating effect on testis function.[28,29] We showed in 25 GH-treated prepubertal boys that 2 years of GH treatment had no effect on serum inhibin B and AMH levels compared to levels in age-matched AGA boys, indicating that GH treatment has no effect on the activity of Sertoli cells in SGA boys. Untreated SGA boys showed, however, significantly higher serum AMH levels compared to GH-treated SGA boys. An explanation for this might be that GH treatment stimulates the Sertoli cell maturation, which results in lower serum AMH levels. It has been reported that before puberty serum AMH levels decrease because of the maturation of the Sertoli cells.[30] On the other hand, serum AMH levels of GH-treated SGA boys were comparable with age-matched AGA boys and GH treatment had no effect on serum inhibin B levels, as has been shown by two other studies.[26,29]

Just before the physical signs of puberty the HPT-axis will be activated. LH stimulates Leydig cells to produce testosterone. FSH and testosterone directly and LH indirectly stimulate Sertoli cells to produce inhibin B and stimulate germ cell development.[6] Whereas serum inhibin B levels in prepubertal boys do not depend on the presence of germinal cells, serum inhibin B levels in postpubertal boys and adult men are closely related to the presence of germ cells from the stage of spermatocytes onwards.[31] In our study we found comparable serum inhibin B levels in SGA men with and without a spontaneous catch-up growth and normal statured AGA men, indicating that gonadal function of SGA men is not reduced due to small size at birth.

Before puberty, serum AMH levels are a specific marker of immature Sertoli cell numbers. The decrease in serum AMH levels at puberty reflects the end-stage maturation of Sertoli cells.[30] Intratesticular testosterone is the major regulator of AMH levels. In early puberty, elevation of serum testosterone levels correlates with a decrease in serum AMH levels. Serum AMH levels are always low in late puberty.[32] Also, in this study serum AMH levels in young men were low. Surprisingly, we found that SGA men had unexplained significantly higher serum AMH levels compared to males born AGA.

Our data showed that serum testosterone and LH levels in SGA men were comparable to those of the AGA group. All three groups had normal serum FSH levels. Elevated serum FSH levels are seen in patients with abnormal Sertoli cell function and/or spermatogenesis because of a lower negative feedback by inhibin B.[6] To assess Sertoli cell function, however, inhibin B has a higher accuracy than FSH because it is a direct product of the Sertoli cell, whereas FSH secretion is not only determined by negative feedback, but also by GnRH, androgens and oestradiol. Cicognani *et al* found that 25 young SGA men had higher serum LH levels and lower serum testosterone levels compared to age-matched boys with short stature born AGA.[4] They concluded that there is a tendency to hypogonadism in SGA boys. When SGA boys with a mean testicular volume < 2 SD were analysed separately, these authors showed significantly lower serum inhibin B levels and higher serum FSH levels compared to controls. However, in the control group they did not make a distinction between subjects with different testis sizes. In addition, 5 out of 25 SGA males had a history of orchidopexy in contrast to the control group. It is known that boys with cryptorchidism are at risk of having decreased Leydig cell function.[33] However, several studies indicated that boys born SGA might have an increased risk for cryptorchidism and hypospadia.[34] In our study these abnormalities were an exclusion criteria.

In conclusion, our study shows that short prepubertal SGA boys do not have reduced serum inhibin B and AMH levels, compared to those of age-matched boys born AGA. In addition, serum inhibin B and AMH levels were similar in GH-treated SGA boys and age-matched AGA boys. Finally, young men born SGA with and without a spontaneous catch-up growth had normal inhibin B, LH, FSH and testosterone levels compared to AGA young men. Serum AMH levels were significantly higher in young men born SGA. Our study indicates that small size at birth, which might reflect fetal growth restriction, does not diminish the number of Sertoli cells and testis function.

References

1. **Wadsworth M, Cripps H, Midwinter R, Colley J** 1985 Blood pressure in a national birth cohort at the age of 36 related to social and familial factors, smoking, and body mass. *British medical journal* (Clin Res Ed) 291:1534-1538
2. **Barker D** 1997 The fetal origins of coronary heart disease. *Acta Paediatr Suppl* 422:78-82
3. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-7.
4. **Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E** 2002 Low birth weight for gestational age and subsequent male gonadal function. *The Journal Pediatrics* 141:376-380
5. **Ibanez L, Valls C, Cols M, Ferrer A, Marcos MV, Zegher de F** 2002 Hypersecretion of FSH in infants boys and girls born small for gestational age. *J Clin endocrinol metab* 87:1986-1988
6. **Pierik FH, Burdorf A, de Jong FH, Weber RF** 2003 Inhibin B: a novel marker of spermatogenesis. *Ann Med* 35:12-20
7. **Sharpe RM, Turner KJ, McKinnell C, Groome NP, Atanassova N, Millar MR, Buchanan DL, Cooke PS** 1999 Inhibin B levels in plasma of the male rat from birth to adulthood: effect of experimental manipulation of Sertoli cell number. *J Andro*l 20:94-101.
8. **Orth JM, Gunsalus GL, Lamperti AA** 1988 Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* 122:787-94.
9. **Atanassova N, McKinnell C, Walker M, Turner KJ, Fisher JS, Morley M, Millar MR, Groome NP, Sharpe RM** 1999 Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood. *Endocrinology* 140:5364-73
10. **Andersson AM, Skakkebaek NE** 2001 Serum inhibin B levels during male childhood and puberty. *Mol Cell Endocrinol* 180:103-7.
11. **Raivio T, Saukkonen S, Jaaskelainen J, Komulainen J, Dunkel L** 2000 Signaling between the pituitary gland and the testes: inverse relationship between serum FSH and inhibin B concentrations in boys in early puberty. *Eur J Endocrinol* 142:150-6
12. **Misra M, MacLaughlin DT, Donahoe PK, Lee MM** 2002 Measurement of Mullerian inhibiting substance facilitates management of boys with microphallus and cryptorchidism. *J Clin Endocrinol Metab* 87:3598-602
13. **Schmiegelow M, Lassen S, Poulsen HS, Schmiegelow K, Hertz H, Andersson AM, Skakkebaek NE, Muller J** 2001 Gonadal status in male survivors following childhood brain tumors. *J Clin Endocrinol Metab* 86:2446-52.
14. **Kubini K, Zachmann M, Albers N, Hiort O, Bettendorf M, Wolfle J, Bidlingmaier F, Klingmuller D** 2000 Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. *J Clin Endocrinol Metab* 85:134-8.
15. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-10.
16. **Roede MJ, van Wieringen JC** 1985 Growth diagrams 1980, Netherlands. Third nation-wide-survey. *T Soc Gezondheidsz* [suppl] 63:1-34

17. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.
18. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.
19. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. *Clin Endocrinol* (Oxf) 59:779-87.
20. **Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE** 1998 Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 83:675-81.
21. **Chemes HE** 2001 Infancy is not a quiescent period of testicular development. *Int J Androl* 24:2-7.
22. **Rey RA, Campo SM, Bedecarras P, Nagle CA, Chemes HE** 1993 Is infancy a quiescent period of testicular development? Histological, morphometric, and functional study of the seminiferous tubules of the cebus monkey from birth to the end of puberty. *J Clin Endocrinol Metab* 76:1325-31.
23. **Bendsen E, Byskov AG, Laursen SB, Larsen HP, Andersen CY, Westergaard LG** 2003 Number of germ cells and somatic cells in human fetal testes during the first weeks after sex differentiation. *Hum Reprod* 18:13-8.
24. **Crofton PM, Thomson AB, Evans AE, Groome NP, Bath LE, Kelnar CJ, Wallace WH** 2003 Is inhibin B a potential marker of gonadotoxicity in prepubertal children treated for cancer? *Clin Endocrinol* (Oxf) 58:296-301.
25. **Andersson AM, Juul A, Petersen JH, Muller J, Groome NP, Skakkebaek NE** 1997 Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels. *J Clin Endocrinol Metab* 82:3976-81.
26. **Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV, Kelnar CJ** 2002 Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clin Endocrinol* (Oxf) 56:215-21.
27. **Teixeira J, Maheswaran S, Donahoe PK** 2001 Mullerian inhibiting substance: an instructive developmental hormone with diagnostic and possible therapeutic applications. *Endocr Rev* 22:657-74.
28. **Hull KL, Harvey S** 2000 Growth hormone: a reproductive endocrine-paracrine regulator? *Rev Reprod* 5:175-82.
29. **Lindgren AC, Chatelain P, Lindberg A, Price DA, Ranke MB, Reiter EO, Wilton P** 2002 Normal progression of testicular size in boys with idiopathic short stature and isolated growth hormone deficiency treated with growth hormone: experience from the KIGS. *Horm Res* 58:83-7.
30. **Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE** 1999 Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. *J Clin Endocrinol Metab* 84:3836-44.
31. **Andersson AM, Muller J, Skakkebaek NE** 1998 Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *J Clin Endocrinol Metab* 83:4451-8.

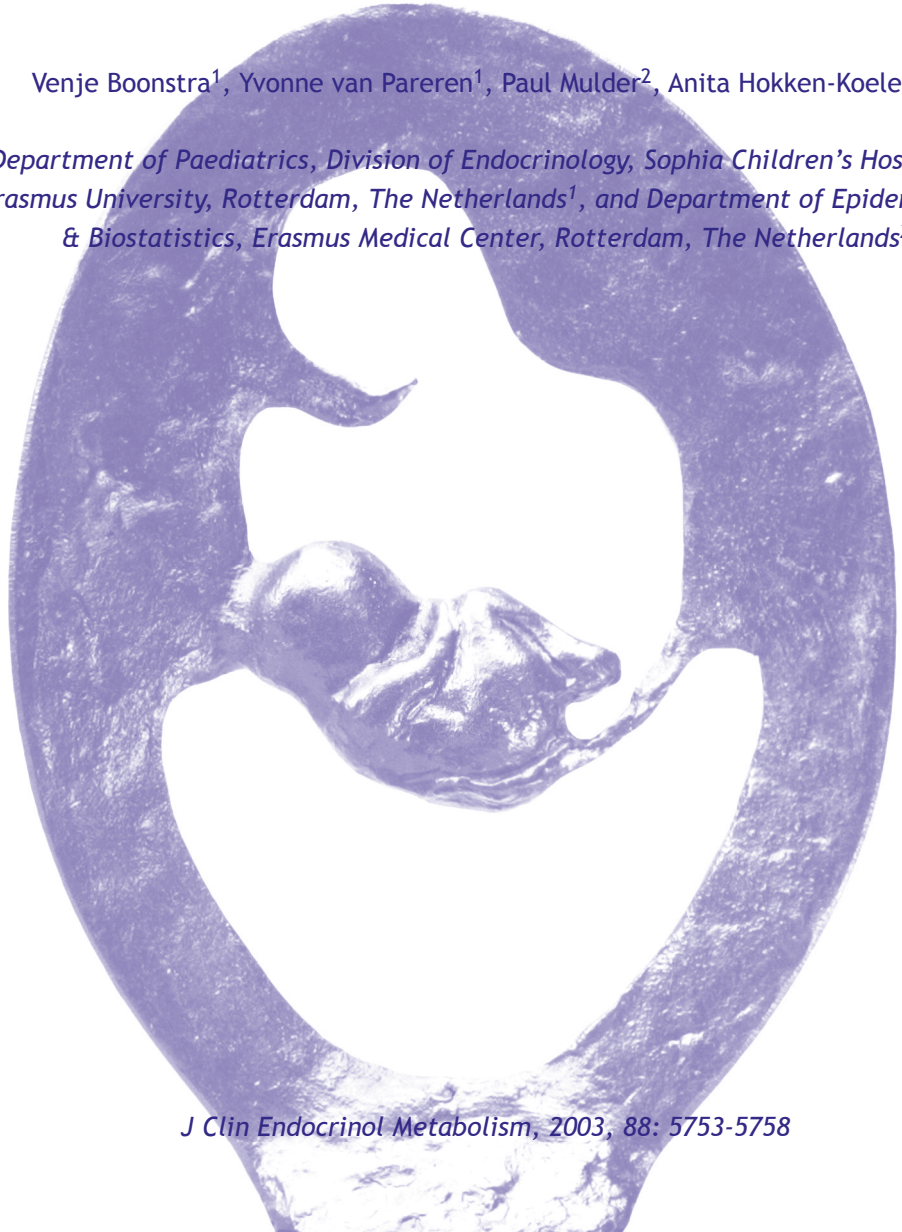
32. **Al-Attar L, Noel K, Dutertre M, Belville C, Forest MG, Burgoyne PS, Josso N, Rey R** 1997 Hormonal and cellular regulation of Sertoli cell anti-Mullerian hormone production in the postnatal mouse. *J Clin Invest* 100:1335-43.
33. **Skakkebaek NE, Rajpert-De Meyts E, Main KM** 2001 Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16:972-8
34. **Gatti JM, Kirsch AJ, Troyer WA, Perez-Brayfield MR, Smith EA, Scherz HC** 2001 Increased incidence of hypospadias in small-for-gestational age infants in a neonatal intensive-care unit. *BJU Int* 87:548-50

Chapter 5

Puberty in growth hormone-treated children born Small for Gestational Age (SGA)

Venje Boonstra[1], Yvonne van Pareren[1], Paul Mulder[2], Anita Hokken-Koelega[1]

Department of Paediatrics, Division of Endocrinology, Sophia Children's Hospital / Erasmus University, Rotterdam, The Netherlands[1], and Department of Epidemiology & Biostatistics, Erasmus Medical Center, Rotterdam, The Netherlands[2]



J Clin Endocrinol Metabolism, 2003, 88: 5753-5758

Abstract

Seventy-five SGA children were studied in a randomised, double-blind, dose-response GH trial with either 1 or 2 mg GH/m^2/day. Mean (SD) age at start of GH therapy was 7.3 (2.2) years. Data were compared with Dutch reference data.

In GH-treated SGA boys, mean (SD) age at onset of puberty was 12.0 (1.0) and 11.6 (0.7) years and in SGA girls it was 10.9 (1.1) and 10.6 (1.2), when treated with 1 and 2 mg GH/m^2/day, respectively. SGA boys treated with the lowest GH dose started puberty later than the AGA controls, for the other GH-dosage groups there was no significant difference in age at onset of puberty compared to AGA controls. The age at menarche and the interval between breast stage M2 and menarche were not significantly different for GH-treated SGA girls compared to their peers. The duration of puberty and pubertal height gain of GH-treated SGA boys and girls were not significantly different between the two GH dosage groups and comparable with untreated short children born SGA.

In conclusion, long-term GH therapy in short SGA children has no influence on the age at onset and progression of puberty compared to AGA controls, regardless of treatment with a dose of 1 or 2 mg GH/m^2/day. Duration of puberty and pubertal height gain were not significantly different between the GH-dosage groups.

Introduction

Spontaneous postnatal catch-up growth occurs in most infants born SGA, but about 10% remain short after the age of two years.[1,2] Important determinants of final height are the height and age at onset of puberty and the magnitude and duration of the pubertal growth.[3-5] Data on puberty in children born SGA are limited. Most studies deal with height and age at onset of puberty and not with duration and progression of puberty. Moreover, study results are difficult to compare due to the various definitions of SGA and the various definitions used for the milestones of puberty. Persson *et al* reported that children born SGA were shorter at onset of puberty than their peers but that the age at onset was the same.[6] A French study reported that the age at onset of puberty, the age at menarche and the pubertal growth spurt in girls born SGA were comparable with the normal population.[7,8] A Swedish population-based study showed that in SGA children with a spontaneous catch-up growth puberty occurred at the normal age in contrast to SGA children with persistent short stature who had a slightly earlier pubertal onset.[1] Most authors do seem to agree that puberty in short SGA children start at a normal age, but relatively early for their short stature.[9]

Several studies have demonstrated that growth hormone (GH) treatment results in a significant catch-up growth in short prepubertal SGA children.[10,11] However, only very limited data are available on puberty and pubertal growth of children born SGA who have been treated with GH for several years. We therefore evaluated puberty, in terms of age and height at onset of puberty, age at menarche, interval between breast development and menarche, duration of puberty and pubertal height gain, in 75 GH-treated children born SGA who participated in a randomised, double-blind, dose-response trial, evaluating the effect of a GH dose of either 1 or 2 mg/m^2/day (0.03 or 0.07 mg/kg/day), in comparison to normal statured children born appropriate for gestational age (AGA).

Patients and methods

Study group

The study group consisted of 75 prepubertal short children born SGA who met the following criteria at start of GH treatment: 1) birth length SDS below –2 SD for gestational age according to the standards of Usher and McLean,[12] 2) Chronological age between 3–11 yr in boys and 3–9 yr in girls at start of the study, 3) height SDS for chronological age (HSDS) below –2 SD according to Dutch references,[13] 4) height velocity SDS for chronological age (HVSDS) ≤ zero,[13,14] to exclude children with spontaneous catch-up growth, 5) prepubertal stage defined as Tanner breast stage I for girls, and testicular volume less than 4 ml for boys,[15] 6) uncomplicated neonatal period, that is without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis neonatorum and without long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, chondrodysplasia) or syndromes, and previous or present use of medication that could interfere with GH treatment. The original group consisted of 79 children. Four children dropped out of the study, before the onset of puberty, for the following reasons: three children were no longer motivated to inject GH daily after 15, 45, and 51 months of GH treatment, respectively, despite ongoing catch-up growth with GH treatment. In 1 prepubertal boy, GH treatment was discontinued after 27 months because of signs of GH insensitivity. As these 4 children were lost to follow-up after discontinuation of GH, their data were not included in the analysis.

Four centres in the Netherlands participated in the study. The study was approved by the Ethics Committee of each participating centre. Due to ethical considerations, the Ethics Committees did not allow for a control group until adult height (AH). Written informed consent was obtained from the parents or custodians of each child.

Study design

All children were randomly and blindly assigned to one of two GH dosage groups: Group A receiving 1 mg GH /m^2/day, Group B receiving 2 mg GH /m^2/day (~0.03 or 0.07 mg/kg/day, respectively). Biosynthetic GH (r-hGH Norditropin (R), Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime with a pen injection (Nordiject 24). Every 3 months the total GH dose was adjusted to the calculated body surface. The study was kept double-blind by using an equal volume of a reconstituted preparation.[10]

Measurements

Height (H) was measured at baseline and subsequently every three months, according to the method of Cameron using a Harpenden stadiometer.[16] Four measurements were made per visit by the same investigators ('91–'95 W. d Waal, '95–'98 Th. Sas, '98–'01 Y. v Pareren) and the mean was used for the analysis. Height was expressed as SDS for chronological age.[13]Target height (TH) was calculated based on Dutch reference data with addition of 3 cm for a secular trend: ½ x ($Height_{father}$ + $Height_{mother}$ + 12) + 3 for boys and ½ x ($Height_{father}$ + $Height_{mother}$ − 12) + 3 for girls.[13] TH and body mass index (BMI) were expressed as SDS using Dutch references.[13] Bone age (BA) was determined by the same investigators according to Tanner and Whitehouse radius, ulna, short-bones score (RUS TW-2).[17] Adult height in GH-treated children was defined as the condition when height velocity (HV) had dropped below 0.5 cm during the previous 6 months and the bone age was ≥ 15 years for girls and ≥ 16.5 years for boys. AH was reached either during GH treatment or during the 2-year follow-up after discontinuation of GH treatment. GH treatment was discontinued after reaching AH or on patient's decision at near-adult height. At each visit pubertal stages were assessed by the same investigators according to the method of Tanner.[15] The onset of puberty was defined as a breast development stage 2 according to Tanner scale for girls[15] and a testicular volume equal or more than 4 ml for boys as determined by means of a Prader orchidometer. At each 3-monthly visit girls were asked if and when they had their menarche. The interval between breast development (M2) and menarche was defined as the time from onset of puberty (breast stage 2) until menarche. The pubertal height gain and the duration of puberty were defined as the adult height minus height at onset of puberty (cm) and the time from onset of puberty until adult height, respectively.

Statistical analyses

The Fourth Dutch National Growth Study (1997) served as reference for age and height at onset of puberty and age at menarche and the interval between M2 and menarche of normal statured children born Appropriate for Gestational Age (AGA controls).[18] In that study the same definitions for pubertal milestones were used as in our study, but as AH was not defined in the Dutch Growth Study we could not compare our data on duration of puberty and pubertal height gain with Dutch references. An independent statistician (PM) performed the statistical analyses. Data are expressed as the mean ± SD, unless indicated otherwise. The null hypothesis of mean SDS values being equal to zero was tested by the one-sample Student's-test. Mean differences of continuous variables between groups were tested using a Student's two sample t-test with variances pooled across all groups. The corresponding 95% confidence interval (95%CI) was used in case of

no significance in the mean difference. Multiple linear regression analyses were used to test the influence of several variables on the age at onset of puberty, interval between M2 and menarche, and pubertal height gain in GH-treated SGA children. A p-value < 0.05 was considered significant. All analyses were performed using SPSS version 10.0.

Results

GH trial

Table 1 lists the baseline clinical data of all 75 children at start of GH treatment. Both GH-dosage groups had similar initial characteristics at start of GH treatment. After the onset of puberty three children dropped out of the study: one girl due to early puberty at the age of 8.4 years after 27 months of GH treatment and two other children were not motivated despite ongoing GH-induced catch-up growth. Their data were only included in the analysis of pubertal onset.

Table 1. Clinical data in 75 children at start of GH treatment.

	Group A 1 mg/m^2/day (n = 39)	Group B 2 mg/m^2/day (n = 36)
Male/female	29/10	21/15
Gestational age (wk)	37.3 (3.2)	36.0 (4.2)
Birth length SDS	−3.5 (1.4)	−3.5 (1.6)
Birth weight SDS	−2.6 (1.2)	−2.6 (1.0)
Chronological age (yr)	7.4 (2.0)	7.3 (2.4)
Bone age (RUS;yr)	6.6 (2.5)	6.9 (3.0)
Height SDS	−3.0 (0.7)	−3.1 (0.7)

Data are expressed as mean (SD).

The onset of puberty

Table 2 lists the age, height (SDS), bone age, BMI (SDS) and duration of GH treatment at the onset of puberty for both GH-dosage groups compared to Dutch AGA controls. Mean age at onset of puberty for boys was 12.0 (1.0) years in group A and 11.6 (0.7) in group B, and for girls 10.9 (1.1) in group A and 10.6 (1.2) in group B, without significant differences between the two GH-dosage groups. Boys of group A were significantly older

at onset of puberty than the AGA controls. For girls the age at onset in the GH-dosage groups versus the AGA controls was not significantly different. Mean height SDS at onset of puberty for boys was −1.3 (0.7) in group A and −0.9 (0.9) in group B, and for girls −1.0 (0.6) in group A and −0.9 (1.4) in group B, without significant differences between the two GH-dosage groups. Height SDS at onset of puberty was significantly lower than for the AGA controls, for boys and girls.

Table 2. Data at onset of puberty in 75 GH-treated SGA children versus Dutch AGA controls.

	SGA		Difference B-A	AGA
	Group A	Group B	(95% CI)	Controls#
Boys				
Number	29	21		2524
Age (yrs)	12.0 (1.0)*	11.6 (0.7)	−0.4 (−0.9 to 0.1)	11.5
Height (cm)	144.7 (7.8)	145.2 (6.2)	0.5 (−3.7 to 4.6)	151.0
Height SDS	−1.3 (0.7)**	−0.9 (0.9)**	0.4 (0.0 to 0.9)	0.0
Bone age (RUS yrs)	12.4 (2.8)	12.7 (1.2)	0.3 (−1.0 to 1.6)	
BMI SDS	−0.5 (1.3)	−0.3 (0.6)	0.2 (−0.4 to 0.8)	
Duration GH therapy (yrs)	4.3 (2.2)	4.0 (2.5)	−0.3 (−1.7 to 1.0)	
Girls				
Number	10	15		2266
Age (yrs)	10.9 (1.1)	10.6 (1.2)	−0.3 (−1.2 to 0.7)	10.7
Height (cm)	141.9 (7.4)	141.5 (10.8)	−0.4 (−8.5 to 7.7)	147.3
Height SDS	−1.0 (0.6)**	−0.9 (1.4)**	0.1 (−0.7 to 1.0)	0.0
Bone age (RUS yrs)	11.3 (1.4)	11.1 (1.6)	−0.2 (−1.5 to 1.1)	
BMI SDS	−0.8 (0.9)	−0.6 (0.8)	0.2 (−0.5 to 0.9)	
Duration GH therapy (yrs)	4.0 (1.9)	3.7 (1.8)	−0.3 (−1.9 to 1.2)	

Data are expressed as mean (SD). Group A and B received 1 and 2 mg GH/m^2/day, respectively.
* Group A versus AGA controls p = 0.02, ** SGA groups versus AGA controls p < 0.05. CI = Confidence Interval # Data of 4th Dutch National Growth Study.[18]

At onset of puberty there was a moderately advanced bone age for boys and girls compared to age, regardless of GH-dosage group. However, only in boys bone age was significantly older than chronological age. The BMI SDS in boys and girls was significantly lower than zero for both GH-dosage groups without a significant difference between the two GH-dosage groups. The duration of GH treatment prior to the onset of puberty in boys and girls was not significantly different between the two GH-dosage groups.

Menarche

The mean age at menarche and the interval between M2 and menarche, between the GH-dosage groups and the AGA controls were not significantly different (Table 3). In addition age at menarche and the interval between M2 and menarche were not significantly different between both GH-dosage groups.

Table 3. Age at menarche and interval between M2 and menarche in GH-treated SGA girls compared to Dutch AGA controls.

	SGA		Difference B-A (95% CI)	AGA controls#
	Group A	Group B		
Number	10	13		3028
Age at menarche (yr)	12.9 (0.8)	13.1 (1.3)	0.2 (−0.7 to 1.2)	13.2
Interval M2 → menarche (yr)	2.0 (0.9)	2.3 (0.9)	0.3 (−0.5 to 1.1)	2.5

Data are expressed as mean (SD). Group A and B received 1 and 2 mg GH/m^2/day, respectively.
CI = Confidence Interval. # Data of 4th Dutch National Growth Study.[18]

The duration of puberty and pubertal height gain

Forty-six children reached adult height. Their baseline data were comparable with those of the 29 SGA children who did not yet reach adult height, with the exception of an older mean age at start of GH treatment (8.5 (1.7) years compared to 5.6 (1.7) years in the 29 SGA children). The duration of puberty and pubertal height gain were analysed for those who reached adult height (Table 4).The duration of puberty was not significantly different for group A and group B, in both sexes. The mean (SD) pubertal height gain for boys was 27.0 (8.4) cm in group A and 31.4 (4.1) cm in group B, in girls 19.0 (7.3) cm in group A and 18.9 (5.7) cm in group B. For boys and girls, mean pubertal height gain was not significantly different between the two GH-dosage groups.

Figure 1 and 2 show the P10, P50 and P90 ages of reaching the milestones of puberty for respectively boys and girls.

Table 4. Pubertal height gain and duration of puberty in 46 GH treated SGA children who reached adult height.

	Group A 1 mg/m²/day	Group B 2 mg/m²/day	Difference B-A (95% CI)
Boys	n = 14	n=12	
Duration of puberty (yrs)	5.0 (1.3)	5.4 (0.8)	0.4 (−0.5 to 1.3)
Pubertal height gain (cm)	27.0 (8.4)	31.4 (4.1)	4.5 (−1.0 to 9.9)
Target height SDS	−1.0 (0.9)	−0.5 (0.7)	0.5 (0.0 to 1.0)
Girls	n = 9	n = 11	
Duration of puberty (yrs)	3.9 (1.0)	4.1 (1.1)	0.2 (−0.8 to 1.2)
Pubertal height gain (cm)	19.0 (7.3)	18.9 (5.7)	−0.1 (−6.2 to 6.1)
Target height SDS	−0.7 (0.7)	−0.4 (1.1)	0.3 (−0.5 to 1.1)

Data are expressed as mean (SD). CI = Confidence Interval.

