SGA and Turner Syndrome

The impact of growth hormone treatment on physical and mental well-being

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SGA and Turner Syndrome

The impact of growth hormone treatment on physical and mental well-being

SGA en Turner Syndroom

De invloed van groeihormoon behandeling op fysiek en mentaal welzijn

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Groeien kan op vele manieren

Aan Michael en mijn ouders

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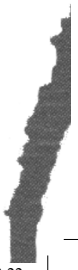
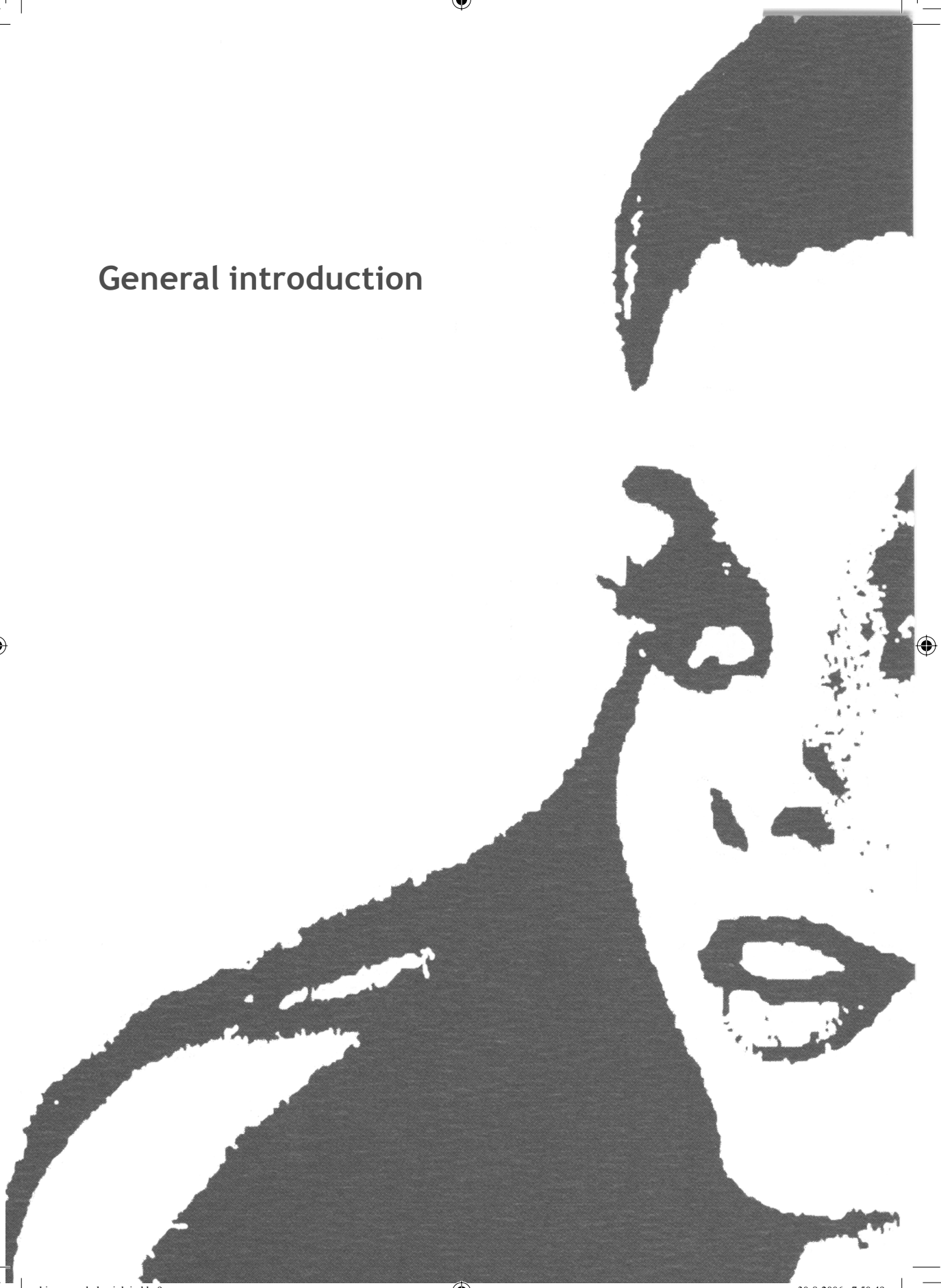


General introduction



Introduction

Growth hormone is available in unlimited volumes since 1986, when growth hormone (GH) could be manufactured. Since then, multiple studies investigated the effect of GH-therapy in children with short stature, either with or without disturbances in the GH-IGF-I axis. In this thesis, two populations of children with short stature are presented: short children being born small for gestational age (SGA) and girls with Turner syndrome (TS). In both populations, GH has proven to be effective, resulting in normalisation of height during childhood, adolescence and adulthood [1-9]. Although several studies have investigated safety aspects and adverse effects of long-term GH therapy, up to today still many questions remain unanswered regarding long-term effects and side effects during and after discontinuation of GH treatment.

This chapter describes definitions and presents background of SGA and Turner syndrome, their prevalence and etiology, and clinical characteristics. It provides the previously reported growth promoting results of GH-therapy and shows safety aspects of growth hormone therapy. Finally, the aims of the study and the outline of the thesis will be presented.

1 Background

— Small for gestational age (SGA)—

Definition

Small for gestational age, or SGA, refers to the size at birth of the newborn adjusted for the gestational age. Both, children born full-term and premature can be born SGA. In literature, different definitions have been used classifying SGA, such as birth weight and/or length below the 3rd or 10th percentile (or below −1.88 or −1.29 SDS). Some investigators use birth weight, others birth length or both birth weight and length. In all Dutch multicenter studies, SGA was defined as a birth length below the −2 SDS using the data of Usher and McLean [10].

Intra uterine growth retardation, or IUGR, refers to a deceleration of fetal growth, which slows down from a preceding growth pattern. To diagnose IUGR, antenatal fetal auxology is required, to demonstrate subnormal prenatal growth velocity. Thus, IUGR does not necessarily result in SGA. SGA and IUGR are not synonyms, although SGA is often a result of growth retardation in utero.

Etiology

SGA comprises a variety of underlying causes. In 40% of the infants born SGA, the underlying mechanism remains unclear. However, the cause of SGA should be identified where possible, as it may have consequences for prognosis and treatment effects. Impaired fetal growth can originate from a number of fetal, maternal, placental, and environmental factors.

Fetal factors include chromosomal disorders (e.g. Down syndrome, Turner syndrome), congenital defects (e.g. cardiac abnormalities), metabolic diseases, and genetic disorders (e.g. achondroplasia).

Maternal factors include age, tobacco smoking, alcohol abuse, and maternal medical conditions, such as infections (e.g. Toxoplasmose, Rubella, Cytomegalovirus, Herpesvirus, Malaria), hypertension, renal disease, diabetes mellitus, collagen vascular diseases (e.g systemic lupus erythematosis), and heart disease.

Placental factors are factors jeopardizing placental function, which is supplying essential nutrients and oxygen from the mother to the fetus. Thus, placental factors include all factors jeopardizing placental perfusion, such as placental hemangiomas, infarcts, and placenta previa.

Environmental factors include infections and exposure to toxins.

Hypotheses regarding the association of SGA with pathology in adult life

SGA is associated with an increased risk of the development of cardiovascular diseases, and metabolic syndrome, or one of its components: hypertension, dyslipidemia, impaired glucose tolerance, or diabetes mellitus [11-13].

'The fetal origin hypothesis', also called 'fetal programming hypothesis', or 'fetal undernutrition hypothesis', was postulated by Barker [14], and suggests that the SGA associated adult diseases are programmed by undernutrition in utero. Impaired fetal growth, especially when timed during mid- to late gestation, would lead to permanent changes in organ-structure and physiology.

'The fetal insulin hypothesis', postulated by Hattersley [15], suggests that genes involving insulin resistance could affect both birth weight and disease in later life. Insulin resistance appears as a key component underlying the metabolic complications. Insulin also plays a crucial role in fetal growth.

The insulin-like-growth factor (IGF) system is involved in pre and postnatal growth and development. Additionally, IGF-I has been suggested to be involved in degenerative common diseases such as atherosclerosis and diabetes [16,17]. Low IGF-I levels have a positive association with cardiovascular diseases, atherosclerosis and diabetes, all associated with short children born SGA. IGF-I levels in short SGA children are reported to be reduced, but within the normal range [2,18,19]. Therefore, a disturbance in the GH-IGF-axis also may play a role in the etiology.

Prevalence

By definition, approximately 3% of the newborns are SGA. As mentioned earlier, SGA newborns may be born full-term or premature. If catch-up growth occurs, it is more or less finalized before 1 year of age [20]. Therefore, the presence of catch up at 2 years of age seems to be relevant for predicting final height outcome. Ten to 15% of the children born SGA do not show a catch-up growth within 2 years, indicating a 7–10 fold increased risk for being short as adult [20,21]. This means that 1:350 to 1:400 live births remain short after being born SGA.

Clinical aspects

Short stature remains in app. 10%–15% of the children born SGA. Children born SGA have a 5 to 7 times higher risk of short stature in adulthood, defined as a height below −2 SDS, than children born appropriate for gestational age [20]. Approximately 22% of the short adults are born SGA [22]. Children born SGA without catch-up growth show primary growth failure, meaning that they have a normal growth velocity. The underlying mechanisms for lacking catch-up growth are still poorly understood. Growth hormone secretion, measured over 24 hours, was subnormal in 60% of the SGA children without catch-up growth, whereas 25% showed low GH peaks during GH provocation tests [23,24]. IGF-I levels are in the lower half of the normal range [2,18]. These findings suggest that disturbances in the GH/ IGF-I axis may play a role.

Chromosomal abnormalities, genetic disorders, or congenital anomalies may cause SGA. For that reason, it is important to search for ***dysmorphic features*** and ***signs of congenital malformations***. In the Dutch multicenter studies, children with multiple dysmorphic features were excluded, except for Silver Russell Syndrome (SRS). Typical features of SRS are severely reduced birth weight and length, short stature during childhood and adulthood, typical craniofacial abnormalities with a large, prominent forehead (frontal bossing), a small triangular face, asymmetry of the limbs, clinodactyly of the fifth finger and some other minor features [25]. It is sometimes difficult to diagnose SRS, as only minor features may be present. As the growth pattern of children with SRS do not differ from short SGA children, most trials investigating SGA included SRS children.

Body composition and diminished appetite. Short SGA children appear small, lean and fragile. They show a significantly reduced lean body mass, fat mass, skin fold measurement and body mass index [26-28]. Parents report often that their child has lack of appetite and low food intake. Short SGA children show a decreased caloric, fat and carbohydrate intake, at a mean age of nearly 6 years, compared to the Recommended Daily Food intake for age-matched controls [28]. The explaining

mechanism is not known. Factors related may be behavioral, organic, or a mixture. Behavioral causes are, for example, cultural expectations of food intake and body figure, parental anxiety for (over)weight, children not fed orally for a long period at a critical age (more common in SGA than in AGA children). These factors may cause feeding problems. Organic causes include swallowing problems, respiratory distress, and lack of appetite due to various systemic diseases. SGA children may have a lower metabolic rate, or a disturbed appetite due to hormonal disturbances influencing the appetite.

Age at start of ***puberty*** in short children born SGA is comparable to normal [29]. A large Swedish study concluded that in SGA boys, the mean age at puberty onset did not differ between normal boys and those with perinatal factors. Girls born SGA were 5 months younger than normal girls at the onset of puberty and menarche, however within the normal range [30]. Associations between intrauterine exposures and disease risk in later life may be confounded by, or mediated through, effects on adolescence.

Intelligence and psychological performance. Short children, born SGA, have a lower self-concept than their Dutch peers [31]. They also showed deficits in divided, focused, and sustained attention. They were less accurate, exhibited more variability in reaction time, and performed more slowly and more impulsively than did the age-matched controls [32]. This indicates that short children born SGA and attention deficits are related [32].

Furthermore, short children born SGA showed a lower estimated total IQ, performal IQ, and verbal IQ compared to their peers [33]. Also in a large Swedish study among 248,051 adult men, being born SGA showed an association with increased risk of subnormal intelligence and psychological performance. Among the SGA males, the most important predictor for subnormal intelligence and psychological performance was the absence of catch-up growth, suggesting that being born SGA without catch-up growth is of disadvantage [34]. Being born SGA, with or without catch-up growth, has also been associated with poor academic performance, poorer mental and emotional well-being, low social competence, and behavioral problems [35-37].

Cardiovascular and insulin-resistance risk factors. Epidemiological studies have found a predisposition for diseases such as diabetes mellitus type 2 and cardiovascular diseases in adults born with a low birth weight [38-40]. In SGA children and adolescents, decreased insulin sensitivity is found [41-44]. In 8% of the children presented in this thesis, impaired glucose tolerance was present before start of GH therapy [42]. Furthermore, SGA children had a significantly higher systolic

blood pressure, but normal diastolic blood pressure and serum lipids compared to peers [45].

— Turner syndrome —

History

In 1938, the American physician Henry Turner described 7 women who were characterized by short stature, absence of pubertal development, and deformity of the elbow (cubitus valgus) [46]. In 1929, Ullrich, a pediatrician from Munich already described the same characteristics in some of his female patients [47]. The term Turner syndrome is used in the Anglo-Saxon literature, but in most German literature Ullrich-Turner syndrome is used. Henry Turner's description has had a significant influence on further research into the disease. In 1959, Ford et al reported a missing chromosome in patients with Turner syndrome [48].

Etiology

Turner syndrome is caused by the total or partial absence of one of the X-chromosomes in all or part of the body cells. Genomic imprinting has been implicated in the pathogenesis of TS. Imprinting occurs when allelic genes or chromosomes are expressed differently depending on the parent of origin. In 70–75% of the patients with 45,X karyotype, the retained X-chromosome was maternal [49]. Conversely, a higher incidence of the paternal X retention has been noted in spontaneously aborted fetuses [49]. Studies continue to establish an association between the retained parental X-chromosome and phenotypic features in TS.

There is a wide variety of the genotype. The phenotype/genotype correlation in TS is generally poor. In general, TS patients with 45,X genotype show the highest incidence of cardiac and renal malformations, and congenital lymphedema [50]. The different karyotypes present in TS are listed in Table 1. Y-chromosomal material is present in 5–10% of patients with TS (like 45,X/46,XY; 45,X/46,X,idic(Y); 45X/46XX/47XXY etc).

Testing for 'hidden' Y chromosomal material should be performed in any TS patient with evidence of virilization or when a marker chromosome (a sex chromosomal fragment of unknown origin, i.e. X *vs.* Y) is found. This can be achieved by DNA studies or fluorescent *in situ* hybridization (FISH). The prevalence and clinical significance of 'hidden' Y material detected only by FISH or DNA analysis needs further investigation. The presence of Y-chromosome material is associated with a 7–10% prevalence of a gonadoblastoma, a benign tumour that may become malignant [51]. Therefore, a gonadectomy is recommended.

Table 1: The different karyotypes present in TS.

Genotype	Percentage	Phenotype
45,X	50—60%	The most severe type with the highest incidence of structural abnormalities.
Mosaic forms (eg 45,X/46XX; 45,X/47XXX etc)	20%	A higher mean height, and spontaneous puberty and menses in up to 40% of the cases.
Isoforms (e.g. 46 X,i(Xq), and ring-chromosomes (e.g. 46,Xr(X)), and deletions (e.g. 46,X,Xp-; 46,X,Xq-),	30%	In TS with isochromosome 46X,i(Xq), common in 6–18% of all TS, structural abnormalities are uncommon, however increased risk of auto-immunity, particularly thyroiditis and inflammatory bowel disease (IBD) exist [52,53]. Mental retardation is associated with the presence of ring chromosomes (r(X)) [54].

Prevalence

Turner syndrome is relatively common, 1:2000–1:2500 female births, and approximately as many as 15% of spontaneous abortions have a 45,X karyotype. About 90% of 45,X pregnancies result in spontaneous abortion. There are no known ethnic or racial factors influencing the incidence. TS is not associated with advanced maternal age [55,56].

Clinical features

The phenotype of Turner syndrome is always female. Short stature and gonadal dysgenesis are the main characteristics in Turner syndrome (TS), present in 80–100%. The features, as described below, can be present in a patient with Turner syndrome, although there is remarkable phenotype variability between individuals. This can partly be explained by the phenomena of X-inactivation and genotype variability. The phenotype/genotype correlation in TS is generally poor. Genes involved with stature, such as the SHOX-gene, lymphatic development, naevus formation, ovarian development, and the cognitive phenotype in TS are expressed on the X-chromosome [57-60]. Variability of inactivation of these genes leading to varying degrees of haploinsufficiency, especially in the pseudoautosomal regions, partly explains the considerable phenotype variation seen in TS.

Short stature (80–100%) is due to a moderate intrauterine growth retardation, a lower growth velocity during childhood and the absence of the pubertal growth spurt. A low birthweight has been associated with a breakpoint between Xq13.3–q22, whereas cases with a breakpoint between Xq22–q27 had a normal birthweight [61]. The mean final height of untreated Caucasian females with TS is between 143–147 cm

(see Figure 1) [62,63]. There is no convincing evidence that patients with karyotype 45,X differ from those with another chromosomal pattern. The underlying mechanisms leading to the stunted growth in girls/women with Turner syndrome are poorly understood. Physiological and stimulated growth hormone levels are normal [64]. IGF-I levels are subnormal, but within the normal range. Furthermore, the short stature is very unlikely due to a form of skeletal dysplasia, since the structure and biochemical composition of the epiphysial cartilage are normal [65].

Short stature and skeletal abnormalities (see below) in Turner syndrome are related to the SHOX gene (***s***hort stature ***ho***meobox containing gene on the ***X*** chromosome) [57,66]. The SHOX gene is part of the pseudo-autosomal region located at the terminal ends of both sex chromosomes (on Xp22). The phenotypes associated with SHOX gene deficiency vary widely, and are dependent on the dose of the deficient gene.

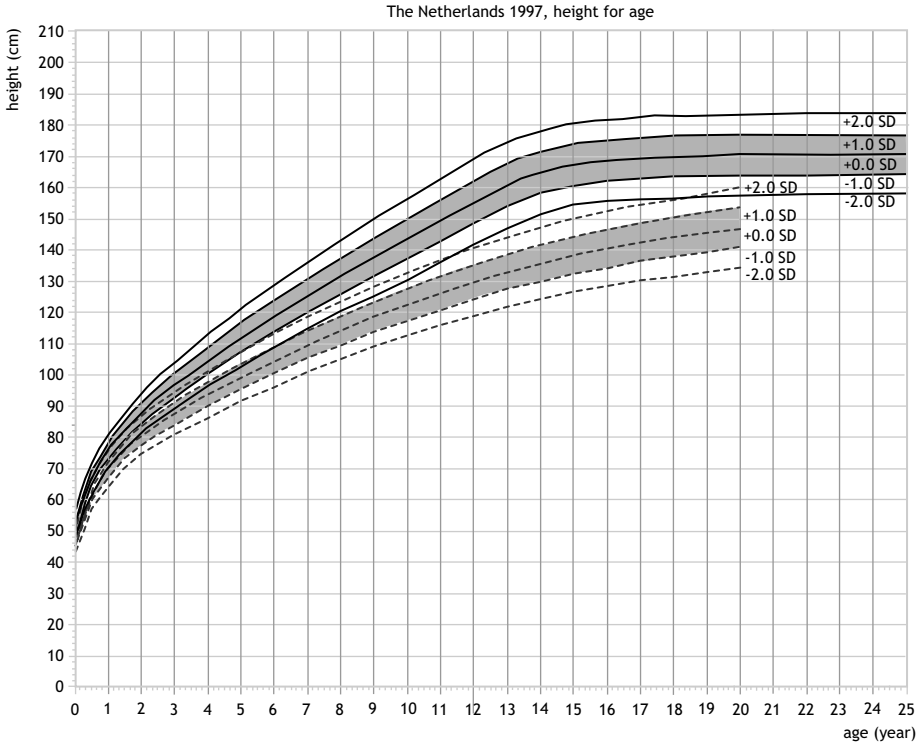
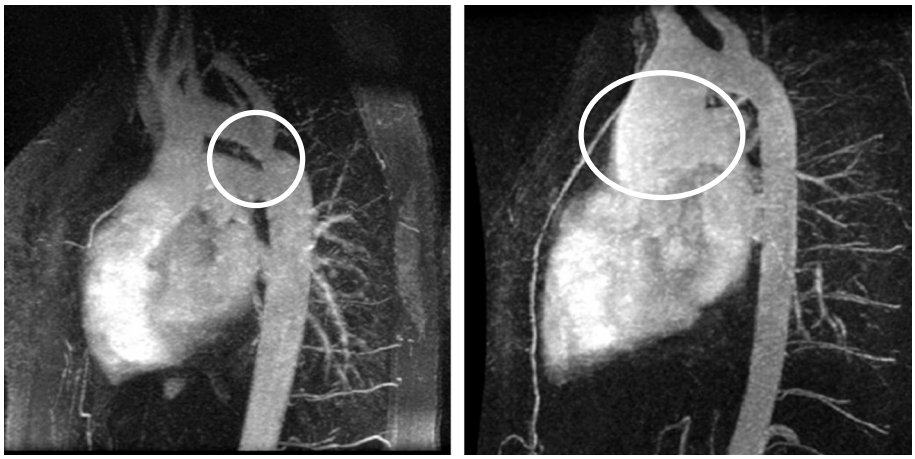


Figure 1: Reference curve for healthy girls (straight line) [67] and for untreated girls with Turner syndrome (North European references, dashed line) [62]. (Figure adapted from the Growth Analyser, www.growthanalyser.org).

Gonadal dysgenesis leading to ovarian failure is present in 80–100% of the Turner patients. During the first 3 months in utero, the ovaries develop normally [68]. Thereafter, a rapid loss of the oocytes occurs, leading to insufficient estrogen production, which is responsible for the absence of normal development of the breasts, uterus, and female body shapes [69]. Most girls with TS don't enter puberty spontaneously. Between 5 and 10% of the women with TS retain sufficient ovarian function to commence puberty spontaneously [70]. The frequency of spontaneous puberty in mosaicism was significantly higher compared to the 45,X karyotype. However, very few women will maintain ovarian function (app. 2–5%), in rare occasions resulting in spontaneous pregnancy [71-73]. There is evidence that a locus (or loci) for ovarian failure maps to one region of the X chromosome, Xp11.21-p22.1 [59].

Structural cardiovascular malformations (CVM) occur in 45–75% of patients with Turner syndrome (TS) [74-79]. Imprinting may play a role in its origin [80]. Congenital bicuspid aortic valve (25–55%) and coarctation of the aorta (30%) (see Figure 2a) are the most common of the cardiovascular malformations. Hypertension is also frequently seen in TS, even without cardiac or renal malformations [81]. These are risk factors for aorta dilatation and aneurysm. In 3–8% of the TS women, aortic dilatation, mainly of the aortic root (see Figure 2b), is present. Aortic root dilatation in TS is even described in children and adolescents with TS [82,83]. In recent studies, however, aortic dilatation was even present in up to 25–50% of patients with TS and CVM [76,79,84]. Aortic dilatation is also frequently seen in TS without other cardiac abnormalities. A rare, but potentially devastating result of aortic dilatation can be aortic dissection. Several reports have been published on aortic dissection in women with TS [77,83]. Especially the number of case-reports on dissection during pregnancy continues to rise [85,86], as oocyte donation is becoming more accessible for TS women. An obvious reason for this finding is that known predisposing factors for aortic dissection such as hypertension and cardiac malformations are seen more frequently in TS than in normal women [75]. Another suggestion to explain the increased risk for arterial dilation and dissection in women with TS is a connective tissue defect [77,87].

Also development of ***cardiovascular disease (CVD)*** is more frequently present in patients with TS. Patients with TS are more prone to develop risk factors for CVD, such as hypertension, dyslipidemia, diabetes mellitus, obesity, and hyperinsulinemia. It has even been reported that CVD is the main cause of their decreased life span [88].



2a: coarctation aortae **2b:** aortic root dilatation

Figure 2: Cardiovascular malformations occurring in Turner syndrome.

Visible features of ***neck, mouth and jaw, chest and skin*** are present in 60–79% of the Turner patients. An obvious and typical feature is a short thick ('webbed') neck, and a low hairline. A cervical vertebral hypoplasia contributes to the short neck appearance [89]. Abnormalities of mouth and jaw are: high arched palatum, small and backward rotated lower jaw, and defective dental development. The thorax can be scutiform, with apparent wide nipples, and nipples are often inverted. Skin features are lymph edema of hands and feet, especially in the neonatal period, increased number of pigmented naevi, dysplasia of finger and toenails, vitiligo, and after surgical operations keloid scarring is more often present [70].

Skeleton abnormalities (40–59%) described in Turner syndrome that are SHOX related are: cubitus valgus (wide angle of arm), Madelungs deformity (present in app. 7%), and short metacarpal bones (e.g. 4^{th}). Possibly the high arched palatum and micrognathia (smaller lower jaw) are also SHOX related [66]. Other skeleton abnormalities often present in Turner patients are: square-shaped "shield" chest, spongiose bone structure, and scoliosis. Osteoporosis is common in the adult patient.

Renal abnormalities are present in 40–59% of the Turner patients, and can exist of structural malformations, such as 'horseshoe kidney', renal aplasia, duplication of the renal pelvis and ureters, unilateral and bilateral malpositioning of the kidneys, and ureters with an abnormal course. Most of these abnormalities do not have serious consequences and do not cause diminished functioning [70].

Ears and eyes abnormalities, including deformed auricles, lower and backwards rotated ear implant, ptosis of the eyes, strabismus, color-vision impairment, and myopia are common in patients with TS [70,90]. Anatomic distortion of Eustachian tubes and impaired middle-ear ventilation predispose patients with TS to otitis media that can lead to permanent hearing loss.

Increased risk for developing auto-immune diseases, such as, thyroid disorders (hypothyroidism, Hashimoto's thyroiditis), celiac disease, and diabetes mellitus exists in the patient with TS [53,91,92]. The prevalence of autoimmune thyroid disease doubles from the 1st to the 3rd decade in life. There is a discrepancy between the high frequency of abnormal laboratory findings and the frequency of clinical illness. Thyroid antibodies can occur in up to 60% of the females with TS, while only between 20–30% of the adult women with TS have hypothyroidism (compared to 1.5% of adult women in the general population). The prevalence of celiac disease in TS is reported to be 6.4% in TS women aged between 8 and 36 years old, compared to < 1% in the general population [92]. The subclinical picture occurs in 60% of the patients. The exact pathogenesis of thyroid and other autoimmune diseases in women with TS is unknown. The isochromosome Xq is associated with a higher risk for the development of autoimmune disorders [50].

Body shape in girls and women with TS is more stocky compared to normal female body shape. Their trunk is relatively large, so are their hands and feet. The shoulders and pelvis are broad compared to height [93,94]. As a group, females with TS have a predisposition to become ***overweight*** compared to normal women, with a higher body mass index (BMI) [93,95].

There is a wide variety of ***skin manifestations*** in TS, such as pigmented naevi, naildysplasia, alopecia areata, keloid tendency, premature skin aging, psoriasis, and vitiligo [96].

Although ***intelligence*** is usually normal in TS, girls and women with TS reported to have more often ***psychosocial problems***, such as impaired social relationships, a lower self-esteem, and less sexual activity. Furthermore, they were more likely to meet criteria for attention-deficit hyperactivity disorder than controls and were often employed in jobs for which they were overqualified. One study in untreated women with Turner syndrome assessed the ***quality of life*** in terms of working status, daily routine and love and marriage [97]. This study reported that most of the unmarried women lived with their parents, were well-educated and worked as normal women. However, they appear to be anxious about their body and marriage. Mental

retardation has only been associated with a small subset with a ring X-chromosome abnormality [98].

Furthermore, Turner patients have more difficulties with mathematics and ***visual-spatial tasks*** compared to their peers [99]. In general, females with TS experience ***motor impairment*** [100]. A small, nonmosaic deletion of the distal short arm of the X-chromosome in adult women is associated with the same cognitive profile seen in women with TS [60], suggesting that haploinsufficiency for genes in the short arm of the X-chromosome(Xp) are responsible for the cognitive features in TS.

2 Treatment in short SGA children and girls with Turner syndrome: Growth hormone and estrogen treatment

— Small for gestational age (SGA) —

Several studies have shown the beneficial effects of growth hormone (GH) treatment on linear growth, and subsequently on adult height in short children born SGA, including the study presented in this thesis [3,5,6,101,102]. In addition, it has been reported that ending GH after a GH-induced catch-up growth and before reaching final height, resulted in a reduced height velocity, returning to pretreatment values [103]. A distinct catch-down growth was observed after discontinuation of the GH treatment. This indicates that GH treatment has to be continued until adult height for an optimal effect.

Results of the first Dutch GH-trial in short SGA children

Efficacy

The GH-trial presented in this thesis is the first Dutch GH trial treating short SGA children. It is a randomized, double blind, dose-response multicenter study, which started in 1991. Seventy-nine short SGA children were enrolled (age: 3–9 yrs for girls, 3–11 yrs for boys), and were treated with either 1 mg GH/m^2/day or 2 mg GH/m^2/day until adult height. All were pre-pubertal at start of GH. Height normalized in the majority of the children within 2 years after start of GH therapy [2], and was continued until start of puberty. GH had no influence on the age at onset and progression of puberty compared to appropriate-for-gestational age controls (AGA), regardless the GH-dosage [104]. Duration of puberty and pubertal height gain were not significantly different between both GH-dosage groups. In this respect there were no significant differences between GH treated SGA girls and untreated SGA girls, as published in the literature [30]. GH treated SGA boys receiving the lower GH dose of 1 mg/m^2/day, were 0.5 years older at pubertal onset than untreated SGA boys, as published in the literature [30].

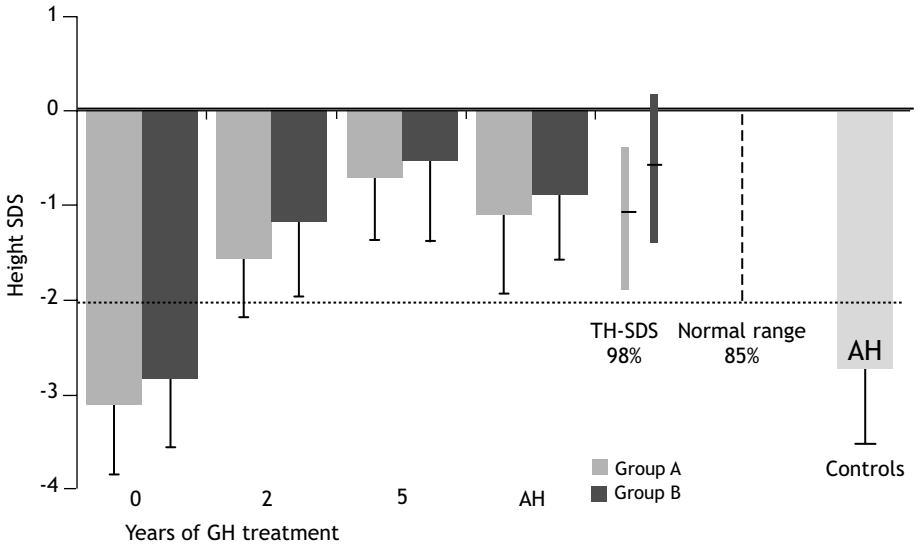


Figure 3: Height SDS of GH-treated short SGA children of the first Dutch GH-trial in short SGA children (adapted from Y. van Pareren [3]). Group A: 1 mg GH/m^2/d, group B: 2 mg GH/m^2/d. 98% Of the GH treated SGA children had an adult height within their Target height range (TH-SDS, based on parental height). 85% Of the GH treated SGA children had an adult height within the normal range. Controls: Parallel group of short SGA children who did not receive GH treatment. AH = Adult height.

Ninety-eight percent of the children reached an adult height within the target-height range, and 85% within the normal range (above −2 SDS) (Figure 3) [3]. The mean gain in height SDS from start of GH treatment until adult height was 1.8 (0.7) SDS for the GH-dose of 1 mg/m^2/d, and 2.1 (0.8) SDS for a GH-dose of 2 mg/m^2/d, which was not significanlty different. The adult height improvement was 11−13 cm in girls and 12−14 cm in boys.

Safety

GH treatment is known to decrease insulin sensitivity [105,106]. A low birth weight has been associated with increased risk of developing diabetes type 2 in adult life [13]. Therefore, extra care has been provided for evaluation of glucose intolerance and signs of insulin resistance. During GH treatment, fasting and stimulated insulin levels increased significantly [42], indicating a relative insulin resistance, which was accompanied by increased insulin release to maintain normoglycaemia. Values of glycosylated hemoglobulin (HbA1c) remained normal. Six months after end of GH treatment, the GH-induced insulin resistance disappeared, and fasting and stimulated insulin levels returned to normal [107].

A low birth weight has also been associated with increased risk of hypertension and dyslipidemia. During GH treatment, the significantly increased systolic blood pressure (0.7 SDS), which was present at start of GH therapy, decreased to a normal systolic blood pressure [45]. Diastolic blood pressure was normal at start of GH-treatment, and also decreased significantly during GH treatment. Serum lipids were normal, and remained stable during GH treatment. Six months after end of GH treatment, these beneficial effects of GH on blood pressure and serum lipids had not changed [107].

A reduced sensitivity to insulin-like growth factor I (IGF-I) may play a role in the poly-endocrinopathy which is associated with short SGA [108]. The IGF-I system plays an important role in growth and development, and IGF-I mediates the growth promoting effects of growth hormone (GH). The IGF-I system has also been suggested to be involved in common diseases such as atherosclerosis, diabetes and neoplasias [16,17,109-112]. Free IGF-I is believed to represent the bioactivity of the IGF-I system. GH therapy increases total and free levels in healthy subjects [113]. During growth hormone (GH) treatment in SGA children, total IGF-I levels increased up to the upper normal range [2]. The effect of GH-therapy on free IGF-I levels in GH-treated short SGA children is not yet known.

Other possible GH effects in SGA

As mentioned earlier, short stature during childhood and adolescence, resulting in a short adult height occurs in 10−15% of the individual born small for gestational age (SGA). Short children can suffer from physical, social and psychological problems. Being born SGA has also been associated with lower intelligence, impaired school performance, low social competence and behavioral problems. After 8 years of GH treatment, performal and total IQ-scores, behavior, and self-perception had improved significantly over time from a significantly lower value to values comparable to Dutch peers [33]. In addition, the taller the child became, the less problem behavior it showed. The effect of GH treatment on quality of life (QoL) is still not known.

— Turner syndrome —

I GH treatment

Efficacy

Several studies have proven the beneficial effects of GH therapy on growth velocity, and subsequently on adult height in girls with Turner syndrome, including the study presented in this thesis [4,7-9,114]. Previously, it has been shown that the parental origin of the retained X-chromosome was of no influence on the response to GH treatment [115].

The GH-trial presented in this thesis is the Dutch GH dose-response study, treating girls with Turner syndrome. It was a randomized, multicenter dose-response study, which started in 1989. Sixty-eight untreated girls with TS (age: 2–11 yrs) commenced in this study. The diagnosis was confirmed by lymphocyte chromosomal analysis. The patients were randomly assigned to group A, B or C, receiving different GH-dosages. Group A (n = 23) received 1.3 mg/m^2 body surface/day (equivalent to 0.045 mg/kg), group B (n = 23) 1.3 mg/m^2/d during the 1st year, followed by 2 mg/m^2/d (≈ 0.0675 mg/kg/d) and group C (n = 22) 1.3 mg/m^2/d during the 1st year, 2 mg/m^2/d during the 2nd year, and thereafter 2.7 mg/m^2/d (≈ 0.090 mg/kg/d).

In most of the TS girls, the GH therapy resulted in normalization of height during childhood and adulthood, with puberty induction at a normal age of 12.7 (0.7 SDS) yrs old. A GH-dosage of 1.3 mg GH/m^2/d (group A) leaded to an average height of 157.6 cm, which is equal to an SD-score of -1.6 when using references for normal Dutch girls [4]. A higher GH-dose resulted in a significantly higher adult height, group B: 162.9 cm (equal to SD-score of −0.7), and group C: 163.6 cm (equal to SD-score of −0.6). The adult height between group B and group C was not significantly different. Treatment resulted in a mean height improvement of 11.9–16.9 cm (Figure 4).