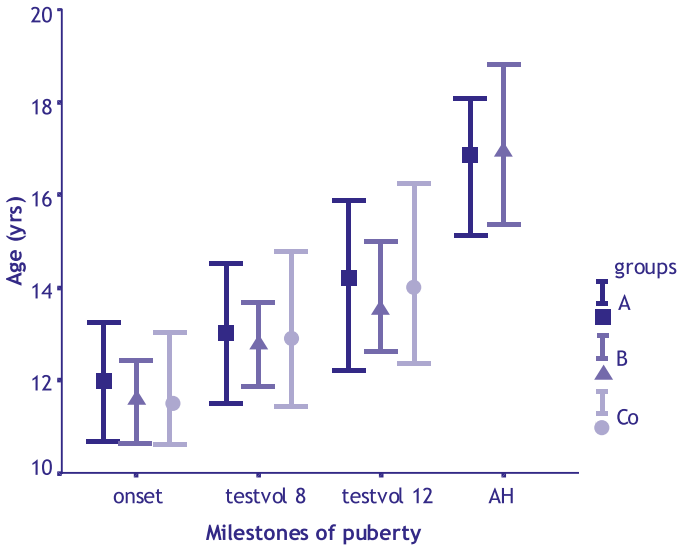


Figure 1. Milestones of puberty for boys. P10, P50 and P90 ages of reaching the milestones of puberty for boys. Onset = Onset of puberty. Testvol 8 = Testicular volume of 8 ml [n = 28 (A), 20 (B)]. Testvol 12 = Testicular volume of 12 ml [n = 26 (A), 20 (B)]. AH = Adult height [n = 14 (A), 12 (B)]. Co = Control.

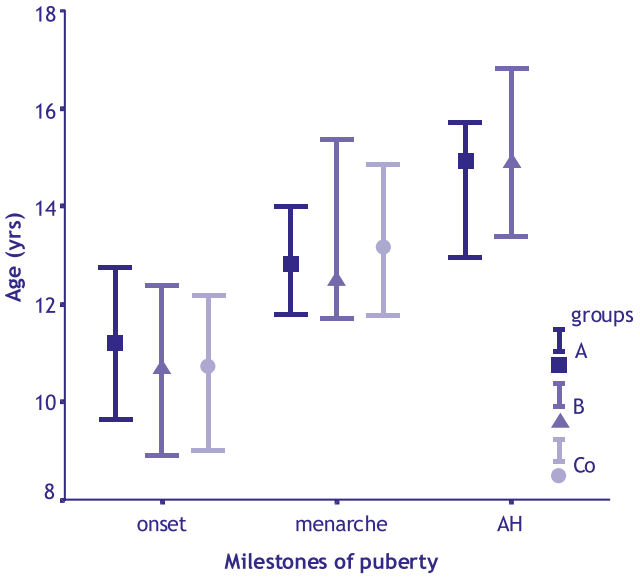


Figure 2. Milestones of puberty for girls. P10, P50 and P90 ages of reaching the milestones of puberty for girls. Onset = Onset of puberty. Menarche = Age at onset of menarche [n = 10 (A), 13 (B)]. AH = Adult height [n = 9 (A), 11(B)]. Co = Control.

Variables

Table 5 shows the results of the multiple regression analysis regarding the age at onset of puberty, interval between M2 and menarche and pubertal height gain.

Variables influencing age at onset of puberty

Boys started their puberty one year later than girls. The longer the duration of GH treatment the older the age at start of puberty. BMI and bone age delay at onset of puberty and GH dosage had no influence on the age at onset.

Variables influencing the interval between M2 and menarche

The older the age at onset of puberty the shorter the interval between M2 and menarche. A higher BMI resulted into a shorter interval and the greater the bone age delay at onset of puberty the greater the interval. The GH dosage had no influence on the interval between M2 and menarche.

Table 5. Multiple regression analysis on age at onset of puberty (yrs), interval between M2 and menarche (yrs) and pubertal height gain (cm).

Dependent variable	Independent variable	Regression Coefficient	SE	P-value
Age at onset of puberty	**Sex (Girls)**	**−1.04**	**0.24**	**< 0.001**
	Duration GH therapy at onset puberty (yr)	**0.13**	**0.06**	**0.02**
	BMI SDS at onset puberty	0.02	0.06	ns
	Bone age delay at onset puberty (yr)	0.20	0.12	ns
	GH Dose 1 *vs* 2mg/m^2/day	−0.25	0.22	ns
Interval M2 → menarche	**Age at onset of puberty (yr)**	**−0.52**	**0.15**	**0.002**
	BMI SDS at onset puberty	**−0.37**	**0.11**	**0.003**
	Bone age delay at onset puberty (yr)	**0.32**	**0.14**	**0.03**
	GH Dose 1 *vs* 2mg/m^2/day	0.45	0.28	ns
Pubertal height gain	**Sex (Girls)**	**−13.78**	**1.60**	**< 0.001**
	Bone age delay at onset puberty (yr)	**2.60**	**0.33**	**< 0.001**
	Height at onset of puberty (cm)	**−0.38**	**0.16**	**0.02**
	Age at onset of puberty (yr)	**−2.68**	**1.17**	**0.03**
	TH SDS	1.17	0.78	ns
	GH Dose 1 *vs* 2mg/m^2/day	2.25	0.14	ns

SE: standard error

Variables influencing pubertal height gain

The difference in height gain between boys and girls was 13.8 cm. A greater bone age delay at onset of puberty increased pubertal height gain and a taller height and older age at onset of puberty reduced the pubertal height gain. TH SDS and GH dosage had no significant effect on pubertal height gain.

Discussion

Our study presents the effects of GH treatment on puberty in short children born SGA. GH treatment with either 1 or 2 mg/m^2/day had no effect on pubertal onset, age at menarche and interval between M2 and menarche, compared to Dutch reference data. Also, there was no GH-dose effect on the duration of puberty and pubertal height gain. Children with an older age, higher BMI and smaller bone age delay at onset of puberty

had a shorter interval between M2 and menarche. Again, the GH dose had no influence. The pubertal height gain was higher in children with a younger age, shorter height and a greater bone age delay at onset of puberty, whereas the GH dose and target height had no effect on the pubertal height gain.Our study shows that there is no GH-dose effect on the age at onset of puberty in SGA children. Also, GH-treated SGA children did not start puberty at a younger age compared to normal statured Dutch children born AGA.[18] SGA boys receiving 1 mg GH /m^2/day started their puberty even significantly later than normal statured Dutch boys born AGA. Thus GH treatment does not result into a younger age at onset of puberty, which is also supported by data of regression analysis, showing that the longer the duration of GH treatment the later the onset of puberty. Both GH-dosage groups had a similar duration of GH treatment prior to the onset of puberty. Bone age delay and BMI had no influence on the age at onset of puberty. Our study also shows that SGA boys start their puberty one year later than SGA girls, which is comparable with the Dutch reference data.[18] Compared to published data of untreated SGA children, we did not find a significant difference regarding age at onset of puberty between our GH-treated SGA girls and Swedish untreated SGA girls.[6] Boys receiving 2 mg were 0.8 years ($p < 0.01$) younger at onset of puberty than untreated Swedish SGA boys. However, in this respect it is important to mention that the definition of onset of puberty in the Swedish study was different from ours, as their onset of puberty was defined as the moment at which the growth velocity starts to be more than 6 cm a year, whereas for our study the onset of puberty was defined as a testis volume of 4 ml in boys, which is known to precede the pubertal growth velocity by 1 year in boys. This means that when we would have applied the Swedish definition of puberty on our data set, the onset of puberty in our group would have been even later. In our study we could not use the increase in growth velocity as the onset of puberty, since we could not determine if the increase of height velocity was induced by either GH therapy or pubertal growth spurt or both.

Height at onset of puberty was not significantly different in both GH-dosage groups, but it was significantly shorter than the height at pubertal onset of the Dutch normal statured AGA children.

Boys had a significantly advanced bone age at onset of puberty compared to the chronological age, in both GH groups. It is known that the bone maturation in children born SGA is different from the normal population and not a reliable estimation in SGA children.[19-22] In addition bone age assessment by RUS TW-2 generally results in a one year older bone age compared to Greulich and Pyle and the chronological age.[23] Furthermore, it might be that GH treatment resulted in an acceleration of bone age in boys. However it appears that the chronological age at onset of puberty and the progression of pubertal development of the GH treated SGA boys and girls were not significantly different from normal statured children born AGA.

One of the milestones of puberty in girls is menarche. Our study shows that the age at menarche and the interval between M2 and menarche, an indicator for the progression of puberty in girls, between both GH-dosage groups were not significantly different and comparable with the age of Dutch AGA controls. The age at menarche and the interval between M2 and menarche were also not significantly different compared to Swedish untreated SGA girls. This suggests that GH treatment has no influence on the progression of puberty in girls. An older age, higher BMI and smaller bone-age delay, however, resulted in a shorter interval between M2 and menarche. Several studies have shown that normal statured AGA girls with an older age at onset of puberty pass faster through pubertal stages than early maturers.[24-27]

It is interesting that in our study group BMI had no influence on the age at onset of puberty but only on the progression of puberty and on the age of menarche (data not shown). In the normal population it is seen that children with overweight mature earlier than non-overweight children.[28] An explanation why BMI in our study group had no influence on the age at onset of puberty but only on the age at menarche and progression of puberty might be that our SGA children were lean with a mean BMISDS significantly lower than zero and that there was only a narrow variation in the BMI (SDS) before puberty. However, it is known that during puberty body composition changes significantly and for that reason might have effect on the age of menarche and progression of puberty in our study group.[29] The reason why BMI has influence on the progression of puberty might be that a higher BMI results in higher serum leptin, estrogens, insulin and IGF-1 levels.[29] Leptin is thought to be one of the hormonal factors which signals to the brain at which time the body is ready for sexual maturation and reproduction.[30-32] Kiess *et al* also reported that leptin is not the primary signal involved in the initiation of puberty but might act as a permissive signal allowing puberty to proceed when metabolic resources are sensed to be sufficient.[30,33] Some studies suggest that insulin and IGF-1 do also have an effect on the mechanism of puberty.[34,35] For future studies it will be very interesting to evaluate the influence of leptin, insulin, and IGF-1 on the progression of puberty.

For the endpoint of puberty we used adult height instead of genital development stage 5 and breast development stage 5 since we experienced that these pubertal stages were not reliable endpoints of pubertal growth. The duration of onset of puberty until adult height in boys and girls was not significantly different between the GH-dosage groups. We couldn't compare the duration of onset of puberty with adult height with the Dutch reference data as adult height was not defined in the Dutch Growth Study. Also, no published data on duration of puberty until adult height in SGA were available. Our study shows that a greater bone age delay at onset of puberty was associated with a longer duration of puberty until adult height, as has been reported for other conditions.[36,37]

The pubertal height gain, in our study defined as the adult height minus the height at onset of puberty, was not significantly less in children receiving 1 mg GH per m^2/day compared to those receiving 2 mg GH per m^2/day. The 95%CI of the mean difference of the pubertal height gain, was, however, rather large for boys and girls indicating that the GH-dose effect on mean pubertal height gain might differ in larger patient groups. The pubertal height gain was less when children were older or taller at onset of puberty. This has also been reported in normal statured children born AGA.[26, 38] Target height had no influence on the pubertal height gain. Children with a smaller bone age delay at onset of puberty had a reduced pubertal height gain, because the duration of puberty was also shorter in these children. A French longitudinal study using comparable pubertal milestones and adult height criteria as we did, reported a mean (SD) pubertal height gain in untreated short SGA children of 23.9 (6.1) and 19.8 (4.9) cm for boys and girls, respectively.[39] This indicates that the pubertal height gain of our GH-treated SGA children was similar or more, being 27.0 (8.4) for group A and 31.4 (4.1) for group B for boys and 19.0 (7.3) for group A and 18.9 (5.7) for group B for girls. As our previously published 5-year data have shown, most of our SGA children had their GH-induced catch-up growth during the first two years.[10] After 4 years of GH treatment the mean height was within the target range for both GH-dosage groups. For that reason it is not surprising that as both groups entered puberty after at least 4 years of GH treatment, children growing within their target range did not further increase their height SDS during puberty. On the other hand, as it has been described that discontinuation of GH might lead to catch-down growth in SGA children it seems advisable to continue GH treatment unless future research would prove otherwise.[40]

In conclusion, age at onset of puberty and menarche and progression of puberty of short children born SGA during long-term, continuous GH treatment, are comparable with normal statured AGA children, regardless of a dose of 1 mg or 2 mg GH/m^2/day. In addition, the duration of puberty and the pubertal height gain were not significantly different between the GH dosage groups.

References

1. **Albertsson-Wikland K, Karlberg J.** 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl* 399:64-70.
2. **Hokken-koelega ACS, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL.** 1995 Children born small for gestational age: Do they catch up? *Pediatr Res* 38:267-271.
3. **Tanaka T, Suwa S, Yokoya S, Hibi I.** 1988 Analysis of linear growth during puberty. *Acta Paediatr Scand Suppl* 347:25-29.
4. **Tanaka T, Komatsu K, Takada G, Miyashita M, Ohno T.** 1996 Prediction of adult height in healthy Japanese children. *Acta Paediatr Suppl* 417:57-60.
5. **Bourguignon JP.** 1988 Linear growth as a function of age at onset of puberty and sex steroid dosage: Therapeutic implications. *Endocr Rev* 9:467-488.
6. **Persson I, Ahlsson F, Ewald U, Tuvemo T, Qingyuan M, von Rosen D, Proos L.** 1999 Influence of perinatal factors on the onset of puberty in boys and girls: Implications for interpretation of link with risk of long term diseases. *Am J Epidemiol* 150:747-755.
7. **Leger J, Levy M, Boch J, Pinet A, Chevenne D, Porquet D, Collin D, Czernichow P.** 1997 Reduced final height and indications for insulin resistance in 20 year old born small for gestational age: Regional cohort study. *BMJ* 315:341-347.
8. **Leger J, Limoni C, Collin D, Czernichow P.** 1998 Prediction factors in the determination of final height in subjects born small for gestational age. *Pediatr Res* 43:808-812.
9. **Hokken-Koelega ACS.** 2002 Timing of puberty and fetal growth. Best Practice & Research Clinical Endocrinology and Metabolism 16:65-71.
10. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A.** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-3070.
11. **de Zegher F, Albertsson-Wikland K, Wollmann HA, Chatelain P, Chaussain JL, Lofstrom A, Jonsson B, Rosenfeld RG.** 2000 Growth hormone treatment of short children born small for gestational age: growth responses with continuous and discontinuous regimens over 6 years. *J Clin Endocrinol Metab* 85:2816-2821.
12. **Usher R, McLean F.** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-910.
13. **Roede MJ, van Wieringen JC.** 1985 Growth diagrams 1980, Netherlands. Third nation-wide survey. *T Soc Gezondheidsz* [suppl] 63:1-34.
14. **Rikken B, Wit JM.** 1992 Prepubertal height velocity references over a wide age range. *Arch Dis Child* 67:1277-1280.
15. **Tanner JM, Whitehouse RH.** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-179.
16. **Cameron N** 1978 The methods of auxological anthropometry. In: Falkner F, Tanner J (eds) Human growth, postnatal growth 2 ed. Tindall, london.
17. **Tanner J, Whitehouse R, Cameron N, Marchall W, Healy M, Goldstein H** 1983 Assessment of skeletal maturity and prediction of adult height (TW2-method) 2 ed. academic press, london.

18. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM.** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-323.
19. **Lee P, Chernausek S, AC H-K, Czernichow P, Board. ISfGAA.** 2003 International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics*. 2003 111:1253-1261.
20. **Tanner J, Lejarraga H, Cameron N.** 1975 The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. *Pediatr Res* 9:611-623.
21. **Job JC, Rolland A.** 1986 Histoire naturelle des retards de croissance a debut intra-uterin. *Arch Fr Pediatr* 43:301-306
22. **Preece MA.** 1997 Puberty in children with intrauterine growth retardation. *Horm Res* 48:30-32.
23. **Bull RK, Edwards PD, Kemp PM, Fry S, Hughes IA.** 1999 Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. *Arch Dis Child* 81:172-173.
24. **Marshall WA, Tanner JM.** 1969 Variations in pattern of pubertal changes in girls. *Arch Dis Child* 44:291-303.
25. **Marshall WA, Tanner JM.** 1970 Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13-23.
26. **Abbassi V.** 1998 Growth and normal puberty. *Pediatrics* 102:507-511.
27. **Marti-Henneberg C, Vizmanos B.** 1997 The duration of puberty in girls is related to the timing of its onset. *J Pediatr* 131:618-621.
28. **Fredriks AM, Buuren S, Wit JM, Verloove-Vanhoick SP.** 2000 Body index measurements in 1996-7 compared with 1980. *Arch Dis Child* 82:107-112.
29. **Travers SH, Jeffers BW, Bloch CA, Hill JO, Eckel RH.** 1995 Gender and Tanner stage differences in body composition and insulin sensitivity in early pubertal children. *J Clin Endocrinol Metab* 80:172-178.
30. **Kiess W, Reich A, Meyer K, Glasow A, Deutscher J, Klammt J, Yang Y, Muller G, Kratzsch J.** 1999 A role for leptin in sexual maturation and puberty? *Horm Res* 51(suppl 3):55-63.
31. **Flier J.** 1998 What's in a name? In search of leptin's physiologic role. *J Clin endocrinol metab* 83:1407-1413.
32. **Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W.** 1997 Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 82:2904-2910.
33. **Galler A, Schuster V, Kiess W.** 2001 Pubertal adipose tissue: is it really necessary for normal sexual maturation? *Eur J Endocrinol* 145:807-808.
34. **He Q, Karlberg J.** 2001 Bmi in childhood and its association with height gain, timing of puberty, and final height. *Pediatr Res* 49:244-251.
35. **Ong K, Kratzsch J, Kiess W, Dunger D.** 2002 Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. *J Clin Endocrinol Metab* 87:1041-1044.
36. **Thamdrup E.** 1961 Precocious sexual development. Copenhagen:Munksgaard.

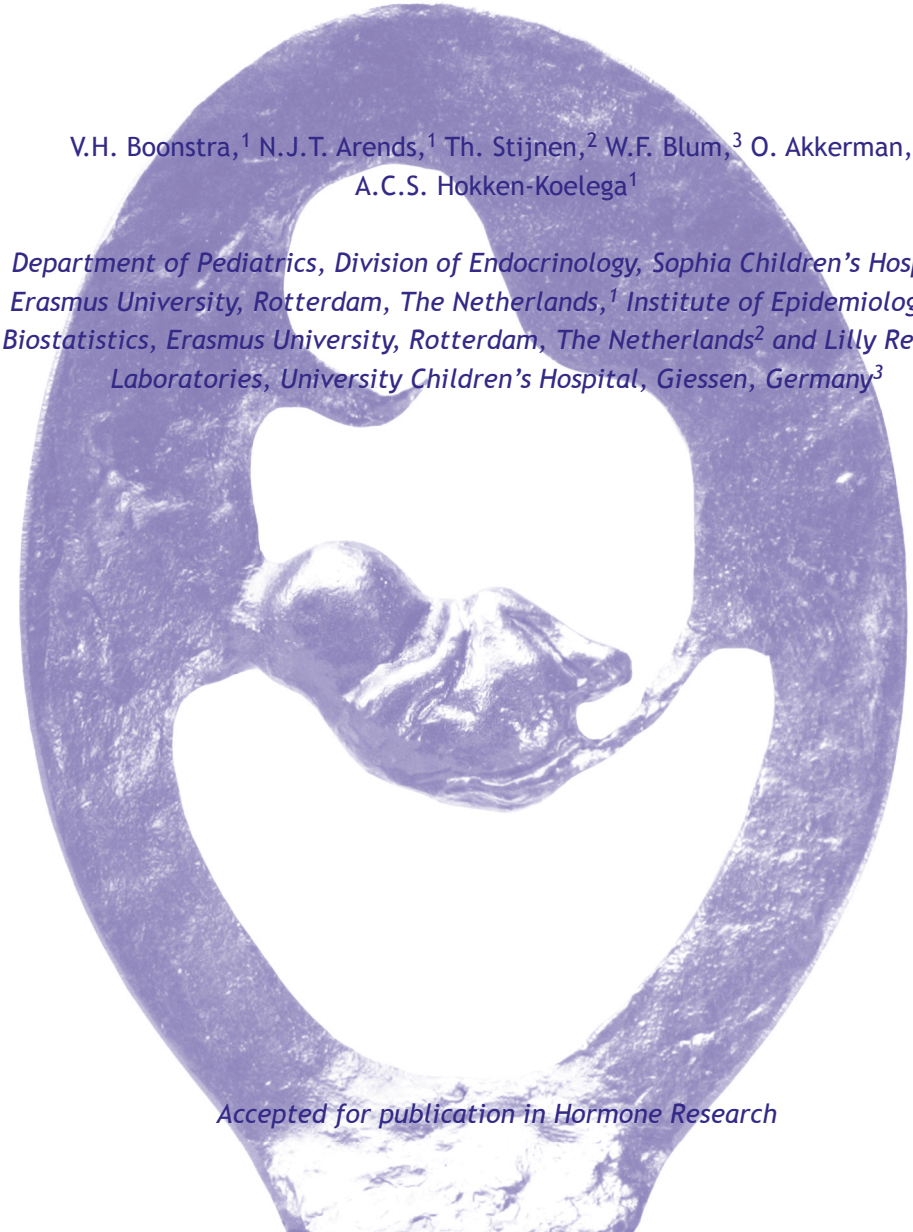
37. **Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Z. l.** 1997 Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-pinneau method. *Horm Res* 47:54-61.
38. **Tanaka T, Suwa S, Yokoya S, Hibi I.** 1988 Analysis of linear growth during puberty. *Acta Paediatr Scand Suppl* 347:25-29.
39. **Lienhardt A, Carel JC, Preux PM.** 2002 Amplitude of pubertal growth in short stature children with intrauterine growth retardation. *Horm Res* 57:88-94.
40. **Zegher de F.** 1998 GH-treatment of SGA children. *Trends Endocrinology and metabolism* 9:233-237.

Chapter 6

Food intake of children with short stature born Small for Gestational Age (SGA) before and during a randomised growth hormone trial

V.H. Boonstra,[1] N.J.T. Arends,[1] Th. Stijnen,[2] W.F. Blum,[3] O. Akkerman,[1] A.C.S. Hokken-Koelega[1]

Department of Pediatrics, Division of Endocrinology, Sophia Children's Hospital/ Erasmus University, Rotterdam, The Netherlands,[1] Institute of Epidemiology and Biostatistics, Erasmus University, Rotterdam, The Netherlands[2] and Lilly Research Laboratories, University Children's Hospital, Giessen, Germany[3]



Accepted for publication in Hormone Research

Abstract

Parents of short children born SGA often report that their children have a serious lack of appetite and a low food intake and that appetite improves after the start of growth hormone (GH) treatment.

In this study we investigated food intake, by using a standardised 7-day food questionnaire, in 88 short SGA children before start of GH treatment. The intake was compared with the recommended daily intake (RDI) of age-matched Dutch children. Later on, the short SGA children were randomised to receive GH (n = 62) or to remain untreated (n = 26). We compared the food intake of the GH-treated children with the randomised controls after 1 year of GH treatment. In addition, we also evaluated the effect of food intake and GH treatment on height, body fat mass, lean body mass (LBM), sum of 4 skinfold (SF), body mass index (BMI) and serum levels of IGF-I, IGFBP-3 and leptin.

Our study shows that caloric intake, fat and carbohydrate intake of short SGA children aged 5.9 (1.6) years was significantly lower compared to the RDI for age-matched children. One year of GH treatment resulted in a significant increase of the caloric, fat, carbohydrate and protein intake compared to baseline. Compared to randomised controls, caloric, carbohydrate and protein intake increased significantly after one year of GH treatment. Short SGA children had significantly lower SDS scores for LBM, fat mass, skinfold and BMI compared to age-matched references. They also had significantly lower serum IGF-I, IGFBP-3 and leptin levels. GH treatment resulted in a significant increase of height, LBM, BMI, IGF-I and IGFBP-3 SDS and a significant decrease of SF SDS and leptin SDS. Caloric and carbohydrate intake showed a positive correlation with LBM and BMI.

In conclusion, our study shows that short SGA children have indeed a lower food intake than age-matched controls. During GH treatment the food intake increased significantly compared to baseline in contrast to the randomised control group.

Introduction

Short stature in children born small for gestational age (SGA) is a well known phenomenon. About 10% of these children fail to show catch-up growth in height above the −2 SD during the first 2 years of life.[1-3] Most short children born SGA remain not only shorter but also have lower lean body mass (LBM) than children who were born appropriate for gestational age (AGA).[4] Parents of short children born SGA often report that their child has a serious lack of appetite and a low food intake and that their child experiences an improvement of their appetite after the start of GH treatment.

Since children with a low food intake might have low serum IGF-I levels and 25–60% of the short SGA children show low serum IGF-I levels it might be that low dietary intake contributes to the failure of complete catch-up growth in these children. In addition, serum levels of leptin, a protein produced by adipose tissue and involved in the regulation of appetite and body weight, are also reduced in short SGA children, which might reflect the suboptimal nutritional stage of these children.[5]

Data on the food intake of short SGA children before and during GH treatment are very scarce. We therefore investigated the food intake, by using a standardised 7-day food questionnaire, in a large group of 88 short children born SGA, in combination with measurements of height, fat mass, LBM, sum of 4 skinfold measurements, BMI, serum IGF-I, IGFBP-3 and leptin levels. We compared the baseline intake with the Recommended Daily Intake (RDI) of age-matched Dutch children. In addition, we investigated the effect of GH treatment on food intake, body composition and hormone levels in comparison with a randomised control group.

Subjects and methods

Subjects

The study comprised 88 prepubertal children with short stature born SGA. Only children who met the following criteria were included: 1) birth length below −2 SD for gestational age according to the standards of Usher & McLean (Usher & McLean, 1969), 2) an uncomplicated neonatal period, 3) chronological age (CA) between 3.00 and 7.99 years at start of the study, 4) height SDS for CA below −2.0 according to Dutch references,[6] 5) height velocity SDS for CA ≤ zero[6,7] to exclude children with spontaneous catch-up growth, 6) prepubertal, defined as Tanner breast stage 1 or a testicular volume < 4 ml,[8] 7) normal liver, kidney and thyroid function. Children with celiac disease, chromosomal abnormalities or syndromes, including Turner syndrome, were excluded except those with Silver-Russell syndrome. Before entering the study the GH status was evaluated using GH stimulation tests. Children with growth hormone deficiency (GHD), which was defined as a GH peak < 10 ng/ml during two GH stimulation tests, were excluded from the present study.

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

Study design

The study design of the GH trial was an open-labelled multicenter study with a randomised control group. The patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group (n = 62; 26 boys and 36 girls) started immediately with GH treatment at a dose of 1mg/m^2 body surface/day (≈ 0.03 mg/kg/day). Biosynthetic GH (r-hGH NorditropinR, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime. The control group (n = 26; 16 boys and 10 girls) remained untreated for 3 years and subsequently received the same GH treatment as the GH-group.

Food intake

Parents were asked to fill out a standardised 7-day food questionnaire for their child in order to measure food intake, prior to the study and 12 months after the start of GH. Since food patterns may differ on school days and weekend days, it was decided to record food intake for 7 days. If a child became ill during the record period, a new 7 day period was arranged. Before starting, parents were given both oral and written instructions on how to fill out the questionnaire in order to achieve maximum accuracy.