Safety

GH treatment is known to decrease insulin sensitivity in normal adults [105,106]. In untreated girls and women with Turner syndrome glucose intolerance, insulin resistance and non-insulin and insulin-dependent diabetes mellitus are more common than in healthy girls and women [88,116-118]. Therefore, concern has been expressed regarding possible effects of supra-physiolocial GH-dosages in girls with TS. During GH treatment, fasting and stimulated insulin levels increased significantly [119]. Fasting glucose levels increased also significantly, but stayed within the normal range, stimulated glucose levels showed no change. Individual values of glycosylated hemoglobulin (HbA1c) remained normal. Six months after GH discontinuation, fasting and stimulated insulin levels decreased to normal levels, however they remained

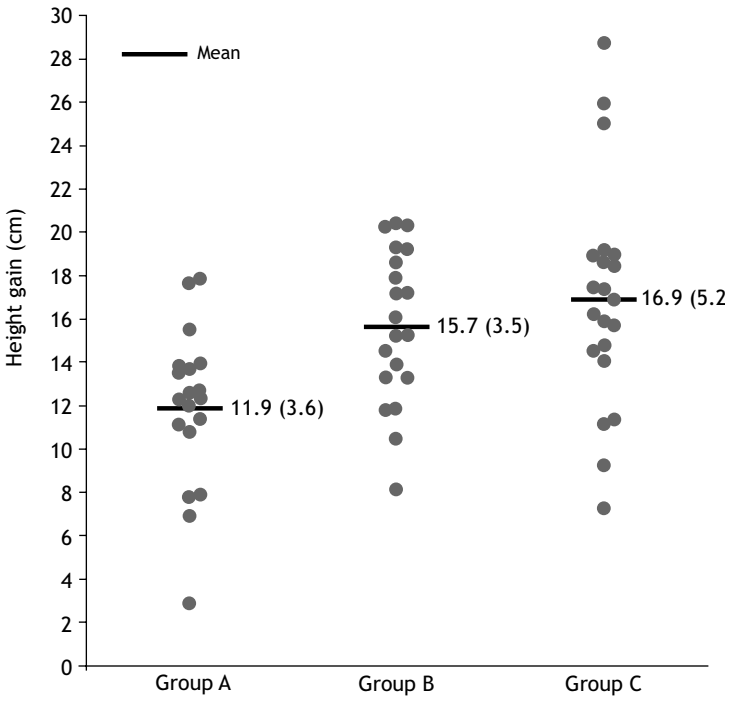


Figure 4: Height gain (difference in cm between adult height and modified projected adult height) of GH treated girls with TS in the Dutch GH dose-response trial (adapted from Y. van Pareren [4]). Group A: 1.3 mg GH/m^2/d, group B: 2 mg GH/m^2/d, group C: 2.7 mg GH/m^2/d. Mean (SD) height gain is given (cm).

above pre-treatment levels [120]. Fasting glucose levels also decreased after GH discontinuation.

Hypertension and dyslipidemia are also more common in patients with TS. During GH treatment the SD-scores of the systolic blood pressure did not change significantly, while the diastolic blood pressure SD-scores decreased [121]. Six months after GH discontinuation, there was a decrease in blood pressure compared to pre-treatment, but it remained slightly higher than in girls matched for age [120]. During the first 4 years of GH-therapy, total cholesterol, low-density lipoprotein (LDL) and atherogenic index (AI) decreased, high-density lipoprotein (HDL) and triglycerides increased [120]. Six months after GH discontinuation compared with 4 yrs lipid levels, total cholesterol and LDL had increased, and triglyceride levels had decreased. Possible explanations for the changes in lipid levels may be the effect of increasing age, or a GH effect.

Patients with TS have a higher risk of congenital or developed cardiovascular malformations and abnormalities. TS patients were treated with supra-physiological

GH dosage, despite normal endogenous GH-levels. In patients with acromegaly GH hypersecretion is associated with an increased morbidity and mortality from cardiovascular disease. An increased left ventricular mass has been reported in acromegalic patients. GH treatment in TS patients, even at the highest dosage of 2.7 mg GH/m^2/d, did not result in left ventricular hypertrophy [121].

IGF-I mediates the growth promoting effects of growth hormone (GH). The IGF-I system has also been suggested to be involved in common diseases such as atherosclerosis, diabetes and neoplasias [16,17,109-112]. Free IGF-I is believed to represent the bioactivity of the IGF-I system. GH therapy increases total and free levels in healthy subjects [113]. During growth hormone (GH) treatment in girls with TS, total IGF-I levels increased, with mean around the upper normal range [4]. Six months after GH discontinuation, total IGF-I levels had fallen to levels comparable to normal age matched girls, but remained higher than pre-treatment levels. The effect of GH-therapy on free IGF-I levels in GH-treated patients with TS is not yet known.

II Estrogen treatment

In order to induce puberty, synthetic or natural estrogens have to be given to girls with Turner syndrome. Route and timing of the start of estrogen therapy, as well as the dose and form, are still a point of discussion. Estrogens have a biphasic effect on growth: stimulatory at low doses and inhibitory at higher doses [122]. Thereby, estrogens cause an acceleration of bone maturation, which subsequently reduces the growth phase and therefore may result in decreased adult height [123]. However, a delay in pubertal development may have serious psychosocial consequences, as the maturation of female identity is also essential at this age in girls. Progestagens should be added for at least 10 days/month, to prevent the development of endometrial carcinoma. Another option is to use both, estrogens and prostagens at the same time continuously. The advantage of the latter therapy is no menstrual bleedings after the use of this therapy for one year. However long term outcome of this therapy is unknown.

Additional effects of estrogens are their positive effects on bone mineral density, lipid profile, liver enzymes, physical fitness [124], cardiovascular system and neurocognitive development [125,126]. Theoretically, starting a low dose of oestrogen therapy before the planned induction of puberty could be favourable for these additional effects, particularly the neurocognitive development. However, starting estrogens before the age-appropriate time is not advantageously for adult height [127,128].

In the Dutch GH dose-response trial, as presented in this thesis, micronized 17ß-estradiol was given to the girls of 12 yrs and older, after at least 4 years of GH

treatment, in order to induce puberty. During GH treatment, in the first 2 years, 5 µg/ kg body weight/ day was given orally, following by a dose of 7.5 µg/kg/d in the 3rd year, and 10 µg/kg/d thereafter. Cyclic progesterone therapy 5 mg/d in the first 14 days of the month was added after 2 years of estrogen therapy. At discontinuation of GH therapy, estrogen treatment was increased to an adult dose of 1 mg/day, and later on to 2 mg/day. Progesterone dosage was increased to 10 mg/d.

Recently, from ours and other studies it appeared that, when GH treatment has been optimized, it is not necessary to delay the induction of puberty [4,129], and in a low dose, estrogens do not negatively influenced height velocity or adult height.

Other possible effects of the GH and/or estrogen treatment in TS

Women with TS have a different body shape, their figure appears more stocky than that of normal women, with relatively short lower extremities, relatively large hands and feet, and relatively broad shoulders and pelvis [93,94,130]. During GH treatment, the pre-existing disproportion between height and sitting height in TS improved moderately, however the disproportionate growth of the feet, partly due to the natural development in TS, may be also influenced by higher GH dosage [94].

Short children can suffer from physical, social and psychological problems. After long-term GH therapy, behaviour of the TS women was comparable to normal Dutch peers [131]. In contrast, their self-perception and their attitude towards their body where slightly less positive, and they had a slightly different coping pattern than their normal Dutch peers. There was no evidence of depression. Thus, some women with TS still experience psychosocial problems, also after reaching a height within the normal range.

The effect of GH treatment or the accompanying gained height on quality of life (QoL) is still not clear.

3 Aims of the study and outline of this thesis

The studies presented in this **doctoral dissertation** were part of two long-term GH-trials in:

1) **Short children born Small for Gestational Age (SGA), started in 1991.**
2) **Girls with Turner Syndrome (TS), started in 1989.**

A time frame of both studies is shown in Figure 5.

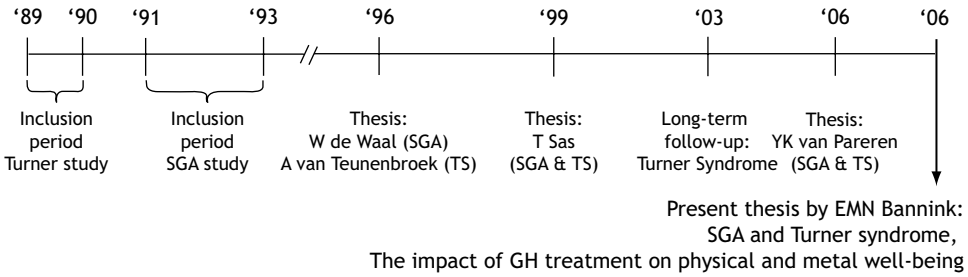


Figure 5: Time frame.

Previous results of both studies were presented in the following theses:
***1996*: "Influencing extremes of growth. Too tall - too small"**, by W. de Waal.
In this thesis preliminary results of the first years of the GH-trial in short SGA children were reported.
***1996*: "Growth hormone treatment modalities in girls with Turner Syndrome"**, by A. van Teunenbroek.
In this thesis the four-year results of the GH-trial in TS girls were reported
***1999*: "Long-term growth hormone treatment in two growth disorders"**, by T.C.J. Sas.
Part: Children with short stature born SGA: reported the five-year results of the GH-trial in short SGA children.
Part: Girls with Turner Syndrome: reported the seven-year results of the GH-trial in TS girls.
***2006*: "Added Centimeters and Their Repercussions. How effective and safe is growth hormone in the treatment of short stature in girls with Turner syndrome and in children born small for gestational age?"**, by Y.K. van Pareren.
In this thesis adult height, psychosocial consequences and 6 month (short-term) follow-up results of both studied populations were reported.

This doctoral dissertation gives a detailed report of various studies performed on the effects of long-term growth hormone treatment during GH therapy and after end

of GH treatment in short children born SGA and girls with TS, as described below. The studies performed are not necessarily in the sequence in which they were carried out.

Serum IGF-I levels (*Chapter 2 and 3*)

Most of the GH effects are due to GH stimulated production of insulin-like-growth-factors (IGF). The IGFs are tightly bound, both in the circulation and in tissues, to a family of 6 proteins, known as the IGFBPs. IGFBP-3 is the major binding-protein of IGF-I. Therefore the molar ratio of total IGF-I/IGFBP-3 is assumed to correspond to free IGF-I levels. Free IGF-I is believed to represent the bioactivity of IGF-I. GH stimulates the production of total IGF-I and IGFBP-3. The total IGF-I levels rise above the normal range in GH-treated short SGA children, as well as in GH treated TS girls. Whether free IGF-I levels are also increased during GH treatment, which may have long-term consequences, remains to be investigated. Furthermore, the value of free IGF-I measurement in addition to total IGF-I and IGFBP-3 is of interest. Both short and long term effects of the exposure of SGA children and TS girls to high circulating IGF-I levels during childhood are not well documented.

The aim of this part of the study was to assess **free-dissociable IGF-I levels** in short SGA children and TS girls before, during and after long-term GH therapy. Additionally, to analyze whether free IGF-I, total IGF-I, IGFBP-3, or the molar ratio of total IGF-I/ IGFBP-3 during GH therapy were related to various IGF-I bioactivity outcome parameters, such as adult height, growth response, glucose and insulin levels. In addition, it was infestigated whether free IGF-I measurements are of value in evaluating the GH treatment.

Quality of life (*Chapter 4 and 5*)

Quality of life is increasingly recognized as an important measure of the impact of a special disorder, disease or therapeutic outcome.

The aim in this part of the study was to evaluate the **quality of life** after long-term GH treatment in the two studied populations presented in this thesis, short SGA children and TS girls.

Puberty induction (*Chapter 6*)

Most girls with TS do not start puberty spontaneously due to ovarian failure. Therefore, puberty has to be induced with exogenous estrogen therapy in most of the TS girls. Route and timing of the of start of estrogen therapy as well as the dose and form are still a point of discussion. Estrogens have a biphasic effect on growth: stimulatory at low doses and inhibitory at higher doses. Thereby, estrogens cause an acceleration of bone maturation, which subsequently reduces the growth phase

and therefore may result in decreased adult height. However, a delay in pubertal development may have serious psychosocial consequences. Previously, a puberty induction starting at an appropriate age, as described in this study, has been proven not to influence height velocity or adult height.

The aim of this part of the study was to describe the effects on **breast development** and **uterine dimensions**, when puberty induction was started at an appropriate age, using a low dose of 17ß-estradiol, and to compare the pubertal development with normal Dutch girls. Additionally, to discuss whether **serum levels of estradiol, gonadotropins, and sex hormone binding globulin** are useful in evaluating induced pubertal development.

Long-term effects after end of GH treatment (*Chapter 7 and 8*)

TS is associated with a number of diseases and conditions, besides short stature and hypogonadism, such as congenital cardiovascular malformations, and risk factors for cardiovascular disease (CVD). Furthermore, untreated TS women show a different, more stocky body than normal women, with relatively short lower extremities, relatively large hands and feet, and relatively broad shoulders and pelvis. The effect of GH on these conditions, during GH therapy and short term after GH discontinuation, has been described previously in the TS population presented in this thesis.

The aim of this part of the study was to investigate the **risks for cardiovascular disease, carbohydrate metabolism, and the body proportions,** in young women with Turner syndrome, **nearly 5 years after end of long-term GH treatment**, and compare them with normal and, if possible, with untreated women with Turner syndrome (C*hapter 7*).

Furthermore, to assess **biophysical properties and dimensions of the aorta** in TS, and to evaluate a possible relation to the long-term GH therapy or GH-dosage applied (C*hapter 8*).

Chapter 9 discusses the significance of this study, and brings data in perspective. ***Chapter 10*** summarizes the results of the complete study, dated from 1991 for the SGA population, and 1989 for the Turner population, and provides an answer to the question: “Was it – and is it – all worthwhile?”. ***Chapter 10*** also includes recommendations for future research. Finally, ***Chapter 11*** summarizes the findings of this thesis in English, as well as in Dutch.

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Chapter 2

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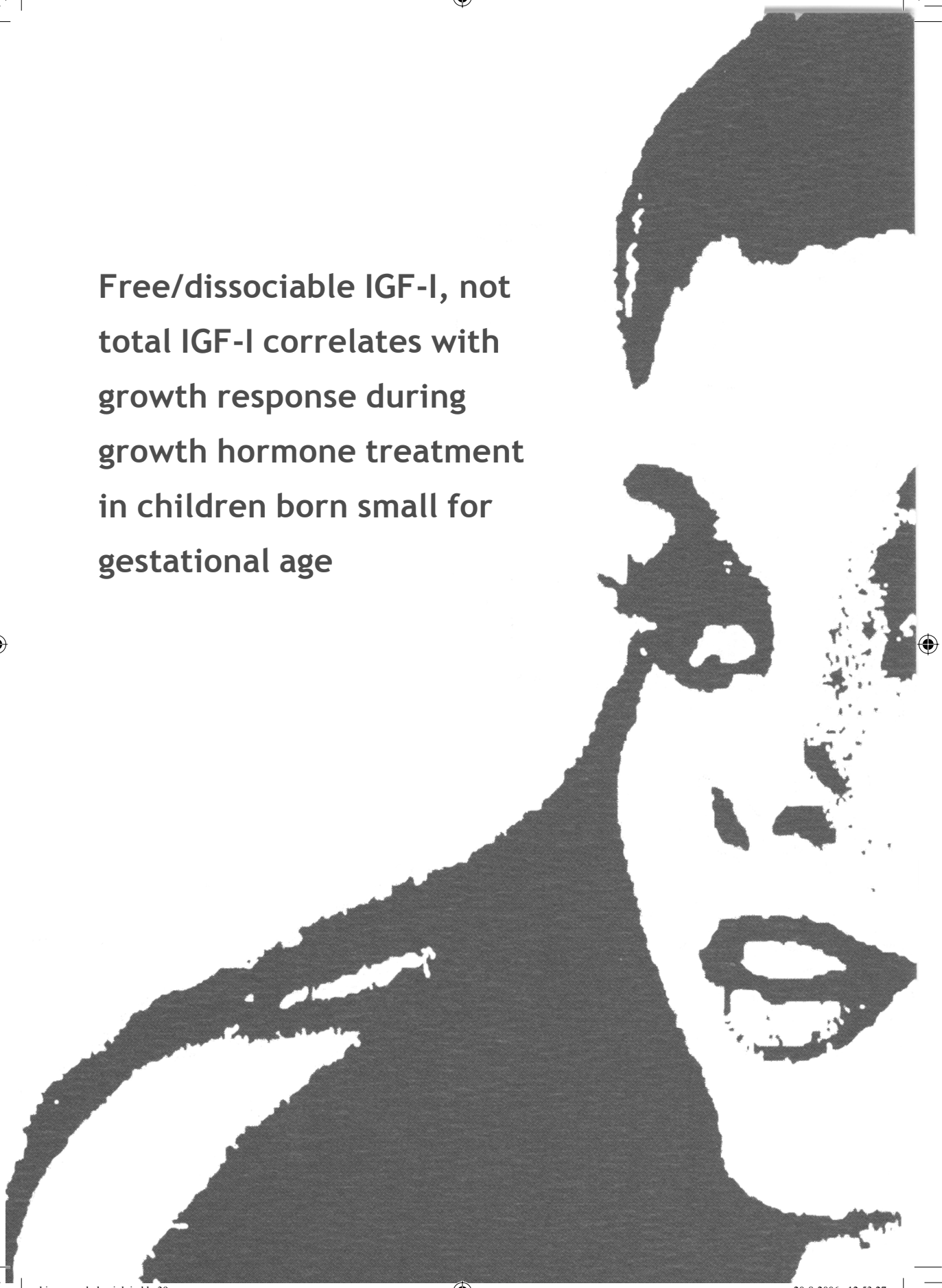
Submitted

Free/dissociable IGF-I, not total IGF-I correlates with growth response during growth hormone treatment in children born small for gestational age



Abstract

Context: IGF-I plays an important role in pre- and postnatal growth. Its serum levels are regulated by metabolic and genetic factors. Mean total IGF-I in short SGA children is reduced, but within the normal range. Free/dissociable IGF-I is the bioactive form of IGF-I.

Objective: Investigating changes in free IGF-I during GH-treatment in short SGA children. Evaluating if free IGF-I levels contribute to predicting first year growth response and/or adult height.

Design, setting & intervention: Randomized double-blind GH dose-response study with either a GH-dose of 1 mg/m^2/d (group A) or 2 mg/kg/d (group B). Free IGF-I, total IGF-I, IGFBP-3 were determined at baseline, after 1 and 5 years, at stop, and 6 months after GH discontinuation.

Patients: 73 (46 male) short SGA children (36 group A), baseline mean age 7.7 (2.2) years, mean GH-duration 8.2 (2.1) years.

Main outcome: Untreated SGA children showed mean free IGF-I SDS of −0.2 (1.2), not related to total IGF-I. During GH-therapy, free IGF-I significantly increased to 1.6 (0.7) SDS, as did total IGF-I and IGFBP-3 (2.0 (0.8), 1.3 (0.9) SDS, resp.). Multiple regression analysis showed a negative correlation between baseline free IGF-I SDS and one-yr growth response and adult height, independently of baseline total IGF-I SDS. Total IGF-I, IGFBP-3, or their ratio showed no correlations with growth response.

Conclusions: Circulating baseline free IGF-I was a better predictor for short-term growth response as well as for adult height in GH-treated SGA children than were total IGF-I, IGFBP-3, or their ratio. Suggesting a possible role for free IGF-I measurement in predicting the effect of GH therapy in short SGA children.

Introduction

About 10–15% of the infants born small for gestational age (SGA) fail to show spontaneous catch-up growth during the first 2 years of life, and have an increased risk of being short as adults [1-3]. Various factors have been mentioned with respect to the stunted postnatal growth, such as intrauterine reprogramming, genetic variations, reduced growth hormone (GH)-secretion, and reduced sensitivity for IGF-I [4-6].

IGF-I plays an important role in both pre- and postnatal growth and its serum levels are regulated by GH and by metabolic and genetic factors. In neonates born SGA, low circulating IGF-I levels have been observed [7-10]. Short SGA children exhibit plasma IGF-I levels that are in the lower-normal range, approximately between −1 to −1.5 standard deviation score (SDS) [11-13].

Several studies demonstrated that GH treatment in short SGA children results in normalization of height during childhood and adulthood [14-18]. During GH treatment in SGA children, a rapid increase in total IGF-I levels and a slower increase in IGFBP-3 levels occurs [19]. Controversies exist on the role of total IGF-I and/or IGFBP-3 levels in the growth response. It has been reported that the growth response showed a positive association with the short-term increase in total IGF-I [19], and an inverse relation to baseline IGF-I levels [19,20]. Other authors reported a positive correlation between the growth response and the change in both total IGF-I and IGFBP-3 in SGA children [21]. However, in another study on short SGA children one could not confirm these results [22]. This apparent discrepancy may be due to the use of different definitions for SGA, leading to differences in inclusion criteria and study groups.

In the circulation, IGF-I is mainly bound to IGF binding proteins (IGFBPs), of which six classes have been identified (IGFBP- 1 to – 6) [23]. The GH-dependent IGFBP-3 is the major carrier protein of IGF-I in the circulation, normally accounting for more than 90% of the IGF-binding [24]. Under normal circumstances, less than 1% of the total plasma IGF-I pool is in the unbound free biologically active form [25,26], which exchanges rapidly with the tissue compartments [23]. To our knowledge, free IGF-I levels have not been studied in SGA children before and during GH treatment. The first aim of our study was to evaluate free IGF-I levels at start and during GH therapy until adult height. Secondly, we wanted to determine if free IGF-I levels would contribute in predicting the first year growth response and adult height.

Patients and Methods

GH trial

Ninety short children born SGA participated in a multi-center GH trial in the Netherlands, which started in 1991. Inclusion criteria for the dose-response trial were: birth length SDS below −2, chronological age (CA) between 3 and 11 years in boys and 3 and 9 years in girls, height SD-score for CA below −2, no spontaneous catch-up growth during last year, pre-pubertal stage, and uncomplicated neonatal period without severe asphyxia, sepsis, or lung problems [12,15].

The GH trial evaluated the effect of GH on long-term growth and adult height. In brief: to stratify for spontaneous GH secretion during a 24-hour GH profile, a group of 79 children was divided into three groups: 'normal profile', 'GH insufficient profile', and 'no profile performed'. Twenty-four-hour GH profiles were performed at baseline in 39 of the children, as previously described [27], an arginine stimulation test was performed in all 79 children. After stratification for age and spontaneous GH secretion during a 24-h GH profile, all children were randomly and blindly assigned to one of 2 GH-dosage groups: group A receiving 1 mg/m^2/d (~33 µg/kg/d), or group B receiving 2 mg/m^2/d (~67 µg/kg/d) [15]. GH was given double blindly. In addition, the remaining 11 short SGA children were treated parallel to the trial with a known dose of 2 mg/m^2/d, because they were older than the maximum age according to the inclusion criteria. Three-monthly, the total GH-dose was adjusted to the calculated body surface. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneous once daily until adult height, defined as height velocity below 0.5 cm during the previous 6 months. Every 3 months height and weight were measured. Height was expressed as SD-score for chronological age and gender, using the references for healthy Dutch children (28). Adult height was expressed as SD-score for gender and an adult age of 20 years.

Study subjects and design

For the present study we only investigated patients who received GH-therapy for ≥ 5 years. Seven of the 11 older subjects were excluded, because they were treated less than 5 years until adult height, whereas 3 other subjects were still being treated with GH at time of analysis, and could therefore not be included. At start of the present study, seven had dropped out of the GH trial, and were lost to follow-up due to the following reasons: lack of motivation (n = 3), moving abroad (n = 2), treatment for precocious puberty (n = 1), and biochemical signs of GH insensitivity (n = 1). Thus, 73 subjects remained eligible for the present study.

Five of them were GH-deficient (GHD), defined as having IGF-1 and IGFBP-3 below −2 SD and peak GH-levels below 20 mU/l after stimulation with arginine. Seven had a

clinical diagnosis of Silver-Russell Syndrome (SRS). All 73 subjects were prepubertal at start and after 1 year of GH therapy.

Both EDTA-plasma and serum samples were taken between 09.00 and 12.00 hours at baseline, 1 year and 5 years after start of GH-therapy, before discontinuation of GH-therapy (final year), and 6 months after ending GH-therapy (post GH). Thus, a maximum of 5 samples per subject was analyzed. The baseline, 1st year and post GH samples were collected after an overnight fasting. All samples were stored appropriately at −80°C until assayed.

Levels of free IGF-I, total IGF-I, and IGFBP-3

Free IGF-I levels were measured in serum with a commercial two-site immunoradiometric assay (IRMA) using a commercial kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA). This IRMA detects both the unbound IGF-I and the easily dissociable IGF-I [29]. The inter-assay CV was 9.7%, calculated from data produced by the investigators, measured at serum levels of 0.26 and 3.41 ng/ml. There was no difference in CV between the low and high level. In order to establish normative range values for circulating free IGF-I, serum samples were collected from healthy children (116 girls and 211 boys, aged between 0 and 17 years) who underwent minor surgical procedures. Serum samples were obtained and stored at −80°C in well-capped tubes until analysis. Smoothed references for free IGF-I were constructed using the LMS method, designed for constructing normalized standards of nonparametric data, described by Cole [30].

The free IGF-I assays were performed by the same investigator (EB), in the same laboratory, under standardized circumstances, i.e. the assays were performed at 5ºC, serum samples were kept at 5ºC, and incubated for exactly 2 hours. The various serum samples investigated in the present study had not been thawed previously. However, we did investigate the effect of repeated freezing and thawing (3 times) of several (n = 13) serum samples on the free-IGF-I levels, but could not find a significant effect (i.e. not larger than the inter-assay variation). Moreover, samples tested again after a year of storage showed the same results. Using 13 healthy adult volunteers working in our department, we compared free IGF-I levels in sera obtained after an overnight fast with those in the non-fasting state. No significant differences were found. This finding is in agreement with a previous report on this subject, showing that, despite a marked elevation in IGFBP-1, overnight fasting did not influence circulating free IGF-I levels [31].

Total IGF-I plasma levels were measured by a semi-automated chemiluminescence technique (Advantage, Nichols Institute Diagnostics, San Juan Capistrano, USA). Plasma IGFBP-3 levels were measured by specific RIA, as described previously [32].

LMS smoothed normative range values for total IGF-I, IGFBP-3 and the molar ratio between total IGF-I andIGFBP-3 have been previously determined in the same laboratory [32,33].

Statistical methods

Unless indicated otherwise, results are expressed as mean (SD). Differences over time and between the GH-dosage groups were analyzed using repeated measurement analyses. Spearman's rho correlations were used to assess correlations between SD-scores of free IGF-I, total IGF-I, IGFBP-3 and total IGF-I/IGFBP3 molar ratio, and 24-hour GH-profiles outcome measures. The outcome measures for the 24-h GH profiles were the number of peaks, mean pulse amplitude, and the area under the curve above zero-level (AUC_0).

Multiple regression analyses were used to assess the relationships between either free IGF-I, total IGF-I, IGFBP-3 or total IGF-I/IGFBP3 ratio, and first year change in height SDS and final height SDS outcome, respectively. Each laboratory parameter was entered separately in the model, along with age at start of GH therapy, target height (TH) SDS, and GH-dose. In the model for adult height SDS we also included height SDS at start of GH treatment. These multiple regression analyses were repeated for the subgroup of patients who underwent a 24-hour GH profile. The 24-hour GH profiles outcome measures were included into the models separately. The percentages of variance explained by the model (R^2 in %) and the adjusted R^2 (%) are given. A P-value of < 0.05 was considered statistically significant. All calculations were performed with SPSS 11.5.

Ethical considerations

The Medical Ethics Committee of each participating center approved the trial protocol. The Medical Ethics Committee of the Erasmus Medical Center approved the collection of reference serum samples in healthy children. Written informed consent was obtained from each participant older than 12 years, and parents.

Results

Study subjects

The clinical characteristics of the 73 children participating in this study are shown in Table 1. There were no significant differences between the two GH-dosage groups. The difference in target height between the two GH-dosage groups nearly reached significance ($P = 0.06$).

Table 1: Clinical characteristics of the two GH groups.

	Group A 1 mg GH/m^2/d n = 36	Group B 2 mg GH/m^2/d n = 37	Total group n = 73
Male (%)	26 (72%)	20 (54%)	46 (63%)
Gestational age (weeks)	37.2 (3.3)	36.3 (4.2)	36.7 (3.8)
Birth length SDS	−3.5 (1.4)	−3.6 (1.6)	-3.5 (1.5)
Birth weight SDS	−2.6 (1.2)	−2.5 (1.0)	-2.5 (1.1)
Age (years): Baseline	7.5 (2.0)	7.9 (2.5)	7.7 (2.2)
At stop GH	16.0 (1.2)	15.8 (1.5)	15.9 (1.4)
Height SDS: Baseline	−3.1 (0.7)	−3.1 (0.7)	−3.1 (0.7)
Adult height	−1.5 (0.9)	−1.2 (1.0)	−1.4 (0.9)
Target height	−0.9 (0.9)	−0.5 (0.9)	−0.7 (0.9)
Height SDS gain[a]	1.6 (0.7)	1.9 (0.7)	1.7 (0.8)
BMI SDS: Baseline	−1.2 (1.3)	−1.2 (1.1)	−1.2 (1.2)
Post GH	0.0 (1.2)	0.1 (0.9)	0.1 (1.1)
Duration of GH therapy (years)	8.5 (1.9)	7.9 (2.2)	8.2 (2.1)

Data expressed as mean (SD) or number (n). Post GH = 6 months after end of GH.

[a]: Adult height SDS − height SDS at start GH therapy

TH_{boys} = ½ * ($Height_{father}$ + $Height_{mother}$ + 12) +3

TH_{girls} = ½ * ($Height_{father}$ + $Height_{mother}$ − 12) + 3

Free IGF-I levels

The serum free IGF-I levels in the reference group showed an age-dependent increase (Figure 1). Pre-pubertal girls had higher free IGF-I levels than boys, but during puberty boys had higher free IGF-I levels than girls.

At baseline, for both groups of short SGA children, serum levels of free IGF-I SD-scores were not different from 0 SDS (mean SDS(SD) group A: -0.3(1.3), group B: −0.2 (1.2)) (Figure 2). There were also no significant differences between the two GH dosage groups during GH treatment and thereafter. During GH therapy, the mean(SD) values of free IGF-I SDS in the total group of SGA subjects increased significantly from -0.2 (1.2) to 1.6 (0.7). After the first year, the free IGF-I SDS did not further increase. After 5 years of GH-treatment, 34.7% of the study subjects had free IGF-I SDS above the +2 SDS. Six months after the end of GH therapy, the mean free IGF-I SD-score had decreased to 0.5(1.0), which was significantly higher than baseline SDS. During GH therapy, 27.5% of all free IGF-I measurements were > + 2 SDS, 0.5% > + 3SDS, and 32 of the 73 patients (44%) never exceeded the +2SDS.

There were no significant differences in free IGF-I levels between SGA patients with or without GHD, neither between SGA patients with or without SRS, at any point in time [34].

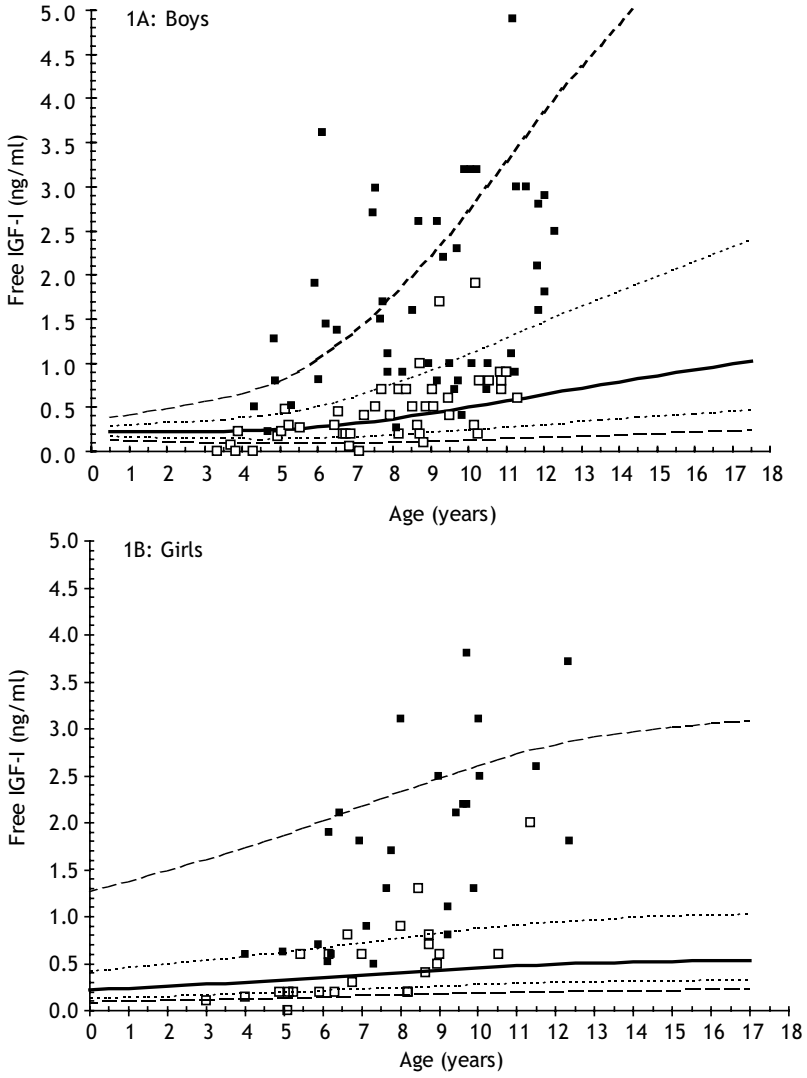


Figure 1: The mean of the Dutch reference population is represented by a solid line (—), the −/+1 SDS by a dotted line (····), and the −/+2 SDS by a broken line (----). Serum levels of free IGF-I in short boys (1a) and girls (1b) born SGA, before start of GH treatment (*open boxes*) and after 1 year of GH treatment (*closed boxes*).

Total IGF-I and IGFBP-3 levels

At baseline, mean total IGF-I SDS and IGFBP-3 SDS were significantly lower than 0 SDS (−0.9 (0.9) and −1.2 (1.2) resp.) (Figure 2), whereas mean SDS for the total IGF-I/IGFBP-3 molar ratio did not significantly differ from zero (−0.1 (0.2)) (Figure 2). Also, for these parameters no significant differences were noted between the two GH dosage groups, at any point in time.

For the total group of SGA subjects, after 1 and 5 years of GH therapy, mean total IGF-1 SDS had increased to 1.4 (1.0) and 1.95 (0.8) SDS, mean IGFBP-3 SDS to 0.5 (1.1) and 1.2 (0.9), and the molar ratio SDS to 1.3 (1.6) and 1.3 (1.0), respectively.

Mean IGFBP-3 SDS after 5 years of GH therapy of 1.2 (0.9) declined to 0.2 (1.0) SDS at GH-discontinuation, which is in absolute values a decline from 139 (31) to 108 (40) nmol/l. Mean IGFBP-3 SDS at GH-discontinuation was significantly lower compared to the value found after 5 years of GH-therapy, but did not significantly differ from that after 1 year of GH-therapy.

Six months after GH discontinuation, SDS for total IGF-I, IGFBP-3, and total IGF-I/IGFBP-3 molar ratio had decreased, but the values of these parameters were still higher than those found at baseline.