Food intake (caloric, fat, carbohydrate and protein) data were converted into energy, expressed in kcal/day, using the software package Komeet (software program developed by the Nutrition Department of Agricultural University of Wageningen) (Komeet, 1989). The individual food intake was compared with the Dutch Recommended Daily Intake (RDI) of age-matched controls according to the National Food Consumption Survey.[9] This recommended intake is a comprehensive description of intake of foods, energy and nutrients of 6,218 persons and is based on the mean minimal need of food substance which is necessary for a normal metabolic functioning.[10] The RDI is expressed in kcal/day for healthy boys and girls. The classification is comparable with that used by the National Research Council and the FAO/WHO (WHO; 1985).

Anthropometric measurements

Standing height (H) was measured 3-monthly by the same person using a Harpenden stadiometer. The mean of 4 measurements was used for analysis. Height was expressed as SD-score for sex and chronological age (HSDS) using Dutch references.[6] Body mass index (BMI) was calculated as weight (in kilogram) divided by square of height (in meters) and was expressed as SD-score for sex and age.[6] Skinfold measurements (SF) of biceps, triceps, subscapular and suprailiacal were measured in all children using one Holtain skinfold caliper.[11] The mean value of two measurements was calculated. For analysis we used the sum of the four measurements, expressed as SD-score using references for healthy Dutch children.[12] To calculate SD-scores, the LMS method was used.[13]

Body composition measured by DEXA

In a subgroup of 30 children (13 boys, 17 girls) visiting Sophia Children's Hospital, body composition was measured by Dual Energy X-ray Absorptiometry (DEXA) type Lunar DPX-L PED using the pediatric medium scan mode. Lean body mass (LBM, in kilogram) and total fat mass (fat mass, in kilogram) were measured. The coefficients of variation for the pediatric body weight were 4.1% for fat mass and 1.0% for lean tissue mass. Both parameters of body composition being dependent on age and sex, the values were transformed into SD-scores using Dutch reference values for children older than 4 years.[14]

Biochemical measurements

Blood samples were taken at the start of the study from all children and subsequently after 12 months for determination of serum levels of IGF-I, IGFBP-3 and leptin. After centrifugation, all samples were frozen (−80°C) until assayed.

Hormone assays

A specific RIA measured IGF-I after acid chromatography as described previously.[15] The serum levels were expressed as SD-scores using reference data from a healthy Dutch population of 600 children.[15] IGFBP-3 was isolated from human plasma according to the method developed by Martin and Baxter[16] and determined by a specific RIA using a polyclonal antiserum derived from New Zealand White rabbits. IGFBP-3 levels were expressed as SD-scores using reference data from a healthy Dutch population of 286 children aged 0–14 years provided by the laboratory.[17] Serum leptin measurements were performed in the laboratory of the University Children's Hospital in Giessen, Germany, using a specific radioimmunoassay (RIA).[18] Sensitivity was 0.03 µg/l and the intra- and inter-assay coefficients of variation (CV) were 0.8% and 8.5% respectively. For calculations leptin was transformed into a natural logarithm. SD-scores adjusting for sex, BMI and pubertal stage were calculated according to formulas described by Blum.[18]

Statistics

Data are expressed as the mean plus or minus the standard deviation (SD). In case of a non-Gaussian shaped distribution data were expressed as median and interquartile range. At start and after 1 year, SDS differences between groups were tested using independent Student's t-tests, In case of a non-Gaussian shaped distribution we used the Mann-Whitney test. ANCOVA was used with sex as covariate to test whether there is a difference in the change of food intake. Pearson's correlation coefficient was used for correlations. SD-scores were compared with zero using Student's one sample t-test. Differences between points in time within the groups were tested by paired Student's t-tests. For correlation and regression analysis the natural logarithm of leptin was taken. Statistical significance was defined as $p < 0.05$. Statistical tests were performed with use of SPSS package (version 10.0).

Results

Clinical data at baseline are shown in Table 1. Birth length, birth weight and height SDS were significantly different from zero ($p < 0.001$). Both study groups had similar initial characteristics at the start of the study, except for the ratio of the number of boys to girls, being higher in the control group.

Table 1. Baseline characteristics of the short SGA children.

	GH group n = 62	Randomised controls n = 26
Boys/girls	26/36	16/10
Gestational age	36.0 (3.8)	36.0 (3.5)
Birth length SDS	−3.4 (1.4)	−3.2 (1.4)
Birth weight SDS	−2.3 (1.2)	−2.7 (1.0)
Age	5.9 (1.6)	5.9 (1.5)
Height SDS	−2.9 (0.7)	−3.1 (0.5)

Data expressed as mean (SD).

Food intake in short SGA children at baseline and after 1 year of GH treatment *vs* no treatment

At baseline the mean total caloric (kcal), fat (kcal) and carbohydrate (kcal) intake of the SGA group (n = 88) were significantly lower ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively) compared to the Recommended Daily Intake (RDI) of age-matched children (Table 2). There was no significant difference in protein intake between the SGA children and the RDI of age-matched children.

In addition, we assessed the intake after 1 year of GH treatment compared to baseline, showing a highly significant increase in caloric, fat, protein and carbohydrate intake ($p < 0.001$). In contrast, the randomised control group did not show a significant difference between the intake after 1 year and the intake at baseline. Table 2 shows that the 1 year change in caloric, protein and carbohydrate intake was significantly higher in the GH group than in the randomised control group, after adjustment for age and sex. The change in fat intake was also higher in the GH group but this did not reach significance.

After 1 year the caloric, fat and carbohydrate intake of both the GH group and randomised control group was still significantly lower compared to the RDI of age-matched children, but the protein intake was not significantly different from the RDI of age-matched controls.

Table 2. Food intake of short SGA children, before and during the randomised GH trial

	At baseline		Change during 1 year study		
	Total SGA group 88	RDI* n=6218	GH group n = 62	Randomised controls n = 26	Δ 1-yr between groups
Food intake:					p-values
Calorie intake (kcal)	1337 (309)#	1697 (237)	148 (29 – 418)##	70 (–40 – 147)	0.02
Fat (kcal)	432 (135)#	558 (78)	90 (3 – 195)##	14 (–20 – 77)	ns
Protein (kcal)	174 (135)	183 (25)	30 (6 – 58)##	11 (–6 – 29)	0.04
Carbohydrate (kcal)	730 (169)#	930 (130)	76 (–23 –192)##	45 (–61 – 99)	0.03

Data expressed as mean (SD) or median and interquartile range, * Age-matched reference data of the Dutch recommended daily intake (RDI) according to the National Food Consumption Survey, # = SGA group *vs* RDI ($p < 0.001$), ## = intake after 1 year compared to baseline ($p < 0.001$).

Table 3. Body composition and hormone levels, before and during the randomised GH trial

	At baseline		After 1 year	
	Total SGA group n = 88	GH group n = 62	Randomised controls n = 26	GH vs controls p-values
Bodycomposition				
Height SDS	–3.1 SDS **	–2.0 (0.7)##	–3.1 (0.5)	< 0.001
DEXA - Fat SDS	–1.4 (0.5) **	–1.6 (0.5)	–1.1 (0.6)	0.03
- LBM SDS	–2.7 (0.5) **	–1.8 (0.5)##	–2.6 (0.4)	< 0.001
SF SDS	–1.2 (0.8) **	–1.9 (0.6)##	–1.0 (1.0)	< 0.001
BMI SDS	–1.3 (0.9) **	–1.2 (0.9)#	–1.0 (0.9)	ns
Hormone levels				
IGF-I SDS	–0.3 (1.0) *	1.2 (1.1)##	–0.2 (0.8)	< 0.001
IGFBP-3 SDS	–1.3 (1.1) **	0.1 (1.1)##	–1.0 (1.5)	< 0.001
Leptin SDS	–0.4 (1.4) *	–1.4 (1.2)#	–0.3 (1.3)	<0.001

Data expressed as mean (SD), * = Compared to 0 ($p < 0.01$), ** = Compared to 0 ($p < 0.001$), # = Compared to baseline ($p < 0.01$), ## = Compared to baseline ($p < 0.001$).

Body composition and hormone levels in combination with food intake

Table 3 shows that the SDS for height, fat mass, LBM, SF, BMI, serum IGF-I, IGFBP-3 and leptin levels were all significantly lower than zero at baseline.

After 1 year the randomised control group showed no change in body composition compared to baseline. In contrast the GH group, showed a significant increase in height SDS, BMI SDS and LBM SDS and a significant decrease of SF SDS compared to baseline. Height SDS, LBM SDS, fat mass SDS and SF SDS were significantly different in the GH-group compared to the randomised control group (Table 3), except for BMI SDS.

After 1 year, the GH group showed significantly higher IGF-I and IGFBP-3 levels and significantly lower serum leptin levels compared to the randomised controls.

At baseline we found that a higher caloric intake (kcal) and carbohydrate intake (kcal) were related to a higher LBM SDS ($r = 0.6$, $p < 0.01$ and $r = 0.6$, $p < 0.001$ resp.), as well to a higher BMI SDS ($r = 0.5$, $p < 0.01$). No correlation was found between the intake of fat and protein and LBM SDS or BMI SDS. Higher caloric, fat, protein and carbohydrate intakes were not related to a higher fat mass, SF or height SDS. We found no correlation between the increase in caloric intake and the increase in height SDS after one year of GH treatment.

No correlations were found between the food components and serum IGF-I, IGFBP-3 and leptin levels. During GH treatment we found a weak correlation between the change in food intake and change in IGF-1SDS ($r = 0.3$, $p = 0.05$). Positive correlations were found between serum leptin levels and fat mass SDS, BMISDS and SFSDS ($r = 0.6$ and $p < 0.001$, $r = 0.4$ and $p < 0.01$, $r = 0.4$ and $p < 0.01$).

No correlation was found between the decline of serum leptin levels and the increase of food intake during GH treatment.

Discussion

Our study investigated food intake in combination with body composition, growth factors and leptin in a large group of short prepubertal children born SGA and the effect of GH in a randomised study design. We show that caloric, fat and carbohydrate intake of short SGA children, with a mean (SD) age of 5.9 (1.6) years was significantly lower compared to the Recommended Daily Food intake of age-matched controls. One year of GH treatment resulted in a significant increase of the caloric, fat, carbohydrate and protein intake compared to baseline. Compared to randomised controls, caloric, carbohydrate and protein intake increased significantly after one year of GH treatment. Short SGA children had a significantly lower LBM SDS, fat mass SDS, skin fold SDS and BMI SDS compared to age-matched controls. In addition, short SGA children had significantly

lower serum IGF-I, IGFBP-3 and leptin levels. GH treatment resulted in a significant increase of LBM SDS, height SDS, BMI SDS, IGF-I SDS and IGFBP-3 SDS and a significant decrease of SF SDS and leptin SDS.

Parents of short SGA children often report that their child has a serious lack of appetite and a low food intake. Measuring food intake is difficult and often not very precise. We therefore used standardised 7-day food questionnaires and gave all parents thorough oral and written instructions how to read food intake and use the questionnaires. Before GH treatment we found that the caloric, fat and carbohydrate intake in short SGA children were significantly lower compared to the Recommended Daily Intake of children of the same age. The exact mechanism of the lower food intake in short SGA children is not known. Several studies have been performed to explain feeding problems in children in general. Factors related to decreased food intake of children may be behavioural, organic or a mixture of both.[19,20] Behavioural problems that affect food intake might have their roots in conditions that may have enhanced the gag reflex (such as prolonged period of orotracheal intubation or a nasogastric tube), failure to establish links between hunger and food intake and satiety in infants who had not been fed orally for a relatively prolonged period of time at a critical age, cultural expectations of food intake and body habits and parental anxiety about weight resulting in power struggles between parent and child that manifest in disturbed eating habits, and whereas anxiety or depression of the child might also play a role. Organic causes leading to decreased food intake include swallowing problems, respiratory distress, excessive fatigability and lack of appetite due to various systemic illnesses. We feel that several factors might contribute to the feeding problems in short children born SGA. One factor might be that parents are concerned about the growth and weight of their child. Because they want them to grow better they will force their child to eat. This might result in a negative parent-child interaction and can cause food aversion by the child. One study reported on the food intake in children with Silver-Rusell Syndrome.[21] From this study it was concluded that children with SRS experienced significantly more feedings problems compared to children without growth disorders. The most important problems were poor appetite, fussiness, slow feeding and problems associated with oral-motor dysfunction. They also found that mealtime interaction between these children and their parents was significantly more negative compared to the control group. The authors concluded that intervention should focus on reduction of the negative parent-child interactions and parental anxiety about feeding, growth, and weight. Another factor contributing to the feeding problem is that a percentage of the SGA children had a nasogastric tube for a prolonged period. It is known that these children are at an increased risk for developing feeding problems.[19] Another factor might be that short SGA children have a lower metabolic rate since they have a low growth rate.

Whatever the initial cause of the lower food intake in these children, in our study we found that during GH treatment food intake increased significantly compared to baseline in contrast to the randomised control group. The last group did not show any significant difference in food intake after 1 year. Our findings agree with the subjective findings expressed by the parents in our first SGA study.[22] They reported a lack of appetite at baseline but an increased appetite and food intake during GH treatment. GH treatment might increase food intake as a result of several changes in body composition and resting energy expenditure, such as an increase in basal metabolic rate, an increase in growth, fat free mass and reduction in body fat mass. Stenlöf *et al* reported that GH increases energy expenditure directly. This was only detectable during night and morning hours, when levels of GH were relatively high.[23,24] Comparably, the low food intake in short SGA children might be explained by a lower energy expenditure because of lower GH and IGF-I levels.[25] In addition, the improved growth itself may also result into a more relaxed parent-child interaction at mealtimes, leading to a better food intake. Blisset *et al* investigated the effect of GH treatment on food intake in a cross-sectional study in 23 children with Turner syndrome (TS) or Silver Rusell syndrome (SRS), aged 2–11 years, compared to 23 age-matched untreated children with TS or SRS. They found a higher food intake in GH-treated children and a better parent child interaction at mealtimes compared to the untreated children.[26]

Many parents consider their short SGA child as lean and fragile compared to their schoolmates. Our study shows that short children born SGA have indeed a lower fat mass, LBM, SF and BMI compared to children of the same age and sex. This is in agreement with other studies.[4,27,28] The fact that LBM increases impressively and total body fat mass reduces during GH treatment is an expected and well documented effect of GH.[29-31] GH causes the body to utilize fat mass stores for energy to aid production of muscle, which is increased in proportion in patients receiving GH. Interestingly, we found that a higher caloric- and carbohydrate intake was related to higher LBM SDS and BMI SDS but not to a higher fat mass SDS. It seems that these food components are used as energy for increasing LBM and not for increasing fat mass. None of the food components had an influence on fat mass, SF SDS and height SDS.

Some studies suggested that low food intake will result in reduced serum IGF-I and IGFBP-3 levels.[32] Findings in previous studies have shown that 25–60% of the SGA children have low serum IGF-I and IGFBP-3 levels.[22,25,33] However, at baseline we did not find a correlation between food intake and serum IGF-I or IGFBP-3 levels. During GH treatment we found only a weak correlation between the change in food intake and change in IGF-I levels.

In our study we found significantly lower serum leptin levels in short children born SGA compared to age-matched Dutch normal population. This is in agreement with other studies.[5,28,34] Leptin is produced by adipocytes and was first described in 1994. It has an

important role in the control of food intake and energy expenditure.[35] Leptin reduces food intake whereas it increases energy expenditure.[36,37] We found positive correlations between leptin SDS and measurements of body fat such as fat mass SDS, BMI SDS and SFSDS. This is in agreement with other studies, showing positive correlations between serum leptin levels and body mass index (BMI) and percentage body fat mass measured by bioelectric impedance measurements (BIA) or dual energy X-ray absorptiometry (DEXA) in healthy adults and children.[18,38,39] Appetite and food intake are stimulated in the presence of low serum leptin levels. Surprisingly, short children born SGA had a low food intake in spite of low serum leptin levels. An explanation for this might be that the low serum leptin levels in these children are not interpreted by the leptin receptors as being too low but as being normal. Therefore food intake is not stimulated but is kept on a low level. Further research should explore if such a lower set point exists and if this might be a result of a different programming during fetal growth restriction in prenatal life.

GH treatment resulted in a significant decline of leptin levels. This was also found in a study by Boguszewski.[5] GH increases basal lipolysis and increases the activity of hormone-sensitive lipase resulting in a rapid decrease of the adipose area and an increase in muscle tissue. It seems therefore likely that the reduction in leptin levels is attributable to the reduction in adipose tissue mass that occurs during GH treatment. Apparently, these lower leptin levels go together with an increase in food intake. At this moment it is, however, not clear if this is a direct effect of leptin or GH, particularly because no correlation was found between the decline of serum leptin levels and the increase of caloric intake.

In conclusion, short prepubertal children born SGA have a lower caloric, fat and carbohydrate intake compared to the RDI of age-matched controls. They have also reduced LBM, fat mass, BMI, SF, IGF-I, IGFBP-3 and leptin compared to age-matched children. GH treatment results in a significant increase in food intake compared to baseline levels and compared to randomised controls. In addition, GH treatment results in a significant increase of height, LBM, BMI, IGF-I, IGFBP-3 and a significant decrease of SF SDS and leptin SDS.

References

1. **Albertsson-Wikland K, Karlberg J** 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl* 399:64-70
2. **Hokken-koelega ACS, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: Do they catch up? *Pediatr Res* 38:267-71.
3. **Chaussain JL, Colle M, Ducret JP** 1994 Adult height in children with prepubertal short stature secondary to intrauterine growth retardation. *Acta Paediatr Suppl* 399:72-3.
4. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. *Clin Endocrinol* (Oxf) 59:779-87.
5. **Boguszewski MC, de Zegher F, Albertsson-Wikland K** 2000 Serum leptin in short children born small for gestational age: dose-dependent effect of growth hormone treatment. *Horm Res* 54:120-5.
6. **Roede MJ, van Wieringen JC** 1985 Growth diagrams 1980, Netherlands. Third nation-wide survey. *T Soc Gezondheidsz* [suppl] 63:1-34
7. **Rikken B, Wit JM** 1992 Prepubertal height velocity references over a wide age range. *Arch Dis Child* 67:1277-80.
8. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.
9. **Survey NFC** 1992 Zo eet Nederland. Results of the Dutch food Consumption Survey 1992. Ministry of Welfare, Public Health and Cultural Affairs. Den Haag: Voorlichtingsbureau voor de voeding.
10. **Voedingsraad. CV** 1984 Nota herbezinning aanbevolen hoeveelheden voor voedingsstoffen. *Voeding* 45:71-74
11. **Cameron N** 1978 The methods of auxological anthropometry. In: Falkner F, Tanner J (eds) Human growth, postnatal growth, 2 ed. Tindall, london
12. **Gerver W, de Bruin R** 1996 Body composition in children based on anthropometric data. A presentation of normal values. *European Journal of Pediatrics* 155:870-876
13. **Cole T** 1989 Using the LMS method to measure skewness in the NCHS and Dutch National height standards. *Ann Hum Biol* 16:407-419
14. **Boot AM, Bouquet J, de Ridder MA, Krenning EP, de Muinck Keizer-Schrama SM** 1997 Determinants of body composition measured by dual-energy X-ray absorptiometry in Dutch children and adolescents. *Am J Clin Nutr* 66:232-8.
15. **Hokken-Koelega AC, Hackeng WH, Stijnen T, Wit JM, de Muinck Keizer-Schrama SM, Drop SL** 1990 Twenty-four-hour plasma growth hormone (GH) profiles, urinary GH excretion, and plasma insulin-like growth factor-I and -II levels in prepubertal children with chronic renal insufficiency and severe growth retardation. *J Clin Endocrinol Metab* 71:688-95
16. **Martin JL, Baxter RC** 1986 Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *J Biol Chem* 261:8754-60
17. **Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM** 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. *Horm Res* 50:166-76

18. **Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W** 1997 Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 82:2904-10.
19. **Lifschitz CH** 2001 Feeding Problems in Infants and Children. *Curr Treat Options Gastroenterol* 4:451-457.
20. **Wright CM** 2000 Identification and management of failure to thrive: a community perspective. *Arch Dis Child* 82:5-9
21. **Blissett J, Harris G, Kirk J** 2001 Feeding problems in Silver-Russell syndrome. *Dev Med Child Neurol* 43:39-44.
22. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.
23. **Stenlof K, Johansson JO, Lonn L, Sjostrom L, Bengtsson BA** 1997 Diurnal variations in twenty-four-hour energy expenditure during growth hormone treatment of adults with pituitary deficiency. *J Clin Endocrinol Metab* 82:1255-60
24. **Stenlof K, Sjostrom L, Lonn L, Bosaeus I, Kvist H, Tolli J, Lindstedt G, Bengtsson BA** 1995 Effects of recombinant human growth hormone on basal metabolic rate in adults with pituitary deficiency. *Metabolism* 44:67-74
25. **de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL** 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. *Clin Endocrinol* (Oxf) 41:621-30.
26. **Blissett J, Harris G, Kirk J** 2000 Effect of growth hormone therapy on feeding problems and food intake in children with growth disorders. *Acta Paediatr* 89:644-9.
27. **Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P** 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. *Journal of Clinical Endocrinology and Metabolism* 78:904-909
28. **Boguszewski M, Dahlgren J, Bjarnason R, Rosberg S, Carlsson LM, Carlsson B, Albertsson-Wikland K** 1997 Serum leptin in short children born small for gestational age: relationship with the growth response to growth hormone treatment. The Swedish Study Group for Growth Hormone Treatment. *Eur J Endocrinol* 137:387-95.
29. **Boot AM, Engels MA, Boerma GJ, Krenning EP, De Muinck Keizer-Schrama SM** 1997 Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. *J Clin Endocrinol Metab* 82:2423-8.
30. **Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P** 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3-year treatment period and after 1 year's withdrawal. *J Clin Endocrinol Metab* 83:3512-6.
31. **Karlsson C, Stenlof K, Johannsson G, Marin P, Bjorntorp P, Bengtsson BA, Carlsson B, Carlsson LM, Sjostrom L** 1998 Effects of growth hormone treatment on the leptin system and on energy expenditure in abdominally obese men. *Eur J Endocrinol* 138:408-14
32. **Katz LE, DeLeon DD, Zhao H, Jawad AF** 2002 Free and total insulin-like growth factor (IGF)-I levels decline during fasting: relationships with insulin and IGF-binding protein-1. *J Clin Endocrinol Metab* 87:2978-83.

33. **Albertsson-Wikland K** 1989 Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. *Acta Paediatrica Scandinavia* 349:35-41
34. **Jaquet D, Leger J, Levy-Marchal C, Oury J, F., Czernichow P** 1998 Ontogeny of Leptin in Human Fetuses and Newborns: Effect of Intrauterine Growth Redartion on Serum Leptin Concentrations. *Journal of Clinical Endocrinology and Metabolism* 83:1243-1246
35. **Zhang XY, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432
36. **Halaas J, L., Gajiwala K, S., Maffei M, Cohen S, L., Chait B, T., Rabinowitz D, Lallone R, L., Burley S, K., Friedman J, M.** 1995 Weight-Reducing Effects of the Plasma Protein Encoded by the obese Gene. *Science* 269:543-545
37. **Campfield L, A., Smith J, Guisez Y, Devos R, Burn P** 1995 Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neutral Networks. *Science* 269:546-549
38. **Considine RV, Sinha MK, Heiman Ml, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292-295
39. **Ellis KJ, Nicolson M** 1997 Leptin levels and body fatness in children: effects of gender, ethnicity, and sexual development. *Pediatr Res* 42:484-8.

Chapter 7

Improved health related quality of life in growth hormone-treated children born Small for Gestational Age (SGA)

V.H. Boonstra,[1] N.J.T. Arends,[1] N.C.M. Theunissen,[2] H.M. Koopman,[3]
A.C.S. Hokken-Koelega[1]

Department of Paediatrics, Division of Endocrinology, Sophia Children's Hospital, Erasmus University Medical Centre Rotterdam, The Netherlands[1], TNO Human Factors, Department of Training & Instruction, Soesterberg, The Netherlands[2], Department of Paediatrics, Leiden University Medical Center, Leiden, The Netherlands.[3]

Submitted

Abstract

In a randomised controlled study we evaluated the effect of 3 years of GH treatment on the health status (HS) and the emotional feelings towards HS, called the health related quality of life (HRQOL). We compared GH-treated short children born SGA (n = 58) with untreated children born SGA (n = 27). At start of the study the children were 5–7 years of age. We used two different questionnaires, the TNO-AZL Children's Quality of life (TACQOL), a generic questionnaire and the TACQOL-Short Stature, a recently developed specific questionnaire for short children. Both questionnaires were completed by the parents.

The generic TACQOL did not show a significant difference between the GH group and control group in HS and HRQOL at start of the study, after 3 years only physical functioning was significantly improved in the GH group. In contrast, the TACQOL-Short Stature showed that SGA children who were treated with GH for 3 years had a significantly better quality of life with respect to their physical abilities, vitality, contact with peers, contact with adults, body image and future prospects compared to the untreated SGA children. The TACQOL-Short Stature showed a positive correlation between height SDS and HRQOL with regard to physical abilities, contact with peers, contact with adults, body image and future prospects. The discrepancy between the outcome of the two questionnaires shows the relevance of using a disorder-specific instrument.

In conclusion, our study shows that short children born SGA have short stature specific problems and for this reason a specific questionnaire is more applicable for measuring quality of life in short children than a generic questionnaire which has more reference to children with a chronic illness. We demonstrated that GH treatment in children born SGA improves several aspects of quality of life in SGA children with short stature.

Introduction

Short stature is a well-known phenomenon in children born small for gestational age (SGA). Although postnatal catch-up growth occurs in most of the SGA newborns, about 10% of these children fail to show catch-up growth in height above the –2 SD.[1,2]

Several studies have demonstrated that growth hormone (GH) treatment results in a significant catch-up growth in SGA children leading to a normal adult height for most of them.[3-5] It is, however, also important to know whether GH treatment has a positive effect on their quality of life.

Some studies have indicated that children with short stature have a low self-concept, are academically and socially handicapped. A part of these studies indicated that GH treatment has a positive effect on psychosocial functioning.[6-9] In contrast, other studies have demonstrated normal psychosocial functioning of short children or they did not support the positive influence of GH treatment on the psychosocial functioning in children with short stature.[10,11] These discrepancies might be due to the use of different study populations or different questionnaires.[11-13] Most studies concentrate on problems and limitations in psychosocial functioning due to a health problem, also called the health status (HS). However, it is also important to investigate the emotional impact that the problem has on the person's life, called the health related quality of life (HRQOL).[13,14] The HRQOL is defined as health status plus emotional responses to health status or, in other words, as a weighting of health status problems by the emotional impact of such problems. It combines assessment of physical, psychological, and social well-being in one outcome measure. If it matters how children feel about their functioning rather than how they are functioning, HS alone does not provide all relevant information.

In addition, several studies evaluating psychosocial problems in children with short stature, applied child behaviour questionnaires which originate from studies in children with a chronic illness. Since these questionnaires were not developed for short children, there might be a lack of sensitivity for short stature related topics.[12,15] Issues, such as being able to reach utensils on shelves and being treated in an age-appropriate manner by relatives are not always evident to persons with a normal stature, however, they are important to individuals of severe short stature.

Recently, a Dutch specific questionnaire for short stature was developed, the TNO-AZL Children's Quality of life (TACQOL)–Short Stature. In the present study, we evaluated the HS and HRQOL in short SGA children participating in a randomized controlled GH-trial, by using two different questionnaires. We used the TACQOL, a generic QOL questionnaire applicable to children with various chronic diseases and handicaps,[16] and the TACQOL-Short Stature, a specific QOL questionnaire developed for children with short stature. Both questionnaires were completed by the parents.

This is the first study evaluating HRQOL with a specific short stature questionnaire in short children born SGA. We hypothesized that the specific questionnaire will measure larger differences between control and treatment groups than the generic questionnaire. In addition we hypothesized that children with more catch-up growth will have a better HRQOL.

Methods

Subjects

Eighty-five Dutch children (37 boys and 48 girls) with short stature born SGA were included in a randomised GH-trial.[17] These children were referred to the hospital because of short stature. At the time of HRQOL and HS measurements the children were between 5 and 7 years of age. The inclusion criteria of the GH-trial were: 1) birth length or birth weight standard deviation score (SDS) below −2.00 SDS for gestational age;[18] 2) an uncomplicated neonatal period, without signs of severe asphyxia (defined as Apgar score below 3 after 5 minutes), sepsis or long-term complications of respiratory ventilation such as bronchopulmonary dysplasia; 3) chronological age (CA) between 3.00 and 7.99 years at start of the study; 4) height SDS for age below −2.00 according to Dutch standards;[19] 5) height velocity SDS for age below zero to exclude children with spontaneous catch-up growth;[19] 6) prepubertal, defined as Tanner stage 1 or a testicular volume < 4 ml;[20] 7) normal liver, kidney and thyroid functions. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, chondrodysplasia) or syndromes except Silver Russell syndrome, and previous or present use of medication that could interfere with GH treatment. All children used Dutch as their primary language.

Nine centres in the Netherlands participated in the study. The study was approved by the Ethics Committees of each participating centre. Written informed consent was obtained from the parents or custodians of each child.

Study design

The study design was an open-labelled, multicenter study with a randomised control group. The patients were randomly assigned to either the GH-group (2/3 of children) or the control group (1/3 of children). The GH-group started with GH treatment at a dose of 1 mg/m^2/day (≈ 33 µg/kg/day). The control group remained untreated for 3 years and subsequently received the same GH treatment as the GH-group.

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

Quality of life measurements

The quality of life was measured by two different questionnaires: The generic TNO-AZL Questionnaires for Children's Quality of life (TACQOL)[16,21] and the TACQOL-Short Stature.

Both questionnaires are based on the same principle. They explicitly offer respondents the possibility of differentiating between their health status (HS) and the way they feel about it (HRQOL). The reference period is formulated as 'the last few weeks my child….'. Each item starts with a specifically formulated HS problem. Response categories are 'never', 'sometimes' or 'often'. If the answer is 'sometimes' or 'often' the item leads to a second part about emotional response: 'During this time my child felt…..' : '(very) good', 'not so well', 'rather bad', or 'bad'. Figure 1 shows an example of such a question. The answers of the HS questions were scored on a 0–2 scale (0 = often, 1 = sometimes, 2 = never), the question scores were counted up excluding the evaluation of the emotional response. HRQOL was scored on a 0–4 scale (added in Figure 1, between brackets), the question scores were counted up including the evaluation of the emotional response. As a result two series of scales were obtained: a HS-score and HRQOL-score. A higher score indicated a better HS or HRQOL. The questionnaires consist of different scales with questions, each with its own specific topic. Two parallel questionnaires were available: a parent form (PF) and a child form (CF), both with good measurement properties.[13] The PF is designed for parents of children aged 6–15 years and the CF is designed for children aged 8–15 years. In this study we used the PF since the children were younger than 8 years at the start of the study.

Did people think that your child was younger than he or she actually was?

❑ never [4] ❑ sometimes ❑ often

During this time my child felt:

❑ (very)good [3] ❑ not so well [2] ❑ rather bad [1] ❑ bad [0]

Figure 1. An example of a question from the TACQOL-Short stature (scale: body image).

The generic TACQOL is a 63-item questionnaire for assessing HS and HRQOL, applicable to children with different diseases and handicaps.[16,21] This questionnaire contains seven

scales: physical functioning, motor functioning, autonomy, cognitive functioning, social functioning, positive emotions and lack of negative emotions. The last two scales did not have a HRQOL part because the questions already include an emotional dimension. In these two scales HS equalled HRQOL. All scales had a Cronbach alpha above 0.70. The parents were asked to fill in the generic TACQOL before randomisation and after 3 years of GH treatment or control period.

The TACQOL-Short Stature is a 47-item questionnaire for assessing HS and HRQOL of children with short stature. This questionnaire contains six scales: physical abilities, vitality, contact with peers, contact with adults, body image and future prospects. Table 1 shows a examples of a question of each scale. In case of a normal stature it is possible to give the response: not applicable or never. All scales had a Cronbach alpha above 0.70 except for the future prospects scale that had an alpha of 0.62. Since the TACQOL-Short Stature was not yet available at start of the study, the parents filled in this questionnaire after 3 years of GH treatment or control period.

Table 1. Example of HS scale and the number of items per scale.

Scales	NI	Example of an item
Physical abilities	7	Were the chairs and tables at school too high?
Vitality	8	Did your child get tired quickly
Contact with peers	8	Did other children lift up your child?
Contact with adults	9	Did someone mistreat your child because of his/her short stature?
Body image	8	Was your child sometimes concerned about his/her short stature
Future prospects	6	Did your child ever think about the fact that he/she will have a shorter stature compared to others in the future?

Each item was followed by an additional question "During this my child felt……." (HRQOL), NI=Number of Items.

Anthropometric measurements

Standing height (H) was measured 3-monthly by two trained investigators (NA and later on VB) using a Harpenden stadiometer. The mean of 4 measurements was used for analysis. Height was expressed as standard deviation score (SDS) for sex and chronological age (HSDSCA) using Dutch references.[19] Target height was calculated using Dutch reference data according to the formula: $1/2 * (H_{father} + H_{mother} + 13) + 4.5$ for boys and $1/2 * (H_{father} + H_{mother} - 13) + 4.5$ for girls, where the addition of 4.5 cm represents the secular trend. TH was expressed as SDS using Dutch references.[19] Pubertal stages were assessed according to Tanner,[20] using an orchidometer in boys.

Statistical analyses

Scale scores were obtained by summing the item scores within scales, and transforming crude scale scores to percentages on a linear 0 to 100 scale, with higher scores indicating better HS and HRQOL. Data were expressed as the mean (SD) at all points of measurements. The differences in clinical characteristics between groups were tested with the independent t-test. The scale score differences between groups at each time were tested by Mann-Whitney test. Differences between HS and HRQOL were tested with the Wilcoxon test. The reliability of the internal consistency of each scale in both questionnaires was evaluated by calculating Cronbach's alpha's.[22] In general a Cronbach alpha range from 0.65 and 0.84 is regarded as satisfactory for comparing different groups.[23,24] The Spearman rank correlation test was used to test the correlations between height SDS and the different scales of the generic TACQOL and TACQOL-Short Stature. A p-value < 0.05 was considered significant. All analyses were performed using SPSS version 10.0.

Results

GH trial

Table 2 shows the clinical characteristics at start and after 3 years for the GH group and control group. Both groups had similar initial characteristics. At start of the study the mean (SD) height SDS of the GH group and control group was −3.0 (0.7) and −3.0 (0.5), respectively, being not significantly different between groups. After 3 years, however, the mean (SD) height SDS was −1.3 (0.7) and −2.7 (0.8) respectively, being significant higher in the GH-treated children ($p < 0.001$).

After 3 years, parents of 8 children did not fill in the questionnaires for the following reasons: 1 child was satisfied with her height and was not motivated to continue GH treatment and the parents of 7 children forgot to fill in the questionnaire. Five children were excluded from the analysis because they were less than 3 years in the study at the moment of evaluation.

Table 2. Clinical characteristics.

	GH group (n = 58)	Control group (n = 27)
Male/Female	25/33	12/15
Gestational age (wks)	36.8 (3.6)	36.7 (3.3)
Birth length SDS	−3.0 (1.3)	−3.3 (1.3)
Birth weight SDS	−2.2 (1.2)	−2.7 (1.0)
Age at start (yr)	6.6 (0.9)	6.6 (0.9)
Height SDS - At start	−3.0 (0.7)	−3.0 (0.5)
- After 3 years	−1.3 (0.7)	−2.7 (0.8)*
Target Height	−0.5 (0.9)	−0.5 (0.8)

Expressed as mean (SD), * $p < 0.001$

Generic HS and HRQOL

Table 3 shows the HS and HRQOL scores obtained by the generic TACQOL for the GH group and control group at start and after 3 years of the study. There were no significant differences between the GH group and the control group at start of the study. After 3 years only the HS and HRQOL score of physical functioning was significantly higher in the GH group compared to the control group. The GH group and control group had significantly higher HRQOL scores than HS scores at start and after 3 years.

Short stature specific HS and HRQOL

Table 4 shows the HS and HRQOL scores of the TACQOL-Short Stature after 3 years for the GH and control group. For all scales the HS scores of the GH group were significantly higher than the HS scores of the control group. Furthermore, in all scales the GH group had a significantly higher HRQOL score compared to the control group. In both groups the HRQOL scores were significantly higher than the HS scores.

Table 3. Generic HS and HRQOL in SGA children at start and after 3 years.

	At start				After 3 years			
	GH group (n = 58)		Control group (n = 26)		GH group (n = 49)		Control group (n =23)	
	HS	HRQOL	HS	HRQOL	HS	HRQOL	HS	HRQOL
Physical functioning	81.3 (14.5)	85.8 (12.2)	77.6 (15.3)	81.6 (14.2)	88.0 (10.9)	89.6 (10.8)	77.9 (19.0)*	80.7 (16.3)*
Motor functioning	90.1 (15.8)	93.7 (10.6)	91.4 (12.7)	94.4 (8.4)	91.6 (14.2)	93.6 (12.8)	94.2 (6.3)	95.9 (5.1)
Autonomy	88.2 (15.7)	92.8 (12.3)	90.3 (12.8)	93.6 (10.4)	94.0 (13.5)	95.7 (11.2)	96.9 (6.3)	98.0 (3.5)
Cognitive functioning	79.1 (21.2)	88.1 (13.7)	81.4 (28.7)	89.9 (14.8)	74.2 (23.0)	82.6 (16.2)	73.3 (26.6)	82.5 (17.0)
Social functioning	89.2 (9.8)	93.0 (6.7)	86.2 (15.8)	91.1 (9.5)	87.6 (12.3)	91 (10.8)	83.2 (12.9)	87.1 (11.3)
Positive emotions		92.7 (10.4)		84.2 (18.5)		89.8 (14.3)		80.0 (21.8)
Negative emotions		67.2 (13.9)		63.0 (18.3)		67.7 (17.0)		68.3 (18.9)

Data expressed as mean (SD) percentage of maximum scores (100%); Higher scores represent better HS and HRQOL, * p = 0.03 GH group *vs* control group.

Table 4. Short stature specific HS and HRQOL in SGA children after 3 years.

	After 3 years			
	GH group (n = 49)		Control group (n = 23)	
	HS	HRQOL	HS	HRQOL
Physical abilities	90.0 (11.6)	92.4 (9.6)	76.2 (18.3)***	85.3(12.8)**
Vitality	87.3 (13.3)	91.4 (10.7)	79.4 (14.6)*	87.1 (9.7)*
Contact peers	82.5 (14.4)	84.2 (14.0)	64.7 (20.3)***	70.3 (17.3)***
Contact adults	82.8 (14.4)	88.9 (10.6)	65.5 (17.7)**	80.4 (13.5)*
Body image	75.7 (23.1)	88.1 (12.8)	52.7 (27.9)**	79.5 (11.3)**
Future prospects	69.6 (20.1)	90.0 (6.8)	56.5 (19.8)**	85.2 (8.0)**

Data expressed as mean (SD) percentage of maximum scores (100%); higher scores represent better HS and HRQOL, * p < 0.05 GH group *vs* control group, ** p < 0.01 GH group *vs* control group, *** p < 0.001 GH group *vs* control group.

Relationship between height SDS and HS or HRQOL

No correlations were found between height SDS and generic HRQOL scores at start and after 3 years. Table 5 shows the correlations between height SDS after 3 years and physical abilities, vitality, contact with peers, contact with adults, body image and future prospects of the TACQOL Short Stature. A highly significant correlation was found between height SDS and physical abilities, contact with adults, body image and future prospects. Figure 2 shows an example of correlation between height SDS and body image. In addition, it can be seen that there are individual differences. A small but significant correlation was found between height SDS and contact with peers. No correlation was found between height SDS and vitality.

None of the HRQOL scores of the TACQOL-Short were correlated with target height SDS, age of the child, and paternal and maternal height SDS (data not shown).

Table 5. Spearman correlation coefficient between height SDS after 3 years and the HRQOL scales of the TACQOL-short stature.

	r	P-value
Physical abilities	0.5	< 0.001
Vitality	0.2	ns
Contact peers	0.3	0.03
Contact adults	0.5	< 0.001
Body image	0.5	< 0.001
Future prospects	0.4	0.003

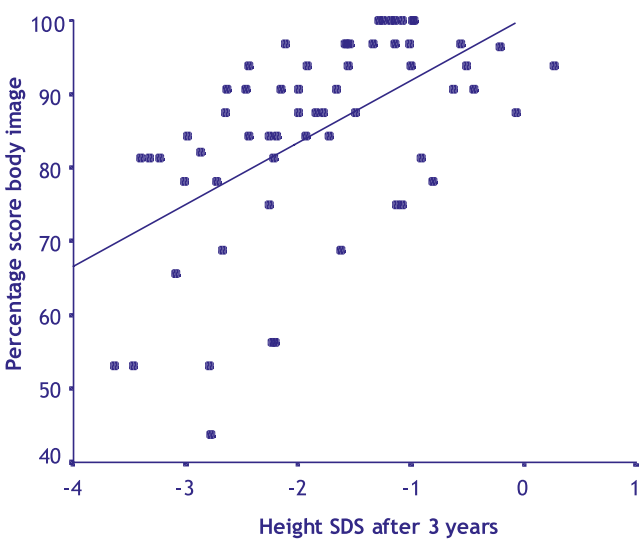


Figure 2. Correlation between height SDS after 3 years and body image.

Discussion

Three years of GH treatment resulted in a significantly taller height compared to the untreated children. At start the generic TACQOL showed no significant difference in HS and HRQOL between the GH group and control group. After three years the generic TACQOL showed that only the physical functioning was significantly improved in the GH group. In contrast, the TACQOL-Short Stature showed that SGA children treated with GH had a significantly better HS and HRQOL with respect to their physical abilities, vitality, contact with peers, contact with adults, body image and future prospects compared to untreated SGA children. Height SDS was positively correlated with HRQOL with regard to physical abilities, contact with peers, contact with adults, body image and future prospects.

The finding that three years of GH treatment results in a significantly taller height in children born SGA is comparable with previous studies in which short children born SGA were treated with GH.[3-5,25] Several studies have also shown that GH has a number of other benefits in short children born SGA. These include an increase in appetite and body mass index (BMI), as well as a reduction in blood pressure and a significant improvement in the serum lipid profile.[26-28] However, the effect of GH treatment of children born SGA on HS and HRQOL has not been studied before. Our study demonstrates that at baseline the generic TACQOL showed no significant difference in HS and HRQOL between the GH group and control group. Three years of GH treatment resulted only into a better HS and HRQOL score for physical functioning. This means for example, that, according to the parents, after 3 years the GH-treated children compared to the untreated children felt less fatigued but were both equally able to run, to climb the stairs, to dress themselves, to wash themselves, to talk, to write, to play, to talk with their parents and were equally angry or scared. In contrast, the specific questionnaire, the TACQOL-Short Stature showed that 3 years of GH treatment induced a significantly better HS and HRQOL regarding physical abilities, vitality, contact with peers, contact with adults, body image and future prospects, indicating that the GH-treated children had a better quality of life than untreated short SGA children. This means that, according to the parents, GH-treated children compared to untreated children did not experience that chairs and tables at school were too high. They had more energy and liked to play and eat better. They had less problems in social contacts with peers such as less teasing, less loneliness and less problems in contact with adults as they were more treated according to their age than short untreated SGA children. They were more satisfied with their height and more confident about their bodily appearance. According to the parents, GH-treated children had more positive thoughts about the future such as that they would attain the same height as their peers.

In this study the parents were asked about their children's health status and their children's emotional reactions to health status. Preferably, HRQOL and HS are self-administered, however, some of the younger children cannot be used as informants because children may lack the necessary language skills, as well as the cognitive abilities to interpret the questions, and the long term view on events. In addition, HRQOL and HS are usually assessed through paper and pencil questionnaires. Alternatively, a proxy respondent can be used and the parent is the most preferable proxy informant about the child's HRQOL.[16,29] As the choice of informant influences the QOL judgements[29-31] we used the same informant, the parent, for all children. Parents are the main decision-makers in respect of the rearing and medical treatment of their children. This makes their perception of HRQOL at least clinically relevant.

The discrepancy between the outcome of the two QOL questionnaires shows that SGA children do not have problems mentioned in a generic questionnaire but have more specific problems related to short stature. This is supported by a study of Haverkamp *et al* which demonstrated that for attaining growth disorder-specific information a special questionnaire is needed.[26] The questions in the TACQOL-Short Stature are more applicable for effects of short stature in contrast to the questions in the generic TACQOL. The last one has more reference to children with a chronic illness or handicap and in case of short stature they ask the "wrong" questions. For this reason significant changes of several aspects of quality of life during GH treatment of short SGA children will not be noticed by the generic questionnaire. Interestingly, the physical functioning scale of the generic TACQOL and the vitality scale of the TACQOL-Short Stature contain both questions that are focused on energy. In the generic TACQOL this scale was the only one which was significantly higher in the GH group compared to the control group. At start of the study no data was available of the TACQOL Short Stature. Theoretical, it might be that the HS and HRQOL scores were already different between the control and GH-treated group at start. However, this seems unlikely since the children were randomly divided to the groups.

In both the GH and control group, the HRQOL scores were higher than the HS scores in all scales. This indicates that health related problems do not necessarily result in a similar reduction of the HRQOL. In the paediatric field, most studies investigating the quality of life have been focused on the HS but not on the HRQOL of the patient. It is important to make a distinction between functioning of children and how they feel about their functioning. This is supported by a study of Verrips *et al* which showed that only half of the reported health status problems were associated with negative emotional reactions in children.[23] The severity of the emotional and social consequences of short stature may vary from one child to another.[32,33] The fact that some short children will not be referred to a hospital does not diminish the seriousness of the problems in those who are referred.[32,34] For this reason, in our study, we evaluated the effect of GH on the HRQOL in referred SGA children by comparing GH-treated to untreated children.

To our knowledge there are no published data on the effect of GH treatment on HRQOL in short SGA children. Two studies have shown that children born SGA with persistent short stature have a higher risk for subnormal intellectual and psychological performance.[6,7] HRQOL was, however, not evaluated in these studies. Theunissen *et al* have investigated the effect of GH on HRQOL in children with idiopathic short stature (ISS) and showed that GH had no effect on the psychosocial well being.[13] They investigated the effect of GH treatment on vitality with a specific questionnaire but vitality did not improve in the ISS treatment group whereas in our study vitality was better for the SGA treatment group. An explanation for this might be that the background of children with ISS is different from that of children born SGA.

The positive correlations between height SDS and HRQOL regarding physical abilities, contact with adults, body image and future prospects, obtained using the TACQOL Short Stature, show that height SDS is important for many aspects of the quality of life of SGA children. GH-treated children had a significantly higher HRQOL score for vitality compared to untreated short SGA children, whereas no correlation was found between height SDS and HRQOL for vitality. An explanation might be that GH treatment increases vitality due to an increase of muscle mass[27] rather than an increment of height SDS.

In conclusion, our study shows that short children born SGA have short stature specific problems. For this reason a specific questionnaire is more applicable for measuring quality of life in short children than a generic questionnaire which has more reference to children with a chronic illness. We demonstrated that GH treatment in children born SGA improves several aspects of quality of life in SGA children with short stature.

References

1. **Albertsson-Wikland K, Karlberg J** 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl* 399:64-70
2. **Hokken-Koelega ACS, De Ridder MA, Lemmen RJ, Den Hartog H, De Muinck Keizer-Schrama SM, Drop SL** 1995 Children born small for gestational age: Do they catch up? *Pediatr Res* 38:267-71.
3. **Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 2003 Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab* 88:3584-90.
4. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.
5. **de Zegher F, Albertsson-Wikland K, Wollmann HA, Chatelain P, Chaussain JL, Lofstrom A, Jonsson B, Rosenfeld RG** 2000 Growth hormone treatment of short children born small for gestational age: growth responses with continuous and discontinuous regimens over 6 years. *J Clin Endocrinol Metab* 85:2816-21.
6. **Lundgren EM, Cnattingius S, Jonsson B, Tuvemo T** 2003 Intellectual and psychological performance in males born small for gestational age. *Horm Res* 59:139-41.
7. **Pryor J, Silva PA, Brooke M** 1995 Growth, development and behaviour in adolescents born small-for-gestational-age. *J Paediatr Child Health* 31:403-7.
8. **Stabler B, Siegel PT, Clopper RR, Stoppani CE, Compton PG, Underwood LE** 1998 Behavior change after growth hormone treatment of children with short stature. *Journal of Pediatrica* 133:366-373
9. **van der Reijden-Lakeman I, Slijper FM, van Dongen-Melman JE, de Waal WJ, Verhulst FC** 1996 Self-concept before and after two years of growth hormone treatment in intrauterine growth-retarded children. *Horm Res* 46:88-94.
10. **Sandberg DE** 2000 Should short children who are not deficient in growth hormone be treated? *West J Med* 172:186-9.
11. **Kranzler JH, Rosenbloom AL, Proctor B, Diamond FB, Jr., Watson M** 2000 Is short stature a handicap? A comparison of the psychosocial functioning of referred and nonreferred children with normal short stature and children with normal stature. *J Pediatr* 136:96-102.
12. **Haverkamp F, Noeker M** 1998 'Short stature in children--a questionnaire for parents': a new instrument for growth disorder-specific psychosocial adaptation in children. *Qual Life Res* 7:447-55.
13. **Theunissen NC, Kamp GA, Koopman HM, Zwinderman KA, Vogels T, Wit JM** 2002 Quality of life and self-esteem in children treated for idiopathic short stature. *J Pediatr* 140:507-15.
14. **Vogels T, Verrips GHW, Koopman HM, Theunissen NC, Fekkes M, kamphuis RP** TACQOL Manuel Parent Form and Child Form. Leiden Center for child health and pediatrics LUMC-TNO.
15. **Perrin EC, Stein RE, Drotar D** 1991 Cautions in using the Child Behavior Checklist: observations based on research about children with a chronic illness. *J Pediatr Psychol* 16:411-21.
16. **Vogels T, Verrips GH, Verloove-Vanhorick SP, Fekkes M, Kamphuis RP, Koopman HM, Theunissen NC, Wit JM** 1998 Measuring health-related quality of life in children: the development of the TACQOL parent form. *Qual Life Res* 7:457-65.

17. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. *Clin Endocrinol* (Oxf) 59:779-87.
18. **Usher R, McLean F** 1969 Intrauterine growth of live-born Caucasian infants at sea level: Standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. *J Pediatr* 74:901-10.
19. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-23.
20. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.
21. **Vogels T, Theunissen NCM, Verrips GHW, Koopman HM, Verloove-Vanhorick SP, Kamphuis RP** 1996 Het meten van kwaliteit van leven bij kinderen met chronische aandoeningen. *Tijdschrift voor adolescenten zorg* 2:104-111
22. **Cronbach L** 1951 Coefficient Alpha and the internal structure of tests. *Psychometrika* 16:297-334
23. **Verrips EGH, Vogels TGC, Koopman HM, Theunissen NC, Kamphuis RP, Fekkes M, Wit JM, Verloove-Vanhorick SP** 1999 Measuring health-related quality of life in a child population. *European Journal of Public Health* 9:188-193
24. **Bland JM, Altman DG** 1997 Cronbach's alpha. *Bmj* 314:572.
25. **Carel JC, Chatelain P, Rochiccioli P, Chaussain JL** 2003 Improvement in adult height after growth hormone treatment in adolescents with short stature born small for gestational age: results of a randomized controlled study. *J Clin Endocrinol Metab* 88:1587-93.
26. **Boonstra V, Arends N, Stijnen T, Blum W, Akkerman O, Hokken-Koelega A** 2005 Food intake of children with short stature born small for gestational age (SGA) before and during a randomised GH-trial. Accepted for publication in *Hormone Research*
27. **Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P** 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3-year treatment period and after 1 year's withdrawal. *J Clin Endocrinol Metab* 83:3512-6.
28. **van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 2003 Effect of discontinuation of growth hormone treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. *J Clin Endocrinol Metab* 88:347-53.
29. **Theunissen NC, Vogels TG, Koopman HM, Verrips GH, Zwinderman KA, Verloove-Vanhorick SP, Wit JM** 1998 The proxy problem: child report versus parent report in health-related quality of life research. *Qual Life Res* 7:387-97.
30. **Achenbach TM, McConaughy SH, Howell CT** 1987 Child/adolescent behavioral and emotional problems: implications of cross-informant correlations for situational specificity. *Psychol Bull* 101:213-32.
31. **Koopman HM, Theunissen NC, Vogels T, Zwinderman KA, Verloove-Vanhoick SP, Wit JM** 1999 Health related quality of life of children with a chronic illness: Parent versus child report. *Gedrag & Gezondheid* 27:118-125

32. **Stabler B, Siegel PT, Clopper RR, Stoppani CE, Compton PG, Underwood LE** 1999 Reply. *J Pediatr* 135:134.
33. **Sandberg DE, Brook AE, Campos SP** 1994 Short stature: a psychosocial burden requiring growth hormone therapy? *Pediatrics* 94:832-40.
34. **Busschbach JJ, Rikken B, Grobbee DE, De Charro FT, Wit JM** 1998 Quality of life in short adults. *Horm Res* 49:32-8.

Chapter 8

General Discussion

General Discussion

This thesis describes several aspects of a large cohort of short children born small for gestational age (SGA). Several epidemiological studies have shown an association between low birth weight and hypertension, diabetes mellitus type II, dyslipidemia and cardiovascular disease at a relatively young age in adulthood.[1] It has been suggested that when changes in the fetal programming occur during critical phases of fetal development this might affect intra-uterine growth and the endocrine axes.[2-5] As a result, this may lead to permanent changes in organ structure and physiology and may subsequently result into several hormonal disturbances during later life.

We evaluated the consequences of being born SGA on adrenarche, pubarche, ovaries and testes and the effects of GH treatment on these consequences. Furthermore, the effect of GH treatment on pubertal development, food intake and quality of life in short children born SGA was studied.

In this chapter we discuss our findings in view of the current literature.

Adrenal glands

Do short children born SGA have a disturbed adrenarche?

SGA, which might be the result of reduced fetal growth, might have altered the function and hormonal production of the adrenal glands. The adrenal glands are responsible for the adrenarche, the rise of serum androgens, which starts a few years before the onset of puberty. During the adrenarche the adrenal glands will, among other androgens, produce dehydroepiandrosterone-sulphate (DHEAS).

We investigated whether children born SGA have a disturbed adrenarche by measuring serum DHEAS levels in a large group of 181 short prepubertal, 3–9 years old children born SGA compared to 171 age-matched controls who were born appropriate for gestational age (AGA). Our data demonstrated that short prepubertal children born SGA have normal serum DHEAS levels. Previously, other studies reported higher serum DHEAS levels in individuals born SGA.[3,6-9] However, most of these studies were much smaller and used different study groups and definitions. To our knowledge our study is the first one investigating serum DHEAS levels in a large group of prepubertal short SGA children compared to age-matched normal statured children born AGA. Recently, one study showed small but significantly lower serum DHEAS levels in 40 SGA children compared to 35 short statured AGA children. However, this was a different group compared to ours as all these SGA children had a birth length < 10^{th} percentile and a normal stature.[10] We found that serum DHEAS levels correlated positively with age, as has been described for healthy children.[11] We also found a weak correlation between serum DHEAS levels and birth weight and birth length, but this correlation disappeared

after correction for age. A Swedish study did also find a weak but significant correlation between serum DHEAS levels and birth weight and birth length but they did not correct for age at investigation, while they also found a significant correlation between serum DHEAS levels and age.[12]

Since many short SGA children are nowadays treated with growth hormone (GH), we also investigated if one year of GH treatment had an effect on serum DHEAS levels. We showed that one year of GH treatment did not influence serum DHEAS levels in SGA children.