Relationships between free IGF-I, total IGF-I, IGFBP-3, and total IGF-I/ IGFBP-3 molar ratio and height

***Pre-treatment*:** At baseline, there was a weak, but significant correlation between free IGF-I SDS and height SDS ($r = 0.25$, $P < 0.05$). Baseline free IGF-I SDS was also positively correlated with baseline IGFBP-3 SDS ($r = 0.41$, $P < 0.001$), but not with baseline total IGF-I and the total IGF-I/IGFBP-3 molar ratio SD-scores ($r = 0.08$, $P = 0.49$ and $r = -0.17$, $P = 0.15$, respectively). Baseline total IGF-I SDS, IGBP-3 SDS, and total IGF-I/ IGFBP-3 molar ratio SDS did not show any correlation with baseline height SDS. At baseline there were no correlations between any of the 24-hour GH profile characteristics or the stimulated GH peak and height SDS, free IGF-1, total IGF-1, IGFBP-3, or total IGF-1/IGFBP-3 ratio.

***During GH treatment*:** After 1 and 5 years of GH treatment, free IGF-I SDS showed a positive relationship with total IGF-I SDS ($r = 0.46$ ($P < 0.001$) and $r = 0.32$ ($P < 0.01$) respectively), and after 1 year of GH with the SDS value of total IGF-I/IGFBP-3 molar ratio ($r = 0.45$, $P < 0.001$). There was no correlation between free IGF-I SDS and IGFBP-3 SDS after 1 and 5 years of GH-treatment.

***First year growth response*:** For the change in height SDS during the first year, the multiple regression model including the independent variables: age at start of GH therapy, TH SDS, and GH-dose, explained 38.8% (R^2) of the variance (Table 2). Adding baseline free IGF-I SDS significantly increased the R^2 to 48.3% ($P < 0.01$), contributing nearly 10% to the prediction of the first year change in height SDS. When, instead of or in addition to free IGF-I, baseline total IGF-I SDS or IGFBP-3 SDS were added, this did not improve the model significantly ($P = 0.4$). First year's change in free IGF-I SDS was not related to first year change in height SDS.

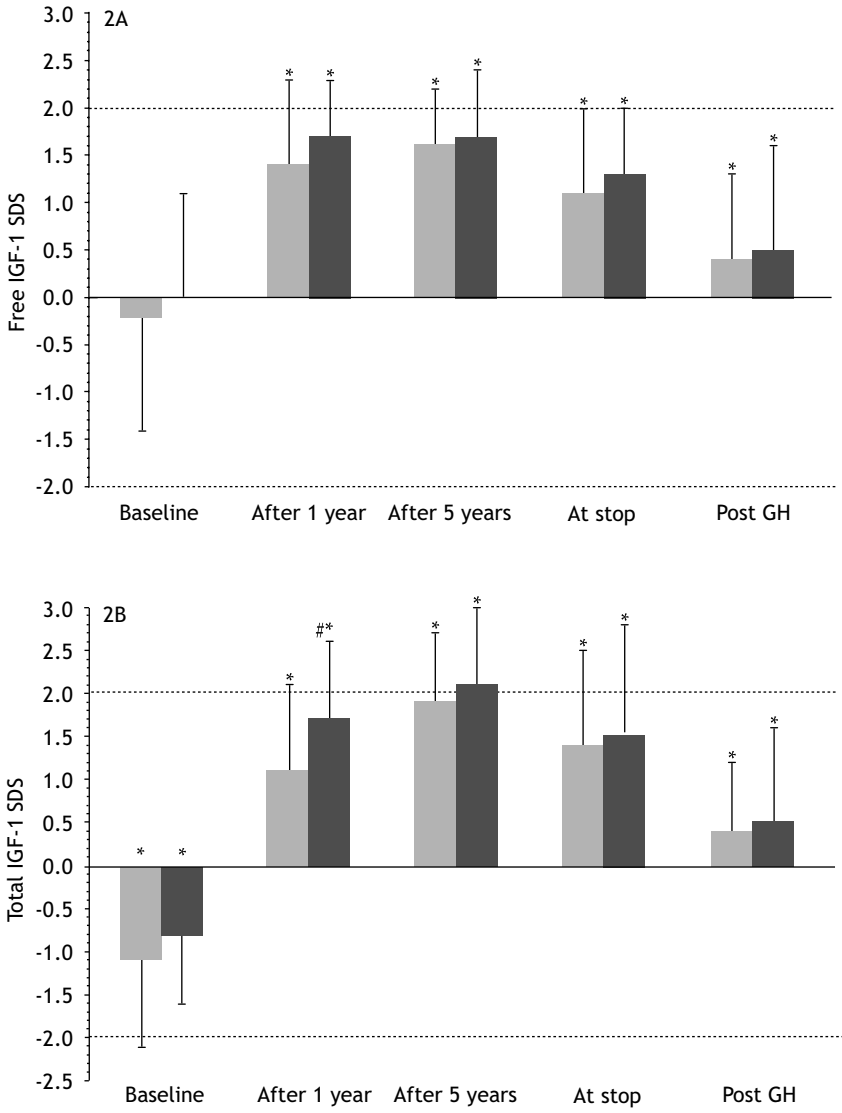
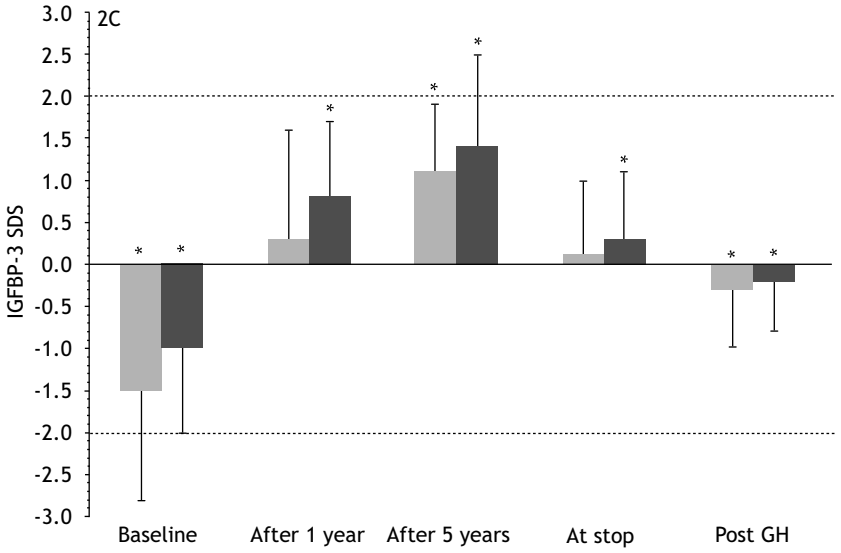
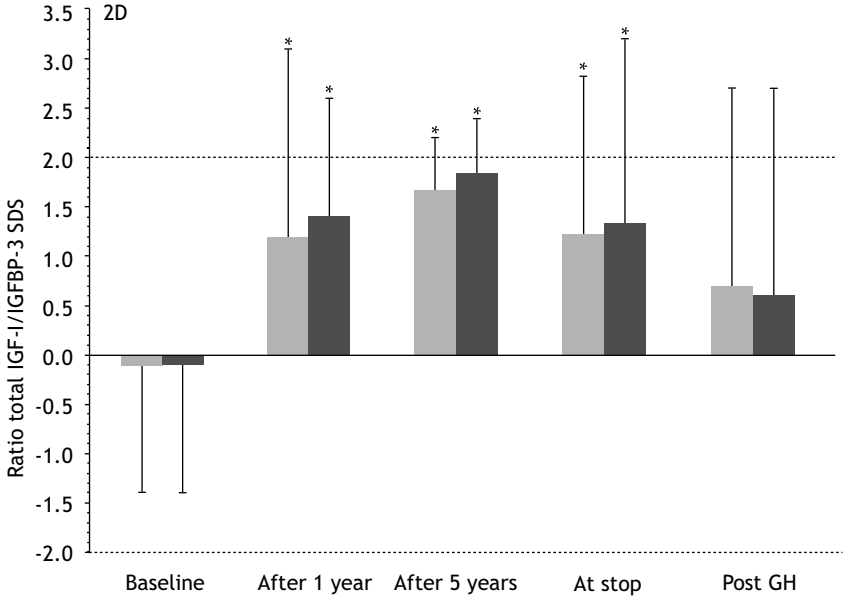


Figure 2: SD-scores of free IGF-I (2A), total IGF-I (2B), IGFBP-3 (2C), and the ratio total IGF-I/IGFBP-3 (2D) for group A (*light grey*) and group B (*dark grey*) are shown before, during and after GH-treatment. Normal range (−/+2 SDS) is shown (*dotted lines*).

*: Significantly different from 0 SDS.

#: Significantly different from group A. (If GH discontinuation was at 5 years, patient was only included in 'at stop' sample, not in both 'after 5 years' and 'at stop')





In the subgroup of 39 SGA patients of whom 24-hour GH profiles were available, the model including baseline free IGF-I SDS along with the independent variables, age at start of GH therapy, TH SDS, and GH-dose, showed an R^2 of 44.6%, and an adjusted R^2 of 37.4%. Adding either the AUC_0 or mean peak amplitude to the model, improved the model significantly, explaining the variance of the first year growth response for 56.4% and 54.2%, respectively (adjusted R^2: 49.1% and 46.6%, respectively), whereas adding the number of peaks was of no influence.

***Adult height SDS prediction*:** For adult height SDS, the model including the independent variables age at start of GH therapy, TH SDS, height SDS at start of GH, and GH-dose explained 40.0% (R^2) of the variance in adult height SDS (adjusted R^2: 36.4%). Adding baseline free IGF-I SDS, the R^2 significantly increased to 46.8% ($P < 0.01$, adjusted R^2 42.6%). Subsequent addition of baseline IGFBP-3 SDS, improved the model even further ($P = 0.01$, R^2 52.7%, adjusted R^2 48.2%). The addition of baseline total IGF-I SDS did not improve the model further ($P = 0.5$). The optimal multiple regression models for first year response and adult height are shown in Table 2.

When free IGF-I was not included in the model, baseline total IGF-I together with the independent variables in a multiple regression model also improved the model significantly, but less pronounced (maximum R^2 was 44%).

None of the 24-h GH-profile characteristics were of significant influence on adult height outcome.

Table 2: Multiple regression models for first year change in height SDS and adult height SDS.

Dependent variable	Independent variables	B	se	ß	P
Change in height SDS after 1 year	Age at start (years)	− 0.048	0.014	− 0.348	0.001
	TH SDS	− 0.055	0.031	− 0.164	0.082
	GH dose (mg/m^2/d)	0.295	0.056	0.487	< 0.001
	Free IGF-I SDS at start	− 0.079	0.028	− 0.293	0.006
R^2 (Adjusted R^2)	**48.3% (45.0%)**				
SD of the residual	**0.226**				
Adult height SDS	Age at start (years)	0.034	0.046	0.079	0.467
	TH SDS	0.184	0.114	0.176	0.111
	Height SDS at start	0.715	0.149	0.525	< 0.001
	GH dose (mg/m^2/d)	0.486	0.174	0.258	0.007
	Free IGF-I SDS at start	− 0.193	0.089	− 0.232	0.033
	IGFBP-3 SDS at start	− 0.224	0.080	− 0.287	0.007
R^2 (Adjusted R^2)	**52.7% (48.2%)**				
SD of the residual	**0.683**				

B: Unstandardized coefficients, ß: standardized coefficients, *P: P* value.

Discussion

Our study describes free IGF-I levels in short children born SGA, before, during and after GH treatment. Mean free IGF-I SD-values were normal in short SGA children, in contrast to the total IGF-1 and IGFBP-3, which were significantly lower than 0 SDS. During GH-treatment, free IGF-I increased, but stayed largely within the normal range, whereas total IGF-I increased to a greater extent than free IGF-1, reaching the +2 SDS. After ending GH-treatment, free IGF-I declined to similar levels as total IGF-1 did, whereas IGFBP-3 declined further. However, all remained slightly higher than baseline levels. The SD-scores are not influenced by differences in pubertal onset with the normal population, as puberty in GH treated short SGA children was comparable to normal children, and started at the same age [34].

Baseline free IGF-I was inversely related to the first year growth response and adult height in contrast to baseline levels of total IGF-I. Also IGFBP-3 SDS was inversely related to adult height SDS. All of the characteristics of the 24-hour GH profiles were inversely related to first year growth response, but not to adult height or any of the baseline IGF-I parameters.

For the present study we used, under strictly controlled conditions, the DSL IRMA for the measurement of free IGF-I levels in serum. In fact, this assay determines the sum of truly free IGF-I and readily IGFBP-dissociable IGF-I which is thought to represents a more biologically relevant pool than free IGF-I alone [25]. There was no difference between fasting and non-fasting state. Serum free IGF-I levels in healthy boys and girls appeared to increase with age and are higher in prepubertal girls than in boys, which is in agreement with earlier reports [25,26,35].

Most samples were analyzed at the end of the study, several years after collecting them. According to our experience total IGF-I and IGFBP-3 in EDTA plasma or serum is very stable, i.e. after more then 10 years of storage at −80°C the values do not change significantly. However, we did not have the opportunity to examine the stability of free-IGF-I upon long-term storage. The samples showed the same results after testing again after up to three years of storage. The samples used had not previously been thawed, and were stored in well-capped tubes at −80°C.

At baseline mean, serum free IGF-I SDS in short SGA children was not significantly different from zero SDS, either with or without GHD. Serum levels of total IGF-I and IGFBP-3 were, however, significantly lower than normal, which has previously been reported [13,19,22,36]. In our study, there were also no differences in free IGF-1 levels between SGA children with and without GHD during GH therapy and thereafter. Since free IGF-I levels are reported to be decreased in untreated GH-deficient children and adults [25,35,37], our results suggests that these children with GHD have a different pathophysiology of short stature than SGA subjects with GHD.

Baseline free IGF-I SDS correlated inversely with first year growth response and adult height SDS, also after adjusting for baseline levels of total IGF-I and IGFBP-3. This suggests that at baseline the free fraction of the total IGF-I level in the circulation has a predictive value not only for first year growth response to GH-therapy, but also for adult height SDS in short SGA children, irrespective of baseline total IGF-I levels.

We did not find a correlation between baseline free IGF-I SDS and baseline total IGF-I SDS. This finding is in contrast with observations under conditions of primary abnormalities in the GH secretion, such as GHD and acromegaly, where usually a close relationship is found between serum free IGF-I levels and total IGF-I levels and total IGF-I/IGFBP-3 molar ratios [38]. One explanation might be that short stature in SGA is due to genetic variations in the IGF-I gene, resulting in altered IGF-I binding to their various binding proteins [39,40].

There was no correlation between the change in free IGF-I and first year growth response. Apparently, the relationship between the concentration of free-IGF-I in the circulation and growth (tissue) response is different before and during GH treatment. As total IGF-I increases more than free IGF-I during GH treatment, it might be that there is an increased clearance of free IGF-I from the circulation to the tissues, due to a very short half life of free IGF-I in the circulation compared with that of total IGF-I [38]. However, the nearly equal SDS levels of total IGF-I and free IGF-I during GH treatment does not support this theory.

One has to consider, however, that the levels of IGF-I or IGFBP-3 measured were circulating levels. Unfortunately, we could not measure locally produced IGF-I levels. In this respect it must be emphasized that circulating concentrations of IGF-I (and IGFBP-3) may not necessarily reflect those occurring at the various tissue compartments, since there is also an (unknown) contribution of locally produced growth factor.

In conclusion, our study showed that untreated short children born SGA have normal free IGF-1 levels, whereas total IGF-I and IGFBP-3 were decreased. Baseline serum free IGF-I was a better predictor for short term growth response as well as for adult height in GH-treated SGA children than were total IGF-I, IGFBP-3, or total IGF-I/IGFBP-3 ratio. This finding suggests a role for free IGF-I measurement in the prediction of growth response of short SGA children at start of GH therapy.

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Chapter 3

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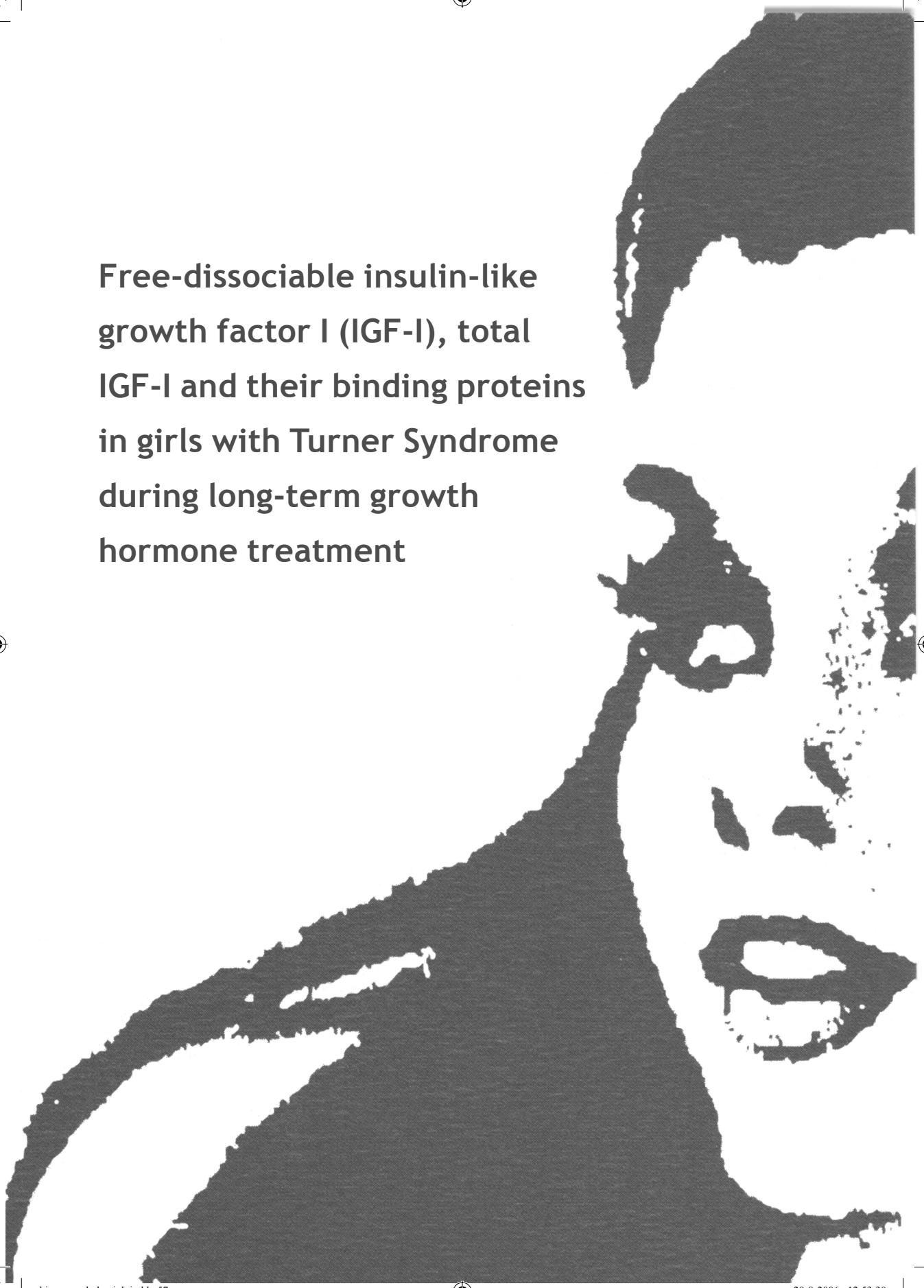
Free-dissociable insulin-like growth factor I (IGF-I), total IGF-I and their binding proteins in girls with Turner Syndrome during long-term growth hormone treatment



Abstract

Objective: To investigate the effect of GH-treatment on free IGF-I levels in TS girls, and to verify relationships between free IGF-I levels and total IGF-I, IGFBP-1, 2 and 3. Additionally, to analyse whether free IGF-I, total IGF-I, IGFBP-3, or its ratio, were related to IGF-I bioactivity outcome parameters.

Design: Sixty-five TS girls were randomly assigned to 3 different GH-dosage groups (1.3, 2.0, and 2.7 mg/m^2/d). Mean duration of GH therapy was 8.7(2.0) years. Free IGF-I, total IGF-I, IGFBP-1,-2,-3 were determined at baseline, first, second, third, and fifth yr of GH therapy, before start of estrogen therapy, final yr of GH treatment, 6 months after GH, and 4.8(2.0) years after GH discontinuation.

Main outcome: During GH treatment, mean free IGF-I levels stayed < +2 SDS, while mean total IGF-I and IGF-I/IGFBP-3 ratio were > +2 SDS. There were no differences in free IGF-I levels between the 3 GH-groups, whereas total IGF-I and ratio levels were significantly higher in the highest GH-group. The following variables contributed significantly to predicting the square-root of free IGF-I levels: age, GH dose, estrogen dose, IGFBP-1, IGFBP-3, BMI, and total IGF-I or total IGF-I/IGFBP-3 ratio. However, the explaining variance did not exceed 55%. Several IGF-I bioactivity outcome parameters positively correlated with total IGF-I and IGF-I/IGFBP-3 ratio, whereas free IGF-I did not.

Conclusions: During long-term GH therapy in TS girls, mean free IGF-I levels stayed within the normal range, while mean total IGF-I and total IGF-I/IGFBP-3 ratio exceeded the upper normal range. Although total IGF-I and the total IGF-I/IGFBP-3 ratio did not accurately represent free IGF-I levels, they seemed to be better representing the IGF-I bioactivity than measured free IGF-I.

Introduction

The insulin-like growth factor (IGF) system plays an important role in growth and development. IGF-I mediates the growth-promoting effects of growth hormone (GH), and exerts negative feedback on pituitary GH secretion. In the circulation, it is bound mainly to IGF binding proteins (IGFBPs), six classes of which have been identified (IGFBP- 1 to – 6) [1].

Under normal circumstances, less than 1% of the total plasma IGF-I pool is considered to be present in the unbound free biologically active form [2,3], which exchanges rapidly with the tissue compartments [1]. The GH-dependent IGFBP-3 is the major carrier protein of IGF-I in the circulation, normally accounting for more than 90% of the IGF-binding [4]. Hence, the total IGF-I/IGFBP-3 ratio has been proposed to be a surrogate measure of biologically available free IGF-I

Since recombinant human GH became available in 1985, increasing numbers of children with short stature have been treated, including those with Turner Syndrome (TS). On average, untreated TS patients reach an adult height that is 20 cm shorter than their healthy peers. The exact cause of the stunted growth is still unknown. Despite normal GH secretion and normal to subnormal IGF-I levels, GH treatment can normalize height during childhood [5,6], and also provide an adult height gain of 12–17 cm [7,8]. Additional estrogen therapy is necessary in most TS patients as, due to ovarian failure, there is no spontaneous puberty.

When TS girls are receiving GH treatment, the total IGF-I levels in their circulation may rise to high-normal or supra-physiological values [5]. Besides increasing total IGF-I levels, GH therapy also causes an increase in plasma IGFBP-3 levels, which may affect the levels of the biologically active free-IGF-I. However, data on free-IGF-I levels are lacking. Neither is there enough documentation on the adverse short-term and long-term effects of exposing TS patients to high circulating IGF-I levels during childhood. In a previous study on girls with TS, we encountered increased fasting and stimulated plasma insulin during GH therapy [9]. After GH discontinuation, there was also a relationship between GH dosage and aortic wall distensibility, the higher GH dose having a beneficial effect [10]. We did not investigate, however, whether these effects are related to plasma levels of total IGF-I and/or free IGF-I.

Therefore, our main objectives were to investigate the effect of long-term GH-treatment, GH dosage, and simultaneously administrated 17-β estradiol on free IGF-I levels, and to establish the relationship between the latter parameter and circulating levels of total IGF-I, IGFBP-1, 2 and 3. We also explored whether free IGF-I, total IGF-I, IGFBP-3, and/or the total IGF-I/IGFBP-3 molar ratio were related to various IGF-I bioactivity outcome parameters, such as adult height, height SDS gain, glucose and insulin levels, and aortic wall distensibility.

Patients and Methods

Study subjects

Sixty-eight previously untreated girls with Turner Syndrome (TS) participated in an open-randomized multi-center growth-hormone (GH) dose-response study in the Netherlands [7].

Diagnosis of TS was confirmed by lymphocyte chromosomal analysis. Subjects were included if their chronological age was between 2–11 years and their height lay below the fiftieth percentile according to normal Dutch references. The exclusion criteria were abnormal thyroid function, associated endocrine and or metabolic disorders, growth failure due to other causes (e.g. emotional deprivation), hydrocephalus, previous use of drugs which could interfere with the GH treatment, and spontaneous puberty. Three girls dropped out of the study due to lack of motivation.

Study design

After stratification for chronological age and height standard deviation scores (SDS), the patients were randomly assigned to group A, B or C, receiving different GH dosages. Group A (n = 23) received 1.3 mg/m^2 body surface/day (equivalent to 0.045 mg/kg); group B (n = 23) received 1.3 mg/m^2/d during the first year, followed by 2 mg/m^2/d (≈ 0.0675 mg/kg/d); and group C (n = 22) received 1.3 mg/m^2/d during the first year, 2 mg/m^2/d during the second year, with 2.7 mg/m^2/d (≈ 0.090 mg/kg/d) thereafter.

Biosynthetic human GH (Norditropin, Norditropin Simplexx, Novo Nordisk A/S, Denmark) was given subcutaneously once daily between 20.00 and 22.00 hours, using a pen-injection system. Every 3 months the dose was adjusted to the calculated body surface. In accordance with the study protocol, the GH treatment was discontinued either when height velocity (HV) was less than 1 cm over 6 months, or when the patient decided she was satisfied with the height she had achieved.

In order to induce puberty, a daily oral dose of micronized 17β-estradiol was administered to girls of 12 years and older, after at least 4 years of GH treatment. In the first 2 years, a dose of 5 µg/kg body weight/day (~0.05 µg ethinyl estradiol/kg/d) was given; in the third year the dose was 7.5 µg/kg/d, and thereafter it was 10 µg/kg/d. After GH discontinuation, the estrogen dosage was increased to 1 or 2 mg/day, i.e. the level usually provided to adults. In addition, cyclic progestagen therapy (Duphaston 5 mg/d, 14 days of the month) was initiated after 2 years of estrogen therapy. If puberty had developed spontaneously (Tanner breast stage ≥ B3) during the GH-therapy, no estrogens were given during GH-therapy.

Determination of circulating levels of free IGF-I, total IGF-I, IGFBP-3, IGFBP-2, and IGFBP-1

Blood samples were taken, between 09.00 and 12.00 hours, at baseline and subsequently every year. Both serum and EDTA plasma were obtained and all samples were stored at −80 °C in well-capped tubes until assayed. Free IGF-I, total IGF-I, IGFBP-1, IGFBP-2, and IGFBP-3 were determined at baseline, 1 year, 2 years, 3 years, and 5 years after the start of GH therapy, at the yearly visit before start estrogen therapy, in the final years of GH treatment, and 6 months after GH discontinuation. At a follow-up visit of 4.8 (2.0) years after GH discontinuation, a blood sample could be obtained from 39 out of 65 patients. A maximum of 9 samples per subject were available for analysis. All free IGF-I, IGFBP-1, IGFBP-2 measurements, most of the IGFBP-3, and some of the total IGF-I measurements were analyzed at the end of the study, i.e. between 2002 and 2003.

Free IGF-I levels were measured in serum with a two-site immunoradiometric assay (IRMA) using a commercial kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA). The IRMA assay measures the unbound IGF-I and the easily dissociable IGF-I [11]. The inter-assay CV was 9.7%. In order to establish normative range values for circulating free IGF-I, serum samples were collected from healthy children who underwent minor surgery. Serum samples from 116 girls (age: 0−17 years) were obtained and stored at −80 °C until analysis. Smoothed references for free IGF-I were constructed using the LMS method, a method for constructing normalized standards of nonparametric data, described by Cole [12].

The various free IGF-I assays were performed by the same analyst (EB), under the same circumstances in the same laboratory. They were performed at 5ºC. Serum samples were kept at 5ºC and incubated for exactly 2 hours. Using 13 healthy adult volunteers working in our department, we compared free IGF-I levels in sera obtained after an overnight fast with those derived in the non-fasting state. No significant differences were found. This finding is in agreement with a previous report on this subject, showing that, despite a marked elevation in IGFBP-1, overnight fasting did not alter circulating free IGF-I levels [13].

Total IGF-I plasma levels were measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). Serum levels of IGFBP-1, IGFBP-2 and IGFBP-3 were measured using specific RIAs, as described previously [14-16]. All assays were performed in the same laboratory.

Since the levels of total IGF-I, IGFBP-3, IGFBP-2, total IGF-I/IGFBP-3 molar ratio, and free IGF-I all depend on age and sex, all values were transformed to SD-scores using the LMS method [12], using reference values for healthy children determined in the same laboratory [15,16]. As no extended reference values of IGFBP-1 were available, plasma IGFBP-1 concentrations could not be transformed to SDS.

IGF-I bioactivity outcome parameters

Height and weight were measured at baseline and subsequently every 3 months in the same department. Height, weight, and body mass index (BMI) were expressed as SD-score using the references for healthy Dutch girls [17] or the references for North European untreated girls with TS [18]. Final height (FH) was defined as the height (in cm) most recently available after discontinuation of GH therapy. Final height SDS gain was defined as [adult height SDS − height SDS at start], according to the references for untreated girls with TS [18].

Oral glucose tolerance tests (OGTT) were performed at baseline, after 4 years of GH therapy and 6 months after GH, as described by Van Pareren *et al* [9]. Outcome measures for OGTT were fasting glucose and insulin levels, the 120-minute area under the curve (AUC) for glucose and insulin, and glucose/insulin ratio at baseline, after 30 and 120 minutes.

Aortic wall distensibilities were determined at 4 levels (ascending aorta and descending aorta at level of the pulmonary artery bifurcation, diaphragm level, abdominal aorta) according to procedures described previously [10]. Distensibilities (10^{-3} $mmHg^{-1}$) were calculated as follows: D = (Amax–Amin)/[Amin*(Pmax − Pmin)]. D = distensibility, A = aortic area (mm^2), P = blood pressure, max = systolic phase, min = diastolic phase.

Statistical methods

Unless indicated otherwise, results were expressed as mean (SD). Spearman's correlation was used to assess the relationship between free IGF-I and age before GH treatment. Differences between the dosage groups were tested with one-way ANOVA analyses, using the Bonferroni method to adjust for multiple comparison analysis. Differences over time were tested using paired *t*-tests. To assess relationships between free IGF-I and other measurable variables, a stepwise forward regression was used to construct a model for free IGF-I. Data on absolute free IGF-I level were square root transformed to achieve a normal distribution. The following variables were used: age, estrogen dose, GH dose, total IGF-I, IGFBP-3, total IGF-I/IGFBP-3 molar ratio, IGFBP-2, IGFBP-1, BMI, and karyotype (45,X yes/no). Interaction variables of GH-dose with the variables total IGF-I, total IGF-I/IGFBP-3 ratio, and IGFBP-3, were added to the model to establish whether the effect of GH on square root of free IGF-I depends on these various variables. Only variables with a $P < 0.05$ were kept in the model. Pearson's correlations were used to assess the relationship of free IGF-I, total IGF-I, IGFBP-3, and its ratio on the four IGF-I bioactivity outcome parameters, which were adult height (cm), height SDS gain, OGTT measurements, and aortic wall distensibilities. Three-year and 5-year delta SDS values were correlated to 3 and 5-year delta height SDS. To characterize the levels of free IGF-I, total IGF-I,

IGFBP-3, and total IGF-I/IGFBP-3 ratio over time, the area under the curve (AUC) from start until discontinuation of GH-therapy was calculated. The calculated AUC was correlated to adult height, height SDS gain, post GH OGTT measurements, and aortic wall distensibilities. The AUC after 4 years of GH therapy was calculated and correlated to the 4-year delta scores of the OGTT measurements. P-values of < 0.05 were considered to be statistically significant. All calculations were performed with SPSS 11.5.

Ethical considerations

The medical ethics committee of each participating center approved the trial protocol. The Erasmus Medical Center medical ethics committee approved the collection of reference serum samples. Written informed consent was obtained from each participant in this study and/or from their parents.

Results

Study subjects

The clinical characteristics of the 65 girls participating in this study (22 in group A and B, and 21 in group C) are shown in Table 1. Fifty-one patients of the 65 (78.5%) received GH for more than 7 yr. There were no significant differences between the groups, except for final height and height gain. Patients in group B and C had all reached a greater adult height than those in group A, whereas group C patients also showed a greater height gain than group A patients.

Circulating levels of free IGF-I, total IGF-I, IGFBP-3, IGFBP-2, and IGFBP-1

In normal healthy girls, the serum free IGF-I levels showed a gradual age-dependent increase (Figure 1). The same phenomenon was found for TS patients before GH treatment ($r = 0.32$, $p = 0.01$), although at that time most of them had lower values.

Free IGF-I: During GH treatment, free IGF-I levels increased (up to 7-fold) (Figure 1), but the mean free IGF-I SDS did not exceed the normal range (Figure 2a). Twenty-four and a half percent of the measurements during GH therapy were above the +2 SDS, whereas 19 of the 65 girls never exceeded the +2 SDS. Mean free IGF-I SDS did not differ significantly among the three GH dosage groups. In all cases, mean free IGF-I SDS six months after GH therapy were still significantly higher than the values encountered at baseline, being comparable to those observed after the first year of GH-therapy. However, at the follow-up visit, several years after GH discontinuation, mean free IGF-I levels had decreased to subnormal levels.

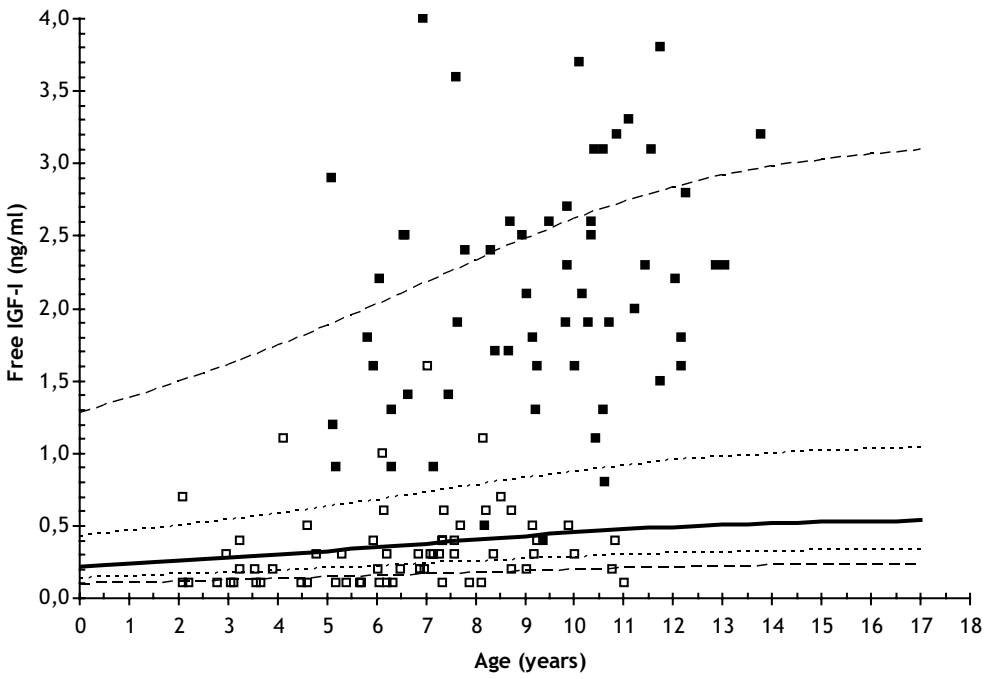


Figure 1: The mean of the Dutch female pupulation is represented by a solid line (—), the −/+ SDS by a dotted line (·····), and the −/+2 SDS by a broken line (– – –). Serum levels of free IGF-I in Turner girls before start of GH treatment (*open boxes*) and 3 years after start of GH treatment (*closed boxes*).

***Total IGF-I and total IGF-I/IGFBP-3 molar ratio*:** During GH treatment, the mean SD-scores both of total IGF-I and of total IGF-I/IGFBP-3 molar ratio were supra-physiological in group B and group C, exceeding +2 SD (Figure 2b, 2c). During GH therapy, 62.7% of all total IGF-I measurements and 56.4% of all ratio measurements were above the +2 SDS. Three of the 65 girls never exceeded the +2 SDS for total IGF-I, and 4 never exceeded the +2 SDS for the ratio.