In addition, we investigated if serum DHEAS levels might be held responsible for the acceleration of bone maturation found in SGA children. Several studies showed that children born SGA have a delay in bone maturation until the age of 6–8 years after which an acceleration of bone maturation occurs more frequently than in other children.[13,14] However, we did not find such an acceleration of bone maturation in untreated SGA children before the age of 9 years (end of the study) and we also did not find higher serum DHEAS levels in SGA children compared to AGA children before the age of 9 years.

During one year of GH treatment the progression of bone maturation was significantly higher in the group receiving 2 mg GH/m^2/day. De Zegher *et al* also found that GH treatment induced a dose dependent acceleration of bone maturation during the first 2 years.[15] Recently, Arends *et al* showed that the acceleration of bone maturation was strongly associated with the magnitude of the catch-up growth during the first 2 years of GH treatment.[16] In addition, van Pareren *et al* showed that there was no GH-dose effect on bone maturation anymore after 5 years of GH treatment.[17]

Our results show that children born SGA do not have a disturbed adrenarche. In addition, GH treatment does not affect serum DHEAS levels.

Do short children born SGA have an increased risk of premature pubarche?

We investigated the incidence of premature pubarche in short SGA children. Since serum DHEAS levels in our SGA group proved to be normal, it is not surprising that the incidence of premature pubarche in children born SGA was also comparable with the normal population. This is in contrast to some retrospective studies reporting a reduced birth weight in girls with a premature pubarche.[18,19] However, these studies have been performed in relatively small patient groups in a specific part of Spain. Their patients presented with premature pubarche and the association with low birth weight was retrospectively found. We feel that prospective evaluation of patients who were selected by their low birth length and birth weight is more appropriate for studying the consequences of a small size at birth on the incidence of premature pubarche. Recently, Neville *et al* published in a retrospective study that the combination of being born SGA and having overweight is associated with higher serum DHEAS levels and premature

pubarche.[20] This was supported by other studies.[21] We did not find a correlation between serum DHEAS levels and BMI SDS. The reason that we did not find such a correlation might be that almost all our short SGA children were lean with only a small variation in BMI SDS.

Our study shows that short children born SGA do not have an increased incidence of premature pubarche.

Ovaries

Do short prepubertal girls born SGA have a reduced size of their ovarian follicle pool?

The most dynamic phase of ovarian development occurs before birth.[22,23] Human follicle development starts in the twelfth week of intra-uterine life and by the fifth month the maximum size of the ovarian follicle pool is reached. During fetal life and childhood, follicles develop through primordial and pre-antral stages, to small antral follicles.[22,24] As a result of reduced fetal growth, girls born SGA might have a reduced size of their ovarian follicle pool. This might result in earlier follicle pool depletion and would increase the risk for infertility and premature ovarian failure. For this reason, we studied if short girls born SGA had a reduced size of their ovarian follicle pool by measuring serum antiműllerian hormone (AMH) levels. AMH is produced exclusively in preantral and small antral follicles and the number of these follicles correlates well with the size of the ovarian follicle pool.[25,26] Our data showed that there is no significant difference in serum AMH levels between prepubertal age-matched untreated short SGA and AGA girls. This indicates that prepubertal short SGA girls have a normal size of their ovarian follicle pool. A recent study of de Bruin *et al* also showed that fetal growth restriction was not associated with a disturbed ovarian development in stillborn fetuses.[23] These findings were in contrast to those of a previous study of de Bruin *et al* showing that prenatal growth restriction was associated with a reduced ovarian percentage of primordial follicles in growth-restricted stillborn fetuses.[27] The authors explained their contrasting findings by the larger number of fetuses and an improved methodology in their most recent study.[23]

Since many short children born SGA are nowadays treated with GH we also investigated the effect of GH on serum AMH levels. We found no difference in serum AMH levels between GH-treated SGA girls and untreated SGA girls.

Prepubertal short SGA girls have normal serum AMH levels, indicating a normal follicle pool size.

Do adolescent SGA girls have a reduced size of their ovarian follicle pool?

In addition, we compared serum AMH levels of adolescent SGA girls, aged 15.3 (14.3–15.9)

years, with those of age-matched AGA girls. The AMH levels appeared to be comparable for SGA and AGA adolescent girls, indicating a comparable number of pre-antral and small antral follicles in both groups. Some studies, however, concluded that girls born SGA have a reduced ovarian follicle pool. This was based on the finding that adolescent girls born SGA had ovarian hyporesponsiveness to follicle stimulating hormone, a reduced size of the uterus and ovaries and oligo-ovulation or anovulation.[28,29] However, the girls in that study were around the age of 14 years and the ovulation frequency was based on a period of 3 months. At this age it is unlikely that girls have regular ovulatory cycles since this may take years to become established.[22] We therefore feel that at this age you cannot draw conclusions about gonadal function in these girls. In our study we investigated serum AMH levels. This hormone is independent of the ovulation cycle since it is produced by preantral- and small antral- follicles, which are independent of gonadotrophin secretion.[30-34] In addition, Ibanez *et al* investigated a different population since their group was a mixture of SGA girls with catch-up and or without catch-up growth. In our study, we investigated the SGA girls without a spontaneous catch-up growth. We feel that it is important to make a distinction between catch up growth and non-catch up growth, since these 2 groups might be different in respect to physiology and development of organs.[35-37]

Ibanez *et al* concluded that girls born SGA are at increased risk for developing Polycystic Ovarian Syndrome (PCOS), especially girls who had shown a premature pubarche.[18,19,38,39] PCOS is characterized by increased serum androgen levels and a large number of small antral follicles. It might be that the increased levels of androgens are responsible for the increase of small follicles since androgens arrest folliculogenesis. It is not surprising that previous studies have shown that women with PCOS have significantly higher serum AMH levels compared to normal controls due to the large number of small antral follicles characterizing PCOS.[40-42] Our data show that adolescent SGA girls have normal serum AMH levels indicating a normal number of pre-antral and small antral follicles. In our patients we also did not find an increased incidence of premature pubarche.[43] This indicates that short adolescent SGA girls in general do not have signs of PCOS. However, it might be that only SGA girls with premature pubarche have an increased risk for having PCOS, as reported by Ibanez. Recently, Neville *et al* concluded from a retrospective study that premature pubarche was associated with SGA and overweight.[20] A possible explanation for this finding might be that increasing weight is associated with decreased insulin sensitivity resulting in higher androgen production and as a result in a premature pubarche.[44] In practice, short SGA children are lean with a low BMI, as the ones in our study. This might explain why we did not find an increased incidence of premature pubarche and signs of PCOS.

Our data show that short adolescent SGA girls do not have a reduced size of their ovarian follicle pool.

Testes

Do short prepubertal boys born SGA have a reduced number of Sertoli cells?

Boys born small for gestational age (SGA) might have an impaired testicular development and consequently a reduced number of Sertoli cells. In the testes, Sertoli cells play an important role in the paracrine control of spermatogenesis. Animal studies have demonstrated that the size of the Sertoli cell population in early life is important for the testicular size and sperm characteristics in adult life.[45-48] Each Sertoli cell can harbour only a limited number of germ cells in different stages of spermatogenesis. As a result the size of the Sertoli cell population determines the number of germ cells in adults. Inhibin B and antiműllerian hormone (AMH) are produced by the Sertoli cells and for this reason their serum levels are important markers of Sertoli cell function in childhood and adulthood.[48-51] Several studies have indicated that prepubertal inhibin B level is a good marker of the number of Sertoli cells.[52-54] Our study showed that serum inhibin B levels of 73 prepubertal short SGA boys, aged 3.0–9.0 years, were not significantly different compared to the levels of age-matched boys born AGA. Serum AMH levels were even higher in SGA boys. This indicated that the number of Sertoli cells in prepubertal SGA boys is not reduced. To our knowledge the present study is the first prospective evaluation of testes function by measuring serum inhibin B and AMH levels in prepubertal SGA boys aged 3 to 9 years. Another study compared serum FSH and inhibin B levels in 13 SGA boys at the age of 4 months with levels of 7 age-matched AGA boys and found significantly higher serum FSH levels and normal inhibin B levels in SGA boys.[55] This young age is characterized by a high level of activity of the pituitary-gonadal axis. The authors hypothesized that the testes of SGA infants need an augmented FSH drive to fulfil the inhibin B requirements because poor fetal growth condition had resulted in a reduced number of Sertoli cells within the gonads. Their data are not comparable with ours because they studied a much younger group including infants with and without postnatal catch-up growth.

Since many short children born SGA are treated with GH, we also analysed if two years of GH treatment had an effect on serum inhibin B and AMH levels of prepubertal short SGA children. After adjustment for age, we found no differences between serum inhibin B and AMH levels before and after 2 years of GH treatment in 25 short SGA boys. Also, serum inhibin B and AMH levels in the GH-treated SGA boys were not significantly different compared to levels in AGA boys. Studies on the effect of GH therapy on testicular development and function are very limited. Hull *et al* reported in a review article that GH is likely to modulate testicular steroidogenesis by IGF-I-independent and possibly by IGF-I dependent mechanism, at least in rats. In addition GH-receptors were found in the male reproductive tract.[56] One study, investigating the effect of GH treatment in boys with idiopathic short stature concluded that GH treatment does not alter testicular growth.[57]

In conclusion, our study shows that short prepubertal SGA boys have normal serum inhibin B levels compared to those of age-matched boys born AGA. Serum AMH levels were higher in SGA boys. In addition, serum inhibin B and AMH levels were similar in GH-treated SGA boys and age-matched AGA boys.

Do young men born SGA have an impaired testicular function?

Just before the physical signs of puberty the hypothalamic-pituitary-testis (HPT) axis will be activated. Hypothalamic gonadotrophin-releasing hormone (GnRH) stimulates the pituitary secretion of FSH and LH in a pulsatile pattern. LH stimulates Leydig cells to produce testosterone. FSH and testosterone directly and LH indirectly stimulate Sertoli cells to produce inhibin B, which in turn, regulate germ cell development. GnRH is under negative feedback control by testosterone, and inhibin B causes selective inhibition of FSH production.[48] Whereas serum inhibin B levels in prepubertal boys do not depend on the presence of germinal cells, serum inhibin B levels in postpubertal boys and adult men are closely related to the presence of germ cells from the stage of spermatocytes onwards.[58]

We found lower serum AMH levels in young SGA and AGA men compared to prepubertal boys. Before puberty serum AMH is a specific marker of immature Sertoli cell numbers. The low serum AMH levels at puberty reflect the end-stage maturation of Sertoli cells.[59] Intratesticular testosterone is the major regulator of AMH levels. In early puberty, increasing serum testosterone levels correlate with the decline in serum AMH levels.[60]

In our study we found comparable serum inhibin B levels in young SGA men and normal statured age-matched AGA men, indicating that the number of Sertoli cells in adolescent SGA boys is not reduced due to small size at birth. We also found that serum testosterone and LH levels in GH-treated and untreated SGA men were comparable to those of the AGA group. All three groups had normal serum FSH levels. Surprisingly we found that young SGA men had unexplained significantly higher serum AMH levels compared to men born AGA. However, since serum inhibin B levels were comparable and serum AMH levels were not lower, we can conclude that young men born SGA do not have a reduced number of Sertoli cells.

Since serum LH and testosterone levels have a weak or absent correlation with other markers of spermatogenesis these hormones are of limited value in the assessment of spermatogenesis. Elevated serum FSH levels are seen in abnormal Sertoli cell function and/or spermatogenesis because of a lower negative feedback by inhibin B.[48] To assess Sertoli cell function, however, inhibin B has a higher accuracy than FSH because it is a direct product of the Sertoli cell, whereas FSH secretion is not only determined by negative feedback, but also by GnRH, androgens and oestradiol. Cicognani *et al* found that 25 young SGA men had higher serum LH levels and lower serum testosterone levels

compared to age-matched men with short stature born AGA.[61] They concluded that there was a tendency to hypogonadism in SGA boys. However, 5 out of 25 men had undergone orchidopexy in the SGA group in contrast to the control group. It is known that boys with cryptorchidism are at risk of having decreased Leydig cell function.[62] In addition, they separately analysed SGA boys with a mean testicular volume < 2 SD, showing significantly lower serum inhibin B levels and higher serum FSH levels compared to controls. SGA boys with a normal testis size showed normal inhibin B levels compared to controls. For the control group they did not make a distinction between subjects with different sizes of the testes.

The boys in our study all had normal testis size, and no cryptorchidism or hypospadia. Some retrospective studies have suggested that boys with small size at birth are at increased risk of having such problems.[62-64] Skakkebaek reported that poor semen quality, testicular cancer, undescended testes and hypospadia are symptoms of one underlying entity, the testicular dysgenesis syndrome, which may be increasingly common due to disruption of embryonal programming and gonadal development during fetal life.[65] Our study was not designed to evaluate the incidence of cryptorchidism or hypospadia in SGA boys as we studied short SGA individuals without cryptorchidism or hypospadia.

Young men born SGA, with and without short stature, have normal serum inhibin B, LH, FSH and testosterone levels compared to AGA young men. Serum AMH levels are significantly higher in young men born SGA. Our study indicates that small size at birth, which might reflect fetal growth restriction, does not diminish the number of Sertoli cells and has no effect on testicular function, in men born SGA.

Puberty

Some studies showed that puberty started at a normal age in untreated short SGA children, but relatively early for their short stature.[66-68] We evaluated whether GH treatment had an effect on the pubertal development of children born SGA in comparison with Dutch reference data of the Fourth National Growth Study.[69] We evaluated the age and height at onset of puberty, age at menarche, interval between breast development and menarche, duration of puberty and pubertal height gain. For this study we included the SGA children participating in the first Dutch SGA GH-trial, since all these children had started puberty. They were treated with either 1 or 2 mg GH m^2/day before the onset of puberty. Both GH-dosage groups had a similar duration of GH treatment prior to the onset of puberty.

Does the age at onset of puberty differ between GH-treated children born SGA and normal statured AGA children?

Our study did not show any GH-dose effect on the age at onset of puberty in SGA children. Also, GH-treated SGA children did not start puberty at a younger age compared to normal statured Dutch children born AGA.[69] These findings were supported by data of regression analyses, showing that the longer the duration of GH treatment the later the onset of puberty. GH-treated SGA boys started their puberty one year later compared to GH-treated SGA girls, which is comparable with the normal population.[69] We found that SGA boys receiving 2 mg/m^2/day started puberty one year earlier than Swedish untreated SGA boys.[70] However, in this Swedish study the definition of onset of puberty was different from our definitions. They defined onset of puberty in boys and girls as the moment at which the growth velocity starts to be more than 6 cm a year. We defined the onset of puberty in boys as a testis volume of 4 ml, which is known to precede the pubertal growth velocity by approximately 1 year in boys. In girls, we defined the onset of puberty as a breast development stage 2 according to Tanner.[71] We found the same age for onset of puberty in girls, which is not surprising since the pubertal growth spurt follows soon after the start of breast development.

Height at onset of puberty was not significantly different in both GH-dosage groups, but the children were significantly shorter at pubertal onset than Dutch normal statured AGA children. This means that puberty in SGA children starts too early with respect to a relatively short height.

Age at onset of puberty of GH-treated children born SGA is comparable with normal statured AGA children, regardless of treatment with 1 mg or 2 mg GH/m^2/day.

Does the age at menarche and the progression of puberty differ between GH-treated SGA girls and normal statured AGA girls?

Our study shows that the age at menarche and the interval between M2 and menarche, which is an indicator for the progression of puberty in girls, were not significantly different between both GH-dosage groups and were comparable with Dutch AGA controls and Swedish untreated SGA children.[70] This suggests that GH treatment has no influence on the progression of puberty in girls. An older age, higher BMI and smaller bone-age delay, however, resulted in a shorter interval between M2 and menarche. This is comparable with the development of normal statured AGA girls.[72-75] In the normal population it has been shown that overweight children mature earlier than non-overweight children.[76] An explanation why BMI in our study group had no influence on the age at onset of puberty but only on the age at menarche and progression of puberty might be that, before puberty, all our SGA children were very lean with only a narrow variation in the BMI (SDS).

Age at menarche and progression of puberty in girls are comparable with the normal population, regardless of treatment with 1 mg or 2 mg GH/m^2/day.

Does the duration of puberty and the pubertal height gain differ between the GH dosage groups?

We defined the pubertal height gain and the duration of puberty as the adult height minus height at onset of puberty (cm) and the time from onset of puberty until adult height, respectively. Adult height was defined as the condition when height velocity (HV) had dropped below 0.5 cm during the previous 6 months and the bone age was ≥ 15 years for girls and ≥ 16.5 years for boys. We did not find a significant difference in duration of puberty and pubertal height gain between the GH-dosage groups. A greater bone age delay at start of puberty resulted in a longer duration of puberty and a greater pubertal height gain, as has been reported for other conditions.[77,78] As in the normal population, the pubertal height gain was less when children were older or taller at onset of puberty.[74,79] We could not compare the duration of puberty and pubertal height gain with the Dutch reference data since different definitions for the endpoint of puberty were used. A French longitudinal study using comparable pubertal milestones and adult height criteria as we did, reported a similar pubertal height gain in untreated short SGA children compared to the SGA children in our study receiving 1 or 2 mg GH/m^2/day. As our previously published 5-year data have shown, most of our SGA children had their GH-induced catch-up growth during the first two years.[80] After 4 years of GH treatment the mean height was within the target range for both GH-dosage groups. For that reason it is not surprising that as both groups entered puberty after at least 4 years of GH treatment, children growing within their target range did not further increase their height SDS during puberty. However, it seems advisable to continue GH treatment till final height since it has been described that discontinuation of GH leads to catch-down growth in many SGA children.[81,82] Recently, Carel *et al* demonstrated that GH treatment of short adolescent SGA children when started at a mean (SD) age of 12.7 (1.4) years will increase mean height with 0.6 SD, bringing half of these short adolescents into the normal adult height range.[83] In contrast, the control group did not show any catch-up growth during puberty. Starting GH treatment at a younger age will result in a higher catch-up growth since the catch-up growth is negatively related to age at start of GH treatment.[17,84]

The duration of puberty and the pubertal height gain were not significantly different between the GH dosage groups.

Food intake and body composition

Our study investigated the food intake of a large group of short SGA children before and during a randomised GH trial, in combination with body composition, and serum levels of growth factors and leptin.

Do short children born SGA have a low food intake?

Parents of short SGA children often report that their child has a serious lack of appetite and a low food intake. Measuring food intake is difficult and often not very precise. We therefore used standardised 7-day food questionnaires and gave all parents thorough oral and written instructions how to record food intake and use the questionnaires. The intake was compared with the recommended daily intake (RDI) of Dutch children with the same age. Before GH treatment we found that the caloric, fat and carbohydrate intake in short SGA children was significantly lower compared to the RDI intake of age-matched Dutch children. There was no significant difference in protein intake between the SGA children and the RDI of children with the same age. The exact mechanism of the lower food intake in these children is not known. Several studies have been performed to explain feeding problems in children in general. Factors related to decreased food intake of children may be behavioural, organic or a mixture of both.[85,86] Behavioural problems that affect food intake might have their roots in conditions that may have enhanced the gag reflex (such as prolonged period of orotracheal intubation or a nasogastric tube), failure to establish links between hunger and food intake and satiety in infants who had not been fed orally for a relatively prolonged period of time at a critical age, cultural expectations of food intake and body habits and parental anxiety about weight resulting in power struggles between parent and child that manifest in disturbed eating habits, whereas anxiety or depression of the child might also play a role. Organic causes leading to decreased food intake include swallowing problems, respiratory distress, excessive fatigability and lack of appetite due to various systemic illnesses. Blissett *et al* also concluded that in families where a child has a growth disorder, parent-child interactions at mealtimes are often negative and feeding problems are often exacerbated by parental mismanagement of feedings problems, which occurs as a result of anxiety about feeding.[87] We feel that several factors might contribute to the feeding problems in short children born SGA. One factor might be that parents are concerned about the growth and weight of their child. Because they want them to grow better they will force their child to eat. This might result in a negative parent-child interaction and can cause food aversion by the child. One study reported on the food intake in children with Silver-Rusell Syndrome (SRS).[88] From this study it was concluded that children with SRS experienced significantly more feedings problems compared to children without growth disorders. The most important problems were poor appetite, fussiness, slow feeding

and problems associated with oral-motor dysfunction They also found that mealtime interaction between these children and their parents was significantly more negative compared to the control group. The authors concluded that intervention should focus on reduction of the negative parent-child interactions and parental anxiety about feeding, growth, and weight. In addition, part of the SGA children has had a nasogastric tube for a prolonged period. It is known that these children are at an increased risk for developing feeding problems.[85] Another factor might be that short SGA children have a lower metabolic rate since they have a low growth rate.

Short prepubertal children born SGA have a lower caloric, fat and carbohydrate intake compared to age-matched controls.

Does GH treatment have effect on food intake in short SGA children?

We found that during GH treatment caloric, fat, protein and carbohydrate intake increased significantly compared to baseline in contrast to the randomised control group. The last group did not show any difference in food intake after 1 year compared to baseline. We also found that the 1 year change in food intake between the two groups was significantly higher in the GH group, except for fat intake. Our findings agree with the subjective findings expressed by the parents in our first SGA study.[80] They reported a lack of appetite before the start of GH treatment and an improved appetite and food intake during treatment. GH treatment might increase food intake as a result of several changes in body composition and resting expenditure, such as an increase in basal metabolic rate, an increase in growth, fat free mass and reduction in body fat. Stenlöf *et al* reported that GH increases energy expenditure directly.[89,90] This was only detectable during night and morning hours, when levels of GH were relatively high. Lower GH levels might be one of the explanations for the low food intake in short SGA children since 25–60% of these children have low GH and IGF-I levels and as a result might have lower energy expenditure.[91] The improved growth itself during GH treatment may result into a more relaxed parent-child interaction at mealtimes, leading to a better food intake. Blisset *et al* investigated the effect of GH treatment on food intake in a cross-sectional study in 23 children with Turner syndrome (TS) or Silver Rusell syndrome (SRS), aged 2–11 years, compared to 23 age matched untreated children with TS or SRS. They found a higher food intake in GH-treated children and a better parent child interaction at mealtimes.[87]

GH treatment results in a significant increase in food intake compared to baseline levels and compared to randomised untreated controls born SGA.

The effect of GH treatment on body composition, IGF-I, IGFBP-3 and leptin in combination with food intake

We measured body composition with 3 different methods: Dual Energy X-ray Absorptiometry (DEXA) (Fat Mass (FM) and Lean Body Mass (LBM)), sum of 4 skinfolds

(SF) and Body Mass Index (BMI). In our study we found that short children born SGA have a reduction in FM, LBM, SF and BMI compared to children of the same age and sex. This is in agreement with other studies.[16,92] The observed increase in LBM and the reduction in total body fat during GH treatment are expected and well documented findings.[93-95] GH causes the body to utilize fat stores for the development of muscles. We found that a higher caloric intake and carbohydrate intake were related to higher LBM SDS and BMI SDS but not to a higher fat mass SDS. It seems that these food components are used as source of energy for increasing LBM and that the increase in food intake does not result in an increase of fat mass. This is a positive finding since some studies reported that adipose children born SGA are at increased risk for the metabolic syndrome.[96,97] None of the food components had an influence on fat mass, SF SDS or height SDS.

Some studies have suggested that low dietary intake results in lower serum IGF-I and IGFBP-3 levels.[98] We previously found that 25−60% of the SGA children have low serum GH and IGF-I and IGFBP-3 levels.[80,91,99] However, at baseline we did not find a correlation between food intake and serum IGF-I levels or IGFBP-3 levels. During GH treatment we found a very weak correlation between the change in caloric intake and the increase in IGF-I levels.

We found significantly lower serum leptin levels in the short children born SGA compared to the normal population. This is in agreement with other studies.[100-102] Leptin is produced by adipocytes and was first described in 1994. It has an important role in the control of food intake and energy expenditure.[103] Leptin reduces food intake whereas it increases energy expenditure.[104,105] We found positive correlations between leptin SDS and BMI SDS, SF SDS and fat mass SDS. Also other studies showed positive correlations between serum leptin levels and BMI and percentage body fat measured by bioelectric impedance measurements (BIA) or by dual energy X-ray absorptiometry (DEXA) in healthy adults and children.[106-108] Low serum leptin levels will stimulate appetite and dietary intake. Surprisingly, the short children born SGA had low dietary intake in spite of their low serum leptin levels. An explanation for this might be that the low serum leptin levels in these children are not interpreted by the leptin receptors as being too low but as being normal because they have another set point. As a result dietary intake is not stimulated but is kept on a low level. Possibly this lower set point may be a result of a different programming during fetal life. Further research should explore if such a lower set point exists and if this is due to a different programming during prenatal life.

GH treatment resulted in a significant decrease of leptin levels. This was also found in a study of Boguszewski *et al.*[102] GH increases basal lipolysis and increases the activity of hormone-sensitive lipase resulting in a rapid decrease of the adipose tissue and an increase in muscle tissue. It seems therefore likely that the reduction in leptin levels is attributable to the reduction in adipose tissue mass that occurs during GH

treatment. It might be that the leptin receptors in the hypothalamic region now respond to the further lowering of serum leptin levels causing a significant increase in dietary intake. On the other hand, in our GH-treated SGA children we did not find a correlation between the reduction of serum leptin levels and the increase of caloric intake.

Short SGA children have reduced LBM, fat mass, BMI, SF and serum levels of IGF-I, IGFBP-3 and leptin compared to normal children. GH treatment results in a significant increase of not only height but also of LBM, BMI, IGF-I, IGFBP-3 levels and a significant reduction of SF and leptin. Caloric and carbohydrate intake were positively correlated to LBM and BMI.

Quality of life

Does GH treatment improve quality of life in short children born SGA?

Our study is the first randomized controlled study reporting the effects of GH treatment versus no treatment on health related quality of life (HRQOL) of short children born SGA by using a specific questionnaire for short children. Most studies investigating psychosocial problems concentrate on problems and limitations in functioning due to a health problem, also called the health status (HS). More important, however, is the evaluation of the emotional impact of the problem on the person's life, called the health related quality of life (HRQOL).[109,110] We evaluated the effect of GH treatment on HS and HRQOL in randomly divided GH-treated short children born SGA (n = 58) compared to untreated children born SGA (n = 27). At start of the study the children were 5–7 years of age. We used two different questionnaires: the TACQOL, a generic QOL questionnaire applicable to children with various diseases,[111] and the TACQOL-Short Stature, a specific QOL questionnaire for children with short stature. The parents filled out the questionnaires before start and after 3 years with or without GH treatment. Three years of GH treatment resulted in a normalisation of height whereas the untreated group remained short. Before the start of GH treatment the generic TACQOL showed no significant difference in HS and HRQOL between the GH group and untreated group. After three years the generic TACQOL showed that only the physical functioning was significantly improved in the GH group. In contrast, the TACQOL-Short Stature showed that SGA children treated with GH for 3 years achieved a significantly better HS and HRQOL with respect to their physical abilities, vitality, contact with peers, contact with adults, self image and future prospects, compared to untreated SGA children. The discrepancy between the outcome of the two QOL questionnaires shows that SGA children do not have the problems addressed in a generic questionnaire but have more specific problems related to short stature. The questions in the generic TACQOL are mainly related to problems due to chronic illness. Recently, Hull *et al* mentioned in a review article that most studies have been based on small sample sizes and on general

health questionnaires which has been criticized for their lack of sensitivity on areas important for short stature.[112] Several studies evaluating psychosocial problems in children with short stature applied child behaviour questionnaires which originate from studies in children with a chronic illness. Since these questionnaires were not developed for short children, there is a lack of sensitivity for short stature related topics.[113,114] Actually, in case of short stature they ask the wrong questions.

Interestingly, the physical functioning scale of the generic TACQOL and the vitality scale of the TACQOL-Short Stature contained both questions that were focused on energy. In the generic TACQOL this scale was the only one which was significantly higher in the GH group compared to the untreated group. It is known that GH treatment improves exercise performance.[115]

The TACQOL-Short Stature showed positive correlations between height SDS and HRQOL regarding physical abilities, contact with adults, body image and future prospects, indicating that height SDS is important for many aspects of the quality of life of SGA children.

In our study we made a distinction between the problems of being short and how the child feels about these problems, the HRQOL score. In both the GH-treated and control group, the HRQOL scores were higher than the HS scores in all scales. This indicates that health related problems do not necessarily result in a similar reduction of the HRQOL for all children.[116-118]

Some studies have compared the quality of life of GH-treated children who were referred to a hospital, with untreated children who were never referred to hospital and concluded that the children who were not referred had no problems.[119,120] However, the fact that some short children are not referred to a hospital does not diminish the seriousness of the problems of those who are referred.[116,121] The severity of the emotional and social consequences of short stature may vary from one child to another. We evaluated the effect of GH treatment on quality of life in a randomised study in referred SGA children, comparing GH-treated children with untreated short SGA children.

Short children born SGA have short stature specific problems and for this reason a specific questionnaire is more sensitive for measuring quality of life in short children than a generic questionnaire. GH treatment improves several aspects of quality of life in SGA children with short stature compared to randomised controls.

Conclusions

One of the aspects we evaluated in our study was the effect of being born SGA on the adrenarche, pubarche, ovaries and testes in short SGA children. Our study showed that small size at birth has no influence on adrenarche. Short children born SGA also did

not have an increased risk of premature pubarche. These findings indicate a normal development and function of the zona reticularis of the adrenal cortex. Our data also showed that small size at birth has no effect on the size of the ovarian follicle pool, indicating that short SGA girls do not have an increased risk for earlier follicle depletion. In addition, our data showed that short SGA boys have a normal number of Sertoli cells and that young SGA men have normal testicular function. In conclusion, small size at birth, which might be a result of fetal growth retardation, does not lead to a permanent change and a disturbed structure and physiology of the zona reticularis of the adrenal cortex, ovaries and testes. No effect of GH treatment was found on the adrenarche, size of the ovarian follicle pool and number and function of Sertoli cells.

We also evaluated the effect of GH treatment on puberty, food intake and quality of life in SGA children. Our study showed that long-term GH treatment in short SGA children has no influence on the age at onset of puberty and progression of puberty compared to normal statured AGA children. There was no GH dose effect on pubertal height gain and duration of puberty. We also found that short SGA children have a low food intake compared to peers. GH treatment resulted in a significant increase of food intake compared to randomised controls. Three years of GH treatment in children born SGA improved several aspects of health status (HS) and health related quality of life which appeared to be specific for children with a short stature.

Considerations and directions for future research

Previous studies, investigating the consequences of being born SGA, showed different and contradictory conclusions and hypotheses. A possible explanation for these discrepancies might be the methodological differences between the studies, such as different definitions for SGA. Studies investigating the consequences of being born SGA, should use the currently accepted definitions for SGA.[122] This would allow better comparisons of results and conclusions that are based on comparable populations.

In addition, it is important to realize that SGA children form a heterogeneous group with different aetiologies for SGA. For example, proportionately small neonates might have a different aetiology compared to disproportionate neonates. Barker hypothesised that undernutrition in early gestation might reset the fetal growth and development to a lower level resulting in proportionate small neonates.[123] This adaptation reduces the subsequent demand for nutrients. It has been reported that this pattern of fetal growth restriction was not associated with adult diseases, in contrast to disproportionate neonates. It might be that the moment of growth restriction during fetal life influences the development of organ physiology and structure. Arends *et al* showed that a percentage of the proportionate small neonates might have a functional variant of the IGF-I gene, located between the promoter region and exon 3 which resulted in significantly lower

serum IGF-I levels.[124] They proposed that the IGF-I gene may provide a link between low birth weight and such diseases in later life, since low IGF-I levels are associated with type 2 diabetes mellitus and cardiovascular diseases. This would be in agreement with the hypothesis of Hattersley *et al* who postulated that changes in certain genes might affect both birth weight and diseases in later life.[125,126] Recently, a homozygous deletion of the IGF-I gene have been described in a 15 year old boy and a missense mutation in a 55 year old man.[127,128] They were born SGA and showed severe pre- and postnatal growth failure, small head circumference and mental retardation. These findings indicate that IGF-I is involved in prenatal growth and that a mutated IGF-I gene might result in small size at birth, small head circumference and short stature. Several studies suggest that there might be a combined effect of genetic and environmental factors.[129,130]

Some studies have suggested that the shorter the gestational age of the SGA child the higher the risk for a disturbed development and function of several organs.[123, 131-133] Siewert-Delle *et al* showed a strong inverse correlation (r = −0.86) between gestational age and adult systolic blood pressure in low birth weight subjects. They also reported that in preterm subjects gestational age had a great impact on adult blood pressure.[127]

Several studies have shown that excessive weight gain and spontaneous catch-up growth in weight in SGA children may provide the trigger for a disturbed development and function of several organs.[20,96,134] It was shown that SGA children with catch up growth in weight had an increased risk of developing type II diabetes mellitus in later life.[97,135,136] Jaquet *et al* found an inverse correlation between insulin resistance and ponderal index at birth and a correlation between catch-up in BMI and increase of serum insulin concentrations in children born SGA.[136] They concluded that a dynamic change in adiposity from fetal to postnatal life is involved in the development of metabolic syndrome in SGA children.

Thus, various factors might have an influence on the postnatal development of SGA children, such as whether they are born as disproportionate or proportionate SGA or whether they have an IGF polymorphism, a higher ponderal index at birth, a shorter gestational age and postnatal catch-up in BMI. Future research is required to evaluate if and to what extent these various factors contribute to the organ development and function in both SGA children and adults.

In addition, another interesting question is whether preterm born AGA children with a weight or length below −2 SDS at term age, due to postnatal growth retardation until that age, are to be considered as SGA as well. Before this can be answered, additional studies should performed to investigate if the short and long-term consequences of fetal growth retardation are comparable with postnatal growth retardation until term age.

It has been suggested that young women born SGA have an increased risk to develop a premature menopause.[28,29,137] Our data, however, do not support this, as we found that short SGA girls and adolescent SGA girls have normal serum AMH levels, indicating a normal size of their ovarian follicle pool.

Some studies reported that SGA girls might develop oligo-anovulation and poly cystic ovarian syndrome (PCOS).[18,19,38,39,137] PCOS is characterised by hyperandrogenism, an increase in antral follicle number and reduced insulin sensitivity. Our study showed that prepubertal short SGA girls had normal serum DHEAS and AMH levels. AMH levels were also normal in adolescent short SGA girls, indicating that they do not have an increased number of antral follicles. In order to evaluate whether SGA women above the age of 17 years have increased risk of anovulation or PCOS, long-term follow up is required. It might also be interesting to investigate the risk in SGA girls with a spontaneous catch up growth who have attained a normal adult height.

Our study showed that short SGA boys and young men have a normal number of Sertoli cells. In our study only SGA and AGA boys with a normal testis size, without hypospadia and without cryptorchidism, were included. In our study group these abnormalities were rare. Some studies have shown that hypospadia and cryptorchidism are associated with fertility problems.[62] A few studies showed a relation between small size at birth and the incidence of these abnormalities.[62-64,138,139] However, these studies used different definitions of SGA and were performed retrospectively. Future prospective research is required to evaluate if SGA boys are at increased risk having hypospadia or cryptorchidism.

The exact mechanism of lower food intake and a different body composition in short SGA children compared to their peers, is not known. Short SGA children have lower leptin levels. Ghrelin, a 28-amino acid peptide, plays a role in energy balance and is closely associated with glucose metabolism and body mass. Recently, higher ghrelin levels were found in SGA neonates, especially those who had a shorter gestational age and those who showed greater infancy weight gain.[140,141] Adiponectin, one of the adipocytokines, exerts important effects on carbohydrate metabolism, improving glucose metabolism by increasing insulin sensitivity. Cianfarani *et al* found that SGA children with a mean age of 8.6 years, had lower adiponectin levels. The lowest levels were found in SGA children with a catch-up growth in weight.[142] Further studies are required to establish whether these hormones have effects on glucose and insulin metabolism, body composition and food intake in short SGA children.

Although most children reached a normal height at onset of puberty, we found that a few children still had short stature at onset of puberty, in spite of a significant improvement of their height during GH therapy. For these children it might be effective to postpone puberty with GnRH analogue treatment for 2–3 years in combination with GH treatment. Research is required to evaluate the effect of postponing puberty on adult height in GH-treated SGA children.

For studies evaluating the quality of life of short children, at least two factors are important. Firstly, specific questionnaires for short stature have to be used since these questionnaires are more sensitive to identify short-stature-related problems than

a generic questionnaire which has more reference to quality of life of children with a chronic illness. Secondly, it is important to make a difference between the practical problems related to short stature, health status (HS) and the emotional feelings to these problems, also called health related quality of life (HRQOL). For example, there are children who are very short but who do not have negative emotional feelings about their short stature.[116,117] Severity of the emotional and social consequences of short stature varies from one child to another. However, the fact that not all short SGA children have negative emotional feelings about their short stature does not diminish the seriousness of the problems for those who have problems with their stature. Sandberg suggested that psychological support in order to learn to cope with the psychosocial problems concerning short stature would be a cheaper and less invasive alternative for GH treatment.[143] One of his motivations is that GH treatment gives disappointing results. However, most of his data and thus conclusions are based on children with idiopathic short stature. Recently, van Pareren *et al* reported that GH treatment in short SGA children resulted in a height gain of 2 SD in 85% of the SGA children.[17] In addition, GH had a positive effect on blood pressure, serum lipid profiles and body composition.[144] One has to realize that very short individuals have to deal with many practical problems in society. The adaptation of daily items such as the houses, cars and furniture to fit very short individuals is very costly. Furthermore, there is no psychological program that has proven to be effective and of practical use. To assess whether a psychological training is an alternative for growth promoting therapy, a randomised trial has to be performed evaluating the long-term physical and psychosocial effects and the cost-benefit analyses of both therapies. In some cases, in which children suffer badly from being short, it might be effective to combine GH treatment with cognitive-behavioural counselling.[145]

In our study the quality of life questionnaires were filled in by the parents, since the children were too young to fill in the questionnaires by themselves. For these reasons, a future study should investigate the effects of GH treatment on the HS and HRQOL quality of life in SGA adolescents who have been treated with GH in comparison with those who did not receive GH treatment. It might also be interesting to investigate the professional achievements in GH-treated SGA adults compared to untreated SGA adults and normal statured adolescents born AGA.

Long-term effects of GH treatment

Long-term GH treatment in short SGA children results in a normalisation of height and proportionate growth of other body parts.[16,17,84,146] It also appears to have a beneficial effect on blood pressure, serum lipids profile, body composition, bone mineral density and head growth. GH treatment appeared a safe treatment since no adverse events occurred during the various studies, including the two Dutch GH-trials.[17,147,148] However, long-term follow-up is still required to evaluate the long-term effects of GH treatment.

Blood pressure and lipids need to be monitored in adulthood since epidemiological studies have shown that type 2 diabetes mellitus, hypertension and cardiovascular diseases, also called the metabolic syndrome, occur more frequently among individuals who were born with a low birth weight.[1,149] Although it has been reported that during GH treatment and a half year after discontinuation of GH treatment blood pressure and lipid atherogenic index decreased significantly, it is interesting and important to monitor if these positive effects remain during adulthood.[144,147]

GH induces relative insulin resistance with an increase in fasting insulin, whereas glucose levels remain normal. Although 6 months after discontinuation of GH, the increased serum insulin levels during GH treatment had returned to normal levels, it is important to regularly evaluate glucose metabolism during GH treatment and after discontinuation of GH treatment in adulthood.

GH treatment induces a significant and sustained rise in mean IGF-I levels, which in most children is between +1 SD and +2 SD during treatment with 1 mg GH and above +2 SD with 2 mg GH/m^2/day.[17] Further research is required to evaluate the effects of high IGF-I levels during several years of GH treatment. It is also important to investigate the serum IGF-I levels after discontinuation of GH treatment and in non GH-treated short or normal statured individuals born SGA.

We found that GH treatment had no effect on the ovarian follicle size and the Sertoli cell function. However, it is also important to investigate fertility during adulthood in GH treated SGA individuals compared to untreated controls.

Some studies indicated that short SGA children are at increased risk of overweight in adult life. We found that GH treatment resulted in an increase of food intake and muscle mass and a decrease of fat mass. It is, however, interesting to further evaluate if GH treatment even after discontinuation does maintain better body composition and thus prevents weight gain in adult life. It might be that the risk of overweight decreases when SGA children have received long term GH treatment. It is also not known if body composition changes after discontinuation of GH treatment. For these reasons it is important to have long term follow up studies in SGA individuals.

Recently, GH treatment has become a worldwide indication for short SGA children, with a birth length and or birth weight below −2.0 SD and an actual height below −2.5 SD. The development of growth prediction models is very important because such models might support making treatment decisions for individual short SGA children. Further studies should aim at optimising GH treatment by developing these advanced prediction models indicating the best treatment options for each child. These studies should include genetic, metabolic or auxological variables which will probably improve the accuracy of these prediction models and the efficacy of future treatment.

References

1. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM** 1993 Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36:62-7.
2. **Barker D** 1994 Mothers, babies and diseases in later life. British medical journal Publishing Group, London
3. **Clark PM, Hindmarsh PC, Shiell AW, Law CM, Honour JW, Barker DJ** 1996 Size at birth and adrenocortical function in childhood. *Clin Endocrinol* (Oxf) 45:721-6.
4. **Chatelain PG, Nicolino M, Claris O, Salle B, Chaussain J** 1998 Multiple hormone resistance in short children born with intrauterine growth retardation? *Horm Res* 49:20-2.
5. **Clark PM** 1998 Programming of the hypothalamo-pituitary-adrenal axis and the fetal origins of adult disease hypothesis. *Eur J Pediatr* 157 Suppl 1:S7-10.
6. **Francois I, de Zegher F** 1997 Adrenarche and fetal growth. *Pediatr Res* 41:440-2.
7. **Ibanez L, Potau N, Marcos MV, de Zegher F** 1999 Exaggerated adrenarche and hyperinsulinism in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 84:4739-41.
8. **Szathmari M, Vasarhelyi B, Tulassay T** 2001 Effect of low birth weight on adrenal steroids and carbohydrate metabolism in early adulthood. *Horm Res* 55:172-8
9. **Ghirri P, Bernardini M, Vuerich M, Cuttano AM, Coccoli L, Merusi I, Ciulli C, D'Accavio L, Bottone U, Boldrini A** 2001 Adrenarche, pubertal development, age at menarche and final height of full-term, born small for gestational age (SGA) girls. *Gynecol Endocrinol* 15:91-7.
10. **Radetti G, Renzullo L, Gottardi E, D'Addato G, Messner H** 2004 Altered thyroid and adrenal function in children born at term and preterm, small for gestational age. *J Clin Endocrinol Metab* 89:6320-4
11. **Miller WL** 1999 The molecular basis of premature adrenarche: an hypothesis. *Acta Paediatr Suppl* 88:60-6.
12. **Dahlgren J, Boguszewski M, Rosberg S, Albertsson-Wikland K** 1998 Adrenal steroid hormones in short children born small for gestational age. *Clin Endocrinol* (Oxf) 49:353-61.
13. **Job JC, Rolland A** 1986 Histoire naturelle des retards de croissance a debut intra-uterin. *Archives of french pediatrics* 43:301-306
14. **Tanner J, Lejarraga H, Cameron N** 1975 The natural history of the Silver-Russell syndrome: a longitudinal study of thirty-nine cases. *Pediatr Res* 9:611-23.
15. **de Zegher F, Butenandt O, Chatelain P, Albertsson-Wikland K, Jonsson B, Lofstrom A, Chaussain JL** 1997 Growth hormone treatment of short children born small for gestational age: reappraisal of the rate of bone maturation over 2 years and metanalysis of height gain over 4 years. *Acta Paediatr Suppl* 423:207-12.
16. **Arends NJ, Boonstra VH, Mulder PG, Odink RJ, Stokvis-Brantsma WH, Rongen-Westerlaken C, Mulder JC, Delemarre-Van de Waal H, Reeser HM, Jansen M, Waelkens JJ, Hokken-Koelega AC** 2003 GH treatment and its effect on bone mineral density, bone maturation and growth in short children born small for gestational age: 3-year results of a randomized, controlled GH trial. *Clin Endocrinol* (Oxf) 59:779-87.
17. **Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 2003 Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial. *J Clin Endocrinol Metab* 88:3584-90.

18. **Ibanez L, Potau N, Francois I, de Zegher F** 1998 Precocious pubarche, hyperinsulinism, and ovarian hyperandrogenism in girls: relation to reduced fetal growth. *J Clin Endocrinol Metab* 83:3558-62.
19. **Ibanez L, Potau N, Marcos MV, De Zegher F** 2000 Adrenal hyperandrogenism in adolescent girls with a history of low birthweight and precocious pubarche [In Process Citation]. *Clin Endocrinol* (Oxf) 53:523-7
20. **Neville KA, Walker JL** 2005 Precocious pubarche is associated with SGA, prematurity, weight gain, and obesity. *Arch Dis Child* 90:258-61
21. **Remer T, Manz F** 1999 Role of nutritional status in the regulation of adrenarche. *J Clin Endocrinol Metab* 84:3936-44.
22. **Macklon NS, Fauser BC** 1999 Aspects of ovarian follicle development throughout life. *Horm Res* 52:161-70.
23. **de Bruin JP, Nikkels PG, Bruinse HW, van Haaften M, Looman CW, te Velde ER** 2001 Morphometry of human ovaries in normal and growth-restricted fetuses. *Early Hum Dev* 60:179-92.
24. **McGee EA, Hsueh AJ** 2000 Initial and cyclic recruitment of ovarian follicles. *Endocr Rev* 21:200-14.
25. **Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J** 2003 Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 18:323-7.
26. **Seifer DB, MacLaughlin DT, Christian BP, Feng B, Shelden RM** 2002 Early follicular serum mullerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertil Steril* 77:468-71.
27. **de Bruin JP, Dorland M, Bruinse HW, Spliot W, Nikkels PG, te Velde ER** 1998 Fetal growth retardation as a cause of impaired ovarian development. *Early Hum Dev* 51:39-46
28. **Ibanez L, Potau N, Enriquez G, de Zegher F** 2000 Reduced uterine and ovarian size in adolescent girls born small for gestational age. *Pediatr Res* 47:575-7.
29. **Ibanez L, Potau N, de Zegher F** 2000 Ovarian hyporesponsiveness to follicle stimulating hormone in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 85:2624-6.
30. **Misra M, MacLaughlin DT, Donahoe PK, Lee MM** 2003 The role of Mullerian inhibiting substance in the evaluation of phenotypic female patients with mild degrees of virilization. *J Clin Endocrinol Metab* 88:787-92.
31. **Fanchin R, Taieb J, Lozano DH, Ducot B, Frydman R, Bouyer J** 2005 High reproducibility of serum anti-Mullerian hormone measurements suggests a multi-staged follicular secretion and strengthens its role in the assessment of ovarian follicular status. *Hum Reprod* 20:923-7
32. **Gruijters MJ, Visser JA, Durlinger AL, Themmen AP** 2003 Anti-Mullerian hormone and its role in ovarian function. *Mol Cell Endocrinol* 211:85-90
33. **Visser JA, Jong de FH, Laven JS, Themmen AP** 2006 Anti-mullerian hormone: a new marker for ovarian function. Reproduction in press
34. **de Vet A, Laven JSE, de Jong FH, Themmen APN, Fauser BCJM** 2002 Antimullerian hormone serum levels: a putative marker for ovarian aging. *Fertil Steril* 77:357-62.
35. **Crowther NJ, Cameron N, Trusler J, Gray IP** 1998 Association between poor glucose tolerance and rapid post natal weight gain in seven-year-old children. *Diabetologia* 41:1163-7.
36. **Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R** 2000 Serum lipid concentrations and growth characteristics in 12-year-old children born small for gestational age. *Pediatr Res* 48:623-8.

37. **Kajantie E, Fall CH, Seppala M, Koistinen R, Dunkel L, Yliharsila H, Osmond C, Andersson S, Barker DJ, Forsen T, Holt RI, Phillips DI, Eriksson J** 2003 Serum insulin-like growth factor (IGF)-I and IGF-binding protein-1 in elderly people: relationships with cardiovascular risk factors, body composition, size at birth, and childhood growth. *J Clin Endocrinol Meta*b 88:1059-65.
38. **Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, De Zegher F** 2002 Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. *J Clin Endocrinol Metab* 87:5702-5.
39. **Ibanez L, Valls C, Potau N, Marcos MV, de Zegher F** 2001 Polycystic ovary syndrome after precocious pubarche: ontogeny of the low-birthweight effect. Clin Endocrinol (Oxf) 55:667-72.
40. **Cook CL, Siow Y, Brenner AG, Fallat ME** 2002 Relationship between serum mullerian-inhibiting substance and other reproductive hormones in untreated women with polycystic ovary syndrome and normal women. *Fertil Steril* 77:141-6.
41. **Laven JS, Imani B, Eijkemans MJ, Fauser BC** 2002 New approach to polycystic ovary syndrome and other forms of anovulatory infertility. *Obstet Gynecol Surv* 57:755-67.
42. **Laven JS, Mulders AG, Visser JA, Themmen AP, De Jong FH, Fauser BC** 2004 Anti-Mullerian hormone serum concentrations in normoovulatory and anovulatory women of reproductive age. *J Clin Endocrinol Metab* 89:318-23.
43. **Boonstra V, Mulder P, de Jong FH, Hokken-Koelega ACS** 2003 Serum DHEAS Levels and Pubarche in Short Children Born Small-For-Gestational Age (SGA) Before and During Growth Hormone Treatment. *J Clin endocrinol metab* (in press)
44. **Hines GA, Smith ER, Azziz R** 2001 Influence of insulin and testosterone on adrenocortical steroidogenesis in vitro: preliminary studies. *Fertil Steril* 76:730-5
45. **Orth JM, Gunsalus GL, Lamperti AA** 1988 Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* 122:787-94.
46. **Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O, Skakkebaek NE** 1998 Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *J Clin Endocrinol Metab* 83:675-81.
47. **Chemes HE** 2001 Infancy is not a quiescent period of testicular development. *Int J Androl* 24:2-7.
48. **Pierik FH, Burdorf A, de Jong FH, Weber RF** 2003 Inhibin B: a novel marker of spermatogenesis. *Ann Med* 35:12-20
49. **Misra M, MacLaughlin DT, Donahoe PK, Lee MM** 2002 Measurement of Mullerian inhibiting substance facilitates management of boys with microphallus and cryptorchidism. *J Clin Endocrinol Metab* 87:3598-602
50. **Schmiegelow M, Lassen S, Poulsen HS, Schmiegelow K, Hertz H, Andersson AM, Skakkebaek NE, Muller J** 2001 Gonadal status in male survivors following childhood brain tumors. *J Clin Endocrinol Metab* 86:2446-52.
51. **Kubini K, Zachmann M, Albers N, Hiort O, Bettendorf M, Wolfle J, Bidlingmaier F, Klingmuller D** 2000 Basal inhibin B and the testosterone response to human chorionic gonadotropin correlate in prepubertal boys. *J Clin Endocrinol Metab* 85:134-8.
52. **Atanassova N, McKinnell C, Walker M, Turner KJ, Fisher JS, Morley M, Millar MR, Groome NP, Sharpe RM** 1999 Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number, and the efficiency of spermatogenesis in adulthood. *Endocrinology* 140:5364-73

53. **Andersson AM, Skakkebaek NE** 2001 Serum inhibin B levels during male childhood and puberty. *Mol Cell Endocrinol* 180:103-7.
54. **Raivio T, Saukkonen S, Jaaskelainen J, Komulainen J, Dunkel L** 2000 Signaling between the pituitary gland and the testes: inverse relationship between serum FSH and inhibin B concentrations in boys in early puberty. *Eur J Endocrinol* 142:150-6
55. **Ibanez L, Valls C, Cols M, Ferrer A, Marcos MV, Zegher de F** 2002 Hypersecretion of FSH in infants boys and girls born small for gestational age. *J Clin endocrinol metab* 87:1986-1988
56. **Hull KL, Harvey S** 2000 Growth hormone: a reproductive endocrine-paracrine regulator? *Rev Reprod* 5:175-82.
57. **Lindgren AC, Chatelain P, Lindberg A, Price DA, Ranke MB, Reiter EO, Wilton P** 2002 Normal progression of testicular size in boys with idiopathic short stature and isolated growth hormone deficiency treated with growth hormone: experience from the KIGS. *Horm Res* 58:83-7.
58. **Andersson AM, Muller J, Skakkebaek NE** 1998 Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *J Clin Endocrinol Metab* 83:4451-8.
59. **Rajpert-De Meyts E, Jorgensen N, Graem N, Muller J, Cate RL, Skakkebaek NE** 1999 Expression of anti-Mullerian hormone during normal and pathological gonadal development: association with differentiation of Sertoli and granulosa cells. *J Clin Endocrinol Metab* 84:3836-44.
60. **Al-Attar L, Noel K, Dutertre M, Belville C, Forest MG, Burgoyne PS, Josso N, Rey R** 1997 Hormonal and cellular regulation of Sertoli cell anti-Mullerian hormone production in the postnatal mouse. *J Clin Invest* 100:1335-43.
61. **Cicognani A, Alessandroni R, Pasini A, Pirazzoli P, Cassio A, Barbieri E, Cacciari E** 2002 Low birth weight for gestational age and subsequent male gonadal function. *The Journal Pediatrics* 141:376-380
62. **Skakkebaek NE, Rajpert-De Meyts E, Main KM** 2001 Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 16:972-8
63. **Moller H, Skakkebaek NE** 1997 Testicular cancer and cryptorchidism in relation to prenatal factors: case-control studies in Denmark. *Cancer Causes Control* 8:904-12
64. **Fredell L, Lichtenstein P, Pedersen NL, Svensson J, Nordenskjold A** 1998 Hypospadias is related to birth weight in discordant monozygotic twins. *J Urol* 160:2197-9
65. **Skakkebaek NE** 2003 Testicular dysgenesis syndrome. *Horm Res* 60 Suppl 3:49
66. **Leger J, Levy M, Boch J, Pinet A, Chevenne D, Porquet D, Collin D, Czernichow P** 1997 Reduced final height and indications for insulin resistance in 20 year old born small for gestational age: Regional cohort study. *British medical journal* 315:341-347
67. **Leger J, Limoni C, Collin D, Czernichow P** 1998 Prediction factors in the determination of final height in subjects born small for gestational age. *Pediatr Res* 43:808-12.
68. **Albertsson-Wikland K, Karlberg J** 1994 Natural growth in children born small for gestational age with and without catch-up growth. *Acta Paediatr Suppl* 399:64-70
69. **Fredriks AM, van Buuren S, Burgmeijer RJ, Meulmeester JF, Beuker RJ, Brugman E, Roede MJ, Verloove-Vanhorick SP, Wit JM** 2000 Continuing positive secular growth change in The Netherlands 1955-1997. *Pediatr Res* 47:316-23.
70. **Persson I, Ahlsson F, Ewald U, Tuvemo T, Qingyuan M, von Rosen D, Proos L** 1999 Influence of perinatal factors on the onset of puberty in boys and girls: Implications for interpretation of link with risk of long term diseases. *Am J Epidemiol* 150:747-55.