After 3 years of GH therapy, there were no differences with respect to these parameters between the three GH dosage groups. In contrast, after 5 years of GH therapy, and also one year before initiation of estrogen treatment, total IGF-I SDS and ratio SDS were significantly higher in patients of group C than the values found for subjects of group A. In the final year of GH therapy, group C exhibited higher total IGF-I SDS values compared to patients in group A. However, 6 months after GH discontinuation and during the follow-up visit, the various differences between the patients of the different GH dosage groups were no longer present.

Table 1: Clinical characteristics at start of the GH-therapy (baseline) and at final year of GH therapy, including other variables of the study participants.

		Group A n = 22	Group B n = 22	Group C n = 21	Total group n = 65
Age (yrs):	Baseline	6.1 (2.1)	6.7 (2.4)	6.5 (2.4)	6.4 (2.3)
	Final year of GH	14.8 (0.9)	14.6 (1.4)	14.7 (0.9)	14.7 (1.1)
Height (cm):	Baseline	104.6 (12.0)	108.6 (11.7)	106.8 (12.6)	106.7 (12.0)
	Adult height	158.3 (6.4)	163.3 (5.6)*	163.8 (6.1)*	161.9 (6.4)
Height SDS[a]:	Baseline	−2.7 (0.9)	−2.4 (1.0)	− 2.6 (1.0)	−2.6 (1.0)
	Adult height	−1.6 (1.0)	−0.8 (0.9)*	−0.7 (1.0)*	−1.0 (1.0)
Height SDS gain[b]		1.7 (1.0)	2.1 (0.7)	2.5 (0.9)*	2.1 (0.8)
BMI sds:	Baseline	0.2 (1.0)	0.2 (1.1)	0.1 (1.0)	0.2 (1.0)
	Final year of GH	0.9 (1.1)	1.2 (0.9)	0.9 (0.8)	1.0 (0.9)
Duration of GH therapy before start E2 (years)		6.5 (1.9)	6.3 (2.2)	6.3 (2.1)	6.3 (2.0)
GH duration (years)		9.0 (1.5)	8.3 (2.4)	8.7 (2.0)	8.7 (2.0)
Karyotype:	45,X	19 (86%)	21 (95%)	16 (76%)	56 (86%)
	Other	3 (14%)	1 (5%)	5 (24%)	9 (14%)

Data represent the mean (SD). GH-dosage: Group A: 1.3 mg/m^2/day, group B: 1.3 mg/m^2/d during the 1st year, followed by 2 mg/m^2/d, and group C: 1.3 mg/m^2/d during the 1st year, 2 mg/m^2/d during the 2nd year, and thereafter 2.7 mg/m^2/d.

[a]: Height for age (references according to healthy Dutch girls [18]).

[b]: Final height sds – height sds at start GH therapy (references untreated Turner girls [19]) Differences between groups: * = P < 0.05 if compared to group A (lowest GH-dose). There were no significant differences between group B and C.

Six months after GH therapy and at the follow-up visit, total IGF-I SDS and ratio SDS had returned to baseline SDS levels, with the exception of the ratio SDS for group C patients that had remained higher than they had been before GH treatment. Several years after GH discontinuation, at the follow-up visit, mean IGF-I values had decreased to subnormal levels, whereas total IGF-I/IGFBP-3 molar ratios were not significantly different from normal.

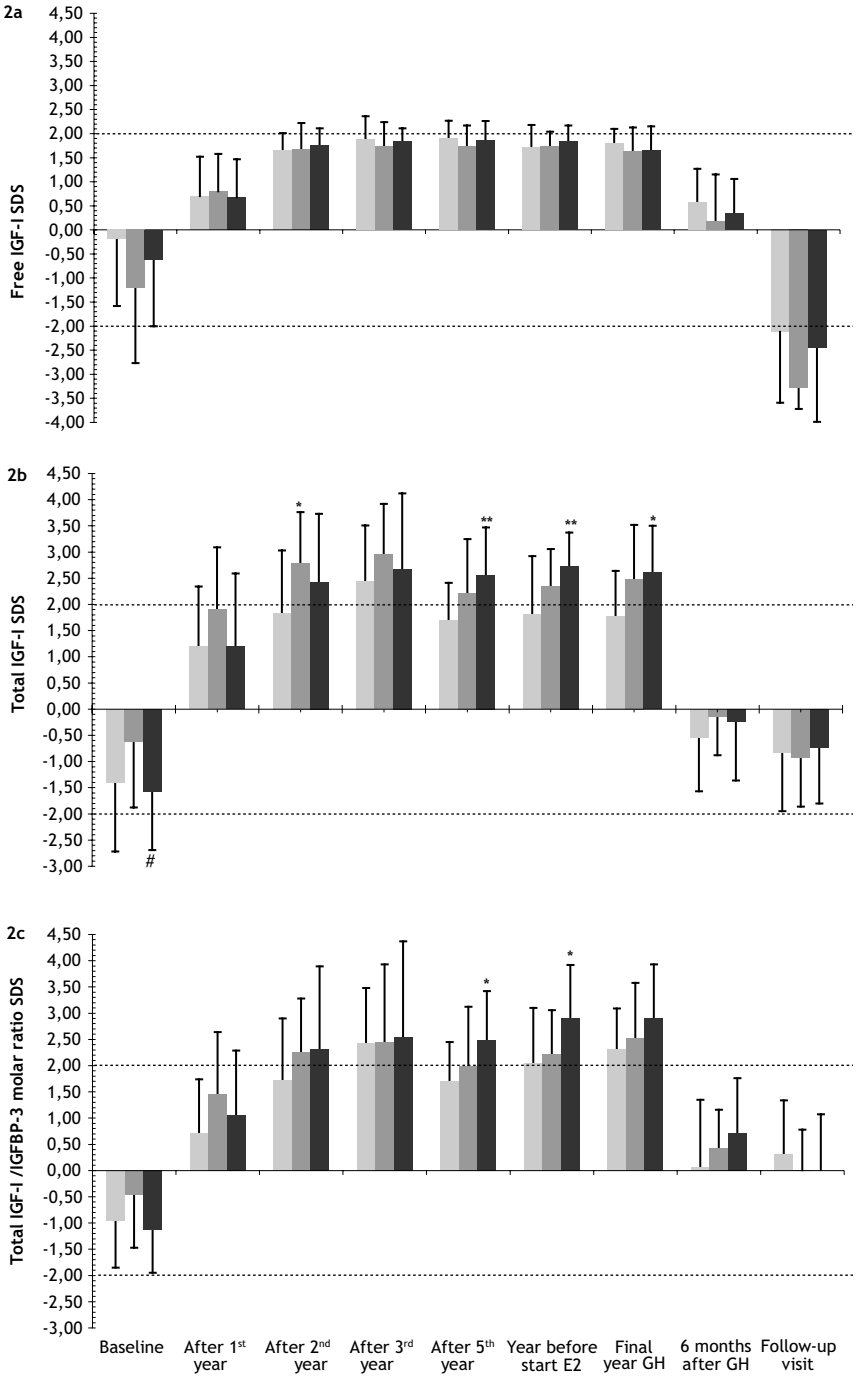


Figure 2: SD scores (SDS) of circulating levels of free IFG-I (2a), total IGF-I (2b), and total IGF-I/IGFBP-3 molar ratio SDS (2c) before, during, and after discontinuation of GH treatment for group A (*white*), group B (*grey*), and group C (*black*), respectively. Data represent the mean +/− 2 SDS. Follow-up visit was 4.8 (2.0) years after GH discontinuation.

* P < 0.05, ** P < 0.01 compared to group A, # P < 0.05 compared to group B.

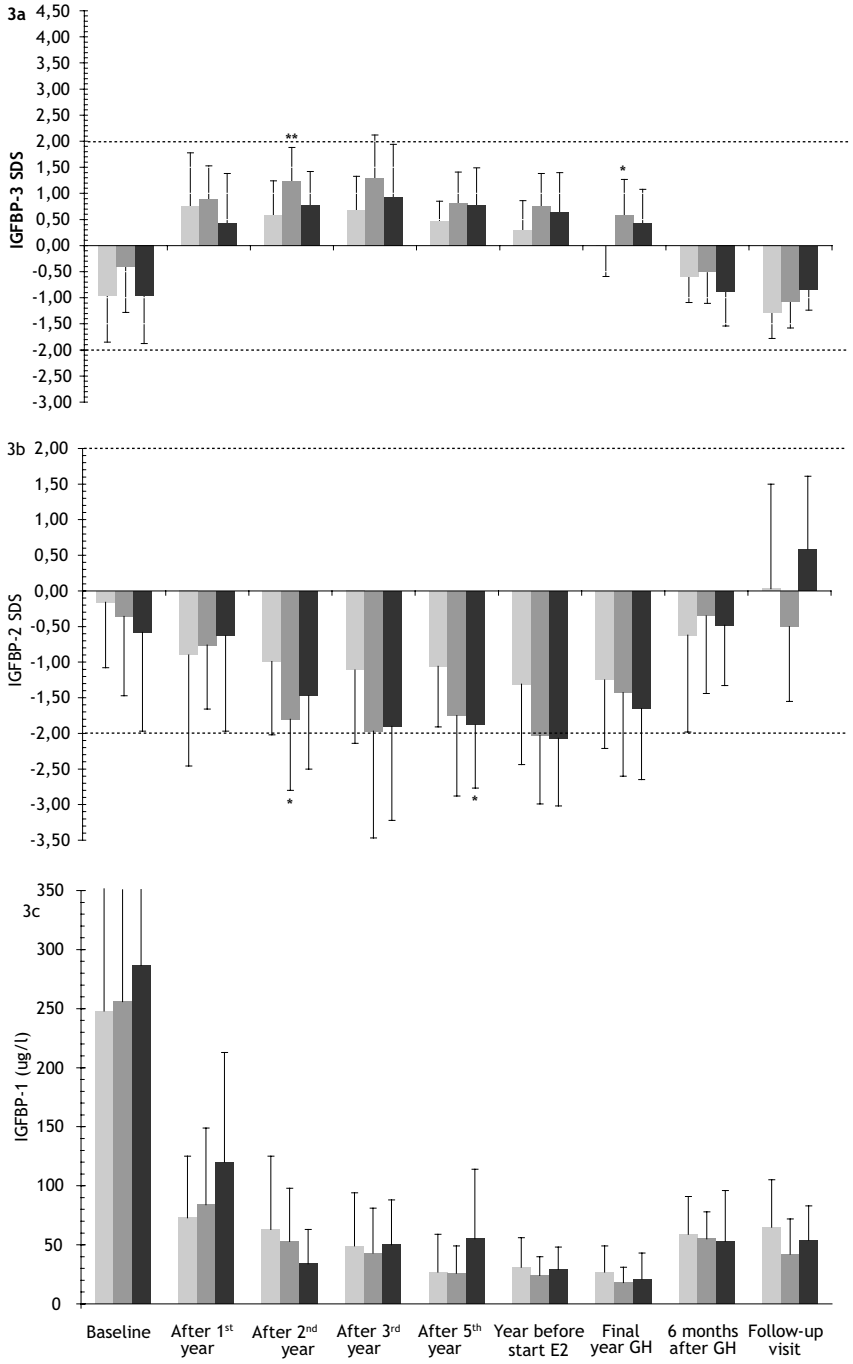


Figure 3: IGFBP-3 SDS (3a), IGFBP-2 SDS (3b), and IGFBP-1 levels (3c) before, during, and after discontinuation of GH treatment for group A (*white*), group B (*grey*), and group C (*black*), respectively. Data represent the mean +/− 2 SDS. Follow-up visit was 4.8 (2.0) years after GH discontinuation.

* P < 0.05, ** P < 0.01 compared to group A, # P < 0.05 compared to group B.

IGFBP-3, 2, 1: The different dosages of GH employed all lead to a significant increase in IGFBP-3 SDS, although less pronounced than the increase in total IGF-I SDS. For most patients, the IGFBP-3 SDS values were around the +1 SD (Figure 3). During GH therapy, only 2.6% of the IGFBP-3 measurements were above the +2 SDS, whereas 56 of the 65 girls never exceeded the +2 SDS. Within all groups, IGFBP-2 SDS and IGFBP-1 levels showed a significant decrease during GH treatment. Six months after GH therapy and at the follow-up visit, SDS values of IGFBP-3 and IGFBP-2 were the same as those measured before the start of GH therapy, whereas IGFBP-1 concentrations remained low. IGFBP-2 and IGFBP-3 levels showed an inverse relationship ($r = -0.50$, $p < 0.001$).

Explaining variables for free IGF-I levels

We investigated which variables showed the closest relationship with free IGF-I levels (Table 2). A stepwise forward regression analysis performed on all free IGF-I measurements resulted in a model using the following variables: age, estrogen dose, GH-dose, BMI, circulating levels of total IGF-I, IGFBP-3, IGFBP-1. This model accounted for 52.4% of the variation in square root of the free IGF-I levels (model 1). Since total IGF-I levels and the molar ratio between total IGF-I and IGFBP-3 were closely related ($r > 0.9$, $p < 0.001$), these parameters were evaluated in separate models to prevent multicolinearity. When the total IGF-I/ IGFBP-3 molar ratio was used instead of total IGF-I, this explained 53.1% of the variation in the square root of free IGF-I levels (model 2). In neither model did IGFBP-2 or the karyotype influence the square root of free IGF-I levels.

As shown in Table 2, the total IGF-I level and the total IGF-I/IGFBP-3 molar ratio predicted free IGF-I levels similarly. In both models, age appeared to be the strongest predictor of free IGF-I levels ($\beta = 0.319$ and $\beta = 0.297$, respectively). Free IGF-I levels were positively related to age, GH dose, IGFBP-3 levels, total IGF-I levels and total IGF-I/IGFBP-3 molar ratio. On the other hand, free IGF-I levels were decreased significantly by estrogen treatment and were inversely related to IGFBP-1 levels and BMI. After adding the interaction variable (GH dose X total IGF-I) and (GH dose X total IGF-I/ IGFBP-3 molar ratio) respectively to both models, the explained variation of the square root of free IGF-I levels increased significantly to 54.0% in model 1 and 54.2% in model 2 (data not shown). In both models, these interaction variables were of significant influence (both $p < 0.001$). This indicates that the square root of free IGF-I depends on the effect of the GH-dose on the total IGF-I levels and total IGF-I/ IGFBP-3 molar ratio.

Table 2: Variables in two linear regression models, with the square root of the serum free IGF-I concentration as dependent variable, either including IGF-I (model 1), or including ratio IGF-I/IGFBP-3 (model 2). **R^2**: explaining % of variance.

Independent variable	Model 1		Model 2	
	Standardized coefficient (ß)	P	Standardized coefficient (ß)	P
Age (years)	0.319	< 0.001	0.297	< 0.001
E2 dose per kg	−0.221	< 0.001	−0.218	< 0.001
GH dose (mg/m^2/d)	0.154	0.007	0.116	0.046
IGFBP-3 (mg/l)	0.189	< 0.001	0.263	< 0.001
IGFBP-1 (ug/l)	−0.168	< 0.001	−0.145	0.002
BMI (kg/m^2)	−0.102	0.041	−0.112	0.023
Total IGF-I (ng/ml)	0.167	0.013		
Ratio total IGF-I/IGFBP-3			0.190	< 0.001
R^2	52.4%		53.1%	
SD of the residual	0.356		0.354	

Relationships between circulating levels of free IGF-I, total IGF-I, IGFBP-3, and total IGF-I/IGFBP-3 molar ratio, and IGF-I bioactivity outcome parameters

Changes in free IGF-I SD-scores as encountered after 3 and 5 years of GH treatment did not correlate with the gain in height SDS at these intervals. In contrast, the alterations in the SD-scores of total IGF-I, IGFBP-3, and their ratio in the circulation after both 3 and 5 years of GH therapy, were significantly related to the respective gain in height SDS, with similar correlation coefficients (Figure 4). In addition, the AUC of free IGF-I levels during GH therapy did not correlate with any of the IGF-I bioactivity outcome parameters, while AUCs of the total IGF-I and total IGF-I/IGFBP-3 ratio levels appeared to influence several of these parameters significantly, including adult height gain and fasting insulin levels after 4 years of GH therapy (Table 3).

As determined 4.8(2.0) years after GH therapy, the AUC during GH therapy of the various variables did not correlate with 6-month after GH OGTT outcome, or with aortic distensibilities.

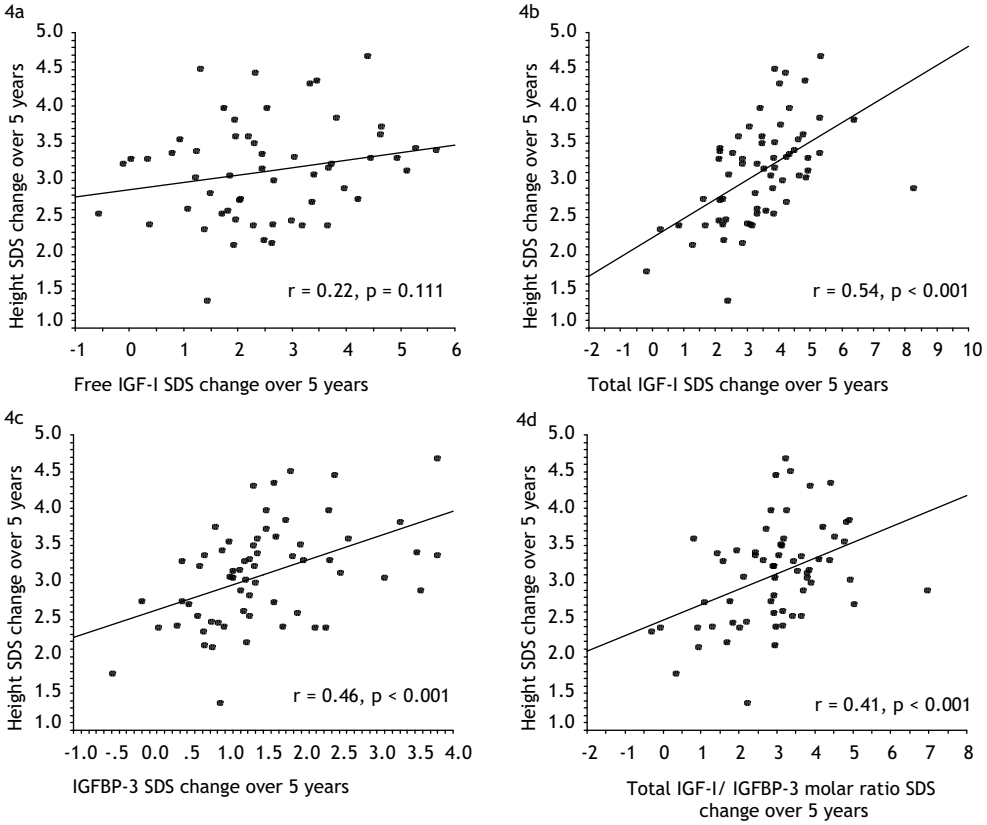


Figure 4: Relationship between five year changes in SDS values of free IFG-I, total IGF-I, IGFBP-3, and total IGF-I/IGFBP-3 molar ratio, and the five year change in height SDS. Correlation *r* is given.

Table 3: Relationships between various clinical variables and circulating levels of free IGF-I, total IGF-I, IGFBP-3, and the total IGF-I/IGFBP-3 molar ratio. Only the significant correlation coefficients (r) are given.

	Free IGF-I total AUC	**Total IGF-I total AUC**	**IGFBP-3 total AUC**	**Total IGF-I/IGFBP-3 total AUC**
Final height (cm)	NS	0.260	NS	0.328
Final height SDS gain[a]	NS	0.376	0.341	0.311
OGTT after 4 years of GH therapy	**free IGF-I 4 years AUC**	**total IGF-I 4 years AUC**	**IGFBP-3 4 years AUC**	**total IGF-I/IGFBP-3 4 years AUC**
Ins at t = 0	NS	0.284	NS	0.248
AUC ins at t = 120 min.	NS	0.280	NS	0.232
Ins/ glc at t = 0	NS	0.258	NS	0.242
Ins/ glc at t = 30 min.	NS	0.353	0.360	0.235

[a]: Final height SDS − height SDS at start GH therapy (references are to untreated Turner girls [18]). AUC = area under the curve from start of GH therapy until end of GH therapy (total AUC) or 4 years of GH therapy (4 yrs AUC). Glc = glucose, Ins = insulin.

Discussion

Absolute serum free IGF-I levels in healthy girls appeared to increase with age, which is in agreement with previous reports on this subject [2,19]. For our measurements, as performed under strictly controlled conditions, we used the DSL IRMA, which employs solid phase antibodies directed against unbound IGF-I. It has been suggested that this assay in fact determines the sum of truly free IGF-I and readily IGFBP-dissociable IGF-I, which has been hypothesized to represent a more biologically relevant pool than free IGF-I alone [2].

To our knowledge, this is the first report on free IGF-I levels in GH-treated TS girls. We found subnormal levels of free IGF-I in TS girls both before and after GH treatment. These results complement data reported previously by Gravholt et al., who found lowered free IGF-I levels in untreated adult TS women [20]. GH treatment in our patients increased the absolute levels of free IGF-I up to 7-fold, although the relative increase from baseline could be explained partly by age, and appeared to be independent of the GH dosage employed. In contrast, total IGF-I levels increased in a GH-dose-dependent manner and at least once in 62 out of the 65 TS girls, it reached levels that were above the normal range. Similar observations have been reported for GH treated children and adults with GHD, although there are also conflicting reports [2,21-23].

At present, it is difficult to explain the apparent discrepancy with respect to the GH-induced longitudinal changes in circulating levels of free IGF-I and total IGF-I. Under several conditions, especially those with primary abnormalities in GH secretion (e.g. acromegaly and GHD), there is usually a close relationship between free IGF-I levels, total IGF-I levels and the total IGF-I/IGFBP-3 molar ratios [21]. For this reason, it has repeatedly been suggested that the total IGF-I/IGFBP-3 molar ratio should reflect changes in free IGF-I levels. Although in our TS girls the levels of total IGF-I and the total IGF-I/IGFBP-3 molar ratio in the circulation were significantly related to free IGF-I levels, the best fitting model only explains less than 55% of the variation in free IGF-I levels.

In this respect it is important to emphasize that the kinetics of free IGF-I in the circulation differs strikingly from those of total IGF-I. Under normal circumstances, free IGF-I has a very short half-life (about 14 min) compared to the much larger pools of binary and ternary complex IGFBP-bound IGF-I. GH-induced changes in plasma levels of IGFBP-1, −2, −3, and −5 may thus have different affects on steady-state concentrations of free-IGF-I and total IGF-I levels.

Interestingly, free IGF-I levels in sera of adult TS patients appeared to correlate inversely with the degree of IGFBP-3 proteolysis [20]. Since enzymatic proteolysis of this IGFBP is considered to reduce its affinity for IGF-I, it was hypothesized that this

paradoxical finding may be explained by an increased clearance of free IGF-I from the circulation, a result of the very short half life of free IGF-I. It may well be that, in our TS girls, an initial GH-dose-dependent rise in free IGF-I levels was compensated by an increased clearance of free-IGF-I from the circulation.

This hypothesis is supported by the findings that, despite GH-dose dependent total IGF-I levels, there were no differences in free IGF-I levels between the different GH dosage groups. It is also supported by the finding that, after adding the interaction variables (GH dose X total IGF-I) and (GH dose X total IGF-I/IGFBP-3 molar ratio), the square root of free IGF-I depends on the effect of the GH-dose on the total IGF-I levels and on the ratio.

As a consequence, we conclude that, although the total IGF-I and the ratio change in the same direction as free IGF-I, and show a relevant correlation, they cannot serve as reliable surrogate markers for free dissociable IGF-I levels during GH treatment in girls with TS

An inverse relationship was found between IGFBP-2 and IGFBP-3. This is a well-known phenomenon that has also been reported for various other states such as increasing age, GH deficiency, acromegaly, fasting, and moderate impairment of liver function [24]. It has been proposed that circulating IGFBP-2 levels are regulated primarily by changes in free IGF-II levels (not determined in the present study).

During GH treatment, estrogen therapy reduced free IGF-I levels. One of the few studies to have evaluated the effect of estrogens on free IGF-I found that high-dose estrogen treatment in constitutionally tall girls leads to a considerable decrease in free IGF-I [25]. On the other hand, an ultrafitration based assay established that, despite an increase in IGFBP-3 proteolysis, adult TS patients undergoing estrogen therapy did not show significant changes in circulating free IGF-I levels [20). However, our own assessment of the influence of estrogen therapy was made in a model along with other variables.

Most samples were analyzed at the end of the study, several years after collecting them. According to our experience IGF-I and IGFBP-3 in EDTA plasma or serum is very stable, i.e. after more than 10 years of storage at −80°C the values do not change significantly. However, we did not have the opportunity to examine the stability of free-IGF-I upon long-term storage. The samples showed the same results after testing them again after a year of storage.

In general, although free dissociable IGF-I is believed to represent the major bioactive form of IGF-I [2,26], one may question whether, from pathophysiological and diagnostic perspectives, it is relevant to assess the concentration of free IGF-I in patients with TS. In the present study, free IGF-I levels showed no clear correlations either with gained height, final height, and height changes after 3 or 5 years of GH therapy, or with changes in insulin sensitivity during GH therapy. In contrast,

circulating levels of total IGF-I and the total IGF-I/ IGFBP-3 molar ratios correlated considerably with the various variables. Thus, with respect to the biological endpoints named above, the determination of free dissociable IGF-I is of no predictive value in the evaluation of GH-treated TS girls.

In conclusion, during GH-therapy in TS girls, mean free IGF-I levels stayed within the normal range, whereas mean total IGF-I levels and ratio levels exceeded the upper normal range. Levels of total IGF-I and the ratio are not good representatives of free IGF-I levels, and the former parameters seem to have a much higher predictive value for several biological endpoints – and hence IGF-I bioactivity. Obviously, this needs to be explored further in vivo and in vitro studies.

The recently developed sensitive IGF-I kinase receptor activation assay (KIRA)[27], which enables the determination of IGF-I-receptor activating IGF-I concentrations, may be helpful in clarifying the bioactive capabilities of circulating IGF-I levels. The very long-term safety of supra-physiological growth factor levels during childhood also remains to be investigated.

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Chapter 4

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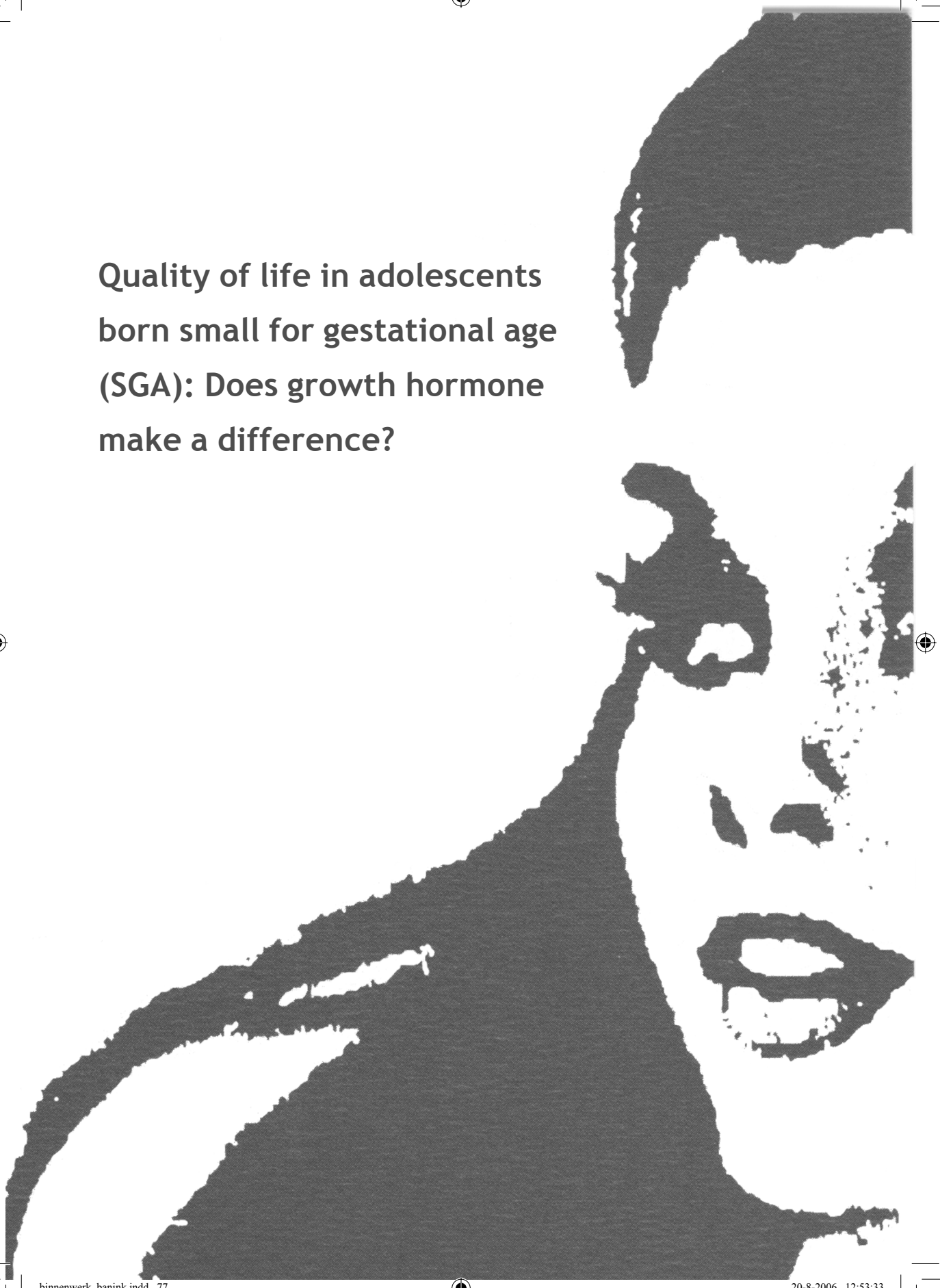
Quality of life in adolescents born small for gestational age (SGA): Does growth hormone make a difference?



Abstract

Background/Aims: To evaluate quality of life (QoL) in adolescents born SGA without spontaneous catch-up growth, treated with and without long-term growth hormone (GH) therapy. Additionally, to assess whether GH treatment has a positive effect on QoL, besides improving adult height and height SDS during childhood.

Methods: Two groups of adolescents born SGA without spontaneous catch-up growth participated in the QoL evaluation; a GH-treated group (n = 44, mean GH duration: 8.8 (1.7) years) and an untreated group (n = 28), both mean age 15.8 (2.1) years. QoL was measured by self-reports of the TACQOL-S, a disorder-specific questionnaire, and the CHQ, a generic questionnaire.

Results: The GH group scored significantly better health status and health-related QoL on several scales of the TACQOL-S. On all TACQOL-S scales the GH group scored better QoL than the untreated group, with effect sizes of moderate to large, not all differences reaching statistical significance. The generic CHQ did not reveal significant differences in QoL between the GH group and the untreated group.

Conclusions: Firstly, adolescents born SGA, with a GH-induced improved height, had in many aspects a better QoL than untreated adolescents born SGA, according to the disorder-specific questionnaire. Secondly, we advise to use, in addition to a generic questionnaire, a disorder-specific questionnaire for measuring QoL in children treated for short stature, as the generic CHQ did not reveal such differences.

Introduction

Quality of life (QoL) is increasingly recognized as an important measure of the impact of a special disorder, disease or therapeutic outcome. Over the past decades health status (HS) and QoL have been studied in a variety of diseases and disorders, including short stature [1-3]. Short stature during childhood and adolescence, resulting in a short adult height occurs regularly after being born small for gestational age (SGA). It is widely held that short children can suffer from physical, social and psychological problems [4]. The physical limitations of short stature and their often younger appearance may result in being treated differently by peers, and they may receive unintentional discrimination from adults. Social interaction, in particular during sports and games with peers, subjects them to taunts and bullying [5]. Adult short stature is often perceived to be a disadvantage. It can be a major physical disability in terms of not being able to drive a car, reach for objects and perform ordinary daily tasks that a person of average height takes for granted. It can also cause difficulties, or even discrimination, in getting the preferred job or career.

There are, however, some inconsistencies in the literature. It is reported that the psychosocial difficulties, associated with being short, seems to be less severe than assumed [1,6]. However, hard data on QoL in short stature SGA children or adults are lacking.

About 10% of the SGA children will not catch-up to a height above −2 SDS and will reach an adult height below −2 SDS [7]. GH-treatment has been proven to be effective for obtaining a normal height during childhood, adolescence and adult height, after being born SGA [8-10], regardless of the serum growth hormone (GH) levels [11,12]. With GH treatment during childhood, short SGA children will reach a normal height within 2 years after start of GH [9], and will acquire an adult height which is in 98% positioned within the target height range, and in 85% within the normal range of the population when treated with long-term continuous GH [10].

Some QoL questionnaires, such as the 'Health Utility Index', focus on the quantity and severity of limitations in executing ordinary daily tasks and psychosocial functioning due to a health problem, handicap, or disorder, the so-called 'health status'. Persons with short stature might not just experience physical limitations, but the accompanying social problems of short stature can overrule the physical limitations. For example, the problem is not that the adolescent can't go to a pub or club, but the problem is that he/she will not be asked by friends or is not permitted to enter due to younger appearance. Therefore, it is important to include the emotional impact of the HS on a person's life. The HS in combination with the emotional impact is called the health-related QoL (HRQoL). It's rating how the adolescent or child feels about their functioning, rather than functioning alone. Recently, a Dutch

questionnaire for short stature was constructed as a disorder specific module of the generic TNO-AZL Children's Quality of life (TACQOL), called the TACQOL-short stature (TACQOL-S). This questionnaire explicitly offers respondents the possibility of differentiating between their ability to function and their associated feelings. The quantity of problems is known as the HS. The HRQOL, qualifies the emotional impact of the problem.

The study aims were to compare QoL scores between 2 groups of adolescents born SGA, a GH-treated group and an untreated group, who all had attained adult or near adult height. This was measured with a disorder specific health-related QoL, the TACQOL-S, specially developed for children with short stature. Additionally, a generic questionnaire, the Child Health Questionnaire (CHQ) was applied. The adolescent completed both, the TACQOL-S and the CHQ.

Patients and Methods

GH-Treated SGA Group (GH Group)

All children with a height < −2SDS were referred to a pediatric endocrinologist, according to the national guidelines for short stature. Seventy-nine short children born SGA were enrolled in a multi-center, double-blind, randomized dose-response GH trial, which started in 1991. The inclusion criteria for participation in the GH-treated SGA group of the QoL evaluation were: completion of the GH trial until final height or satisfied height, age ≤ 18 years, be able to fill in the questionnaire, no growth interfering treatment other than GH therapy. Seventeen adolescents did not meet the inclusion criteria due to: > 18 years old (n = 11), treatment for precocious puberty (n = 1), dropped out of the GH-trial within 4 years after start of GH therapy due to GH insensitivity (n = 1), due to moving abroad (n = 2), due to lack of motivation (n = 2). This leaves 62 of the 79 adolescents eligible for participation in the QoL evaluation. Forty-four adolescents out of the 62 (71%) agreed to participate, mean (SD) age 15.7 (2.1) years. No differences in clinical characteristics were found between the adolescents who participated and the adolescents who did not participate.

The GH trial evaluated the effect of GH on long-term growth and ultimately on adult height, as well as psychosocial development, cognition and QoL. Inclusion criteria for the dose-response trial were: birth length SDS below −2, chronological age (CA) between 3 and 11 years in boys and 3 and 9 years in girls, height SD-score for CA below −2, no spontaneous catch-up growth, prepubertal stage, uncomplicated neonatal period without severe asphyxia. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneous once daily. GH was given double blindly in a dosage of 1 (group A) or 2 (group B) mg GH/m^2 body surface/day (~ 33

or 67 µg/kg/day). In 2001, at time of QoL evaluation, none of the participants knew their GH dosage. GH treatment was discontinued after reaching adult height (height velocity < 0.5 cm in 6 months) or on patient's decision after reaching satisfactory height (near adult height). Twenty-three of the 44 participants (52%) had reached adult height or near adult height and had discontinued GH treatment.