71. **Tanner JM, Whitehouse RH** 1976 Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. *Arch Dis Child* 51:170-9.
72. **Marshall WA, Tanner JM** 1969 Variations in pattern of pubertal changes in girls. *Arch Dis Child* 44:291-303.
73. **Marshall WA, Tanner JM** 1970 Variations in the pattern of pubertal changes in boys. *Arch Dis Child* 45:13-23.
74. **Abbassi V** 1998 Growth and normal puberty. *Pediatrics* 102:507-11.
75. **Marti-Henneberg C, Vizmanos B** 1997 The duration of puberty in girls is related to the timing of its onset. *J Pediatr* 131:618-21.
76. **Fredriks AM, Buuren S, Wit JM, Verloove-Vanhoick SP** 2000 Body index measurements in 1996-7 compared with 1980. *Arch Dis Child* 82:107-112
77. **Thamdrup E** 1961 Precocious sexual development. Copenhagen:Munksgaard
78. **Kauli R, Galatzer A, Kornreich L, Lazar L, Pertzelan A, Z. I** 1997 Final height of girls with central precocious puberty, untreated versus treated with cyproteron acetate or GnRH analogue. A comparative study with re-evaluation of predictions by the Bayley-pinneau method. *Hormone Research* 47:54-61
79. **Tanaka T, Suwa S, Yokoya S, Hibi I** 1988 Analysis of linear growth during puberty. *Acta Paediatr Scand Suppl* 347:25-9
80. **Sas T, de Waal W, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 1999 Growth hormone treatment in children with short stature born small for gestational age: 5-year results of a randomized, double-blind, dose-response trial. *J Clin Endocrinol Metab* 84:3064-70.
81. **Zegher de F** 1998 GH-treatment of SGA children. *Trends Endocrinology and metabolism* 9:233-237
82. **Fjellestad-Paulsen A, Simon D, Czernichow P** 2004 Short children born small for gestational age and treated with growth hormone for three years have an important catch-down five years after discontinuation of treatment. *J Clin Endocrinol Metab* 89:1234-9
83. **Carel JC, Chatelain P, Rochiccioli P, Chaussain JL** 2003 Improvement in adult height after growth hormone treatment in adolescents with short stature born small for gestational age: results of a randomized controlled study. *J Clin Endocrinol Metab* 88:1587-93.
84. **Dahlgren J, Wikland KA** 2005 Final height in short children born small for gestational age treated with growth hormone. *Pediatr Res* 57:216-22
85. **Lifschitz CH** 2001 Feeding Problems in Infants and Children. *Curr Treat Options Gastroenterol* 4:451-457.
86. **Wright CM** 2000 Identification and management of failure to thrive: a community perspective. *Arch Dis Child* 82:5-9
87. **Blissett J, Harris G, Kirk J** 2000 Effect of growth hormone therapy on feeding problems and food intake in children with growth disorders. *Acta Paediatr* 89:644-9.
88. **Blissett J, Harris G, Kirk J** 2001 Feeding problems in Silver-Russell syndrome. *Dev Med Child Neurol* 43:39-44.
89. **Stenlof K, Johansson JO, Lonn L, Sjostrom L, Bengtsson BA** 1997 Diurnal variations in twenty-four-hour energy expenditure during growth hormone treatment of adults with pituitary deficiency. *J Clin Endocrinol Metab* 82:1255-60
90. **Stenlof K, Sjostrom L, Lonn L, Bosaeus I, Kvist H, Tolli J, Lindstedt G, Bengtsson BA** 1995 Effects of recombinant human growth hormone on basal metabolic rate in adults with pituitary deficiency. *Metabolism* 44:67-74

91. **de Waal WJ, Hokken-Koelega AC, Stijnen T, de Muinck Keizer-Schrama SM, Drop SL** 1994 Endogenous and stimulated GH secretion, urinary GH excretion, and plasma IGF-I and IGF-II levels in prepubertal children with short stature after intrauterine growth retardation. The Dutch Working Group on Growth Hormone. *Clin Endocrinol* (Oxf) 41:621-30.
92. **Leger J, Carel C, Legrand I, Paulsen A, Hassan M, Czernichow P** 1994 Magnetic resonance imaging evaluation of adipose tissue and muscle tissue mass in children with growth hormone (GH) deficiency, Turner's syndrome, and intrauterine growth retardation during the first year of treatment with GH. *Journal of Clinical Endocrinology and Metabolism* 78:904-909
93. **Boot AM, Engels MA, Boerma GJ, Krenning EP, De Muinck Keizer-Schrama SM** 1997 Changes in bone mineral density, body composition, and lipid metabolism during growth hormone (GH) treatment in children with GH deficiency. *J Clin Endocrinol Metab* 82:2423-8.
94. **Leger J, Garel C, Fjellestad-Paulsen A, Hassan M, Czernichow P** 1998 Human growth hormone treatment of short-stature children born small for gestational age: effect on muscle and adipose tissue mass during a 3-year treatment period and after 1 year's withdrawal. *J Clin Endocrinol Metab* 83:3512-6.
95. **Karlsson C, Stenlof K, Johannsson G, Marin P, Bjorntorp P, Bengtsson BA, Carlsson B, Carlsson LM, Sjostrom L** 1998 Effects of growth hormone treatment on the leptin system and on energy expenditure in abdominally obese men. *Eur J Endocrinol* 138:408-14
96. **Eriksson JG** 2005 The fetal origins hypothesis--10 years on. *Bmj* 330:1096-7
97. **Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D** 2000 The fetal and childhood growth of persons who develop type 2 diabetes. *Ann Intern Med* 133:176-82
98. **Katz LE, DeLeon DD, Zhao H, Jawad AF** 2002 Free and total insulin-like growth factor (IGF)-I levels decline during fasting: relationships with insulin and IGF-binding protein-1. *J Clin Endocrinol Metab* 87:2978-83.
99. **Albertsson-Wikland K** 1989 Growth hormone secretion and growth hormone treatment in children with intrauterine growth retardation. *Acta Paediatrica Scandinavia* 349:35-41
100. **Boguszewski M, Dahlgren J, Bjarnason R, Rosberg S, Carlsson LM, Carlsson B, Albertsson-Wikland K** 1997 Serum leptin in short children born small for gestational age: relationship with the growth response to growth hormone treatment. The Swedish Study Group for Growth Hormone Treatment. *Eur J Endocrinol* 137:387-95.
101. **Jaquet D, Leger J, Levy-Marchal C, Oury J, F., Czernichow P** 1998 Ontogeny of Leptin in Human Fetuses and Newborns: Effect of Intrauterine Growth Redartion on Serum Leptin Concentrations. *Journal of Clinical Endocrinology and Metabolism* 83:1243-1246
102. **Boguszewski MC, de Zegher F, Albertsson-Wikland K** 2000 Serum leptin in short children born small for gestational age: dose-dependent effect of growth hormone treatment. *Horm Res* 54:120-5.
103. **Zhang XY, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM** 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425-432
104. **Halaas J, L., Gajiwala K, S., Maffei M, Cohen S, L., Chait B, T., Rabinowitz D, Lallone R, L., Burley S, K., Friedman J, M.** 1995 Weight-Reducing Effects of the Plasma Protein Encoded by the obese Gene. *Science* 269:543-545
105. **Campfield L, A., Smith J, Guisez Y, Devos R, Burn P** 1995 Recombinant Mouse OB Protein: Evidence for a Peripheral Signal Linking Adiposity and Central Neutral Networks. *Science* 269:546-549

106. **Considine RV, Sinha MK, Heiman Ml, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334:292-295
107. **Blum WF, Englaro P, Hanitsch S, Juul A, Hertel NT, Muller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W** 1997 Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 82:2904-10.
108. **Ellis KJ, Nicolson M** 1997 Leptin levels and body fatness in children: effects of gender, ethnicity, and sexual development. *Pediatr Res* 42:484-8.
109. **Theunissen NC, Kamp GA, Koopman HM, Zwinderman KA, Vogels T, Wit JM** 2002 Quality of life and self-esteem in children treated for idiopathic short stature. *J Pediatr* 140:507-15.
110. **Vogels T, Verrips GHW, Koopman HM, Theunissen NC, Fekkes M, kamphuis RP** TACQOL Manuel Parent Form and Child Form. Leiden Center for child health and pediatrics LUMC-TNO.
111. **Vogels T, Verrips GH, Verloove-Vanhorick SP, Fekkes M, Kamphuis RP, Koopman HM, Theunissen NC, Wit JM** 1998 Measuring health-related quality of life in children: the development of the TACQOL parent form. *Qual Life Res* 7:457-65.
112. **Hull KL, Harvey S** 2003 Growth hormone therapy and Quality of Life: possibilities, pitfalls and mechanisms. *J Endocrinol* 179:311-33.
113. **Haverkamp F, Noeker M** 1998 'Short stature in children--a questionnaire for parents': a new instrument for growth disorder-specific psychosocial adaptation in children. *Qual Life Res* 7:447-55.
114. **Perrin EC, Stein RE, Drotar D** 1991 Cautions in using the Child Behavior Checklist: observations based on research about children with a chronic illness. *J Pediatr Psychol* 16:411-21.
115. **Leal Cerro A** 2004 Long-term challenges in growth hormone treatment. *Horm Res* 62 Suppl 4:23-30
116. **Stabler B, Siegel PT, Clopper RR, Stoppani CE, Compton PG, Underwood LE** 1999 Reply. *J Pediatr* 135:134.
117. **Sandberg DE, Brook AE, Campos SP** 1994 Short stature: a psychosocial burden requiring growth hormone therapy? *Pediatrics* 94:832-40.
118. **Wygold T** 2002 Psychosocial adaptation to short stature - an indication for growth hormone therapy? *Horm Res* 58:20-3.
119. **Kranzler JH, Rosenbloom AL, Proctor B, Diamond FB, Jr., Watson M** 2000 Is short stature a handicap? A comparison of the psychosocial functioning of referred and nonreferred children with normal short stature and children with normal stature. *J Pediatr* 136:96-102.
120. **Voss LD** 1999 Short but normal. *Arch Dis Child* 81:370-1.
121. **Busschbach JJ, Rikken B, Grobbee DE, De Charro FT, Wit JM** 1998 Quality of life in short adults. *Horm Res* 49:32-8.
122. **Lee P, Chernausek S, Hokken-Koelega A, Czernichow P** 2003 International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics*. 2003 111:1253-61
123. **Barker D** 1997 The fetal origins of coronary heart disease. *Acta Paediatr Suppl* 422:78-82
124. **Arends N, Johnston L, Hokken-Koelega A, van Duijn C, de Ridder M, Savage M, Clark A** 2002 Polymorphism in the IGF-I gene: clinical relevance for short children born small for gestational age (SGA). *J Clin Endocrinol Metab* 87:2720.

125. **Hattersley A, Tooke T** 1999 The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 353:1789-1792
126. **Hattersley A, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S** 1998 Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat genet* 19:268-270
127. **Woods KA, Camacho-Hübner C, Savage MO, Clark AJL** 1996 Intrauterine growth retardation and postnnatal growth failure associated with deletion of the insulin-like growth factor I gene. *Br.Report* 335:1363-1367
128. **Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, Chen JW, Mohan S, Denley A, Forbes B, van Duyvenvoorde HA, van Thiel SW, Sluimers CA, Bax JJ, de Laat JA, Breuning MB, Romijn JA, Wit JM** 2005 Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. *J Clin Endocrinol Metab* 90:2855-64
129. **Jaquet D, Tregouet DA, Godefroy T, Nicaud V, Chevenne D, Tiret L, Czernichow P, Levy-Marchal C** 2002 Combined effects of genetic and environmental factors on insulin resistance associated with reduced fetal growth. *Diabetes* 51:3473-8.
130. **Barker DJ** 2002 Fetal programming of coronary heart disease. *Trends Endocrinol Metab* 13:364-8.
131. **Kajantie E, Eriksson J, Barker DJ, Forsen T, Osmond C, Wood PJ, Andersson S, Dunkel L, Phillips DI** 2003 Birthsize, gestational age and adrenal function in adult life: studies of dexamethasone suppression and ACTH1-24 stimulation. *Eur J Endocrinol* 149:569-75.
132. **Siewert-Delle A, Ljungman S** 1998 The impact of birth weight and gestational age on blood pressure in adult life: a population-based study of 49-year-old men. *Am J Hypertens* 11:946-53.
133. **Jarvelin MR, Sovio U, King V, Lauren L, Xu B, McCarthy MI, Hartikainen AL, Laitinen J, Zitting P, Rantakallio P, Elliott P** 2004 Early life factors and blood pressure at age 31 years in the 1966 northern Finland birth cohort. *Hypertension* 44:838-46
134. **Eriksson JG, Forsen T, Tuomilehto J, Osmond C, Barker DJ** 2001 Early growth and coronary heart disease in later life: longitudinal study. *Bmj* 322:949-53
135. **Veening MA, Van Weissenbruch MM, Delemarre-Van De Waal HA** 2002 Glucose tolerance, insulin sensitivity, and insulin secretion in children born small for gestational age. *J Clin Endocrinol Metab* 87:4657-61
136. **Jaquet D, Deghmoun S, Chevenne D, Collin D, Czernichow P, Levy-Marchal C** 2005 Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. *Diabetologia* 48:849-55
137. **Ibanez L, Potau N, Ferrer A, Rodriguez-Hierro F, Marcos MV, de Zegher F** 2002 Reduced ovulation rate in adolescent girls born small for gestational age. *J Clin Endocrinol Metab* 87:3391-3
138. **Gatti JM, Kirsch AJ, Troyer WA, Perez-Brayfield MR, Smith EA, Scherz HC** 2001 Increased incidence of hypospadias in small-for-gestational age infants in a neonatal intensive-care unit. *BJU Int* 87:548-50
139. **Hussain N, Chaghtai A, Herndon CD, Herson VC, Rosenkrantz TS, McKenna PH** 2002 Hypospadias and early gestation growth restriction in infants. *Pediatrics* 109:473-8
140. **Farquhar J, Heiman M, Wong AC, Wach R, Chessex P, Chanoine JP** 2003 Elevated umbilical cord ghrelin concentrations in small for gestational age neonates. *J Clin Endocrinol Metab* 88:4324-7
141. **Iniguez G, Ong K, Pena V, Avila A, Dunger D, Mericq V** 2002 Fasting and post-glucose ghrelin levels in SGA infants: relationships with size and weight gain at one year of age. *J Clin Endocrinol Metab* 87:5830-3

142. **Cianfarani S, Martinez C, Maiorana A, Scire G, Spadoni GL, Boemi S** 2004 Adiponectin levels are reduced in children born small for gestational age and are inversely related to postnatal catch-up growth. *J Clin Endocrinol Metab* 89:1346-51
143. **Sandberg DE** 2000 Should short children who are not deficient in growth hormone be treated? *West J Med* 172:186-9.
144. **van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A** 2003 Effect of discontinuation of growth hormone treatment on risk factors for cardiovascular disease in adolescents born small for gestational age. J Clin Endocrinol Metab 88:347-53.
145. **Stabler B, Siegel PT, Clopper RR, Stoppani CE, Compton PG, Underwood LE** 1998 Behavior change after growth hormone treatment of children with short stature. *Journal of Pediatrica* 133:366-373
146. **Hokken-Koelega A, van Pareren Y, Arends N, Boonstra V** 2004 Efficacy and safety of long-term continuous growth hormone treatment of children born small for gestational age. *Horm Res* 62 Suppl 3:149-54
147. **Sas T, Mulder P, Aanstoot H** 2001 Carbohydrate metabolism during long-term growth hormone treatment in children with short stature born small for gestational age. *Clinical Endocrinology* 54:243-251
148. **Sas T, Mulder P, Hokken-Koelega A** 2000 Body composition, blood pressure, and lipid metabolism before and during long-term growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. *J Clin Endocrinol* Metab 85:3786-92.
149. **Barker DJ, Bull AR, Osmond C, Simmonds SJ** 1990 Fetal and placental size and risk of hypertension in adult life. *Bmj* 301:259-62.

Chapter 9

Summary

Summary

Epidemiological studies showed an association between low birth weight and hypertension, diabetes mellitus type II, dyslipidemia and cardiovascular disease at a relatively young age. It has been suggested that the programming of the endocrine axes and the development of the organs occurs during critical phases of fetal development which will be affected by intra-uterine growth retardation. As a result children born SGA might have several hormonal disturbances and permanent changes in organ structure and physiology. In this thesis we present the results of studies on the adrenarche, pubarche, ovaries and testes in short children born small for gestational age (SGA) (Chapter 2, 3 and 4). Since many short children born SGA are now being treated with GH, we also describe the effects of GH treatment on adrenarche, pubarche, ovaries and testes. Furthermore we present data on puberty, food intake, body composition, and quality of life in short SGA children and the effects of GH treatment on these consequences (Chapter 5, 6 and 7).

Chapter 1

This chapter gives an overview on literature data regarding the definitions of SGA, the prevalence and aetiology of SGA, the growth factors and hormones playing a role in fetal growth and possible consequences of being born SGA. In addition, it gives a summary of previously reported data on the effects and safety of GH treatment in short children born SGA. At the end of this chapter the aims of the thesis and the various study designs are described.

Chapter 2

Children born SGA might have several hormonal disturbances. One of the unresolved questions was whether short children born SGA have a disturbed adrenarche, the prepubertal rise in the secretion of the adrenals steroids dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulphate (DHEAS) and androstenedione. We investigated this by measuring serum DHEAS levels since these levels are most appropriate for evaluation of adrenarche. Serum DHEAS levels of 181 prepubertal 3–9 years old short children born SGA were compared with a control group of 170 prepubertal age-matched normal statured children born Appropriate for Gestational Age (AGA). We also investigated whether these children are at increased risk for a premature pubarche, defined as the appearance of pubic hair growth before the age of 8 years in girls and 9 years in boys. Since it had been reported that children born SGA have an acceleration of bone maturation around the age of 6–8 years, we studied whether there was an association

between serum DHEAS levels and bone maturation in SGA children. In addition the effect of one year of GH treatment was investigated.

Our data showed that short prepubertal children born SGA have normal serum DHEAS levels compared to AGA children and that one year of GH treatment had no influence on the serum DHEAS levels. We did not find a correlation between serum DHEAS levels and birth weight and birth length after correction for age. Premature pubarche was found in 2.2% of the girls and in none of the boys, which is comparable with the normal population. We did not find an acceleration of bone maturation in the untreated SGA children.

In conclusion short SGA children have normal serum DHEAS levels before the age of 9 years. The incidence of premature pubarche is comparable with the normal population. In addition, one year of GH treatment has no effect on serum DHEAS levels.

Chapter 3

Impaired fetal growth may lead to permanent changes in ovarian development. As a consequence girls born SGA might have a reduced size of the ovarian primordial follicle pool, which might result in earlier follicle pool depletion. This would increase the risk for infertility and premature ovarian failure for these girls.

Since serum Antimullerian Hormone (AMH) levels correlate well with the size of the ovarian follicle pool, we measured serum AMH levels in these girls. Thirty-five prepubertal short SGA girls were compared with 16 age-matched prepubertal normal statured AGA girls. Since many short children born SGA are now treated with growth hormone (GH) we also compared serum AMH levels of GH-treated SGA girls (n = 30) with age-matched untreated SGA girls (n = 35) and normal AGA girls (n = 16). In addition we compared serum AMH levels of post-menarchal SGA girls (n = 31) with age-matched AGA girls (n = 27).

Our data showed that there is neither a significant difference in prepubertal AMH levels between untreated SGA and AGA girls nor in GH-treated prepubertal SGA girls. During adolescence AMH levels appeared comparable for SGA and AGA girls. We also did not find a correlation between birth length and serum AMH levels.

In conclusion, our study shows that short SGA girls have normal serum AMH levels. In addition, two years of GH treatment has no effect on serum AMH levels. Finally, AMH levels in adolescent SGA girls are comparable to those of AGA girls. These data indicate that short SGA girls do not have a reduced size of the ovarian follicle pool.

Chapter 4

Impaired fetal growth may lead to permanent changes in testicular development. As a consequence boys born SGA might have a disturbed gonadal development resulting in reduced testis function later in life. Recently, low birth weight in boys was associated with impaired gonadal development.

Before puberty the best way to evaluate the testis development is the assessment of the number of Sertoli cells, by estimation of inhibin B and antimullerian hormone (AMH). From the onset of puberty levels of testosterone, LH, FSH and inhibin B can serve as markers of the intactness of the hypothalamic-pituitary-testis (HPT) axis.

We compared serum inhibin B and AMH levels of 73 prepubertal short SGA boys with 72 age-matched AGA boys. Since many short children born SGA are treated with GH, we also analysed if two years of GH treatment had an effect on serum inhibin B and AMH levels of prepubertal short SGA children. In addition, we compared serum inhibin B, AMH, testosterone, LH and FSH levels between young adults: 21 short SGA men, 15 normal statured SGA men and 25 normal statured AGA men.

No difference in serum inhibin B levels was found between the two prepubertal groups. Serum AMH levels were even higher in the SGA boys. We found no difference between serum inhibin B and AMH levels before and after 2 years of GH treatment after adjustment for age. Also, serum inhibin B and AMH levels in the GH-treated SGA boys were not significantly different compared to levels in AGA boys. The serum hormone levels, inhibin B, testosterone, LH and FSH between the 3 groups of young men were not significantly different, except for serum AMH levels, being higher in all SGA men.

In conclusion, short SGA boys have a normal number of Sertoli cells. In addition, serum inhibin B and AMH levels are the same in GH-treated SGA boys and age-matched AGA boys. Finally, young men born SGA have normal testis function.

Chapter 5

In this chapter we studied the effect of GH treatment, by comparing two different dosages, on the pubertal development of SGA children. The pubertal development was evaluated in terms of age and height at onset of puberty, age at menarche, interval between breast development (M2) and menarche, duration of puberty and pubertal height gain. We investigated the participants of the first Dutch GH-trial in short SGA children, since all 75 children had started puberty. We also compared our data with Dutch reference data of the fourth National Growth Study, except for duration of puberty and pubertal height gain since different definitions for adult height were used.

GH-treated SGA boys had a mean (SD) age at onset of puberty of 12.0 (1.0) and 11.6 (0.7) years and SGA girls of 10.9 (1.1) and 10.6 (1.2), when treated with 1 and 2 mg GH/m^2/day, respectively. No significant difference was found between the GH dosage groups. Compared to AGA controls, we found that SGA boys treated with the lowest GH dose started puberty significantly later. For the other GH-dosage groups there was no

significant difference in age at onset of puberty compared to AGA controls. The age at menarche and the interval between breast stage M2 and menarche was not significantly different for GH-treated girls compared to their peers, all regardless of the GH dosage. The duration of puberty and pubertal height gain of GH-treated SGA boys and girls were also not significantly different between the two GH dosage groups. A French study showed comparable data in untreated short children born SGA.

In conclusion, long-term GH treatment in short SGA children has no influence on the age at onset and progression of puberty compared to AGA controls, regardless of treatment with a dose of 1 or 2 mg GH/m^2/day. Duration of puberty and pubertal height gain are not significantly different between the GH-dosage groups and are comparable with untreated short children born SGA.

Chapter 6

Parents of short children born SGA often report that their children have a serious lack of appetite and a low food intake and that appetite improves after the start of GH treatment. We evaluated in a randomised GH controlled study the food intake at baseline and after 1 year with or without GH treatment in 88 SGA children. The food intake was also compared with the recommended daily intake (RDI) of age-matched Dutch children. In addition we measured body composition, IGF-I, IGFBP–3 and leptin. Body composition was measured by using 3 different methods: Dual Energy X-ray Absorptiometry (DEXA), skinfolds (SF) and body mass index (BMI).

Our study showed that caloric, fat, protein, and carbohydrate intake in short SGA children aged 5.9 (1.6) years was significantly lower compared to the RDI for age-matched Dutch children. One year of GH treatment resulted in a significant increase of the caloric, fat, carbohydrate and protein intake compared to baseline. Compared to randomised controls, caloric, carbohydrate and protein intake increased significantly after one year of GH treatment. Short SGA children have significantly lower Lean Body Mass (LBM) Standard Deviation Score (SDS), fat mass SDS, SF SDS, BMI SDS, serum IGF-I SDS, IGF-BP-3 SDS and leptin SDS levels compared to age-matched references. GH treatment resulted in a significant increase of height, LBM, BMI, IGF-I and IGFBP3 SDS and a significant decrease of SF SDS and leptin SDS.

In conclusion, our study shows that short SGA children have indeed a lower food intake than age-matched controls. During GH treatment, food intake increases significantly compared to baseline in contrast to the randomised control group.

Chapter 7

We evaluated the problems and limitations of having a short stature on daily life, called health status (HS) and the emotional feelings towards HS, called the health related quality of life (HRQOL) in a randomised GH controlled study in short SGA children. Studies evaluating psychosocial problems in short children are mostly based on HS without emotional feelings and on generic questionnaires which has more reference to children with a chronic illness.

We compared GH-treated short children born SGA (n = 58) with untreated short children born SGA (n = 27). At start of the study the children were 5–7 years of age. Two different questionnaires were used, the TNO-AZL Children's Quality of life (TACQOL), which is a generic questionnaire and the TACQOL-Short Stature, a specific questionnaire.

The generic TACQOL did not show a significant difference between the GH group and control group in HS and HRQOL at start of the study, whereas after 3 years only physical functioning was significantly improved in the GH group. In contrast, the TACQOL-Short Stature showed that SGA children who were treated with GH for 3 years had a significantly better quality of life with respect to their physical abilities, vitality, contact with peers, contact with adults, body image and future prospects compared to the untreated SGA children. The TACQOL-Short Stature showed a positive correlation between height SDS and HRQOL with regard to physical abilities, contact with peers, contact with adults, body image and future prospects. The discrepancy between the outcomes of the two questionnaires shows the relevance of using a disorder specific instrument.

In conclusion, our study shows that GH treatment in children born SGA improves several aspects of quality of life that are specific for children with a short stature.

Chapter 8

In the general discussion our findings are discussed in the context of the most recent literature data. The chapter ends with conclusions, clinical implications and suggestions for future research.

Chapter 10

Samenvatting

Samenvatting

Epidemiologische studies hebben laten zien dat hypertensie, diabetes mellitus type II en cardiovasculaire ziekten vaker optreden bij personen die geboren zijn met een laag geboortegewicht. Men neemt aan dat de programmering van endocriene assen en ontwikkeling van de organen plaats vindt tijdens kritische fases van de foetale ontwikkeling. Factoren die leiden tot intra-uterine groei vermindering zouden dus effect kunnen hebben op deze programmering. Kinderen die geboren worden met een te kleine lengte voor de zwangerschapsduur (SGA) hebben mogelijk een verhoogd risico op een afwijkende orgaanontwikkeling en hormonale stoornissen.

In dit proefschrift worden de mogelijk gevolgen van SGA op de ontwikkeling van de adrenarche, pubarche, ovaria en testes besproken aan de hand van onze onderzoeksresultaten op die gebieden. Aangezien tegenwoordig de meeste SGA kinderen met een te kleine lengte behandeld worden met groeihormoon (GH) bespreken we ook de effecten van GH behandeling op de ontwikkeling van deze organen (hoofdstuk 2, 3 en 4). Verder tonen we de onderzoeksresultaten op het gebied van puberteitsontwikkeling, voedingsinname en kwaliteit van leven van te kleine SGA kinderen en de effecten van GH behandeling hierop (hoofdstuk 5, 6 en 7).