For analysis of the QoL questionnaires we combined the 2 randomized GH dosage groups (GH group), as they were samples from the same underlying population at baseline because of the randomization. As a result there were no significant differences in height SDS at time of QoL evaluation, nor was there a difference in height SDS gain between the two dosage groups.

Untreated SGA Group (Untreated Group)

In 1990, 107 children, born in three academic hospitals in the Netherlands during the same time period as the GH group (1980 until 1989) with a birth length below −2 SDS, were included in a cohort study to evaluate natural growth in SGA children with short stature in parallel to the GH-trial [7]. The inclusion criteria for participation in the untreated SGA group of the QoL evaluation were: age ≤ 18 years, be able to fill in the questionnaire, no growth interfering treatment. Fifty adolescents did not meet the inclusion criteria due to: > 18 years old (n = 28), GH therapy (n = 19), treatment for precocious puberty (n = 1), not being able to fill in the questionnaire due to moving abroad (n = 2). This leaves 57 of the 107 adolescents eligible for participation in the QoL evaluation. Twenty-eight out of 57 (49%) adolescents agreed to participate (mean age (SD) 15.8 (2.1)).

Design and Clinical Evaluation Dose-Response GH Trial

Children of the study group were assigned to either group A, with a GH dose of 1 mg/m^2/day (33 µg/m^2/day), or group B with a GH dose of 2 mg/m^2/day (67 µg/m^2/day) [9,10,13]. A daily subcutaneous injection of biosynthetic GH (r-hGH Norditropin, Novo Nordisk A/S, Copenhagen, Denmark) was given at bedtime. Three-monthly, the total GH dose was adjusted to the calculated body surface.

Every 3 months height was measured using a Harpenden stadiometer [14]. Four measurements per visit were taken and the mean was used for analysis. Target height (TH) was calculated with Dutch reference data with addition of 3 cm for secular trend: ½ x ($Height_{father}$ + $Height_{mother}$ + 12) + 3 for boys and ½ x ($Height_{father}$ + $Height_{mother}$ − 12) + 3 for girls [15]. Height and TH were expressed as an SD score for chronological age (CA) and gender [15].

Measurements

All QoL data were collected in the year 2001. For measuring the QoL, the child form (CF87) of the Child Health Questionnaire (CHQ) was used for adolescents ≤ 18 years. Participants ≤ 15 years had in addition to the CHQ, the TNO-AZL Children's Quality of Life Short stature module (TACQOL-S), a questionnaire developed by The Netherlands Organisation for Applied Scientific Research (TNO) in conjunction with the Academic Hospital in Leiden (AZL).

TACQOL-S: The TACQOL-S is a disorder specific questionnaire and was specially designed in the Netherlands to measure the impact of short stature on QoL for children aged 5–15 years [16]. The questionnaire was constructed as a disorder-specific module of the generic TNO-AZL Children's Quality of Life questionnaire (TACQOL) [17-19]. The items were based on years of clinical and research experience and interviews with children with short stature. As such, it explicitly offers respondents the possibility of differentiating between their functioning; the *quantity* of problems, called the HS and their associated feelings with it, the *emotional impact*, called the HRQOL. We used the TACQOL-S child form (CF), a self-report questionnaire consisting of 37 items (all referring to the last few weeks) divided in 5 scales: Physical abilities (e.g. Did you experience the tables at school as being too high?), Vitality (e.g. Have you been getting tired quickly?), Contact with peers (e.g. Have other children been bullying you?), Contact with adults (e.g. Were adults surprised after they heard your age?), Body image (e.g. Would you like to look different?). A higher test score indicates a better QoL on that specific scale. The TACQOL-S was performed in a subgroup of the total group, in participants ≤ 15 years of age (n = 39). Internal consistency of the scales of the TACQOL-S, the Cronbach's alpha [20] was studied in a larger group of short children (n = 63). The Cronbach's alphas were all above 0.70, except for vitality HS (0.57). In general, a Cronbach's alpha ranging from 0.70 to 0.84 is regarded as satisfactory for comparing different groups [21]. If a scale has a Cronbach's alpha < 0.70 and significance occurs, conclusions should be drawn carefully.

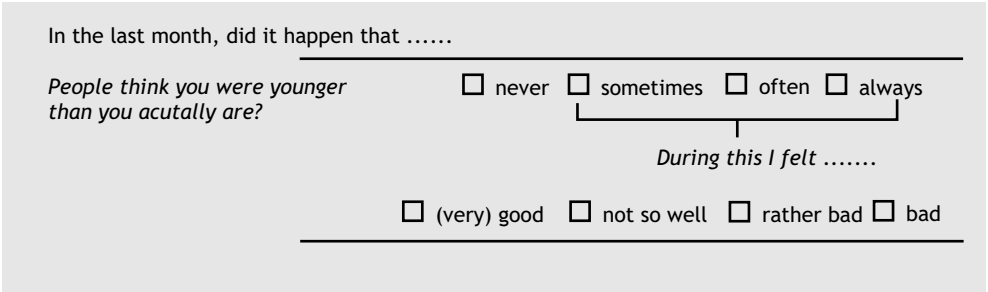


Figure 1: Example of the format of the TACQOL-S.

Child Health Questionnaire (CHQ): The CHQ is a generic questionnaire developed in the USA [22,23] and since then widely used in the US, Australia, Slavic countries, Germany, Denmark, France, Belgian, China and the Netherlands. The child form (CF87) of the CHQ is a self-report form and is designed to measure generic HS covering physical and psychosocial domains in children and adolescents ≤ 18 years of age. A Dutch version [12], consisting of 87 items divided into 12 scales, was used. A higher test score indicates a better quality for that specific scale, with a maximum score of 100. The reference population consisted of 444 children of four representative schools in Rotterdam, the Netherlands, as prescribed previously by Raat *et al*. [12]

Socioeconomic Status: Data on occupational level were provided by the parents. Parental occupational level (SES) ranged from 1 (lower occupation) to 3 (higher occupation). When both parents were employed the highest of the two SES levels was used. For unemployment the lowest SES was used [24].

Statistical Analysis

All data are expressed as mean (SD) unless otherwise specified. Independent *t*-tests were used to test for differences between participants and non-participants to the QoL study, analyzed per GH dosage group. Only in participants to the QoL study were independent *t*-tests used to test for differences in clinical data between the two dosage groups (A and B) and compared to untreated group, and independent *t*-tests were used to test for differences between the group ≤ 18 yrs and ≤ 15 yrs. Mann-Whitney U tests were used to test for differences in TACQOL-S and CHQ outcome between GH group, untreated group and references.

The magnitude and meaning of the differences in QoL outcome between groups can be calculated as Cohen's effect size (d) [25,26]. The effect size d is calculated as follows: [mean(a) − mean(b) / largest standard deviation score (SDS)]; this means that differences between groups are expressed in units of the largest within-group standard deviation. According to Cohen, effect sizes between 0.2 and 0.5 indicate a small effect, an effect size between 0.5 and 0.8 indicates a moderate effect, whereas effect sizes of 0.8 or larger indicate a large effect [25,26].

Multiple linear regression analyses were performed to assess possible factors influencing the QoL outcome. Each questionnaire scale was tested separately. For this purpose, the variables sex (M = 1, F = 2), age at time of QoL evaluation (in yrs), and height SDS or height SDS corrected for target height SDS (Ht SDS − TH SDS) at time of start GH treatment and at time of QoL evaluation, with and without SES (range 1–3), were tested. The percentages of variance explained by the model (R^2 in %) are given. $P < 0.05$ was considered significant. All calculations were performed with SPSS 10.1.

Ethical Considerations

The Medical Ethics Committees approved the evaluation of QoL. Due to ethical considerations the Medical Ethics Committees did not allow a randomized control group the long-term dose response GH trial. Written informed consent was obtained from the parents or custodians and from each adolescent.

Results

Clinical Data

Table 1 shows the clinical characteristics of all participating adolescents at baseline and at time of the QoL evaluation. The mean (SD) height SDS gain was significantly higher in the GH-treated group (2.4 (1.1)) versus the untreated group (0.5 (0.6)). Mean (SD) height SDS was in 2001 significantly higher in the GH-treated group (−0.6 (1.2)), than in the untreated group (−1.8 (0.8)). This difference was larger in the subpopulation ≤15 years, who participated in the TACQOL-S. The GH group showed significantly better growth than the untreated group, resulting into better height SDS, height SDS gain and target height corrected height SDS ($P < 0.01$).

There were no significant differences in clinical characteristics between the complete group ≤ 18 years (CHQ group) and the subgroup ≤ 15 years (TACQOL-S group), apart from age. The TACQOL-S group was 1.6 year younger than the CHQ group, due to a younger age at start of the GH dose-response trial. Regarding parental occupational levels, the GH group was not significantly different compared to the untreated group.

TACQOL-Short Stature

Table 2 shows HS and HRQOL scores of the self-report TACQOL-S, measured in 2001. For 'physical abilities' and 'contact with adults', HRQOL and HS were significantly higher in the GH group compared to the untreated group. HS of 'body image' was significantly higher in the GH group compared to the untreated group, whereas HRQOL scored also higher but this difference was not significant.

Table 1: Mean (SDS) clinical data of all the adolescents who participated in the evaluation of the quality of life evaluation, at baseline (1991) and at time of QoL evaluation (2001).

	Whole group, CHQ participants all ≤ 18 years		Subgroup, TACQOL-S participants all ≤ 15 years	
	GH-treated SGA (n = 44)	Untreated SGA (n = 28)	GH-treated SGA (n = 24)	Untreated SGA (n = 15)
Male : Female (% male)	28 : 16 (64)	12 : 16 (43)	14 : 10 (58)	6 : 9 (40)
Gestational age	36.8 (4.0)[b]	34.3 (3.6)	36.4 (4.0)[a]	33.5 (3.0)
Birth length SDS	−3.4 (1.3)	−3.8 (1.3)	−3.4 (1.5)	−3.9 (1.5)
Birth weight SDS	−2.5 (1.1)[a]	−3.0 (0.8)	−2.5 (1.2)[a]	−3.1 (0.8)
Target height SDS	−0.7 (0.9)	−0.5 (1.0)	−0.7 (0.8)	−0.5 (0.9)
Age				
At time of start study	6.6 (2.0)[c]	5.9 (2.3)[c]	5.0 (1.1)	4.4 (1.4)
At QoL evaluation	15.8 (2.1)[c]	15.8 (2.1)[c]	14.2 (1.2)	14.2 (1.1)
Height SDS				
At time of start study	−3.1 (0.7)[b]	−2.3 (0.7)	−3.1 (0.7)[a]	−2.5 (0.7)
At QoL evaluation	−0.6 (1.2)[b]	−1.8 (0.8)	−0.1 (1.1)[b]	−1.7 (0.8)
Height SDS gain	2.4 (1.1)[b]	0.5 (0.6)	2.9 (1.1)[b]	0.8 (0.5)
Corrected height SDS (Hsds-THsds):				
At time of start study	−2.4 (0.8)[a]	−1.8 (0.9)	−2.3 (0.7)	−2.0 (0.8)
At QoL evaluation	−0.1 (1.2)[b]	−1.3 (0.7)	0.6 (1.1)[b]	−1.3 (0.7)
Duration of GHRx at QoL evaluation	8.8 (1.7)[b]	–	10.1 (1.2)[b]	–

Data are expressed as the mean (±SD).

Independent *t*-test: [a] $P < 0.05$; [b] $P < 0.01$; GH-treated compared to untreated group; [c] $P < 0.01$, CHQ participants compared to TACQOL-S participants.

In Table 2, the effect sizes of the differences in HS and HRQOL between the GH group and the untreated group, as reported by the children, are shown. A positive effect size indicates better QoL scores in the GH group, whereas a negative effect size, which did not occur, indicates better QoL scores in the untreated group. The effect size of the difference between the GH-treated group and the untreated group regarding 'physical abilities' was 0.83 ($P = 0.004$) for HS and 0.9 ($P = 0.002$) for HRQOL, which indicates a large effect. The effect size of the difference between the GH-treated group and the untreated group regarding 'contact with adults' was 0.76 ($P = 0.02$) for HS and 0.85 ($P=0.002$) for HRQOL. This indicates a large effect in quantity of problems (HS), and an even larger effect on the emotional impact (HRQOL). The effect size of HS on 'body image' was 0.59 ($P = 0.04$) and the effect size of HRQOL of 'body image' was 0.47 ($P = 0.2$).

Table 2: Results of the TACQOL-S.

		GH-treated SGA group (n = 24)	Untreated SGA group (n = 15)	Effect size[1] (*d*)	P value
Physical abilities	HS	90.8 (9.8)[b]	74.3 (19.8)	0.83	0.004
	HRQoL	93.6 (7.4)[b]	78.6 (16.6)	0.90	0.002
Vitality[2]	HS	75.0 (18.4)	65.8 (21.9)	0.42	0.234
	HRQoL	79.2 (16.5)	69.6 (23.8)	0.40	0.283
Contact with peers	HS	81.0 (18.5)	73.8 (21.9)	0.33	0.296
	HRQoL	85.7 (16.4)	76.3 (19.9)	0.47	0.110
Contact with adults	HS	85.2 (10.6)[a]	70.9 (18.8)	0.76	0.017
	HRQoL	92.9 (4.4)[b]	80.0 (15.1)	0.85	0.002
Body image	HS	88.4 (8.0)[a]	74.1 (24.1)	0.59	0.038
	HRQoL	91.2 (6.9)	81.8 (20.1)	0.47	0.191

Data are expressed as the mean (±SD).

There were no differences between the 2 dosage groups. [a] $P < 0.05$; [b] $P < 0.01$, compared to the untreated group (0 mg/m^2/d). Higher scores = Better health related QoL (HRQoL)/ Health status (HS). [1] Effect size = positive effect size indicate better QoL in GH-treated SGA group; $0.2 \leq d < 0.5$ = small effect; $0.5 \leq d < 0.8$ = moderate effect; $d \geq 0.8$ = large effect. Negative effect size (not present) indicates better QoL in untreated SGA group. [2] Cronbach's alpha was 0.57.

CHQ Data

As shown in Table 3, the children of the untreated group scored significantly lower on 'family cohesion' than their normal peers, 64.6 (26.9) *vs.* 75.7 (23.2) respectively, whereas those of the GH group were not significantly different from their normal peers. The children of both SGA groups (GH-treated and untreated) scored significantly lower than their normal peers on 'role social-emotional', 88.4 (16.2) and 82.7 (17.5) respectively, *vs.* 92.3 (16.8). There were no significant differences between the GH group and the untreated group.

As shown in Figure 2, the GH group showed on all the scales of the CHQ higher scores than the untreated group; however, these differences between the GH-treated and untreated groups did not reach statistical significance.

Table 3: Results of the CHQ (CF87).

	GH treated SGA (1 or 2 mg/m^2/d) (n = 44)	Untreated SGA (0 mg/m^2/d) (n = 28)	Reference Population[1] (n = 441)
Physical Functioning	95.5 (6.4)	93.0 (12.2)	96.8 (5.4)
Role Social – Physical	96.0 (9.6)	90.5 (18.6)	96.5 (11.6)
Role Social – Emotional	88.4 (16.2)[a]	82.7 (17.5)[b]	92.3 (16.8)
Role Social – Behavior	93.4 (14.8)	88.9 (15.7)	91.4 (13.7)
Bodily pain	75.0 (20.9)	74.6 (23.0)	78.2 (19.5)
Behavior	84.8 (9.4)	82.8 (12.4)	83.6 (10.2)
Mental Health	79.2 (11.3)	75.1 (15.1)	78.2 (13.0)
Self Esteem	77.6 (11.3)	74.0 (12.9)	75.4 (12.5)
General Health	73.2 (11.9)	72.2 (16.4)	74.6 (15.9)
Family Activities	80.0 (15.0)	77.1 (19.7)	ND
Family Cohesion	72.4 (20.4)	64.6 (26.9)[a]	75.7(23.1)

Data are expressed as mean (SD).

No significant differences between GH-treated and the untreated group;

[a] $P < 0.05$, [b] $P < 0.01$, compared to reference values.

ND = Not Done, this scale was not fielded in this study.

[1]'Reliability and validity of the CHQ-CF87 in a Dutch population', by Raat *et al*. [12]

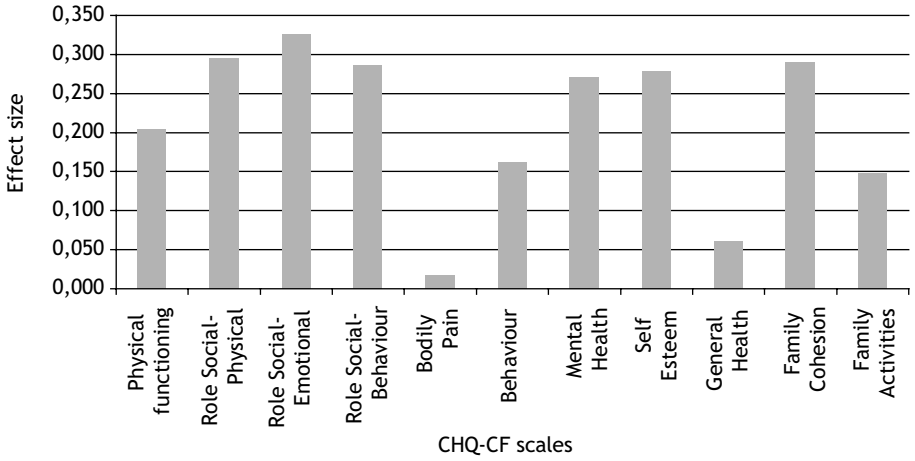


Figure 2: Effect sizes of the differences on the QoL scales between the GH-treated SGA group and the untreated SGA group as measured with the CHQ and reported by the children. Higher score indicates better QoL in the GH-group. Effect size (d): $0.2 \leq d < 0.5$ = small effect; $0.5 \leq d < 0.8$ = moderate effect; $d \geq 0.8$ = large effect.

Factors Influencing QoL Outcome

TACQOL-S. Using multiple regression, the HRQoL score in the GH group for the scale 'contact with adults' increased with 3.0 units per 1 SDS of the current height, adjusted for sex, age and height SDS at start GH treatment (R^2 = 43.5%). This means the taller they were, the more they felt age-appropriately treated by adults. In the same GH group, the HRQoL score of 'body image' increased with 5.3 units per 1 height SDS corrected for target height (Ht SDS − TH SDS), after adjustment for sex, age and height SDS corrected for TH SDS at start (R^2 29.4%). This indicates the taller they were compared to their parents, the more they felt satisfied and happy with their own body. Lastly, in the GH group the HS score of 'vitality' decreased with 14.7 units per 1 height SDS corrected for TH SDS at start of the GH-therapy, adjusted for sex, age and current height SDS corrected for TH SDS (R^2 44.3%). This indicates that the smaller they were at start of the GH therapy compared to their parents, the more vital they currently felt. In the untreated adolescents, height SDS or height SDS corrected for TH SDS were not associated with QoL, measured with the TACQOL-S. Socio-economic status was not of significant influence.

CHQ. In the adolescent reports of the CHQ-CF87, the QoL score on the scale 'role social behavior' increased with 6.8 units and 12.6 units per 1 SDS of the current height in the GH group and untreated group, respectively (R^2 18.1% and R^2 29.8%). This means the taller the adolescents were, the less problems they experienced in daily life (like doing schoolwork or sports with friends) with their behavior. In addition, the QoL score of 'family cohesion' in the GH group increased with 11 units per 1 height SDS at the start, adjusted for sex, age and current height SDS (R^2 14.5%). None of the CHQ scales were affected by height SDS corrected for TH SDS.

Discussion

This study delineates various aspects of QoL in short children born SGA after long term GH treatment. Although generic instruments are widely used, they may include irrelevant aspects or miss certain aspects of importance for specific groups of patients, here with short stature, and therefore being insensitive when measuring the effect of short stature on QoL. We therefore included a QoL questionnaire specially developed for children and adolescents with short stature, the TACQOL-S.

Before reviewing these results one has to consider the following issues to appreciate the outcome. As we do not have baseline data, QoL was not evaluated longitudinally. The GH trial started in 1991, when QoL was not as much recognized as an important measure outcome as it is today. Moreover, the QoL questionnaires were developed in the early and mid-1990s, after the start of this GH trial. Due to

the lack of information about QoL before GH treatment, possible influencing factors on QoL outcome were analyzed. Second, subjects were not randomized, but from 2 separate studies, with similar inclusion criteria and age. It appeared that the untreated group had a higher height SDS than the GH group at the start of the GH study. This might have positively influenced their QoL during childhood, as their height was always nearer to the normal range. Despite a shorter height at start of study, the GH-treated group reported a better QoL, after 10 years of GH treatment, on several domains of the disorder-specific questionnaire compared to the untreated group. Third, there is a limited experience with the disorder specific questionnaire the TACQOL-S. However, the TACQOL-S is constructed from the generic TACQOL, which is broadly validated and used. The items were based on years of clinical and research experience and interviews with children with short stature. Fourth, a placebo effect could be argued. It is well known that a placebo effect on growth in GH-controlled trials only lasts for 3 months after start of GH treatment [27,28]. As our study analyzed the long-term effect, after nearly 9 years of GH treatment, we do not expect any placebo effect. Finally, in the GH-treated SGA group, 62 of 79 enrolled in the early 1990s, were eligible for the health-related QoL evaluation, of which 44 (71%) completed the questionnaires. While clinical characteristics of the 44 responders did not differ significantly from nonresponders, we are unaware of other potential sources of selection bias. In the untreated SGA group 57 of 107 enrolled in the early 1990s, were eligible for health-related QoL evaluation, of which 28 (49%) completed the questionnaires. GH therapy that was applied outside the context of clinical studies (n = 19) was a source of not being eligible for QoL evaluation; although to our knowledge (psychological) problems did not correlate to GH therapy in that subgroup, drop-out as well as nonresponders may have introduced bias of which we are unaware.

The specific self-report questionnaire, the TACQOL-S, showed that the GH-treated SGA adolescents had a significantly better HS and HRQOL regarding 'physical abilities' and 'contact with adults' and 'body image' HS compared to untreated SGA adolescents. The better QoL in 'physical abilities' means that the adolescents in the GH group had experienced an improvement in sports, in reaching things which are high up, were able to sit comfortable at tables and in chairs at school without experiencing them too high. The 'contact with adults' was better in the GH group. The GH group reported a positive influence of height on the 'contact with adults', indicating that the taller they were, the better they felt age-appropriately treated by adults. The effect size of the quantity of problems in contact with adults was moderate, but the emotional impact was large. This means that the adolescents in the GH group were more age-appropriately treated than the adolescents in the untreated group, and the adolescents experienced this respect for age by the adults

as very positive. The problems the SGA adolescents experienced with 'body image' was positively influenced by their height corrected for target height, meaning the closer the height was to that of their parents, the more happy they were with their bodies. The influences of height were not found in the untreated group. This might be related to the smaller variation in height SDS and smaller group size of the untreated group.

Our study shows effect sizes which were moderately to markedly better in the GH group compared to the untreated group on all scales of the TACQOL-S, both with respect to HS as well as the HRQOL, although some did not reach a significant difference. This might be due to the limited size of our study population. However, Kazis *et al* [26] reported that not only statistical outcome, but also the effect size contribute to an understanding of the differences between groups. The authors demonstrated that statistically significant differences might not be synonymous with what is clinically important.

The generic self-report questionnaire, the CHQ, showed a near-normal QoL in SGA adolescents after long-term GH treatment. The GH group was extremely short at the start with a height SDS far below the normal range and had normalized its height at the time of QoL evaluation. For the CHQ results, no significant differences were shown between the GH group and the untreated group. We have to realize, however, that the untreated group had never been as extremely short as the GH group, and always had a height SDS just below the normal range for many years and at time of QoL evaluation just within the normal range. In both groups, GH and untreated, height influenced their 'role social – behavior' positively, meaning they experienced less social problems related to behavior when they were taller. These findings are in line with a recent paper by our group, revealing a significant decline in problem behavior in SGA children during GH-treatment [29].

Several studies have previously shown that using a generic questionnaire for a special disorder or disease, one might miss relevant QoL issues [16,30-33]. Not only the most obvious daily life issues, like walking up the stairs, closing the buttons on your shirt, are relevant for an optimal QoL, less obvious daily life issues, like reaching up for kitchen cupboards, going shopping for clothes or hanging out with friends, are important. These issues might seem less obvious to someone who has a chronic disease or handicap, but their contribution to a persons QoL can be of great merit. A generic questionnaire focuses mainly on daily life issues, which are limited in case of a chronic disease or handicap. The items of the TACQOL-S are more sensitive for effects of stature on QoL outcome. So, in addition to generic QoL measurements, height-specific QoL measurements should be applied in growth studies.

It has been suggested that psychological training in coping with the psychosocial problems related to short stature would be a less-invasive alternative for GH

treatment. Regarding QoL, it would have been appropriate to test the difference in QoL outcome in a prospective randomized study model. Unfortunately, to date no structural psychological program has been evaluated to be effective and of practical use. Recently, it has been shown that GH-treated children had significantly reduced total problem behavior, externalizing behavior and a better self-perception after 10 years of GH treatment compared to pretreatment [34,35]. Taller children had less problem behavior over time. These findings were parallel to their height improvement [34]. This indicates that GH treatment is capable of improving several aspects of short SGA children, not only height.

In conclusion, our study shows that children born SGA, when treated with long-term GH therapy, show significantly better QoL on physical abilities and contact with adults than untreated children born SGA, when measured with the disorder-specific TACQOL-S. Additionally, also fewer problems with body image were reported in the GH group. Furthermore, our study shows a larger effect size of QoL in the GH-treated versus the untreated group when tested with the disorder-specific TACQOL-S. The generic CHQ did not reveal such significant differences. Our results are, however, preliminary and need to be verified in a larger randomized, placebo-controlled study design. Furthermore, we advise to use, in addition to a generic questionnaire, a height-specific questionnaire for measuring the influence of GH treatment on QoL.

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Chapter 5

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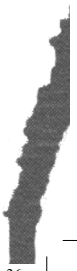
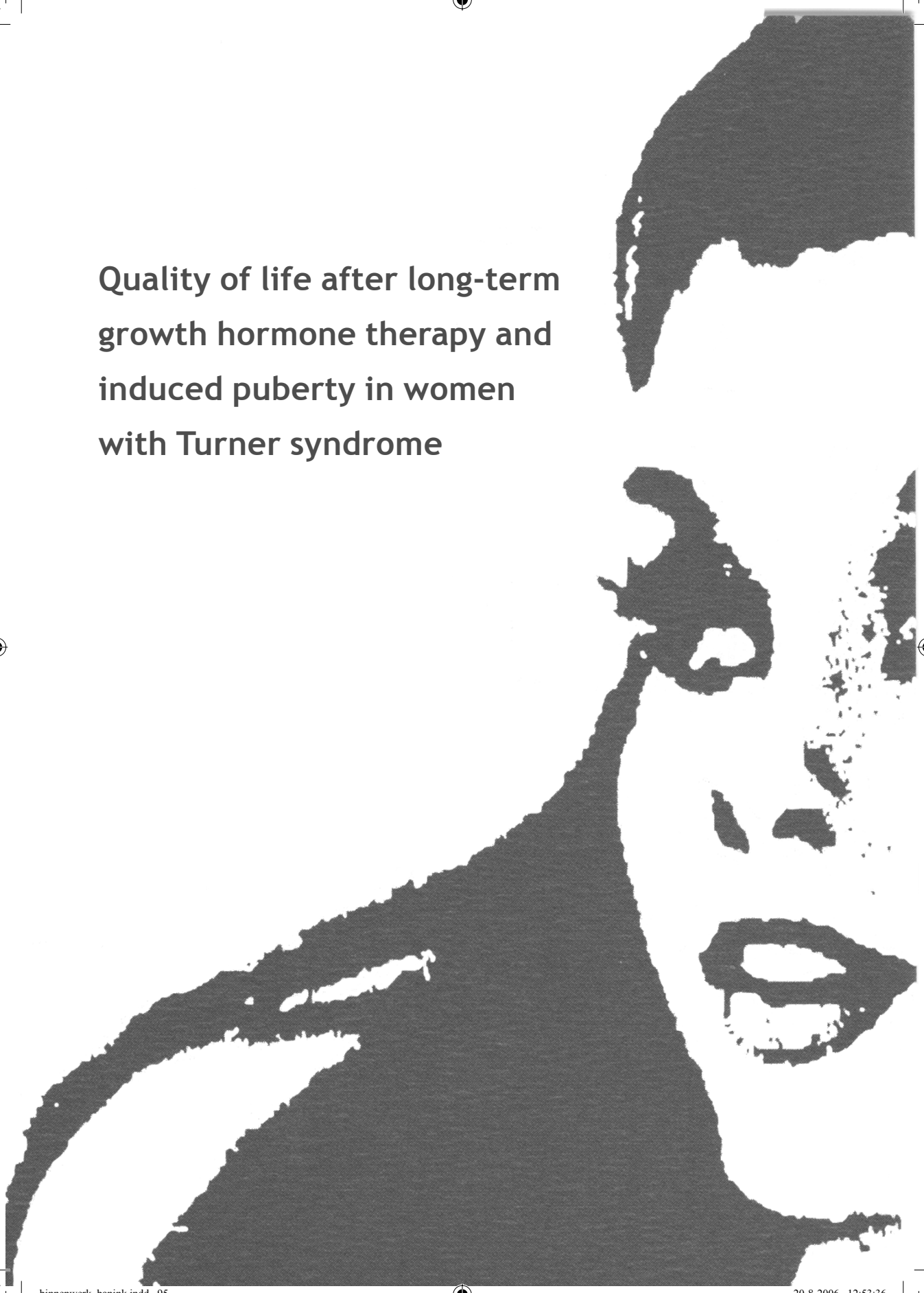


Quality of life after long-term growth hormone therapy and induced puberty in women with Turner syndrome



Abstract

Objective: To evaluate health-related quality of life (HRQOL) in young women with Turner syndrome (TS) after long-term growth hormone (GH) therapy and induced puberty and to analyze whether HRQoL was influenced by auxologic parameters, pubertal development or subjective parameters.

Study design: The study group comprised 49 women with TS, mean (standard deviation) age 19.6 (± 3.0) years, all former participants of 2 GH studies, ≥ 6 months after GH discontinuation. Puberty was induced by estrogen treatment, at mean age 12.9 (± 1.1) years. HRQoL was measured by self-reports of the 2 generic questionnaires, SF36 and TAAQOL. As an additional source of information on HRQoL, we applied parental proxy-reports.

Results: HRQoL of the women with TS was normal. Remarkably, the women with TS had higher HRQoL scores on some of the scales, including 'social functioning' and 'role–emotional'. Satisfaction with height and breast development had a positive influence on several HRQoL scales.

Conclusions: The young women with TS who reached normal height and had age-appropriate pubertal development reported normal HRQoL. The relatively high scores on some of the HRQoL scales can be explained by an estrogen effect or by a possible response shift, indicating a different internal reference in women with TS. We hypothesize that GH and estrogen treatment positively influenced HRQoL in young women with TS.

Introduction

It is generally accepted that a main goal of any medical treatment, therapy or intervention is improving quality of life. The 2 major characteristics of Turner syndrome (TS) are short stature and absence of pubertal development because of gonadal dysgenesis. It is widely held that short children can suffer from physical, social and psychological problems [1]. Currently, growth hormone (GH) treatment for short stature in children with TS is now an accepted indication in many parts of the world. GH treatment in TS increases growth velocity and normalizes height during childhood and adolescence. This treatment results in a height gain to an adult height within the normal range [2-5]. Another feature of TS is an absence or delay in development of bodily feminization, which can cause psychosocial problems in affected teenage girls [6]. Age-appropriate induction of puberty with estrogens, which mimics normal pubertal development without compromising adult height, is possible [3,7].

Several studies have reported psychosocial problems in girls and women with TS, including impaired social relationships, poor self-esteem, and decreased sexual activity [8-12]. Furthermore, these patients are more likely to meet criteria for attention-deficit hyperactivity disorder than controls and are often employed in jobs for which they are overqualified. One study in untreated women with TS assessed their quality of life in terms of working status, daily routine, and love and marriage and reported that most of the unmarried women lived with their parents, that many were well educated and worked as normal woman, and that they appeared anxious about their bodies and marriage [13]. Carel *et al* reported normal HRQoL in GH-treated women with TS, without any influences of height or other variables associated with GH-treatment [14]. This study did not include subjective measures in terms of height, pubertal development, and visible TS features, however.

In the present study we evaluated the HRQoL in young women with TS after long-term GH treatment and puberty induction at an age-appropriate time. We compared HRQoL scores of the young women with TS to scores in samples from the general population. We also analyzed whether HRQoL outcome was influenced by anthropometric parameters, pubertal development and subjective parameters, such as satisfaction with height and breast development.

Methods

Subjects

All young women with TS who had participated in 2 earlier GH trials were invited to participate in an HRQoL evaluation. The 2 earlier trials were a dose-response GH trial (DRS) and a frequent-response GH trial (FRS) to investigate the effects of long-term GH therapy, given in different dosages and at varying frequencies (see below). To be eligible, a woman had to have discontinued GH treatment for at least 6 months and to be able to fill in the questionnaires. Forty-nine women agreed to participate (response rate 49/69: 71%), with 20 women not participating either for practical reasons or because they had lost interest in participating in such studies.

GH-trials

The study designs of the DRS and the FRS have been described by Sas *et al* [2,15]. Both of these studies started in 1989. Sixty-nine cases were eligible for participation in the HRQoL evaluation, including 52 of the DRS and 17 from the FRS.

Dose-response study: Sixty-eight Dutch girls with TS, age 2 to 11 years, were enrolled in the open randomized multi-center GH DRS. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S Denmark) was given subcutaneously once daily at bedtime at a dose of 1.3, 2, or 2.7 mg GH/m^2 body surface area/ day (~ 0.045, 0.067, or 0.09 mg/kg/day). To induce puberty, micronized 17β-estradiol was given to the girls age 12 years and older after at least 4 years of GH treatment. During the first 2 years 5 µg/ kg body weight/ day was given orally, following by a dose of 7.5 µg/kg/d in the third year, and 10 µg/kg/d thereafter. Cyclic progestagen therapy (Duphaston, 5 mg/d for the first 14 days of each month) was added after 2 years of estrogen therapy. If puberty had developed spontaneously (as marked by Tanner breast stage $\geq$ 2) during the study period and before start of estrogen therapy, then no estrogen was given during GH-therapy. Of the 68 subjects, 6 girls dropped out of the study and were lost to follow-up, 6 girls were still receiving GH treatment, and 4 girls were unable to complete the questionnaires because of mental retardation. This left 52 girls eligible for participation in the HRQoL evaluation; 34 agreed to participate.

Frequency-response study: Nineteen Dutch girls with TS, age 11 years and older, were enrolled in an open randomized multicenter FRS. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S Denmark) was given subcutaneously once or twice daily in a dosage of 2 mg GH/m^2 body surface area/ day (~ 0.067 mg/kg/ day). To induce puberty, ethinyl estradiol was given orally at a dose of 0.05 µg/ kg body weight/ day at start of the trial. After the first 2.25 years of GH treatment,

the ethinyl estradiol dose was increased to 0.10 µg/kg/d, and cyclic progestagen therapy was added. Two girls were unable to fill in questionnaires because of mental retardation, leaving 17 girls eligible for participation in the HRQoL evaluation; 15 agreed to participate.

Health-Related Quality of Life Evaluation

The HRQoL evaluation was performed after GH treatment had been discontinued for at least 6 months and final height had been reached. The HRQoL evaluation consisted of 2 generic self-report questionnaires, the Medical Outcome Study Short Form-36 Health Survey (SF36) and the TNO/AZL Adult Quality Of Life (TAAQOL). As an additional source of information on QoL of the study population, we applied proxy-reports. The parents were used as proxies because women with Turner syndrome are considered more immature than their peers [10].

SF36: We used the validated Dutch translation of the SF36 [16]. Developed by Ware and Sherbourne in 1992 [17], the SF36 measures physical and mental health and social functioning. It is a self-report questionnaire consisting of 35 items divided into the following 8 domains (with the number of items in each domain in parenthesis): *Physical functioning (10), Role limitations due to physical health problems (4), Bodily pain (2), General health perceptions (5), Vitality (4), Social functioning (2), Role limitations due to emotional health problems (3), and Mental health (5)*. For each domain, the results were scored from 0 to 100, with a higher score indicating a better subjective quality of life.

***TAAQOL*:** The TAAQOL explicitly offers respondents the ability to differentiate their ability to function and their associated feelings. This concept, known as HRQoL, qualifies the quantity of the problem with its emotional impact. The TAAQOL was designed in the Netherlands and validated in the general population [18,19]. It is a self-report questionnaire consisting of 45 items which refers to the preceding few weeks, divided into the following 12 domains (with the number of items in parenthesis): *Gross motor skills (4), Fine motor skills (4), Cognition (4), Sleep (4), Bodily Pain (4), Social functioning (4), Daily activities (4), Sexual functioning (2), Vitality (4), Positive emotions (4), Negative passive emotions (eg depression)(4), Negative active emotions (eg aggression)(3)*. For each domain, the results are scored from 0 to100, with a higher score indicating a better subjective quality of life. An example of the format of the questionnaire is shown in Figure 1.

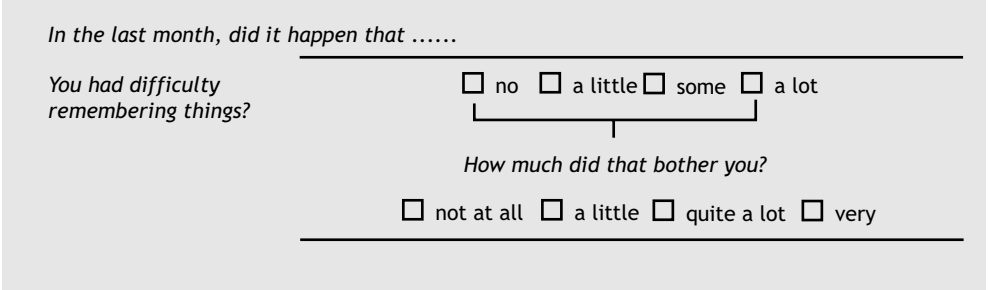


Figure 1: Example of the format of the health related questionnaire: TNO/AZL Adult Quality of Life (TAAQOL).

Reference population

SF36 and TAAQOL data of the Dutch general female population are available and were used for comparison. The population for the female reference data for the SF36 had an age range of 16 to 25 years (n = 121) [16], and that for female reference data for the TAAQOL had a mean age of 19.7 (2.0) and a range of 16 to 22 years (n = 116) [18].

Additional measurements

The mailed questionnaires contained additional items on subjective parameters of nationality and socio-economic status (SES). Information provided by the parents included parent's occupation, parent's educational level, daughter's nationality, and daughter's current and past health problems. The SES scores ranged from 1 (low) to 3 (high). When both parents were employed, the highest of both SES levels was used. For unemployment, the lowest SES score was used [20]. Reported health problems during life were counted.

Both the women with TS and their parents answered the following questions about the women with TS:

- Are you satisfied with your (*your daughter's)* adult height?
- Are you satisfied with your (*your daughter's*) breast development?
- Do you think that other people can see that you (*your daughter*) have (*has*) Turner syndrome?
- If answered yes to the previous question, how do you think people can see that you (*your daughter*) have (*has*) Turner syndrome?
- Do you think your (*your daughter's)* appearance will cause limitations? If "yes", which limitations?

Furthermore, they were asked where the woman with TS was living, with their parents or elsewhere.

The following variables were scored dichotomously (0 = no, 1 = yes): being satisfied with height and breast development, having visible features of TS, and having self reported limitations due to their physical appearance. In addition, the following spontaneously reported limitations due to physical appearance were also scored dichotomously (0 = no, 1 = yes): having limitations in relationships, having limitations in job perspectives, having feelings of insecurity, lacking charisma, and difficulties buying shoes or clothes. To determine whether the quantity of self-reported features of TS had a linear effect or a dichotomous effect, the self-reported visible features of TS were counted and scored from 0 to 3, with 0 representing no visible features and 3 representing 3 or more visible features.

Statistical analysis

All data are expressed as mean (standard deviation) unless otherwise specified. Independent *t*-tests were used to test for differences between self-reports or parent reports and the normal references based on a review of HRQoL measures of the TAAQoL and the SF36 scales. Independent *t*-tests were also used to test for differences between participants and non-participants of the HRQoL study. A paired *t*-test was used to test for differences between self-reports and parent-reports. The McNemar test and the Wilcoxon signed-rank test were used to test for differences in reported subjective data on features of TS and limitations between the women with TS and their parents.

The effect of physical features of TS and limitations on HRQoL outcome, reported by the patients with TS was analyzed per HRQoL scale in a regression model. Each of the following explanatory variables was entered separately in the model, along with age and SES: satisfaction with height, satisfaction with breast development, visible features of TS, self-reported limitations (ie, limitations in relationships, limitations in job perspectives and, feelings of insecurity), number of health problems, age at start of puberty, height standard deviation score (SDS) gain, and adult height. Age at start of puberty was defined as age (years) at first visit with Tanner breast stage B2 [21], spontaneous or induced. Height was scored in cm.

Results from the regression analyses are given as unstandardized coefficients (B) along with their 95% confidence interval and their 2-tailed P-value. The estimated B-coefficient is the relevant effects adjusted for age and SES. Only B-coefficients with a P-value < 0.15, and only variables with a significant influence on at least one of the HRQoL scales are reported. The effects of age and SES are not presented. P-values < 0.05 were considered significant. All calculations were performed with SPSS 11.5 software.

Ethical Considerations

The institution's Medical Ethics Committee (MEC) of each participating center approved earlier GH trials and the MEC of our institution the subsequent HRQoL evaluation. Written informed consent was obtained from the parent or custodian of each child for the GH study and from each participant for the HRQoL evaluation.

Results

Clinical data

Table 1 presents the clinical characteristics of the 49 women with TS who participated in the HRQoL evaluation. The average height of 160.7 (±6.5) cm is −1.2 (±1.1) SDS lower than the normal Dutch references [22] and 2.2 (±1.0) SDS higher than the North European untreated women with TS [23]. The mean age at onset of puberty, defined as first visit with breast stage B2, was 12.9 years compared to 10.8 years in the normal population [7,24]. The progression through puberty, defined as the time intervals from breast stage B2 to B3, B2 to B4, and B2 to B5, was comparable to that of normal references (data not shown)[7]. Three of the 49 participating women with TS (6%) experienced a spontaneous start of puberty. All of the 49 women were receiving hormone replacement therapy in adult dosages. The duration of GH treatment was 7.1 (±2.7) year. At the time of the HRQoL evaluation, the mean (SD) time after GH-discontinuation was 2.9 (±1.6) years. No significant differences in breast development or other clinical characteristics as listed in Table 1 were found between the women who participated in the HRQoL evaluation and the women who did not participate.

HRQoL evaluation

SF36: Table 2 gives the HRQoL results of the SF36 of young women with TS. The women with TS reported a significantly better HRQoL in the 'Social functioning', 'Role limitations – Emotional' and 'Bodily pain' domains compared with the normal population. For the remaining domains, the HRQoL scores were equal to those of the normal population.

***TAAQOL*:** The TAAQOL (Table 3) revealed significantly better HRQoL scores for 'Pain', 'Daily activities', 'Sexuality' and 'Aggressive emotions' in the women with TS compared with normal references. For the remaining domains, the HRQoL scores were comparable between with the 2 populations. For the 'social functioning' domain, the self-reports and the parent-reports were significantly different in terms of HRQOL.

Table 1: Clinical data of young women with Turner syndrome, who participated in the evaluation of the quality of life.

	Turner population (n = 49)
Age (yrs) at QoL evaluation	19.6 (3.0)
Range (years)	14.8–25.8
Adult height (cm)	160.7 (6.5)
Adult height SDS[a]	−1.2 (1.1)
Height SDS gain[b]	1.7 (1.0)
Age start puberty (years)	12.9 (1.1)
Karyotype 45,X	39 (80%)
'Other'	10 (20%)
Living situation:	
With parents	39 (85%)
With partner	2 (4%)
On their own	5 (11%)

Data are expressed as the mean (± SD) or n (%).

[a]: Height for age: references healthy Dutch girls [22]

[b]: Final height SDS − height SDS at start GH therapy (references for untreated Turner girls [23])

Table 2: Health related QoL (HRQoL) results of the SF36, expressed as the mean (± SD).

	Self-report n = 48	Parent-report n = 35	References[1] n = 121	P value[¶]
Physical Functioning	91.3 (12.4)	90.0 (17.8)	92.2 (11.0)	0.630
Role − Physical	91.1 (20.3)	92.1 (22.5)	86.8 (28.3)	0.145
Bodily Pain	86.4 (16.3)**	91.1 (20.0)**	79.0 (20.0)	0.003
Vitality	68.0 (18.0)	71.7 (13.5)	68.0 (16.2)	0.994
General Health	79.0 (16.3)	74.3 (19.9)	76.3 (16.6)	0.253
Social Functioning	92.4 (15.2)*	90.4 (17.4)	86.7 (16.9)	0.012
Role − Emotional	90.3 (24.8)**	91.4 (26.0)*	79.2 (33.5)	0.003
Mental Health	77.2 (17.0)	79.0 (12.8)	75.8 (14.4)	0.580

[1]: Aaronson NK, *et al* J Clin Epidemiol 1998;51(11):1055-68.[16]

Higher scores = better HRQoL.

* P < 0.05, ** P < 0.01 compared with reference values.

¶ P value of the difference between the self-report and the references.

Table 3: Health related QoL (HRQoL) results of the TAAQOL, expressed as the mean (±SD).

	Self-report n = 49	Parent-report n = 36	References[1] n = 116	P value[¶]
Gross motor function	92.6 (15.0)	90.3 (21.0)	89.3 (19.6)	0.299
Fine motor function	98.5 (5.3)	96.0 (8.9)	95.7 (13.1)	0.054
Cognitive functioning	79.7 (23.7)	84.2 (19.2)	83.2 (21.2)	0.356
Sleep	78.2 (23.2)	75.5 (26.9)	71.7 (25.7)	0.129
Pain	86.6 (16.7)**	85.9 (18.7)*	76.7 (21.7)	0.005
Social functioning	90.3 (17.6)	85.1 (15.9)[§]	89.3 (17.4)	0.731
Daily activities	91.1 (16.4)**	85.8 (23.2)	82.6 (22.8)	0.008
Sexuality	98.9 (5.3)**	92.1 (21.9)	89.8 (21.7)	< 0.001
Vitality	63.9 (20.4)	68.6 (20.0)	62.1 (22.6)	0.630
Positive emotions	69.6 (17.9)	69.4 (15.9)	73.3 (20.7)	0.275
Depressive emotions	78.1 (18.1)	78.7 (16.5)	75.1 (19.0)	0.351
Aggressive emotions	90.9 (13.5)**	89.8 (9.0)**	82.7 (17.8)	0.002

[1]: Manual TAAQOL, Leiden: TNO & LUMC [18]. Higher scores = better HRQoL.

* P < 0.05, ** P < 0.01 compared with reference values. [§] P = 0.01, self-reports compared to the parent-reports. [¶] P value of the difference between the self-report and the references.

Table 4: Additional data reported by Turner women and their parents.

	Turner women (n = 45)	Parents (n = 47)	P value
Satisfied with height:			
yes	42 (93%)	44 (94%)	1.00
no	3 (7%)	3 (6%)	
Satisfied with breasts:			
yes	26 (58%)	27 (57%)	0.77
no	17 (38%)	18 (38%)	
no opinion	2 (4%)	2 (4%)	
Self-reported visible Turner features:			0.007
None	12 (26%)*	28 (61%)	
Yes: n = 1	21 (46%)	11 (24%)	
n = 2	11 (24%)	6 (13%)	
n ≥ 3	2 (4%)	1 (2%)	
Self-reported limitations due to physical appearance:			
None	35 (78%)	30 (67%)[b]	
Yes:	10 (22%)	15 (33%)	0.66
Relationships	3a *	1	0.024
Work	4	3	0.36
Feelings of insecurity	4[a]	3	0.32
Lack of charisma	–	6	0.16
Buying clothes/ shoes	–	2	1.00

Data expressed as n (%). [a]: 2 Limitations reported by 1 patient. [b]: 2 Parent reports were missing.

*: Significant difference between the Turner women and their parents.

Additional data

Of the women with TS, 26% had a low SES level, 34% had an intermediate level and 40% had a high SES level, not significantly different from the SES levels in the normal population (ie, 33% low, 34% intermediate, and 33% high).

Table 4 gives data for subjective measures reported by the TS patients and their parents. Four of the women with TS and 2 of the parents did not return the questionnaire. The 3 patients who were not happy with their height would have liked to be taller. Of the 17 patients who were not happy with their breast development, 3 (18%) judged their breasts of uneven in size, 5 (29%) judged them too small, and 9 (53%) judged them too big. The parents scored significantly fewer features of TS than their daughters. The most common features of TS reported by the women with TS were short stature (n = 12), neck webbing (n = 8), and pigmented nevi (n = 4). Also reported were abnormal nails, characteristic facial expression, and slower motor performance.

The frequency of medical problems reported by the parents of the women with TS is 0 in 3 cases (7%), 1 in 12 cases (27%), 2 in 15 cases (33%), 3 in 10 cases (22%), 4 in 5 cases (11%). Two respondents did not answer this question.

Factors influencing HRQoL outcome

The explanatory variables that were evaluated separately are listed in Table 5. The estimated B coefficients, the relevant effects adjusted for age and SES, are also given. The table shows that the women who where satisfied with their height had a significantly better HRQoL (approximately 25 on a scale of 0 to 100) on the physical performance scales of both SF36 and TAAQOL. The height SDS gain had a significant positive effect on the HRQoL outcome of 'Role limitations due to physical health problems', with an 8.3-point higher HRQoL score per unit SDS height gain. SDS height gain also positively influenced daily activities. The absolute height only significantly influenced the HRQoL-outcome of 'vitality' of the TAAQOL. The women who had feelings of insecurity due to their physical appearance had a lower HRQoL in the 'Social functioning' domain. The difference was 18.5 points (95% confidence interval [CI] = 2.2 to 34.8) according to the SF36 and 30.6 points (95% CI = 13.5 to 47.7) according to the TAAQOL. Remarkably, the age at onset of puberty did not significantly influence any of the HRQoL outcomes. However, the satisfaction with breast development at time of the HRQoL evaluation did influence several of the HRQoL scales.

Having visible Turner features had a significant positive effect on scores for the 'Pain' domain of the TAAQOL (Table 5). This indicates that *having* reported visible features of TS is associated with a higher HRQoL with respect to 'pain', but the *quantity* of the reported visible Turner features was of no affect (data not shown).

Table 5: Effect of each of the explanatory variables: satisfaction with height and breast development, visible Turner features, limitations, final height, and height sds gain on HRQoL-outcome of the SF36 (5a) and of the TAAQOL (5b), corrected for age and SES.

5a: SF36		Satisfied with height[a]	Satisfied with breasts[a]	Visible Turner features[b]	Feelings of insecurity[c1]	Final Height (cm)	Height SDS gain[2] (SDS)
Physical functioning	B	13.5 (−1.8; 28.9)	8.1 (1.0; 15.3)				
	p	0.082	0.026				
Role – Physical	B	25.2 (0.3; 50.2)	13.2 (−0.6; 27.1)				8.3 (1.4; 15.2)
	p	0.047	0.060				0.019
Bodily pain	B		10.4 (0.2; 21.1)				4.3 (−1.4; 9.9)
	p		0.054				0.135
General health	B		9.7 (−0.8; 20.2)				
	p		0.069				
Vitality	B		11.1 (−0.3; 22.6)			−0.74 (−1.6; 0.1)	
	p		0.057			0.084	
Social functioning	B		13.1 (3.5; 22.7)		−18.5 (−2.2; −34.8)		
	p		0.009		0.027		
Role – Emotional	B		15.4 (−0.2; 31.1)				
	p		0.053				
5b: TAAQOL							
Gross motor functioning	B	24.8 (6.8; 47.7)		9.8 (20.6; −1.0)			
	p	0.008		0.074			
Fine motor functioning	B				−5.6 (0.03; −11.2)		
	p				0.051		
Sleep	B		15.3 (0.6; 30.1)				
	p		0.042				
Pain	B			11.7 (0.9; 22.5)			
	p			0.035			
Social functioning	B				−30.6 (−13.5; −47.7)		
	p				0.001		
Daily activities	B						4.3 (0.02; 8.5)
	p						0.049
Sexuality	B						1.0 (−0.3; 2.4)
	p						0.135
Vitality	B		10.9 (−2.3; 24.1)			−1.1 (−2.0; −0.2)	
	p		0.103			0.020	

Legend to Table 5: Results with a P value > 0.15 are not shown. Unstandardized coefficients are given, B (95% Confidence Interval).

[a]: Positive score indicates better QoL when being satisfied; negative score indicates better QoL when not being satisfied.

[b]: Positive score indicates better QoL when reported having TS features.

[c]: Negative score indicates lower QoL when having feelings of insecurity.

[1]: Feelings of insecurity due to their physical appearance. The variables of self-reported limitations due to physical appearance in relationships and work were of no significant influence on the QoL-outcome.

[2]: Height SDS gain = Final height SDS − height SDS at start GH-therapy (ref. untreated Turner girls [23]).

SES significantly positively influenced scores in the 'Depressive emotions' domain of the TAAQOL (data not shown). Karyotype, reported number of medical problems in the past, and age at onset of puberty had no significant influence in either of the HRQOL instruments. The SF36 HRQoL domains 'vitality', 'mental health', 'general health', and 'role − emotional' were not significantly influenced by the tested variables. Furthermore, for the TAAQOL HRQoL domains 'cognition', 'positive emotions', 'depressive emotions' and 'aggressive emotions' were not influenced by the variables evaluated.

Discussion

The women with TS reported higher HRQoL scores in the domains 'Social functioning' and 'Role limitations due to emotional health problems'. This means that women with TS felt less restricted in social contact and/or daily life due to their physical health or emotional problems than the reference population. The reported results of better HRQoL in social functioning in the TS women is remarkable; earlier data indicated that women with TS scored significantly lower on social acceptance than the normal population [8,25]. But the present study indicates that HRQoL is not influenced by this factor, as the women with TS reported normal or even better HRQoL in terms of in social functioning.

A possible explanation for this finding is that the women with TS no longer feel different from their peers as they attain normal height and normal feminization during the growth phase and adulthood. Satisfaction with breast development and a lack of feelings of insecurity due to physical appearance have a significant positive influence on social functioning. These data emphasize the importance of feeling equal to normal peers. GH and estrogen treatment resulted in normal height and breast development

in most of the girls [3,7] and diminished differences from peers. Because short stature and delayed pubertal development can be of significant negative influence on HRQoL [1,6], we hypothesize that GH treatment and appropriate induction of puberty can normalize HRQoL in women with TS.

Our group of women with TS showed even a *better* HRQoL than the reference population on some of the HRQoL scales. The women with TS had less aggressive feelings, demonstrating significantly better HRQoL for the TAAQOL domain 'Aggressive Emotions'. These results confirm earlier reports that girls with TS have less delinquent and aggressive behavioral problems [8,9]. This might be an effect of estrogen treatment, because estrogens significantly decrease aggressive and delinquent behavior in girls with TS [9], whereas GH has no influence [26]. It also may be influenced by the diminished androgens levels in TS [27], caused by lack of ovarian androgen production, because androgens increase aggressiveness [28]. However, the higher percentage of patients with karyotype 45,X in our study in comparison with other studies (approximately 80% *vs.* 65%) also must be taken into account. Spontaneous puberty, indicating (some) ovarian function, is less common in patients with karyotype 45,X than in patients with mosaic type

As a group, the women with TS had a normal HRQoL for motor performance. The women with TS who were satisfied with their height and those with a greater height gain had a better HRQoL in areas of physical/ motor functioning, suggesting a positive effect of the GH-induced height gain. The effect of estrogen treatment also may contribute to this finding, because estrogens also improve motor performances and speed in women with TS [29,30].

The possibility exists that women with TS have undergone a 'response shift', a change in internal standards or values [31]. This phenomenon has been reported in elderly persons who exclude some age-related problems or morbidities when they self-assessed their susceptibility to illness [32]. In terms of the present study, response shifting in our subjects may mean that the women with TS have different internal references than normal women. Facts supporting this hypothesis can be found not only in the reported relatively better HRQoL for 'social functioning' and 'role – emotional', but also in the better HRQoL for 'pain' and 'sexuality'. Based on our previous research and on reports in the literature, we did not expect these results. Because women with TS have more medical problems than the normal population, they tend to experience more pain and discomfort than those other women; also, women with TS are reported to be less sexually active then their normal peers [12]. Thus we remain uncertain whether the relatively high HRQoL scores for these domains in our women with TS reflects actual health effect or results from response shifting. Future studies that include cognitive interviewing will aid investigation of this hypothesis.

In general, the parent reports yielded similar results to those of their daughters with TS, except for assigning lower scores for their daughter's social functioning on the TAAQOL questionnaire (see Results). This finding confirms the validity of the HRQoL outcomes reported by the women with TS. The parents of the daughters with TS can be considered as first-choice proxy raters because they typically have intensive contact with their daughters, a phenomenon often seen in young adults with chronic diseases or disabilities and their parents.

Age at onset of puberty was of no significant influence on several HRQoL outcomes. Absolute height negatively influenced 'vitality'; however, the effect was small (B = −1.1; range −2.0 to −0.2). The normal height and pubertal development of our study population, as well as the small differences in final height and age range of pubertal onset, may explain the lack of a significant affect of these measures on HRQoL. These results confirm the recently published findings of Carel *et al* [14] that indicate normal HRQoL in TS with no significant influence of GH treatment-related variables, such as adult height or estimated height gain. However, our data reveal a significant influence of height gain on the 'role limitations due to physical health problems' and 'daily activities' domains. Furthermore, in our study, satisfaction with height and/ or breast development had a significant positive influence on several HRQoL domains, and those women with TS who reported feelings of insecurity due to their physical appearance had lower HRQoL in the 'social functioning' domain.

To appreciate our results one has to consider the following. HRQoL was not evaluated longitudinally, because the HRQoL questionnaires were developed in the early and mid 1990s and the GH-studies began in 1989, when HRQoL became recognized as an important measure of the impact of a special disorder, disease or therapeutic outcome. Secondly, our study did not specifically evaluate the consequences of infertility on HRQoL. This might be a relevant factor with increasing age, because infertility is present in the majority of adults with TS and can have a significant influence the HRQoL [33-36]. Future studies are needed to evaluate the influence of infertility on HRQoL in women with TS. Thirdly, it would have been ideal to compare the treated TS population to an untreated TS population. However, given that GH treatment for girls with TS is common practice since early 1990s, no untreated population in the same age range was available for study. Finally the response rate was 71%. There might have been a selection bias, but we believe that this unlikely, because there were no significant differences in clinical data, such as age, height, height gain, age at start of puberty and karyotype, between the respondents and non-respondents.

In conclusion, our study shows a normal health related quality of life in young women with TS after long-term GH treatment and puberty induction at an age-appropriate time. The relative high scores on some of the HRQoL scales can be explained by an estrogen-effect or by a possible response shift, indicating that the

TS women might have a different internal reference. Additionally, satisfaction with height and with breast development had a significant positive influence on several HRQoL scales, including social functioning and physical functioning. Therefore we hypothesize that GH and estrogen treatment positively influenced HRQoL in young women with TS.

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Chapter 6

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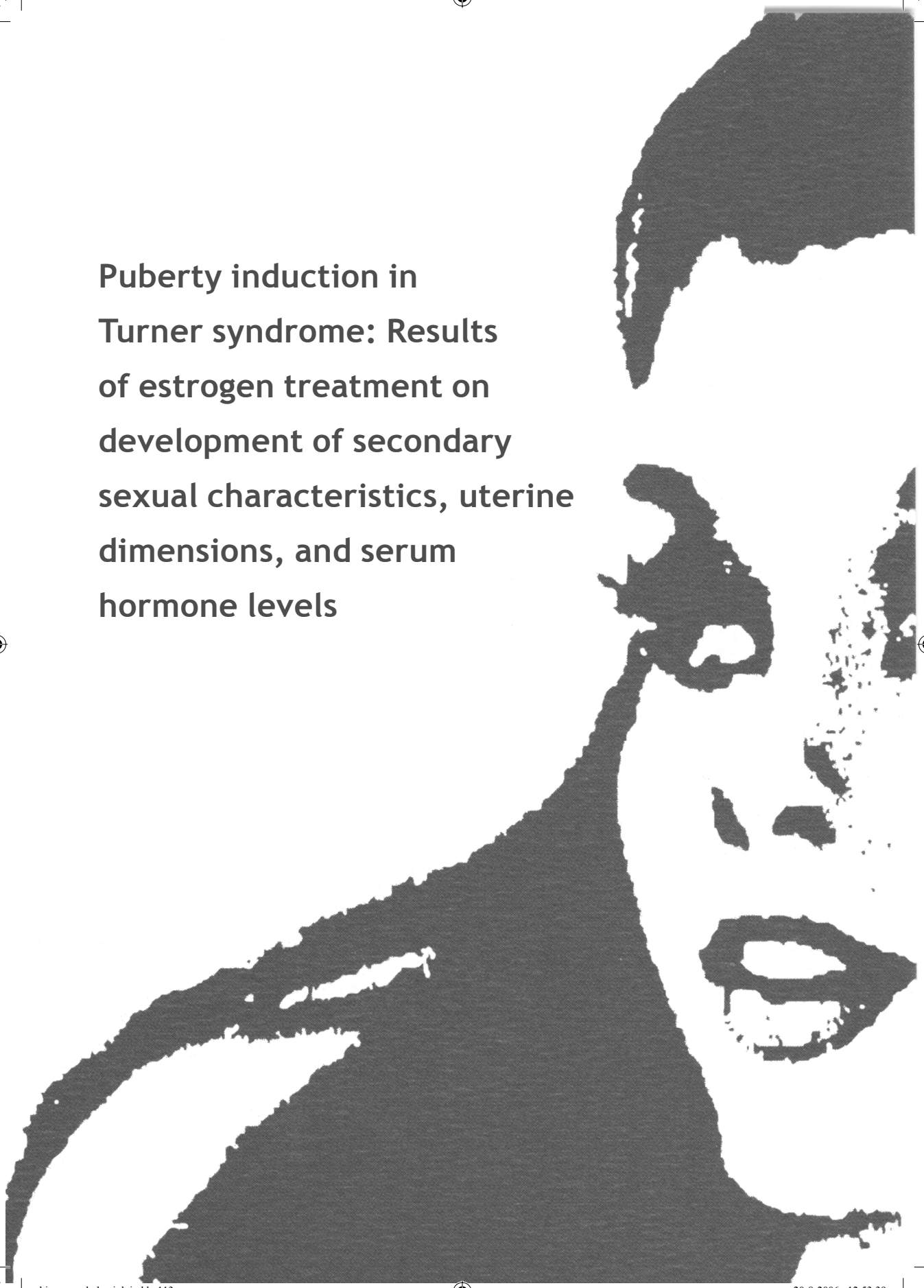
Submitted

Puberty induction in Turner syndrome: Results of estrogen treatment on development of secondary sexual characteristics, uterine dimensions, and serum hormone levels



Abstract

Background: Besides short stature, gonadal dysgenesis leading to a lack of estrogens is one of the main characteristics of Turner Syndrome (TS). In most TS girls, puberty is induced with exogenous estrogens.

Objective: To describe the pubertal development and uterine dimensions achieved by low-dose 17β-estradiol orally started at an appropriate age. Additionally, to determine whether serum hormone levels aid evaluation of pubertal progression.

Design: In 56 TS girls, we studied pubertal stage, serum E2, LH, FSH, SHBG, and E1, starting estrogen treatment with a low dose 17ß-estradiol (5 µg/kg/day) during GH-treatment at age 12.7(0.7). Hormone levels were measured at start, 3 months after start and increasing 17ß-estradiol dosage. Uterine dimensions were measured in 39 TS women at age 19.9 (2.2).

Main outcome: Although breast and pubic-hair development were similar to that in normal Dutch girls, breast development was 2 years delayed. Before estrogen therapy, E2-levels were comparable to those in prepubertal girls. With 17ß-estradiol dose of 5 µg/kg/d, these levels increased significantly, becoming comparable to normal late pubertal or adult concentrations, whereas SHBG-levels were unchanged. At adult 17ß-estradiol dose, SHBG had increased significantly. Uterus shape was infantile in 4 (10.2%), cylindrical in 4, and heart-shaped in 31 (79.5%) of TS patients.

Conclusions: During GH-treatment in TS girls, normal breast development can be mimicked, but with a 2-year delay. In a clinical setting, serum hormone levels provide no additional information for evaluating pubertal progression. After age-appropriate pubertal induction, uterine dimensions in women aged nearly 20 were subnormal. It remains unclear whether this was related to E2-dosage or duration, or factors related to TS.

Introduction

Besides short stature, gonadal dysgenesis is one of the main characteristics in Turner Syndrome (TS). It leads to lack of estrogens, which play an essential role in changes occurring during female puberty, such as the development of secondary sexual characteristics, the establishment of fertility, and the pubertal growth spurt.

Between 5% and 10% of women with TS start pubertal development spontaneously, the frequency being higher number in women with TS with mosaicism than in those with TS with the 45,X karyotype (40% *vs*. 8%). However, very few of these women maintain ovarian function, and spontaneous pregnancies are rare (app. 2–5%) [1-3]. In most of the girls with TS, puberty has to be induced with exogenous estrogens.

Uterine dimensions in untreated girls and young women with TS are small for age, and are described as pre-pubertal [4,5]. While some studies have reported that estrogen therapy in early to mid-adolescence leads to normal uterine development [6,7], others have reported that development of the uterus after estrogen therapy is suboptimal [8-10]. However, the differences between these studies – which mainly involved different age ranges, the route of estrogen treatment, and the form of estrogen therapy – mean that their results are difficult to compare. Most of these studies did not have a standardized puberty-induction treatment protocol.

The debate on TS has often focused on the effect of estrogen therapy on adult height, and on whether estrogen therapy should be started during GH treatment or afterwards [11-14]. Recently, various studies (including our own) have shown that when GH treatment has been optimized, it was not necessary to delay the induction of puberty [15-17], and that estrogens in a low dose did not negatively influence height velocity or adult height.

Little is known about their breast development compared to normal breast development, when estrogens are used in a low dose, started at an appropriate age. The present study therefore describes the breast development and uterine dimensions that followed after puberty induction, started at an appropriate age, using low doses of oral 17β-estradiol. We also discuss whether measurements of serum levels of estradiol (E2), estrone (E1), gonadotropins, and sex-hormone binding globulin (SHBG) are useful in evaluating induced pubertal development.

Patient and Methods

Study design

This study was performed in a population of patients with Turner Syndrome (TS) who participated both in a GH trial, and additionally in a follow-up study.

GH trial: As previously described by Van Pareren *et al* [15], sixty-eight previously untreated girls with TS started in an open-randomized multi-center growth hormone (GH) dose-response study that began in the Netherlands in November 1989. Subjects were included if their chronological age was between 2 and 11 years, and their height below 50th percentile according to normal Dutch references [18]. Every three months during the GH trial, their height and weight were measured, and their pubertal stage according to Tanner [19] was assessed. The GH treatment was discontinued when final height had been attained; in the study protocol, this was defined as a height velocity of less than 1 cm over 6 months. Height and weight were expressed as SD-score using the references for healthy Dutch girls [20] or the references for North European untreated girls with TS [21].

In order to induce puberty, a daily oral dose of micronized 17ß-estradiol was given to girls aged 12 and over who had already undergone at least four years of GH treatment. In the first two years, a dose of 5 µg/kg body weight/day (equivalent to 0.05 µg ethinyl estradiol/kg/d) was given; in the third year the dose was raised to 7.5 µg/kg/d, and thereafter it was 10 µg/kg/d (tablets containing 0.1 mg micronized 17ß-estradiol being supplied for the study by Novo Nordisk A/S, Bagsvard, Denmark). After two years of estrogen therapy, cyclic progestagen therapy 5 mg/d was added in the first 14 days of the month (dydrogesterone (Duphaston®) Solvay Pharmaceuticals BV, Weesp, the Netherlands). If start of puberty had developed spontaneously into Tanner breast stage B2, the start of estrogen therapy was postponed for one year. If, one year later, Tanner breast stage was still B2, estrogen therapy was started according to the schedule as described above. If Tanner breast stage was ≥ B3, no estrogens were given.

Post GH trial: Six months after GH therapy ended, one more visit took place as part of the GH trial. Thereafter, the regular check-ups was performed by the girls' pediatric endocrinologist. In 2003, a follow-up study took place, a mean of 4.8 (2.0) years after the end of GH therapy. As well as other assessments, this involved assessment of the pubertal stages and pelvic ultrasound of the internal genitalia.

After the end of GH therapy, estrogen treatment was increased to an adult dose of 1 mg/day, and additionally to 2 mg/day. Cyclic progesterone dosage was increased to 10 mg/d.

At the end of GH therapy, pubertal development was re-evaluated in girls who had had spontaneous start of puberty and no estrogen treatment during GH therapy. If progression of breast development was insufficient in these girls, estrogen substitution therapy was initiated.

Study subjects

As described above, 68 girls with TS started in the GH-trial. Four girls dropped out before the start of estrogen therapy, and could not be included in this evaluation. Fifty-five girls out of the 64 had karyotype 45,X, and eight had a variant karyotype.

Six girls entered puberty spontaneously, four with karyotype 45,X and two with a variant karyotype; none of these 6 girls were included in the evaluation of breast development and evaluation of serum hormone levels (see below). Two girls dropped out of the study three and six months after start of estrogen therapy, and could not thus be included in the evaluation of the pubertal development and hormone levels. This left n = 56 for analysis (Figure 1), 50 of whom had karytotype 45,X, and six a variant karyotype.

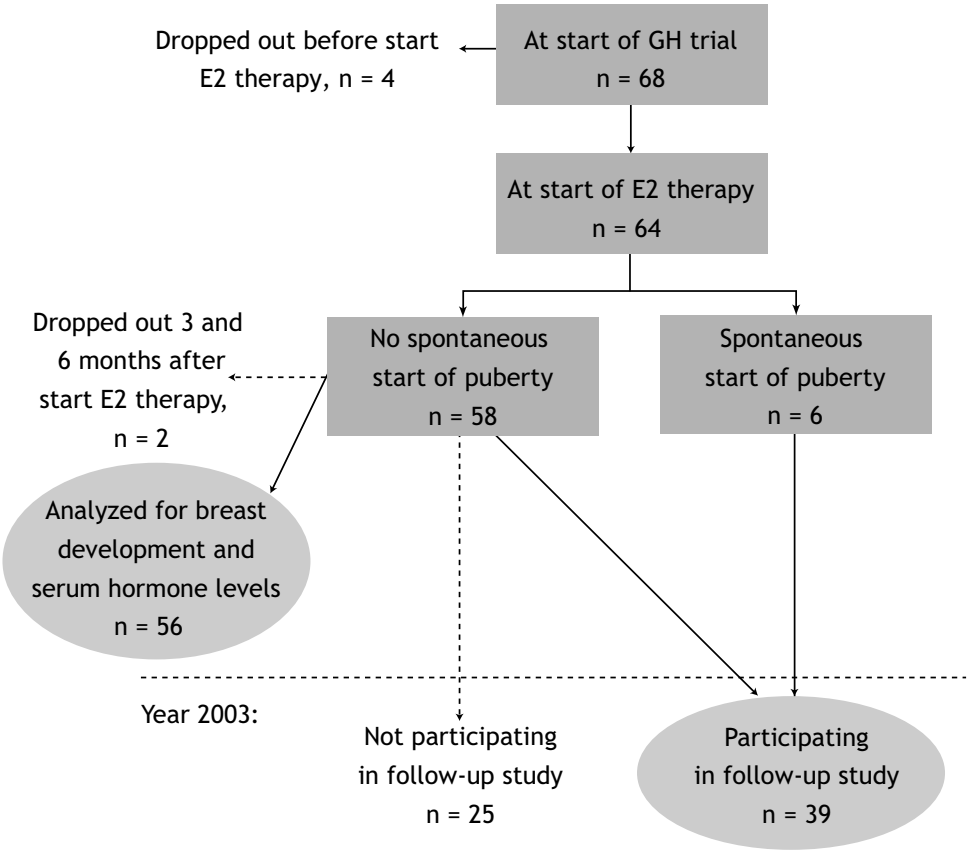


Figure 1: Flow diagram of the patients in the initial GH-trial and the follow-up study.