Hoofdstuk 1

Dit hoofdstuk geeft een overzicht van de literatuur betreffende de definitie van SGA, de prevalentie en etiologie van SGA, groeifactoren en hormonen die een rol spelen bij de foetale groei en de mogelijke gevolgen van SGA. Tevens wordt een literatuuroverzicht gegeven van de meest belangrijke studies bij SGA kinderen met een te kleine lengte. Tenslotte worden de doelstellingen van de studies, de patiëntenpopulaties en het design van de studies beschreven.

Hoofdstuk 2

SGA kinderen met een te kleine lengte hebben mogelijk een verhoogde kans op verschillende hormonale afwijkingen. Een van de vragen was of te kleine SGA kinderen een verstoorde adrenarche hebben. Dit is de prepubertaire stijging van de adrenale steroïden in het bloed, dehydroepiandrosteron (DHEA), dehydroepiandrosteron-sulfaat (DHEAS) en androsteendion. We hebben dit onderzocht door DHEAS spiegels te meten in het serum, hetgeen de meest adequate meting is voor de evaluatie van adrenarche. We hebben serum DHEAS spiegels van 181 te kleine SGA kinderen tussen de 3 en 9 jaar vergeleken met DHEAS concentraties van 170 kinderen tussen de 3 en 9 jaar die een normale lengte bij de geboorte hadden (AGA). Tevens hebben we onderzocht of SGA kinderen een verhoogde kans hebben op een premature pubarche. Dit is de ontwikkeling

van pubishaar vóór de leeftijd van 8 jaar bij meisjes en 9 jaar bij jongens. Aangezien studies hebben beschreven dat SGA kinderen rond de leeftijd van 6-8 jaar een versnelde botrijping hebben, hebben we ook geëvalueerd of er een relatie is tussen serum DHEAS spiegels en de botrijping. Tenslotte hebben we gekeken of GH effect heeft op serum DHEAS spiegels.

Onze data lieten zien dat te kleine SGA kinderen normale serum DHEAS spiegels hebben en dat 1 jaar GH behandeling geen effect had op deze spiegels. We vonden ook geen correlatie tussen serum DHEAS spiegels en geboorte-gewicht of -lengte. Premature pubarche werd gevonden in 2.2% van de meisjes en niet bij de jongens, wat vergelijkbaar is met de normale populatie. In onze studiegroep vonden we in de onbehandelde SGA kinderen geen versnelde botrijping.

Wij concludeerden dat SGA kinderen met een te kleine lengte geen te vroege of gestoorde adrenarche hebben vóór de leeftijd van 9 jaar. De incidentie van premature pubarche is vergelijkbaar met de normale populatie. Eén jaar GH behandeling heeft geen effect op serum DHEAS spiegels.

Hoofdstuk 3

Afwijkende foetale groei zou kunnen leiden tot permanente veranderingen in de ovaria. Om deze reden zouden te klein geboren meisjes een kleinere ovariële follikelvoorraad kunnen hebben. Dit zou kunnen leiden tot een verhoogd risico op infertiliteit en vroege uitputting van de follikelvoorraad (premature menopauze).

Aangezien serum anti-műllerian hormoon (AMH) spiegels goed correleren met de grootte van de follikelvoorraad hebben we serum AMH spiegels van 35 prepubertaire te kleine SGA meisjes vergeleken met die van 16 AGA meisjes met een normale lengte van dezelfde leeftijd. Aangezien tegenwoordig te kleine SGA kinderen behandeld worden met GH, hebben we ook gekeken of GH invloed heeft op de ovariële follikelvoorraad, in 30 GH-behandelde prepupertaire SGA meisjes. Tevens hebben we serum AMH spiegels van 31 oudere SGA meisjes vergeleken met AMH spiegels van 27 AGA meisjes van dezelfde leeftijd.

Onze data lieten zien dat er geen significant verschil is in serum AMH spiegels van prepubertaire SGA meisjes met een te kleine lengte, AGA meisjes en GH-behandelde meisjes. Gedurende de adolescentie blijven serum AMH spiegels ook vergelijkbaar voor SGA en AGA meisjes. We vonden ook geen correlatie tussen serum AMH spiegels en geboortelengte.

Concluderend, een te kleine lengte bij de geboorte heeft geen effect op serum AMH spiegels, zowel bij prepubertaire SGA meisjes met een kleine lengte als bij adolescente SGA meisjes. Tevens heeft GH behandeling geen invloed op serum AMH spiegels. Deze data laten zien dat SGA meisjes en jonge vrouwen geen kleinere ovariële follikelvoorraad hebben.

Hoofdstuk 4

Afwijkende foetale groei zou kunnen leiden tot een verstoring van de ontwikkeling van de testikels. Dit zou kunnen leiden tot een verminderde testisfunctie op latere leeftijd. Enkele studies hebben beschreven dat laag geboortegewicht geassocieerd is met een afwijkende ontwikkeling van de testes.

De beste marker voor de testisontwikkeling bij prepubertaire kinderen is het aantal Sertoli cellen. Dit aantal is sterk gecorreleerd met de serum spiegels van inhibine B en AMH. Vanaf de puberteit kunnen ook serum testosteron, LH, FSH en inhibine B spiegels in het bloed worden bepaald om de functie van de testes te bepalen.

In ons onderzoek hebben we serum inhibine B en AMH concentraties van 73 prepubertaire SGA jongens vergeleken met die van 72 AGA jongens van dezelfde leeftijd. Omdat tegenwoordig veel kleine SGA kinderen behandeld worden met GH hebben we ook gekeken of GH invloed heeft op het aantal Sertoli cellen. Tenslotte hebben we de serum inhibine B, AMH, testosteron, LH en FSH spiegels vergeleken van 21 jonge SGA mannen met een te kleine lengte met 15 jonge SGA mannen met een normale lengte en 25 jonge AGA mannen.

De serum inhibine B spiegels waren gelijk in beide prepubertaire groepen, de AMH spiegels waren zelfs hoger in de SGA groep. De serum inhibine B en AMH spiegels waren na 2 jaar GH vergelijkbaar met die bij start. Serum inhibine B en AMH spiegels van GH-behandelde SGA jongens en AGA jongens waren gelijk. Er was geen verschil in de serum inhibine B, testosteron, LH en FSH spiegels tussen de drie groepen jonge mannen, alleen de serum AMH spiegels waren hoger in de twee SGA groepen.

Concluderend, een te kleine lengte bij de geboorte heeft geen invloed op serum inhibine B en AMH spiegels in prepubertaire jongens. Serum inhibine B en AMH spiegels zijn gelijk in GH-behandelde SGA kinderen en AGA kinderen. Tenslotte is de testes functie normaal in jonge mannen die SGA werden geboren. Deze data laten zien dat SGA jongens en jonge mannen niet minder Sertoli cellen hebben en geen verminderde testis functie.

Hoofdstuk 5

In dit hoofdstuk worden de effecten beschreven van GH behandeling op de puberteitsontwikkeling van te kleine SGA kinderen. We hebben de leeftijd en lengte bij start van de puberteit, de leeftijd van de menarche, het interval tussen het ontstaan van de borstontwikkeling (M2) en de menarche, de duur van de puberteit en de toename in lengte tijdens de puberteit geëvalueerd. We hebben onze data ook vergeleken met Nederlandse referentie data van de 4e nationale groeistudie.

Onze data lieten zien dat GH-behandelde SGA jongens de puberteit op een gemiddelde leeftijd (SD) van 12.0 (1.0) en 11.6 (0.7) jaar starten en GH-behandelde SGA

meisjes op de leeftijd van 10.9 (1.1) en 10.6 (1.2) jaar, na behandeling met 1 of 2 mg GH/m^2/dag, respectievelijk. Er werd geen significant verschil gevonden tussen beide GH-dosis groepen. Vergeleken met AGA jongens, waren SGA jongens die werden behandeld met de laagste dosis GH significant ouder bij de start van de puberteit. De leeftijd in de andere GH groepen was niet significant verschillend van die in AGA kinderen. De leeftijd waarop de menarche begon en het interval tussen het ontstaan van borstontwikkeling en menarche was in beide GH groepen gelijk. Er was ook geen significant verschil met de AGA meisjes. De duur van de puberteit en de lengtewinst tijdens de puberteit was niet significant verschillend tussen beide GH groepen, zowel voor jongens als voor meisjes.

Deze resultaten laten zien dat langdurige GH behandeling van SGA kinderen geen invloed heeft op de leeftijd van de start en de progressie van de puberteit in vergelijking met de Nederlandse referentie data, ongeacht of een GH dosering van 1 of 2 mg/m^2/dag werd gebruikt. De duur van de puberteit en de lengtewinst tijdens de puberteit zijn niet significant verschillend tussen beide groepen.

Hoofdstuk 6

Ouders van kleine SGA kinderen melden vaak dat hun kind een slechte eetlust heeft en erg weinig eet. In dit hoofdstuk worden de bevindingen getoond van de voedingsinname van 62 te kleine SGA kinderen vóór en na 1 jaar groei hormoon (GH) behandeling in vergelijking met 26 te kleine SGA kinderen zonder groeihormoon behandeling. Tevens hebben we de voedingsinname vergeleken met de aanbevolen hoeveelheden voor Nederlandse kinderen van dezelfde leeftijd (RDI= recommended daily intake). Aanvullend hebben we de lichaamssamenstelling, serum IGF-I, IGFBP3 en leptine spiegels bepaald vóór en na GH behandeling. De lichaamssamenstelling werd op 3 manieren gemeten: Dual Energy X-ray Absorptiometry (DEXA), huidplooi (skin fold=SF) en body mass index (BMI).

Onze studie liet zien dat de voedingsinname van te kleine SGA kinderen met een leeftijd van 5.9 (1.6) jaar significant lager was vergeleken met de RDI van kinderen met dezelfde leeftijd. Een jaar GH behandeling liet een significante toename zien van calorie-, vet-, eiwit- en koolhydraat- inname vergeleken met die bij start van GH behandeling. GH-behandelde SGA kinderen hadden na een jaar een significant hogere inname van calorieën, eiwitten en koolhydraten vergeleken met de gerandomiseerde controle groep. SGA kinderen met een te kleine lengte hadden een significant lagere vetvrije massa- (Lean Body Mass=LBM), vetmassa-, SF-, BMI- , IGF-I-, IGF-BP-3- and leptine- Standaard Deviatie Score (SDS) spiegels vergeleken met leeftijdsgenoten. GH behandeling leidde tot een significante toename van lengte-, LBM-, BMI-, IGF-I- en IGFBP-3 SDS en een afname van SF SDS en leptine SDS.

Concluderend laat ons onderzoek zien dat SGA kinderen met een te kleine lengte een verminderde calorie-, vet-, koolhydraat- en eiwit- inname hebben in vergelijking met leeftijdsgenoten. GH behandeling resulteert in een significante toename van voedingsinname vergeleken met de situatie voor GH behandeling en met de gerandomiseerde controle groep.

Hoofdstuk 7

Dit hoofdstuk toont de effecten van het te klein zijn op het dagelijks functioneren (HS) en de emotionele gevoelens hierbij (HRQOL) in een gerandomiseerde GH gecontroleerde studie van SGA kinderen. De meeste studies die gedaan zijn naar kinderen met een te kleine lengte zijn gebaseerd op algemene vragenlijsten die ontwikkeld zijn voor kinderen met een chronische ziekte. Tevens wordt in die vragenlijsten vaak gevraagd naar de problemen die het kind ondervindt door zijn ziekte en niet naar de emotionele gevoelens daarbij. Wij onderzochten 58 GH-behandelde SGA kinderen in vergelijking met 27 onbehandelde SGA kinderen met een te kleine lengte. Bij start van de studie hadden de kinderen een leeftijd tussen de 5 – 7 jaar. Er werden 2 vragenlijsten gebruikt, een algemene vragenlijst, de TNO-AZL Children's Quality of life (TACQOL) en een specifieke vragenlijst voor kinderen met een te kleine lengte, de TACQOL-Short.

De algemene vragenlijst liet bij start geen significant verschil zien tussen de GH groep en de gerandomiseerde controle groep. Deze vragenlijst liet na drie jaar GH behandeling zien dat alleen het onderdeel lichamelijke ongemakken verbeterd was. De specifieke vragenlijst daarentegen liet zien dat de kwaliteit van leven zowel voor HS als HRQOL significant verbeterd was na 3 jaar GH behandeling wat betreft lichamelijke ongemakken, omgaan met andere kinderen, omgaan met volwassenen, lichaamsbeeld van het kind, vitaliteit en toekomstbeeld in vergelijking met de gerandomiseerde controle groep. De specifieke vragenlijst liet ook een positieve correlatie zien tussen de lengte en lichamelijke gemakken, contact met leeftijdsgenoten, contact met volwassen, lichaamsbeeld en toekomstverwachting. Het verschil in bevindingen tussen de 2 vragenlijsten laat de relevantie zien van het gebruik van een specifieke vragenlijst bij kinderen zonder een chronische ziekte.

Concluderend laat ons onderzoek zien dat GH behandeling de kwaliteit van leven van te kleine SGA kinderen significant verbetert.

Hoofdstuk 8

In de algemene discussie hebben we de bevindingen van onze studie bestudeerd met betrekking tot de recente literatuur. Dit hoofdstuk eindigt met conclusies en suggesties voor toekomstige onderzoeken.

Dankwoord

In december 1999 begon ik aan mijn promotieonderzoek. Nu, bij het schrijven van dit dankwoord is er ruimte voor reflectie en een terugblik. Ik kom al snel tot de conclusie dat ik in de afgelopen zes jaar enorm ben gegroeid.

Het promotieonderzoek is bijna afgerond, ben inmiddels getrouwd, heb twee super dochters en zit al weer in mijn tweede jaar van de opleiding kindergeneeskunde.

Mijn onderzoek heeft mij het inzicht gegeven dat groei niet altijd gelijk opgaat en dat er soms hulp bij nodig is. Ook voor mijn ontwikkeling en groei kunnen diverse stimulerende factoren worden geïdentificeerd. Veel mensen hebben mij geholpen, gesteund en gestimuleerd. Ik wil dan ook al diegenen hartelijk bedanken. Dit is een grote groep en een opsomming van namen zou nooit compleet kunnen zijn. Ik richt mij met naam en toenaam dan ook tot een beperkter aantal mensen die ik om diverse redenen in het bijzonder wil bedanken.

Alle kinderen en ouders uit het onderzoek wil ik hartelijk danken voor hun medewerking. Mede dankzij jullie is dit proefschrift tot stand gekomen en ik vond het leuk om dit onderzoek te doen, met en voor jullie.

Mijn promotor, Prof.dr. A.C.S. Hokken-Koelega. Beste Anita, ik voel me helemaal vereerd en vind het erg leuk dat ik je eerste promovendus ben. Ik bewonder je enorme enthousiasme en je werklust. Je bent een grote stimulans voor me geweest. De besprekingen met jou waren altijd erg motiverend en laagdrempelig. Over en weer konden we goed onze gedachten ventileren. Door je volle programma kon je er niet altijd zijn. We spraken elkaar soms op reguliere tijden maar juist vaak ook tijdens jouw diensten, de late avonden, weekenden, tijdens vakanties, ouderschaps- en zwangerschapsverloven. Zo konden we beiden een toch wat vol programma controleren en naast de vakinhoudelijke dingen hadden we dan ook ruimte om de dagelijkse bezigheden te bespreken.

Prof.dr. S.L.S. Drop, beste Sten, bedankt voor het nakijken van mijn artikelen en de samenwerking binnen de endo-groep. De wekelijkse journalclub was een leuke en leerzame aanvulling op het promotieonderzoek.

Prof.dr. F.H. de Jong, beste Frank, je was bij vele onderdelen van mijn promotieonderzoek betrokken. Ik vond onze besprekingen altijd erg verhelderend en motiverend. Bedankt voor het altijd snel en kritisch beoordelen van mijn artikelen en het beoordelen van mijn manuscript. Ik vond het plezierig om met je samen te werken.

Dr. A.P.M. Themmen, beste Axel, bedankt voor alle antwoorden op mijn vragen waar ik je soms over mailde of voor belde. Hartelijk dank voor het meedenken en je commentaren.

Dr. J.S.E. Lave en Dr. R.F.A. Weber, beste Joop en Rob, bedankt voor de fijne en enthousiaste samenwerking. Ik vond het altijd boeiend en leerzaam om met jullie te brainstormen over jullie vakgebied. Onderwerpen die veraf lijken te staan van de kindergeneeskunde, maar niets is minder waar.

Dr. H.M. Koopman en Dr. N.C.M. Theunissen, beste Hendrik en Nicolet, ik heb veel geleerd van jullie over onderzoek doen naar kwaliteit van leven. Bedankt voor de goede en plezierige samenwerking.

De kans is uitermate klein dat ik mijn promotie tot een goed einde zou hebben gebracht zonder de hulp van de statistici, Prof.dr. Th. Stijnen en Dr. P.G.H. Mulder. Beste Theo en Paul, heel veel dank en ik heb grote bewondering voor hoe jullie je steeds weer verplaatsten in al mijn onderzoeken en hoe jullie die statistiek keer op keer de baas waren. Ook Maria de Ridder wil ik persoonlijk bedanken voor het altijd bereid zijn om op het laatste moment nog statistische problemen op te lossen.

Prof.dr. J.M. Wit en Prof.dr. J.B. van Goudoever wil ik hartelijk bedanken voor het beoordelen van mijn manuscript.

Alle kinderarts-endocrinologen van de verschillende centra die mee hebben gewerkt aan dit onderzoek wil ik ook hartelijk bedanken. Prof. dr. H. Dellemarre-van der Wal, Dr. E.C.A.M. Houdijk, Drs. P. Voorhoeve, Dr. M. Jansen, Drs. J.C. Mulder, Dr. R.J.H. Odink, Dr. H.M. Reeser, Dr. C. Rongen-Westerlaken, Dr. W.H. Stokvis-Brantsma en Dr. J.J.J. Waelkens.

Hierbij wil ik ook Prof.dr. H.M. Oudesluys-Murphy en Dr. M. de Hoog bedanken. Beste Annemarie en Matthijs, bedankt voor het aandragen van het idee en het verlenen van toestemming om ouderschapsverlof op te nemen om zodoende de promotie tijdens het opleidingstraject toch goed af te kunnen ronden. Het ouderschapsverlof is oorspronkelijk niet bedoeld om te promoveren, maar aangezien zowel promoveren als ook het volgen

van de opleiding een grote inzet en betrokkenheid vergen is de combinatie dan ook vrijwel onmogelijk. Dit verlof bood zodoende gelukkig een uitkomst.

Novo Nordisk Farma BV Nederland en Novo Nordisk A/S Denemarken wil ik hartelijk bedanken voor de plezierige samenwerking en de financiële ondersteuning van het onderzoek en dit proefschrift.

De mensen van Lab. interne III wil ik hartelijk bedanken voor het altijd snel bepalen van de monsters.

Tevens wil ik al de (ex)arts-onderzoekers bedanken voor de gezellige tijd.

Wat had ik gemoeten zonder de leuke collega's van de endo. Es en Marian jullie waren mijn steun en toeverlaat. We hebben leuke reisjes door het land gemaakt. Veel gelachen en gekletst. Ik mis jullie wel hoor! Marieke, Marije, Ellen en Robbert, ik heb een super tijd met jullie gehad. We hebben vele uren hard samengewerkt maar ook vele uren gekletst, gelachen en gemopperd over van alles. De ESPE was altijd een groot feest. Wietske en Ruben bedankt voor de overname van het onderzoek. Ingrid, Janneke, Dini en alle andere endo-collega's bedankt voor de steun, gezelligheid en samenwerking!

Ook wil ik mijn lieve familie; Klaas, Peter, Akkelies, Rumsiah, Sadiah, Ester, Jeroen, Wilte, Mustafa, Marleen, Geert en Wendela bedanken voor al de steun en begrip voor mijn drukke leven. Om gelijke reden wil ik ook al die mensen bedanken waar ik de afgelopen jaren ondanks de drukte telkens weer vriendschap van mocht ontvangen en ik heb goed nieuws, want met het lezen van dit proefschrift kan ik garanderen dat er weer meer tijd is om vaker af te spreken en lol te maken.

Lieve "oma Tiny" en "oma Nelleke" bedankt voor al het oppassen en altijd maar weer klaar staan voor mij. En dan Harry, het feit dat ik me niet meer tot je kan richten is verschrikkelijk jammer temeer omdat ik weet dat je ook hier enorm van genoten zou hebben.

Lieve Nico, jij was mijn eerste contact binnen de endo-groep, mijn grote voorbeeld en je hebt in vele opzichten de weg voor me vrij gemaakt. Je voldoet daarmee, of je wilt of niet, aan het profiel van de perfecte paranimf en ik ben blij en vind het super dat je voor mij nog één keer in de promotiestress wilt geraken.
Nico, je relativeringvermogen en wereldse nuchterheid zijn uniek. Ik zie uit naar onze etentjes en vakantie!

Lieve Akkelies. Zus. Vriendin. Collega. Paranimf. Wat heb ik het met je getroffen.

Lieve Pa en Ma, zonder jullie…….. ach, wat zal ik een onmetelijk rijk mens zijn als ik op latere leeftijd van mijn kinderen de waardering mag ontvangen die ik jullie zo graag zou willen geven. Mijn probleem op deze bladzijde is echter dat woorden ontoereikend zijn om mijn waardering en dank volledig te communiceren. Bereid je dus maar voor op een dikke kus en een vette knuffel. Vol liefde.

Lieve Femke en Meintje, door jullie aanwezigheid weet ik wat echt belangrijk in het leven is. En Femke, als jij niet zo'n fantastische lieve en makkelijke meid geweest was, had ik niet geweten hoe ik het proefschrift had moeten afmaken. Met zekerheid hoef ik nu niet meer zo vaak te zeggen: "nu even niet want mama moet lezen".

Lieve Goof, je hebt altijd veel geduld en begrip voor mij en mijn werk opgebracht, vooral het laatste anderhalf jaar in combinatie met de opleiding. Zonder jouw warmte, humor, creativiteit, relativeringsvermogen en eeuwige steun op allerlei vlakken had ik de groei van de afgelopen 6 jaar niet kunnen volbrengen.

Tijd voor een feest!

Curriculum Vitae

Venje Boonstra was born October the 2nd, 1970 in Mqanduli, South Africa, and moved to the Netherlands when she was two years old. After finishing high school (VWO-B) in Groningen in 1989, she went on to study for theatre assistant. In 1991, she started her medical training at the Groningen University (RUG). In 1994, she participated in a project that aimed to determine the effect of introducing guidelines on the postoperative pain management in children at the department of Pediatric Surgery and Anesthsiological, University Medical Centre Groningen (supervisor Dr. R. Huntink and Prof.dr. R.P. Zwierstra). In 1996, she researched the effect of under nutrition on the morbidity in children with a Wilms' tumor at the department of Pediatrics Oncology, University of Stellenbosch in South Africa (supervisor Prof.dr. W.A. Kamps and Dr. G. Wessels). These international experiences were followed by her internships at the hospital Medisch Spectrum Twente in Enschede. At the end of this practical training she performed literature research on the mutations responsible for hypovirilisation of the XY-karyotype at the department of Pediatric Endocrinology, University Medical Center Nijmegen (supervisor Dr. B.J. Otten). After obtaining her medical degree in 1998, she started to work as a resident at the Department of Pediatrics of the Amphia hospital in Breda. From December 1999 until March 2004 she worked as a research-fellow at the Department of Pediatrics, Subdivision of Endocrinology in Erasmus Medical Center, Sophia Children's Hospital in Rotterdam (supervisor Prof.dr. A.C.S. Hokken-Koelega and Prof.dr. S.L.S. Drop) which has resulted in the present thesis. In April 2004, she started her clinical pediatric training ship in Erasmus Medical Center, Sophia Children's Hospital in Rotterdam (head: Dr. M. de Hoog and Prof.dr. A.J. van der Heijden).

List of Abbreviations

ACTH	=	Adrenocorticotrophic Hormone
AGA	=	Appropriate for Gestational Age
AH	=	Adult Height
AMH	=	Anti-Müllerian Hormone
BA	=	Bone Age
BMI	=	Body Mass Index
CA	=	Chronological Age
DEXA	=	Dual Energy X-ray Absorptiometry
DHEA	=	Dehydroepiandrosterone
DHEAS	=	Dehydroepiandrosterone-sulphate
FM	=	Fat Mass
FSH	=	Follicle Stimulating Factor
GH	=	Growth Hormone
GHD	=	Growth Hormone Deficiency
HPT	=	Hypothalamic-pituitary-testis
HRQOL	=	Health Related Quality of Life
HS	=	Health Status
IGF-I	=	Insulin-like growth factor-I
IGFBP3	=	IGF-binding protein-3
LBM	=	Lean Body Mass
LH	=	Lutheine Stimulating Factor
PCOS	=	Polycystic Ovarian Syndrome
RDI	=	Recommended Daily Intake
SDS	=	Standard Deviation Score
SGA	=	Small for Gestational Age
SF	=	Skinfold
TACQOL	=	TNO-AZL Children's Quality of life
TH	=	Target Height

Stellingen

Behorend bij het proefschrift

Short Children Born Small for Gestational Age (SGA)

Adrenarche, pubarche, gonadal function, pubertal development, food intake, quality of life and effects of growth hormone treatment

1. De incidentie van premature pubarche onder te kleine kinderen die bij de geboorte reeds te klein waren (SGA) is vergelijkbaar met de normale populatie. (Dit proefschrift)

2. De leeftijd waarop de puberteit start en de duur van de puberteit is bij groeihormoon behandelde SGA kinderen gelijk aan die van kinderen met een normale geboortelengte en lengte. (Dit proefschrift)

3. De normale serum Anti-Müllerian Hormoon spiegels van prepubertaire te kleine SGA meisjes en jong volwassen SGA vrouwen indiceert dat ze een normale ovariële follikelvoorraad hebben. (Dit proefschrift)

4. De normale serum inhibine B spiegels van prepubertaire te kleine SGA jongens en jong volwassen SGA mannen indiceert dat ze een normaal aantal sertolicellen hebben. (Dit proefschrift)

5. Om het effect van kleine lengte op de kwaliteit van leven te meten zijn specifieke vragenlijsten met betrekking tot lengte nodig. (Dit proefschrift)

6. Target Height (“doellengte”) dient met grote terughoudendheid te worden gehanteerd indien één van de ouders een afwijkende lengte heeft op basis van een chromosomale afwijking, syndroom, chronische ziekte of SGA geboren zijn.

7. Promoveren en meerdere jaren AGNIO-schap als voorwaarde voor een opleidingsplaats resulteren in een drastische afname van het aantal productieve specialistenjaren, mede omdat de opleiding daardoor naar een leeftijdsfase wordt verschoven waarin vrouwen door gezinsvorming vaker een langer durende parttime opleiding volgen.

8. Omdat verzet tegen het moslimextremisme door niet-moslims vaak polariserend werkt, zou het verzet hiertegen meer van de moslims moeten komen.

9. Ontwikkelingshulp is een farce wanneer de rijke landen wel gelden doneren maar tegelijkertijd de eigen markt beschermen voor goede producten uit de ontwikkelingslanden.

10. De stelling: “Het voordeel van het met potlood schrijven van een manuscript valt niet uit te vlakken” is geschiedenis, tegenwoordig valt het voordeel van het werken met een computer namelijk niet meer te deleten. (Zie stelling 16 proefschrift Boonstra H. 1987)

11. Promoveren tijdens een zwangerschapsverlof is een bevalling op zich.