Thirty-nine girls participated in the follow-up study, a mean of 4.8 (2.0) years after the end of GH treatment. Twenty-five did not participate in the follow-up study, due either to lack of motivation (n = 18) for reasons including psychological problems, practical considerations or lack of interest. One was lost to follow up due to emigration, and six not included due to mental retardation or autism. Of the remaining participants, six had undergone a spontaneous start of puberty, and in 33, puberty was induced according to the study protocol (Figure 1). Five of the six girls with spontaneous start of puberty had started estrogen treatment at GH discontinuation. Thirty-one girls out of the 39 had karyotype 45,X, and eight had a variant karyotype.

The GH trial protocol was approved by the Medical Ethics Committees of each participating center, and the follow-up study protocol was approved by the Medical Ethics Committees at Erasmus University Medical Center. Written informed consent was obtained from the girls and/or their parents.

Measurements

Secondary sexual characteristics: During the GH trial, pubertal stage was determined every three months using the criteria and definitions described by Tanner [19]. After the GH trial, data was collected from the hospital information systems of the different hospitals, up to a mean age of 16.7 (1.2), range 14.5–19.8 years. Breast development was compared to that in healthy Dutch girls (n = 3562) [2].

Serum hormone levels: The serum concentrations of E2, E1, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and SHBG were measured at start of estrogen therapy, three to six months after start of estrogen treatment, and after each increase in estrogen dosage. Estrogens were administered on the morning of the hospital visit. To measure the serum E2 levels at the highest expected serum concentration, blood samples were taken 4–6 hours after estrogen administration [23]. Concentrations of serum E2 and E1 were measured using radioimmuno-assay kits provided by Diagnostic Products Corporation (Los Angeles, CA) and Diagnostic Systems Laboratories (Webster, TX). LH, FSH and SHBG were estimated using luminescence-based immunometric assays (Immulite 2000, Diagnostic Products Corporation).

Serum E2 levels were compared to normal levels as described by Sehested *et al* [24], who described a normal female population (n = 403) without oral contraceptives during pubertal development. Serum E2, LH and FSH were reported according to breast-stage, median age and range.

Pelvic ultrasound: Pelvic ultrasound was part of a follow-up study performed 4.8 (2.0) years after the end of GH therapy, when the girls were aged 19.9 (2.2).

Mean duration of estrogen therapy at ultrasound measurement was 7.1 (2.2) years. The ultrasonographic examination was performed transabdominally according to the conventional full-bladder technique. The ovaries were measured when visible, and their volume was calculated. The fundo-cervical (FC) ratio was calculated as: (anterior-posterior (AP) diameter of the fundus) / (AP diameter of the cervix). Uterine shape was designed as infantile (cervix larger than fundus), cylindrical (mid-childhood with cervix and fundus approximately the same), and heart-shaped (mature-adult shape with fundus larger than cervix) [25]. Uterine volume was calculated according to the formula for a prolate ellipsoid: maximal depth X maximum width X maximum length X 0.523 [25]. Girls who had entered puberty spontaneously were included in the analysis of the pelvic ultrasound.

Statistical methods

Unless indicated otherwise, results were expressed as mean ± SD. Repeated measurement models were used to compare serum hormone levels with the different oral estradiol dosages over time. As oral estrogen dose was increased more rapidly to an adult dose after discontinuation of GH, not all girls had an estrogen dose of 7.5 µg/kg/d (n = 38) and 10 µg/kg/d (n = 21). Hormone levels at the adult estradiol dosage of 1 and 2 mg/day were taken together for analyses. As serum could not be collected from all patients at the adult oral estradiol dose, the repeated measurement was performed separately in the subgroup concerned (n = 19) for purposes of comparison with the last measurement available (5, 7.5 or 10 µg/kg/d) and with baseline measurement. Geometric mean and 95% confidence interval of the estimates are given. Spearman's correlations were used to assess the relation between breast stage and serum E2 levels; between duration from breast stage B2-B4 and age at start of estrogen therapy; and between serum levels of E2 and serum levels of LH, FSH, SHBG, and E1. Student's *t*-tests were performed to analyze the differences in uterine length, shape and volume between women having karyotype 45,X and those with a variant karyotype, and between woman who had spontaneous pubertal onset and those who had not. P-values < 0.05 were considered to be statistically significant. All calculations were performed with SPSS 11.5.

Results

Study subjects

In girls without spontaneous start of pubertal development, the mean age at start of puberty induction was 12.7 (0.7) years, with a range 11.8–15.0 yr. The mean GH duration before start of estrogen therapy was 6.2 (1.9) years. The mean GH

duration after start of estrogen therapy was 2.6 (0.9) years. Six girls entered puberty spontaneously, one of whom had regular menstrual cycles. The remaining five started estrogen therapy at GH discontinuation, at a mean age of 15.1 (1.1) years.

Secondary sexual characteristics

The average age (50th percentile) (P10-P90) for attaining the different stages of breast and pubic hair development are shown in Table 1. The different breast stages advanced gradually, and were comparable to development in the normal Dutch female population [22], albeit with a two-year delay (Figure 2, Table 1). Six of the 56 girls had some breast development (B2) without further spontaneous progression, and started estrogen treatment one year later according to the protocol.

Table 1: Mean ± SD (range) age at which the different stages of secondary sexual characteristics were reached.

	Turner population P50 (P10–P90)		**Normal Dutch population[a] n = 3562 P50 (P10–P90**	
Stage:	Breast stage	Pubic hair stage	Breast stage	Pubic hair stage
2	n = 56 12.67 (12.06–13.53)	n =56 10.32 8.28–12.36)	10.72 (9.0–12.2)	11.01 (9.4–12.5)
3	n = 54 13.76 (12.79–15.76)	n = 54 11.87 (10.07–13.67)	11.90 (10.5–13.1)	11.89 (10.6–13.2)
4	n = 49 15.13 (13.61–18.12)	n = 55 13.15 (11.57–14.72)	12.84 (11.5–14.5)	12.68 (11.4–14.3)
5	n = 23 19.23 (15.19–NA)	n = 46 15.13 (13.25–NA)	14.34 (12.5–19.5)	13.76 (12.1–17.7)

[a]: Mul, D., *et al*, Pediatr Res, 2001. **50**(4): p. 479-86. [22]

The development of pubic hair in the Turner girls was similar to that in the normal Dutch female population (Figure 3, Table 1). Seventeen of the TS girls started to have withdrawal bleedings 1.5 (1.2) years after start of dydrogesterone 5 mg/d, and 30 started to have withdrawal bleedings 0.5 (0.6) years after start of a dydrogesterone dose of 10 mg/d.

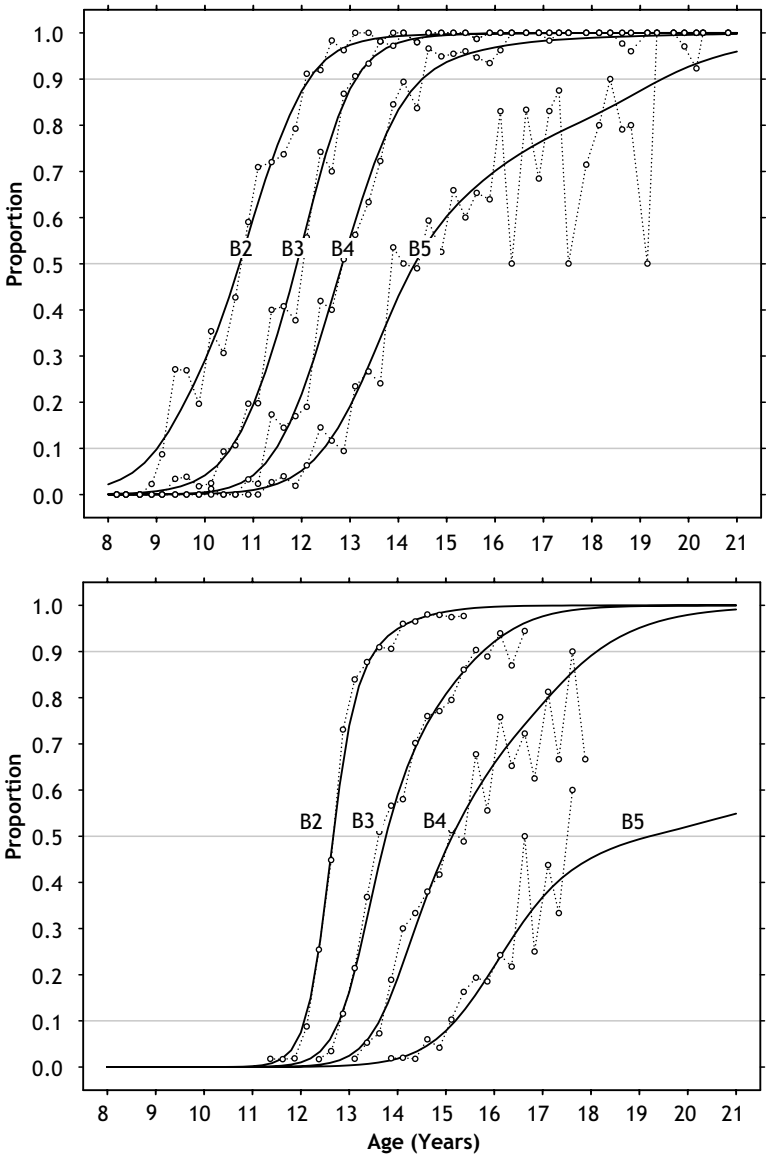


Figure 2: Breast stages according to Tanner in a normal Dutch reference population (*top panel*) [22] compared to that in girls with Turner Syndrome (*lower panel*) who started 17β-estradiol at an age-appropriate time. Adapted from Mul *et al* [22] with permission.

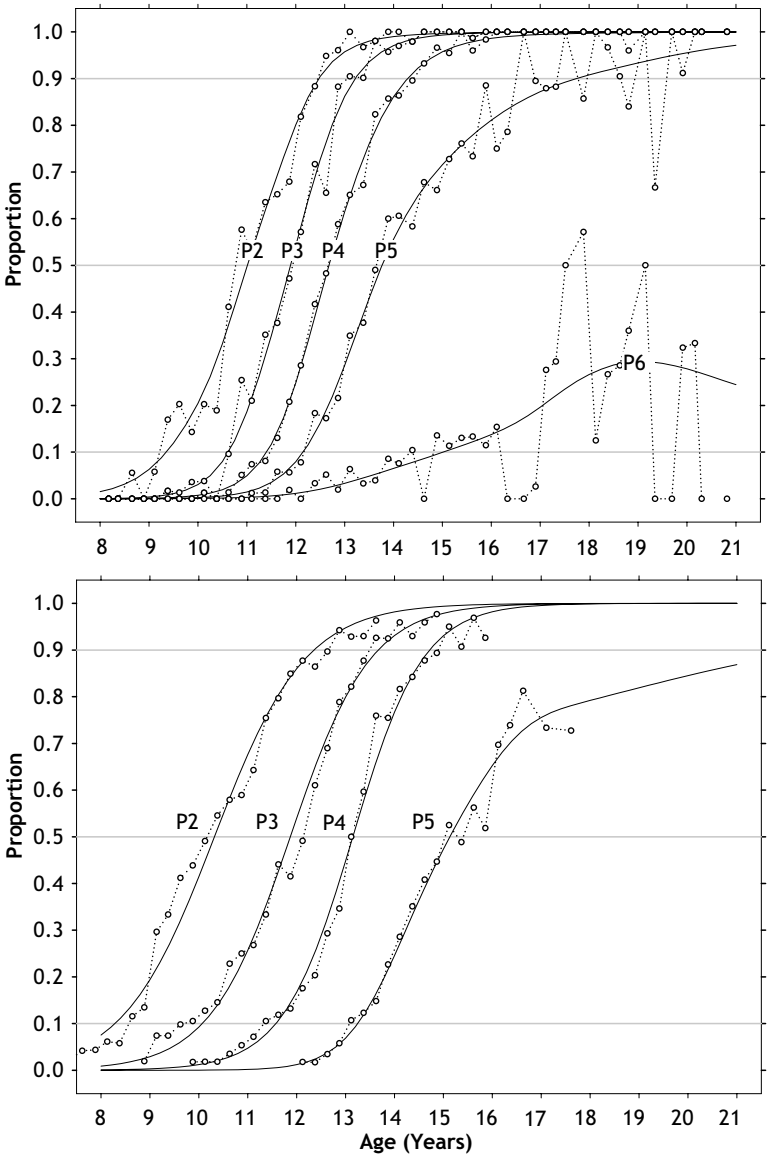


Figure 3: Pubic-hair stages according to Tanner in a normal Dutch reference population (*top panel*) [22] compared to that in girls with Turner Syndrome (*lower panel*) who started 17β-estradiol at an age-appropriate time. Adapted from Mul *et al* [22] with permission.

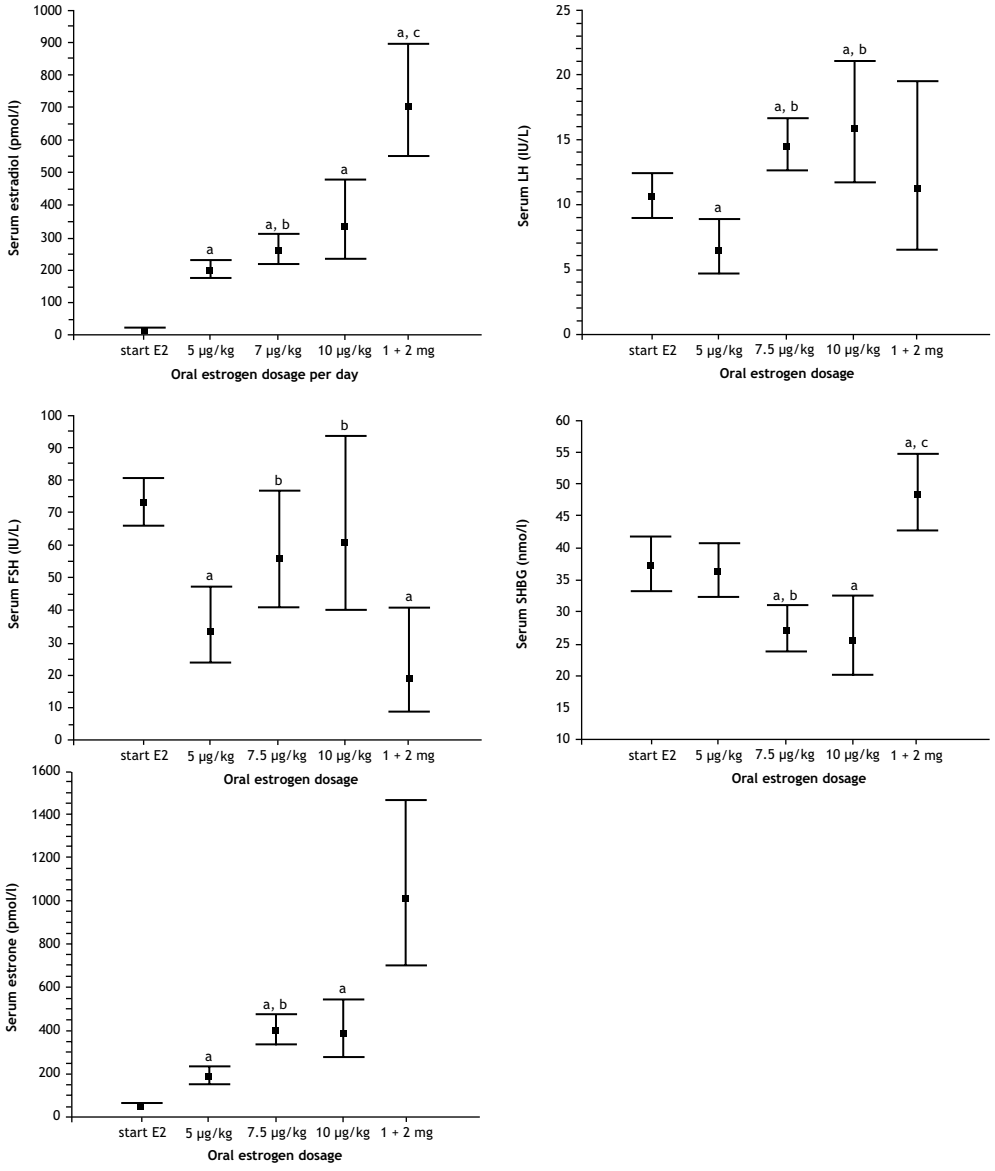


Figure 4: Geometric mean and 95% confidence interval at different oral 17ß-estradiol dosages of serum estradiol (pmol/l)(5a), LH (IU/L)(5b), FSH (IU/L)(5c), SHBG (nmol/l)(5d), and estrone (pmol/l)(5e).

Analysis of repeated measurements produced the following significant differences (P value < 0.05): ***a***: compared to start E2, ***b***: for the oral dosage of 7.5 and 10 µg/kg/d compared to preceeding dosage of 5 respectively 7.5 µg/kg/d, ***c***: adult dose of 1 or 2 mg/day compared to the levels at the last oral dosage taken.

Serum hormone levels

Figure 4 shows the geometric mean and the 95% confidence interval of serum E2, E1 LH, FSH, and SHBG concentrations at the different oral 17ß-estradiol dosages. At 24.7 pmol/l (95%CI 22–28), the mean serum E2 concentration at start of estrogen therapy (mean age 12.7(0.7) years) was comparable to that in prepubertal girls (mean age 9.1 yr) with serum estradiol levels of 22 pmol/l (95%CI < 18–52) [24]. Measured at the expected peak concentration, the serum E2 concentration increased significantly to 202 (95%CI 176–231) pmol/l at a 17ß-estradiol dose of 5 µg/kg/d, which is comparable to normal late pubertal (breast stage 4/5) or adult serum E2 concentrations (B4: 162 pmol/l 95%CI < 18–1094 pmol/l, B5: 182 pmol/l 95%CI 27–1108 pmol/l, or adults: 289 pmol/l 95%CI 74–1075 pmol/l, respectively) [24]. The serum E2 levels at the adult oral dose of 1 or 2 mg/d (mean 703 pmol/l 95%CI 552–895 pmol/l) were significantly higher than the serum E2 levels at the preceding dose. Serum E1 concentrations rose significantly along with the increase in 17ß-estradiol dosage.

Mean LH concentrations at an oral 17ß-estradiol dose of 5 µg/kg/d were lower than before start of 17ß-estradiol (6.4 IU/l 95%CI 4.7–8.8 IU/l and 10.6 IU/l 95%CI 9.0–12.4 IU/l, respectively). The LH levels at oral 17ß-estradiol dosages of 7.5 and 10 µg/kg/d were higher relative to the levels at the 5 µg/kg/d, and also significantly higher than before start. FSH concentrations showed a significant decrease after the start of estrogen therapy. SHBG concentrations did not change after starting the lowest dose of 5 µg/kg/d, and decreased significantly with a dose of 7.5 and 10 µg/kg/d. The serum SHBG levels at the adult oral dose of 1 or 2 mg/d (mean 48 nmol/l 95%CI 43–55 nmol/l) were significantly higher than the serum SHBG levels before start (37nmol/l 95%CI 33–42 nmol/l) and than during the preceding estrogen dose.

Table 2: Mean ± SD (range) of uterine dimensions during follow-up study 7.1 (2.2) years after start of estrogen therapy, and 4.8 (2.0) years after discontinuation of GH.

	Total group n = 39	Karyotype	
		45,X n = 31	'variant' n = 8
Age (years)	19.9 ± 2.2 15.0 – 23.4	19.7 ± 2.1 15.0 – 23.2	20.7 ± 2.4 16.6 – 23.4
Uterus length (mm)	60.1 ± 15.9 25.0 – 87.0	58.9 ± 16.7 25.0 – 87.0	66.9 ± 11.4 49.0 – 83.0
Uterus volume (ml)	24.8 ± 15.0 4.4 – 57.9	22.3 ± 13.0* 4.4 – 57.5	34.5 ± 19.0 7.9 – 57.9
Fundo-cervical ratio	5.5 ± 0.5 0.8 – 2.8	1.4 ± 0.5 0.8 – 2.8	1.5 ± 0.3 1.1 – 2.0

*: Significant difference between patients with karyotype 45,X and 'variant' with P < 0.05.

Pelvic ultrasound

***Uterus*:** Uterine dimensions are shown in Table 2. Uterine volume in patients with karyotype 45,X was significantly smaller than in patients with variant karyotype. There were no significant differences between patients with spontaneous start of puberty and those without. Fundo-cervial ratio (FCR) was infantile (FCR < 1) in four patients (10.2%), cylindrical (FCR≈1) in four, and heart-shaped (FCR > 1) in 31 (79.5%). None of the patients with variant karyotype had an infantile shaped uterus, and one out of the nine had a cylindrically shaped uterus.

***Ovaries*:** Streaks or no ovaries were detected in 17 girls out of the 39. In four girls (one with spontaneous puberty) one ovary was detectable, while in 18 girls (two with spontaneous puberty) two ovaries were visualized. The mean ovarian volume of all ovaries measured (n = 40) was 2.5 (2.2) ml. The volumes of the ovaries detected in the girls with start of spontaneous puberty were not significantly larger than those measured in the girls without spontaneous start of puberty. The girl with regular menstrual cycles had ovarian volumes of 1.3 and 1.5 ml.

Correlations

Age at start of estrogen therapy was negatively correlated with the duration from B2 to B4 ($r = -0.30$, $P < 0.05$). Serum E2 concentration was positively correlated with breast stage ($r = 0.71$, $P < 0.001$). Serum E2 concentration was negatively correlated with serum FSH ($r = -0.31$, $P < 0.001$), but not with serum LH ($r = 0.01$, $P = 0.87$), nor with serum SHBG ($r = 0.04$, $P = 0.625$). It was also strongly correlated with serum E1 concentration ($r = 0.81$, $P < 0.001$).

Discussion

The main purpose of estrogen therapy in girls with Turner Syndrome (TS) is to induce puberty and feminization as physiologically as possible, without sacrificing adult height. The present study shows the results of puberty induction during GH treatment in girls with TS, starting at an age of 12.7 (0.7) years. Induction was started with a very low dose of 17β-estradiol for two years before the estrogen dose was slowly increasing. 17β-Estradiol is a natural estrogen, which has less pronounced effects on the coagulation factors, lipid profiles and blood pressure than synthetic estrogens [26]. The low dose did not affect height velocity [15].

Although it has previously been reported that normal progression through the breast stages is possible in girls with TS [7,11], the results are difficult to compare, as the form, dosage, and route of the estrogen treatment were not the same as in our study. Chernausek et al used conjugated equine estradiol in a daily dose of 0.3 mg

(~0.48 mg 17ß-estradiol) over six months, following this with 0.625 mg daily – a higher estrogen dose than used in our study [27]. As their dosage was thus relatively high with a faster dosage increase, it was not beneficial for adult height [11]. At start of puberty induction, the other study referred to above had a large age range (10.7 to 17.7 years) [7].

Our own study showed that with a very low dose of oral estrogen therapy over two years, normal breast development occurs in the majority of Turner women, but two years later than in their peers; the estradiol dosage is then slowly increased. As adult height is not affected by this low dose, it is possible to start this estrogen regimen during GH therapy at an appropriate age.

Our results also showed that the older the TS girls at start of estrogen therapy, the faster the progression through puberty. This can probably be explained by the faster increase in estrogen dose, which was increased to an adult dose after final height had been attained. This phenomenon mimics the natural situation in normal girls, where an earlier onset of puberty corresponds with a longer duration [28].

Pubic hair developed similar to normal, and had started in most girls before estrogen treatment was initiated. This indicates that, although ovarian androgen production is lacking, adrenarche in girls with TS proceeds normally, which is in agreement with earlier reports [29].

The levels of serum E2 at the lowest oral estrogen dose were comparable to normal late pubertal or adult serum E2 concentrations [24]. However, the E2 levels in these healthy girls and women showed a wide confidence interval, possibly because blood samples were taken irrespective of the day of the menstrual cycle. The lowest E2 (5 µg/kg/d) dose suppresses LH and FSH. However, the double E2 dose (10 µg/kg/d), also providing double serum E2 levels, LH and FSH levels increased. This effect may be a result of changed sensitivity of the E2 feedback, possibly due to an increased age or to a higher tolerance. Furthermore, the E2 and E1 levels were measured at the expected highest serum concentration, which means that the *mean* serum value is lower. The lack of an increase in serum SHBG after starting estrogen substitution in the Turner girls, and the relatively high gonadotropin levels, both indicate that the overall serum E2 effect at the low estradiol dose was small.

In postmenopausal women receiving 2 mg estradiol per day, gonadotropins decreased significantly [30,31], resulting in LH and FSH levels which are comparable [31] or lower [30] than the levels during treatment with the adult dose reported in our study. Whereas the lower 17ß-estradiol dosage during GH therapy did not significantly increase SHBG levels, these levels increased significantly after the adult dose. This was comparable to the situation in postmenopausal women taking 2 mg of 17β-estradiol, in whom SHBG levels also increased significantly [31].

In our Turner population, uterine volume was smaller (mean 24.9 ml, range 4.4–57.9 ml) than that reported in normal female students of the same age who had

never been pregnant (mean 61 ml, range 37–130 ml) [32]. Furthermore, the authors reported that the uterus had continued to grow several years after menarche, resulting in a larger uterine volume at young adulthood than in fully matured girls at the age of approximately 15 years. Most reports on uterine dimensions provide normative data up to the age of 15–16 years [33-36]. The uterine volume, length and shape in our Turner population were comparable to uterine dimensions in normal girls who have reached breast stage B5, and/or those aged 14–16 [33-35]. As normal uterine volume increases after breast stage B5 has been reached, or after the age of 15, uterine length and/or shape will increase simultaneously. This may indicate that uterus development in our Turner population at age 19.9 (2.2) was suboptimal compared to that in women of the same age. However, there is no normative data on uterine length and shape in women aged 15–25. Although Paterson et al reported similar uterine length in an estrogen-treated Turner population of a similar age range, uterine shape was less mature than in our population [8]. One explanation for the differences between their study and ours may be the age at initiation of the estrogen replacement and the period of estrogen therapy before ultrasound measurement, which were not reported in their paper. Furthermore, the form of estrogen treatment may have been of influence, as they used ethinyl estradiol, whose pharmaco-kinetics are different from those of 17ß-estradiol.

TS women participating in an ovum-donation IVF program were reported to have a lower successful pregnancy rate per embryo-transfer [37,38]. If TS women attain normal adult uterine dimensions, this may reduce the number of pregnancy-related problems they face in an ovum donation IVF program. It has been suggested that lacking small amounts of estrogens during childhood or starting estrogen replacement later than at the physiological age of the serum estradiol increase, resulting in a hypoplastic uterus, may be irreversible [8,9]. However, Snajderova *et al* [10] reported that in adult TS women with a median age of 21.4, a higher daily dose of estradiol was associated with a greater uterine length, and that uterine length was positively associated with uterine shape. These data suggest that possibly a higher estrogen dose may result in normal uterine dimensions at an adult age, and be more suitable for embryo transfer and pregnancy. An earlier report had already proposed that, for optimum endometrial response and improved outcome, higher constant estrogen replacement was needed before embryo transfer [37]. It is possible that the dose in our study was not sufficient for increasing uterus dimensions. Alternatively, other factors related to TS may underlie uterine dimensions that remain subnormal. But as puberty induction started two years after the onset of physiological pubertal, it is also possible that uterine development is subject to delay.

Successful pregnancy requires an optimal response by the endometrium. In our population we did not measure endometrial thickness, as we did not standardize the timing of the ultrasound before the timed withdrawal bleeding.

In conclusion, when a low dose of oral 17ß-estradiol is started at an appropriate age to induce puberty in girls with TS, the breast development that follows is comparable to normal breast development, but with a two-year delay. Serum hormone levels do not provide additional information for evaluating the progression through puberty in a clinical setting. In addition, uterine dimensions in TS women aged nearly 20 years were comparable to those in normal fully matured girl aged 15. It nonetheless remains unclear whether subnormal uterine dimensions in young TS women are related to the dosage or duration of estrogen administration, or to other factors related to Turner syndrome.

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Chapter 7

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Submitted

Long-term follow-up after growth hormone therapy in women with Turner Syndrome

Abstract

Objective: To investigate whether long-term GH treatment influenced the risk factors for cardiovascular disease, carbohydrate metabolism, and body proportions in young TS women, several years after ending long-term GH treatment, and to compare them with normal and, if possible, untreated TS women.

Design: Follow-up study after randomized GH dose-response trial.

Methods: Thirty-nine patients with TS (20.0 $\pm$ 2.1 year) participated, 4.8 (1.9) year after GH discontinuation. Mean GH duration 8.7 (2.0) year; in 3 different GH-dosages (1.3, 2.0, and 2.7 mg/m^2/d).

Measurements: fasting glucose, insulin, serum lipids, blood pressure, body proportions.

Results: Several years after GH-discontinuation, insulin sensitivity remained lower; ß-cell function and fasting insulin levels remained higher than pre-treatment. ß-Cell function was positively influenced only by BMI. During GH treatment, diastolic BP had decreased. Several years after end of GH, diastolic BP had increased and was similar to pre-treatment levels. Diastolic BP was negatively influenced by GH-dose. Systolic BP was not influenced by GH dose or duration. Serum TC, LDL and HDL had further increased compared to 6 months after GH, resulting in higher TC, but also higher HDL levels compared to controls. AI remained constant, which is lower than controls. The BMI increased gradually during and after GH-therapy. During GH therapy, shape values of sitting height decreased, of the foot increased, and of the hand, biiliacal and biacromial diameter remained constant.

Conclusions: GH therapy in girls with TS has, besides height, additional beneficial effects on BP, serum lipids, and body proportions, except on foot length. Nearly five years after GH discontinuation the favorable effect of GH on BP and serum lipids was still noticeable. The GH-induced decreased insulin sensitivity, however, remained unchanged, possibly due to having TS.

Introduction

Girls with Turner syndrome (TS) have two main characteristics: short stature and ovarian failure. Although they are not growth-hormone (GH) deficient, GH treatment is common in many countries [1]. This improves height during childhood and adulthood, and results in an adult height within the normal range [2-4]. Due to ovarian dysfunction, puberty has to be induced in most TS girls by exogenous estrogen treatment.

Since TS was first described in seven female patients by Dr. H. Turner in 1938 [5], it has become increasingly clear that the syndrome is associated not only with short stature and hypogonadism, but also with a number of diseases and conditions. As well as having a higher incidence of congenital cardiovascular malformations, TS women are also predisposed to cardiovascular disease (CVD), as CVD risk factors such as hypertension, hyperlipidemia, and insulin resistance occur more often in TS [6,7]. TS patients tend to be overweight [8], untreated TS women being more stocky than women in the normal population, with relatively short lower extremities, relatively large hands and feet, and relatively broad shoulders and pelvis [8-10].

Various investigators have examined the effect of GH treatment on these conditions. Our group previously reported [11] that increased GH-induced fasting insulin levels had fallen significantly six months after GH ended, but they were still higher than at start of GH therapy. Six months after GH, systolic and diastolic blood pressure SD-scores (SDS) were both significantly lower than before GH, but remained higher than normal. The serum lipid levels had also changed into more cardio-protective values. Although the pre-existing disproportion between standing height and sitting height in TS improved moderately during GH treatment, the disproportionate growth of the feet may have been negatively influenced by higher GH dosages [9].

The aim of this study was to investigate whether long-term GH treatment influenced the carbohydrate metabolism, risk factors for cardiovascular disease, and body proportions, several years after ending GH-treatment in young women with TS. Comparisons were made with normal women, and, if possible, with untreated TS women.

Patient and Methods

Study subjects

Thirty-nine patients with TS, all participants in an earlier long-term GH trial, participated in this follow-up study.

The original GH trial had initially involved 68 previously untreated TS girls [2], whose diagnosis was confirmed by lymphocyte chromosomal analysis. At inclusion, the chronological age (CA) of these girls ranged between 2 and 11 years; their height lay below the fiftieth percentile according to normal Dutch references. The exclusion criteria were abnormal thyroid function, associated endocrine and or metabolic disorders, growth failure underlain by other causes (e.g. emotional deprivation); hydrocephalus, previous use of drugs which could interfere with the GH treatment, and spontaneous puberty. Four girls dropped out of the study due to lack of motivation.

Thirty-nine of the remaining 64 girls participated in the follow-up study, which took place 4.8 (1.9) years after GH treatment ended. Twenty-five did not participate in the follow-up study, 18 due either to lack of motivation caused by psychological problems, practical factors or lack of interest, one due to emigration, and six due to minor mental retardation or autism. Thirty-one girls of the 39 had karyotype 45,X, and eight had a variant karyotype.

The Medical Ethics Committees approved both the GH trial and the protocol for the follow-up study. Written informed consent was obtained from the girls and/or their parents.

Study design

GH trial: After stratification for chronological age and height standard deviation scores (SDS), the patients were randomly assigned to group A, B or C, which were assigned the following GH dosages: group A (n = 23) received 1.3 mg/m^2 body surface/day (equivalent to 0.045 mg/kg); group B (n = 23) received 1.3 mg/m^2/d during the first year, followed by 2 mg/m^2/d (≈ 0.0675 mg/kg/d); and group C (n = 22) received 1.3 mg/m^2/d during the first year, 2 mg/m^2/d during the second year, and 2.7 mg/m^2/d (≈ 0.090 mg/kg/d) thereafter. Biosynthetic human GH (Norditropin, Norditropin simplex, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime, the dose being adjusted every three months according to the calculated body surface. On the basis of the study protocol, GH treatment was discontinued when height velocity (HV) had been less than 1 cm over the past 6 months, or when the patient decided she was satisfied with the height she had reached.

In order to induce puberty, micronized 17ß-estradiol (E2) was given to all girls aged 12 yrs and older who had had at least four years of GH treatment. During the

first two years of E2, 5 µg/kg body weight/day was given orally, following by a dose of 7.5 µg/kg/d in the third year, and 10 µg/kg/d thereafter. After two years of estrogen therapy, all these girls received cyclic progestagen therapy consisting of 5 mg/d for 14 days of the month. Upon discontinuation of GH therapy, estrogen treatment was increased to an adult dose of 1 or 2 mg/day, and progestagen dosage was increased to 10 mg/d.

Follow-up of the GH trial: The GH trial involved short-term follow-up (ST-fu) of all girls six months after the end of GH. Long-term follow-up study (LT-fu) took place in 2003, mean (SD) 4.8 (1.9) years after discontinuation of GH. The LT-fu study existed of different parts, among quality of life evaluation, MRI-scans of the aorta, and uterine ultrasound, as described elsewere [12,13].

Measurements

The following measurements were performed at the start of the GH trial, at the end of GH, and at LT-fu: blood pressure (BP), height, weight, sitting height (SH), left hand length (Hand), left foot length (Foot), biacromial diameter (Biac), and bi-iliac diameter (Biil). Body mass index (BMI) was calculated. BP and BMI were also measured at ST-fu.

Fasting blood samples were taken at start of the GH trial, after four years of GH therapy, at ST-fu, and at LT-fu to determine the following: glucose, insulin, glycosylated hemoglobin (HbA1c), serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL). The atherogenic index (AI) was calculated (TC/HDL). After centrifugation, all samples were frozen (−80°C) until assayed.

To estimate insulin sensitivity and ß-cell function, we used the HOmeostasis Model Assessment (HOMA) as described previously by Matthews *et al* [14,15]; this was subsequently validated [16]. The HOMA model is a structural model of glucose/insulin interaction, with mathematical equations describing the functioning of the major effector organs. HOMA-B (%) represents ß-cell-function, and HOMA-S (%) represents insulin sensitivity, with high HOMA-S indicating high insulin sensitivity.

Systolic and diastolic BP was determined four times with a single Dynamap Critikon 1846SX in sitting position using a cuff size corresponding to arm size. BP was expressed as an SD-score using age and sex-specific reference values [17]. TC, HDL, LDL, and AI were compared with those of a Dutch control group of the same age and sex [18].

The anthropometric measurements were performed according to Cameron [19], using a Harpenden anthropometer. The mean of two measurements was used for analyses. At end of GH-treatment and at LT-fu, we calculated the SD scores for height

and body proportions using the reference values for 18-year-old girls [20]. In addition, the SDS of the measurements SH, Biac, Biil, Hand, and Foot were expressed as a shape value, adjusted for height SDS, using the formula [10]: *Shape SH = (SH SDS − height SDS) /√(2-2r)*, in which r is the correlation coefficient between height and SH in the reference population. Values < −2 and > +2, were considered to lie outside the normal range. The data of Gravholt et. al. were used as a reference for untreated TS women [8]. To compare Gravholts data with ours, we calculated the mean shape values from the SDS of that study, since the same reference population was used as in our study.

Assays

The plasma glucose level was measured at the local hospital laboratories, and plasma insulin was determined in one laboratory by IRMA (Medgenix, Biosource Europe; fasting normal range: 4-15 mU/l). HbA1c levels were measured in one laboratory as described previously [21]. References for lipid levels were measured using the same assays in the same laboratory as described previously [11].

Statistics

Unless indicated otherwise, results were expressed as mean (SD). For the analyses at baseline and during GH therapy, only the LT-fu participants (n = 39) were included. Repeated measurement models were used to compare the outcome variables over time and between the GH dosage groups. At LT-fu, backward multiple linear regression analyses were used to assess multivariable relationships. The dependent variables were systolic and diastolic BP SDS, fasting insulin, HOMA-B, HOMA-S, TC, HDL, and LDL. The following independent variables were entered into the model: GH dosage ($mg/m^2/d$), GH duration (yrs), duration after GH discontinuation (yrs), BMI (kg/m2), karyotype (1 = 45,X, 2 = variant), and age (yrs). In the model for BP SDS, age and BP at start were included, as there was a significant difference between the GH-groups at start. In the model for the shape value of the foot, only the variables GH duration and GH dosage were entered. Independent variables with P-values < 0.1 were kept in the model; those with P-values < 0.05 were considered statistically significant. All calculations were performed with SPSS 11.5.

Results

Study subjects

Table 1 shows the characteristics of all participants in the long-term follow-up (LT-fu) study. They were 20.0 (2.1) years old, ranging 15.7–23.4 years. Apart from the greater adult height attained in group C than in group A, there were no significant differences between the three GH dosage groups.

Table 1: Characteristics of the TS women participating in the long-term follow-up study.

	Group A	Group B	Group C	Total group
Number of girls/women	14	13	12	39
Age at long-term follow-up (years)	20.5 (1.6)	19.8 (2.1)	19.6 (2.5)	20.0 (2.1)
Age at GH discontinuation (years)	15.6 (0.7)	14.8 (1.4)	15.2 (1.1)	15.2 (1.2)
Age at start of E2 therapy (years)	12.9 (1.2)	13.0 (1.0)	12.9 (1.4)	12.9 (1.2)
Duration of GH therapy (years)	8.6 (1.3)	8.4 (2.6)	9.2 (1.9)	8.7 (2.0)
Duration after end of GH (years)	4.9 (1.4)	5.1 (2.4)	4.4 (2.0)	4.8 (1.9)
Height (cm)	157.9 (7.2)	163.8 (5.8)	164.7 (6.0)*	162.0 (6.9)
Karyotype (n) – 45,X : variant	12 : 2	12 : 1	7 : 5	31 : 8

Data expressed as mean (SD). * $P < 0.05$ compared to group A.

Measurements

LT-fu was 4.8 (1.9) years (range 1.5–9.1) after GH discontinuation, while short-term follow-up (ST-fu) was standardized at six months after GH discontinuation.

***Carbohydrate metabolism*:** During the GH trial, none of the girls developed diabetes mellitus (DM). At LT-fu, one of the 39 girls showed signs of DM (fasting glucose 10.5 mM, HbA1c 7.9%), and was referred for further analyses. She was excluded from the LT-fu carbohydrate evaluation.

Over time, there were no differences between the three groups regarding changes in any of the parameters measured, or in differences between the GH dosage groups at any of the time points. All individual HbA1c values remained within the normal range throughout the years during and after GH treatment.

Mean fasting glucose and insulin levels increased from start up to year 4 of GH therapy ($p < 0.01$, $p < 0.001$ respectively), and were lower at ST-fu than at year 4 of GH-therapy ($p = 0.001$, $p < 0.05$ respectively) (Figure 1). At ST-fu, glucose levels had returned to pre-treatment levels, whereas mean insulin levels had remained higher than those pre-treatment ($p < 0.001$). From ST-fu to LT-fu, mean glucose levels increased ($p < 0.01$), reaching levels that were not significantly different from those

at year 4 of GH therapy (P = 0.09). However, mean glucose levels remained within the normal range. At LT-fu, mean fasting insulin levels had not changed significantly compared to ST-fu levels (P = 0.271), and were still higher than pre-treatment levels (p < 0.001).

Insulin sensitivity (HOMA-S) decreased from start to year 4 of GH therapy (P < 0.001) (Figure 1), remaining constant thereafter (i.e., during ST-fu and LT-fu) at 75–85%. Beta-cell function (HOMA-B) increased from start to year 4 of GH therapy (P < 0.001), and remained constant thereafter (150–160%).

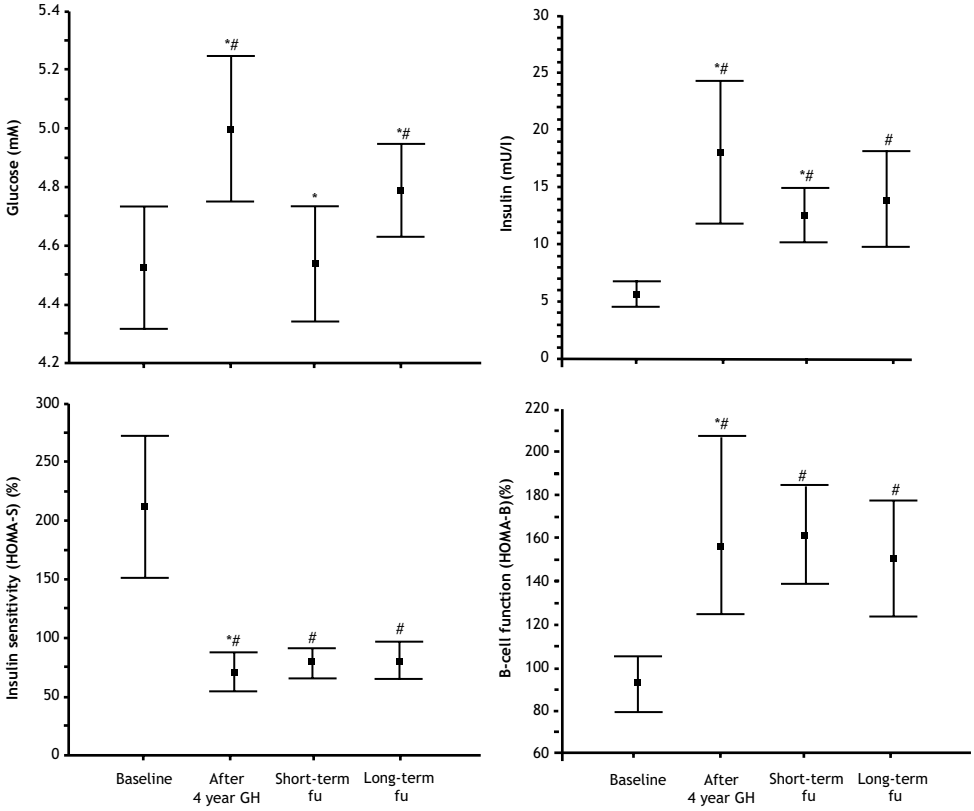


Figure 1: Mean and 95% confidence interval for fasting glucose, insulin levels, insulin sensitivity (HOMA-S), and ß-cell function (HOMA-B) in Turner Syndrome patients (n = 38) at start of GH therapy, after 4 year of GH therapy, 6 months after GH (Short-term fu), and 4.8 (1.9) years after GH (Long-term fu).

There where no differences between the three GH-dosage groups at any measured points in time.

* P < 0.01 compared to preceding levels, # P < 0.01 compared to pre-treatment levels

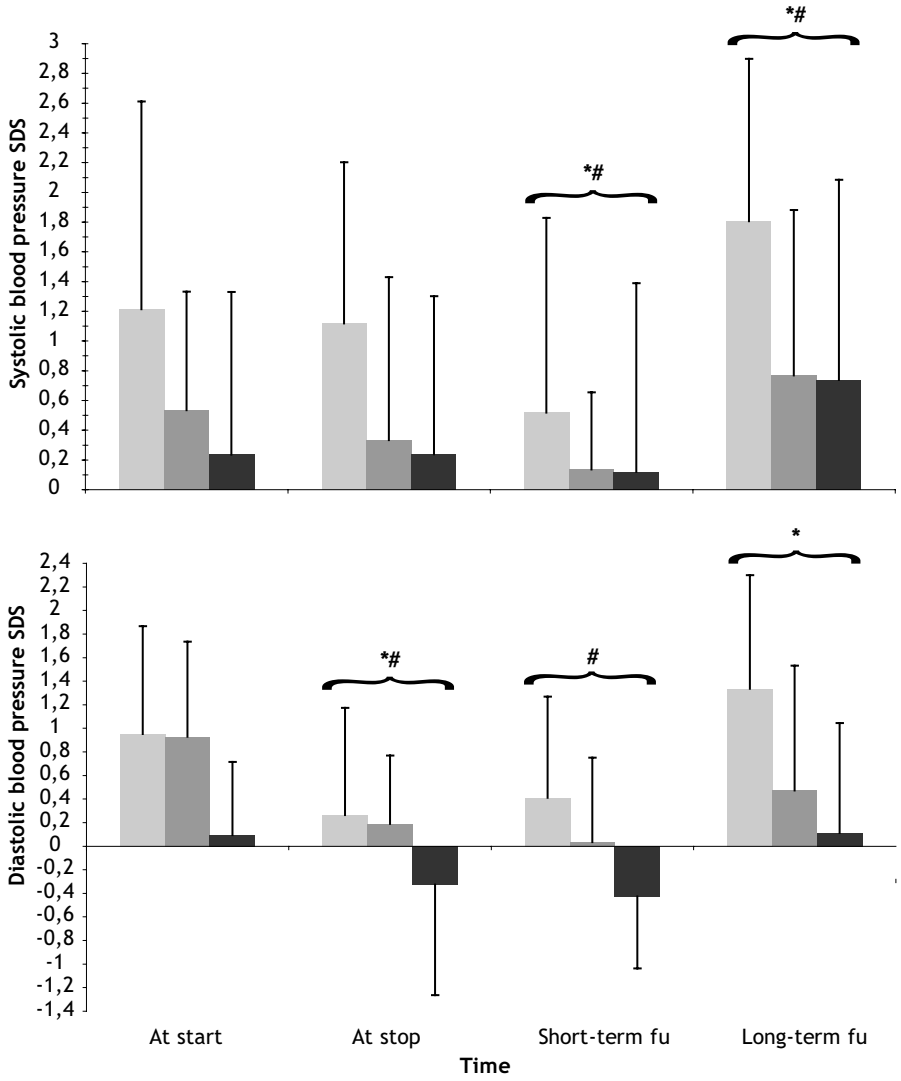


Figure 2: Systolic and diastolic blood pressure SDS in Turner Syndrome patients (n = 39) at start of GH therapy, end of GH, 6 months after GH (Short-term fu), and 4.8 (1.9) years after GH (Long-term fu).

* P < 0.05 compared to preceding levels, # P < 0.05 compared to pre-treatment levels

***Blood pressure*:** Changes over time in systolic blood pressure (BP) did not differ between the three GH dosage groups (P = 0.8). Systolic BP did not change during GH treatment (P = 0.57) (Figure 2). At ST-fu, systolic BP was lower than at the end of GH (P < 0.05) and than pre-treatment values (P < 0.05). Between ST-fu and LT-fu, mean systolic BP increased significantly from 111.4 (12.5) to 122.1 (14.2) mmHg, resulting in SD-scores higher than pre-treatment SD-scores (p < 0.05).

Changes over time in diastolic BP did not differ between the three GH dosage groups (P = 0.4). Diastolic BP showed a small decrease during GH treatment (P < 0.001) (Figure 2). Diastolic BP remained constant during the first six months after GH (Short-term fu)(P = 0.94), and was lower at ST-fu than pre-treatment (P < 0.001). Between ST-fu to LT-fu, mean diastolic BP increased from 67.0 (8.4) to 79.1 (11.4) mmHg, resulting in SD-scores no different from pre-treatment levels (P = 0.9).

At LT-fu, 17 (44%) of the 39 women had a systolic BP above +1.3 SD-score (ninetieth percentile), six of whom had a variant karyotype. Eleven of them also had a diastolic BP above +1.3 SD-score (four with variant karyotype).

***Serum lipids*:** Changes over time in TC, LDL and triglycerides were not significantly different between the three GH dosage groups. At LT-fu, TC and LDL were higher than at ST-fu, resulting in TC levels higher and LDL levels lower than controls (Table 2).

Triglycerides increased significantly after year 4 of GH therapy compared to pre-treatment, after which there were no significant changes.

HDL was significantly different between the three groups at baseline, being 0.58 (0.04) in group A, 0.69 (0.05) in group B, and 0.88 (0.04) in group C. From start to year 4 of GH therapy, HDL increased significantly in all three groups, resulting in 1.03 (0.06) for group A, 1.09 (0.07) for group B, and 1.25 (0.06) for group C. At year 4, groups A and C were no longer different from group B, though the difference between A and C remained. Thereafter, there were no longer any differences between the three GH dosage groups. At LT-fu, HDL was higher than at ST-fu, and than in controls.

Table 2: Lipid levels before, during and after GH treatment.

	Start	After 4 years GH therapy	Short-term follow-up	Long-term follow-up	Controls[a] (n = 703)
TC	4.20 (0.66)	3.91 (0.50)^{b}	4.20 (0.69)^{c}	4.98 (0.83)^{bbccdd*}	4.7 (0.7)
HDL	0.71 (0.19)	1.12 (0.23)^{bb}	1.42 (0.34)^{bbcc}	1.70 (0.40)^{bbccd**}	1.3 (0.3)
LDL	2.65 (0.70)	1.98 (0.43)^{bb}	2.23 (0.59)^{bbc}	2.64 (0.63)^{ccdd*}	2.9 (0.7)
AI	6.20 (7.91)	3.61 (4.49)^{bb}	3.07 (3.70)^{bbc}	3.05 (3.95)^{bbc**}	3.6 (4.8)
Trigl	0.95 (1.48)	1.30 (2.11)^{bb}	1.17 (1.76)^{b}	1.22 (2.04)^{b}	

[a]: Dutch control group [18]. Data are expressed as mean (SD). TC: total cholesterol, AI: Atherogenic index = TC/HDL, Trigl: triglycerides.

Repeated measurement analyses: No differences in changes over time between the three groups in TC, LDL, and Trigl.

Differences between groups in HDL and AI at baseline as described in the text. Differences over time: ^{b}: P < 0.05, ^{bb}: P < 0.001 vs start; ^{c}: P < 0.05, ^{cc}: P < 0.001 *vs* 4 years GH; ^{d}: P < 0.05, ^{dd}: P < 0.001 *vs*. ST-fu. *: P < 0.01, **: P < 0.001 LT-fu *vs*. controls.

At baseline, atherogenic index (AI) was also significantly different between the three groups, being 7.3 (0.2) in group A, 6.0 (0.2) in group B, and 5.0 (0.2) in group C. From start to year 4 of GH therapy, AI decreased significantly in all three groups. A and C were no longer different from B, but the difference between A and C remained. At LT-fu, AI was the same as at ST-fu, and was lower than in controls.

BMI: Over the years there were no significant differences between the three GH dosage groups with regard to the standard deviations scores for BMI. BMI (kg/m^2) was 22.6 (5.0) at end of GH, 23.3 (5.3) at ST-fu, and 26.5 (5.1) at LT-fu, a significant increase ($P < 0.001$). At LT-fu, BMI was between 20–25 kg/m^2 in 15 of the 39 women (38.5%), between 25–30 kg/m^2 in 18 women (46%) and > 30 kg/m^2 in six women (15.5%).

***Body proportions*:** Although the change over time between the three GH dosage groups was significantly different for SH shape values (SV), the changes for SV of Biac, Biil, Hand and Foot were not. During GH treatment, SH SV decreased ($P < 0.01$), foot SV increased ($P < 0.001$), and SV of hand, Biac and Biil did not change significantly ($P = 0.08$, $p = 0.44$, and $P = 0.86$ resp.) (Figure 3).

Between end of GH and LT-fu, SH SV in group A increased slightly, but significantly, while it did not change significantly in group B and C. Biac and Biil SV also increased after end of GH (both $P < 0.001$). Hand and foot shape values did not change after GH discontinuation.

Multiple regression analyses

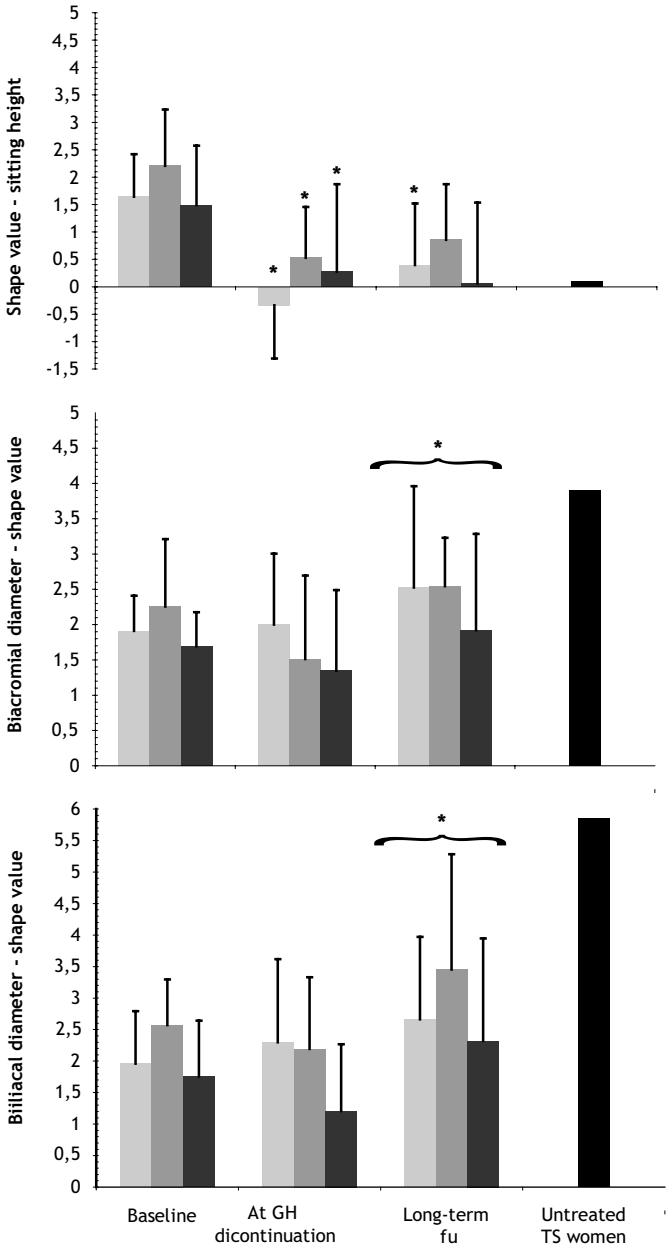
At LT-fu, the following relationships were found.

HOMA-S was not associated with any of the tested variables. HOMA-B was positively influenced only by BMI (B 6.5, $P < 0.01$), explaining 18.5% of the variability. Fasting insulin levels were positively associated with BMI (B 1.4, $P < 0.001$).

Age and systolic BP at start of GH-treatment influenced systolic BP. GH dose or duration was not of influence on systolic BP at LT-fu. Diastolic BP was negatively influenced by GH dose (B −1.0, $P = 0.001$), indicating that the higher GH dose, the lower the diastolic BP at LT-fu, corrected for the diastolic BP at start. After adding age at start of the GH treatment to the model, the GH dosage influence remained significant. Karyotype (45,X *vs.* variant) influenced diastolic BP, with a higher diastolic BP in patients with variant karyotype, with a difference of 0.86 SDS.

Serum TC and LDL were positively associated with the time-period after GH, also after adjustment for age. Thus, the longer the time-period after GH, the higher the serum TC and LDL. HDL was not associated with any of the variables tested.

The SV of the foot was positively influenced by GH dose ($P < 0.01$, $R^2 = 23.1\%$), but not by GH duration ($P = 0.18$). Thus, the higher the dose, the greater the relative size of the foot.



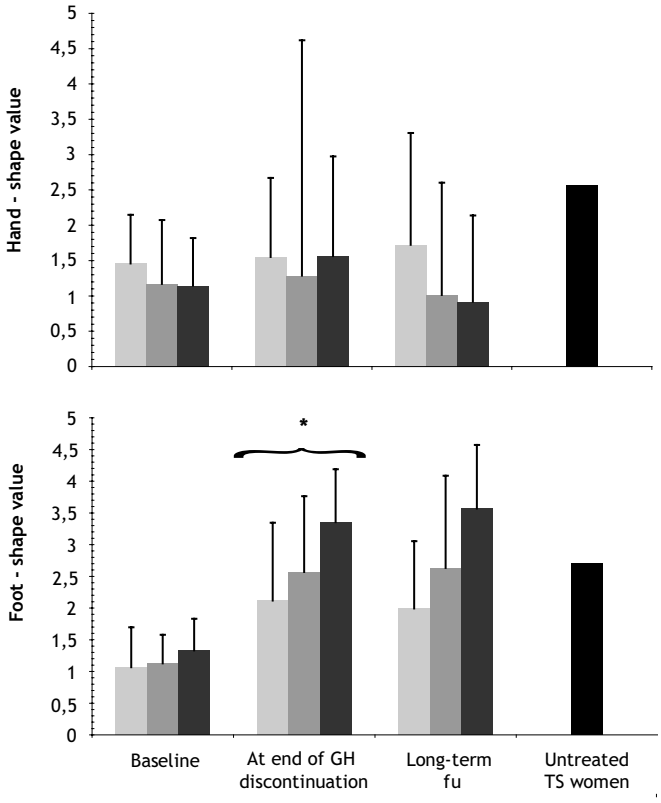


Figure 3: Mean (SD) body proportions at baseline, end of GH, and long-term follow-up for group A (*white*), B (*light grey*), and C (*dark grey*). Black bars shows reference for untreated TS women (n = 79) with a mean age of 35.7 (0.9) years [8].
* P < 0.05 compared to preceding levels.

Discussion

Several years after GH, insulin sensitivity remained low, while ß-cell function and fasting insulin levels remained high. Previously, we hypothesized that the higher insulin levels after GH might result from a higher BMI [22,23], as several studies showed a positive correlation between insulin levels and BMI in normal children and adults [22,23]. A positive association of BMI with ß-cell function, and with fasting insulin levels at LT-fu several years after GH therapy supports this possible explanation. Now, however, because untreated young TS women have been reported to have a higher

prevalence of insulin resistance and impaired glucose tolerance [24], we believe this phenomenon may also be related to TS. Salgin et al recently presented data of insulin and glucose levels in untreated TS women, aged 29.9 (16.5–46.6) years. They showed an insulin sensitivity of 103 (78.6)%, which is not significantly different compared to 81.6 (46.5)% in our GH treated TS women, aged 20.0 (2.1) years [25]. Recently it was suggested that haploinsufficiency for an unknown X-chromosome gene (or genes) may be responsible for impaired β-cell function in TS [26]. Alternatively, the persistence of low insulin sensitivity and high β-cell function may be explained by the GH therapy administered. Although no association with GH dosage or GH duration was known, such an effect could not be ruled out.

During GH therapy, diastolic BP had decreased significantly. Nearly five years after ending GH therapy, diastolic BP had increased, and was similar to pre-treatment levels. Systolic BP did not change during GH treatment. For untreated TS women there is no comparable data: in one study the untreated TS woman were older than our TS women [8]; in a second it was unclear whether the population studied had received GH [7].

It is reasonable to assume that GH treatment had a positive effect on diastolic BP, as the diastolic BP had decreased during and increased after end of GH therapy. This assumption is supported by the fact that, even several years after the GH, its effect on diastolic BP had been sustained: patients who had received a higher GH dose, had a lower diastolic BP, also after adjusting for the BP at start. The lack of significant difference between the GH dosage groups may be explained by a non-dose-response effect of GH on diastolic BP. GH-dose and duration was of no influence on systolic BP. The increased BMI at LT-fu can not explain the increased blood pressure, as is was not a significant variable. GH therapy has also been reported to lower diastolic and systolic BP in adult GH-deficient patients [27].

During the first four years of GH therapy we found a decrease in TC, LDL, and atherogenic index (AI), and an increase in HDL and triglycerides. Six months after GH, TC, LDL and HDL had increased, resulting in a decreased AI. Similar results were reported after GH in GH-deficient patients [28]. There was a similar trend in serum lipid levels in small children born small for gestational age during and six months after GH treatment [29]. Several years later, at the LT-fu, serum TC, LDL and HDL had further increased, resulting in higher TC, but also higher HDL levels than in controls. However, LDL and the AI remained lower than in controls. The TC, HDL and LDL levels were similar to those reported in untreated TS women aged 33.2 (7.9) years [30]. A longer time-period after end of GH was associated with higher TC and LDL levels. This finding supports the hypothesis that GH may have had protective benefits in TS girls, which were still noticeable several years after ending GH treatment, as the AI was lower than in controls.

BMI increased gradually during and after GH therapy. At an average age of 20, fewer than 40% of the TS women had a normal BMI (below 25). The average BMI of our TS group was similar to that of an untreated adult group of TS women, although the age difference was 7–15 years [7,8]. A large study in the U.S.A. (n = 2468) reported that the BMI SDS in girls with TS increased with age, starting approximately at age nine [31]. The authors did not find any influence of GH therapy on the magnitude of increase. These findings suggest that mechanisms specific for TS are responsible for age-related increase in BMI SDS. In a randomized placebo controlled study in TS girls by Gravholt et al, two months of GH therapy significantly reduced fat mass (FM), and increased lean body mass (LBM), whereas BMI remained unchanged [32]. Unfortunately, in our study we did not investigate body composition.

Previously, our group reported a moderate improvement in the disproportion between height and sitting height (SH), and a further increase in the disproportion between foot length and height. After a GH duration of nearly nine years, up to adult height, we can confirm these earlier reports. The disproportion between SH and height nearly normalized, whereas disproportion between hand, biacromial diameter (Biac), biiliacal diameter (Biil), and height remained unchanged during GH therapy. The disproportion of the foot increased during GH therapy, as stated previously. While it was not possible to test this statistically, untreated adult TS women seemed to have larger disproportion of Biac and Biil (Figure 3)[8]. A possible explanation of this discrepancy may be a GH effect. Although shape values of Biac and Biil did not change significantly during the years of GH treatment, GH therapy may have prevented deterioration. Another possibility may be measurement errors, as the bodily fat may influence the measurements. However, the untreated women reported a BMI similar to that of our TS population. The increased BMI also may be responsible for the differences between measurements of Biac and Biil noted during ST-fu and LT-fu.

The increased proportion between height and sitting height at LT-fu may be due to further growth of the spine after GH and to a higher estrogen dose. Another explanation may be, again, the increased BMI, which led to more bodily fat on the buttocks. In untreated adult women there was also a disproportion between foot length and height [8], and in our GH-treated TS women there was a GH dosage effect. We can thus confirm our previous conclusions that the disproportionate growth of the feet is part of the natural development in TS, albeit influenced by higher GH dosages.

When reviewing the results of this study, one has to consider the following. Firstly, as not all participants of the GH trial participated in the LT-fu, there may be a selection bias. This seems unlikely, however, as the results of BP, fasting insulin, glucose, and serum lipid levels of the LT-fu participants during GH therapy, were similar to those previously reported for the whole group on these parameters [11].

Secondly, it would have been ideal to compare these results to those in untreated young TS women. However, as most countries have regarded GH therapy as an accepted treatment in TS for several years, it almost impossible to find an age-comparable untreated TS population; in general, most studies in untreated TS women are performed in an older TS population.

On the basis of our results, we conclude that, as well as height gain, GH therapy brings women with TS additional benefits regarding diastolic BP, serum lipids, and some body proportions. Nearly five years after GH the favourable effect of GH on serum lipids was still noticeable, but waning. The effect on diastolic BP had disappeared, as it had returned to pre-treatment levels. The lower GH-induced insulin sensitivity, however, remained unchanged, possibly due to having TS.

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Chapter 8

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Aortic Distensibility and Dimensions and the Effects of Growth Hormone Treatment in the Turner Syndrome

Abstract

In Turner's syndrome (TS), an increased risk for cardiovascular malformations exists, including aortic dilation of unknown cause. Abnormal biophysical wall properties may play an important role. Magnetic resonance imaging has been successfully used to assess aortic size and wall distensibility.

The aim of this study was to assess aortic biophysical properties and dimensions in TS.

Thirty-eight former participants of a growth hormone (GH) dose-response study in TS (mean age 12 ± 2 years, mean body surface area 1.7 ± 0.2 m^2) and 27 controls (mean age 21 ± 2 years, mean body surface area 1.8 ± 0.1 m^2) were enrolled. Previously, patients had been assigned to 1 of 3 groups treated with different GH-dosages: group A (0.045 mg/kg/day), group B (0.067 mg/kg/day), and group C (0.09 mg/kg/day). All underwent magnetic resonance imaging ≥ 6 months after GH discontinuation to determine aortic dimensions and distensibility at 4 predefined levels: (1) the ascending aorta, (2) the descending aorta, (3) the level of the diaphragm, and (4) the abdominal aorta.

Patients had larger aortic diameters at all but level 4 and tended to have reduced distensibility at level 3. Distensibility in group A was significantly less compared with that in group C at level 4. Compared with controls, patients in group A had larger aortic diameters at all but level 4 and reduced distensibility at level 4. The results for patients in groups B and C were not different from those for controls.

In conclusion, patients with TS formerly treated with GH have dilated aortas and signs of impaired wall distensibility. The severity of abnormalities seems related to the GH-dose, with a beneficial effect of a larger GH-dose on the abnormalities.

Introduction

Structural cardiovascular malformations occur in up to 76% of patients with the Turner's syndrome (TS) [1,2]. Coarctations of the aorta and bicuspid aortic valve are most common and represent, respectively, 25% to 30% and 25% to 35% of cardiovascular malformations in TS [1-3]. Aortic dilation has been regarded as less common (6.5%) [2]. However, in recent studies using magnetic resonance imaging (MRI), aortic dilation was found in 25% to 50% of patients with TS and cardiovascular malformations [1,3,4]. The most common non-structural cardiovascular disease in TS is arterial hypertension [5]. The number of reports on aortic dissection in TS is increasing [2,6]. A function of the large arteries is the transformation of pulsatile flow into continuous flow, known as the "Windkessel" phenomenon. This phenomenon is determined by the biophysical properties of the aortic wall. Biophysical wall properties can be assessed using non-invasive imaging tools, allowing the in vivo quantification of the effects of altered arterial wall composition [7]. MRI has been proven accurate and reproducible for this purpose [7]. The aim of this study was to assess biophysical properties and dimensions of the aorta in TS.

Methods

Patients

For this prospective MRI evaluation study, we approached all 64 participants who had completed our previous growth hormone (GH) dose-response study in TS [8]. Thirty-eight patients (59%) agreed to participate. During the GH trial, biosynthetic human GH (Norditropin, Novo Nordisk A/S, Bagsvaerd, Denmark) was given in 3 different dosages: patient in group A received 1.3 mg, those in group B 2 mg, and those in group C 2.7 mg/m^2 body surface area (BSA)/day (approximately 0.045, 0.067, and 0.09 mg/kg/day, respectively), as described previously by van Pareren *et al* [8]. To induce puberty, micronized 17β-estradiol was given orally from the age of 12 years, after ≥ 4 years of GH treatment. At the start of GH therapy, diastolic blood pressure was significantly lower in group C than in the other 2 subgroups, resulting in a lower mean blood pressure in group C than in group A. GH therapy was continued until final height. All patients had discontinued GH treatment for ≥ 6 months.

Twenty-seven healthy age-, gender- and BSA-matched controls were included. Height, weight and blood pressures were measured, and BSA was calculated. Apart from the generally accepted contraindications for MRI, no exclusion criteria were used. The medical ethics committee approved MRI evaluation. Written informed consent was obtained from all participants.

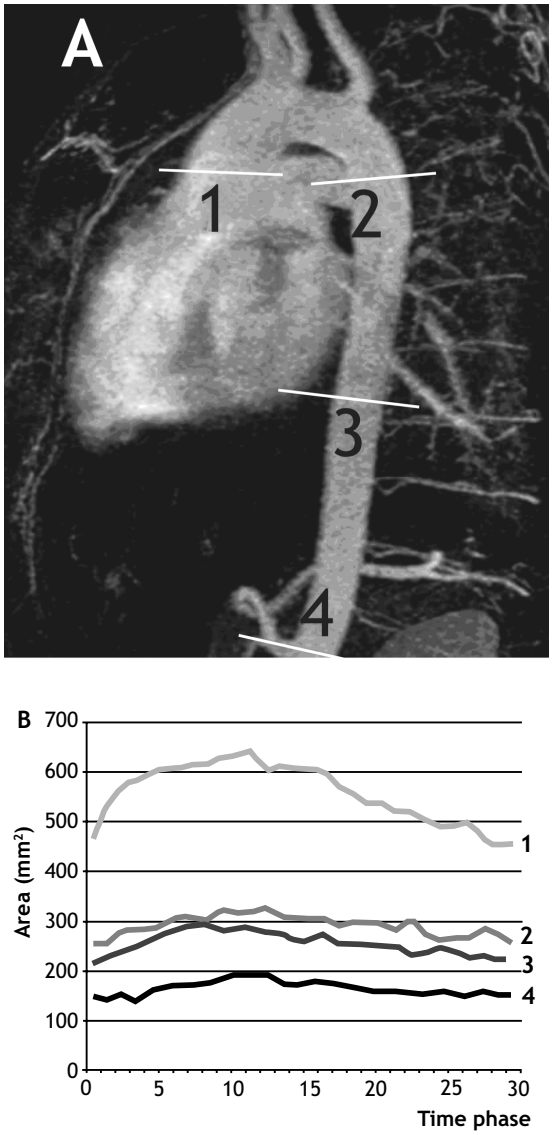

Figure 1: MR angiography of the aorta in TS: a typical example.
A. The predefined levels of measurement: 1. ascending aorta. 2. descending aorta. 3. level of the diaphragm ,and 4. abdominal level
B. Aortic area (mm^2) variation over the cardiac cycle

MRI protocol

Imaging was performed on a GE 1.5-T CV/i scanner with software version 9.1 (GE Healthcare, Milwaukee, USA) using a 4-channel, phased-array torso coil. An anatomic overview of the area of interest was obtained in multiple directions

using a bright-blood imaging protocol on the basis of an electrocardiographically gated, fat-suppressed, 2-dimensional, single-shot, steady-state, free precession technique. These images were used for planning subsequent scans. Images of the aortic anatomy were obtained for reference using a high-resolution magnetic resonance 3-dimensional angiographic protocol and a double dose of gadolinium-diethylenetriamine pentaacetic acid (0.2 mmol/kg, Magnevist, Schering AG, Berlin, Germany). Flow measurements were performed using a standard 2-dimensional flow-sensitized scan. The scans were planned perpendicular to the aorta at 4 predefined locations: (1) the ascending aorta at the level of the pulmonary artery bifurcation, (2) the descending aorta at the level of the pulmonary artery bifurcation, (3) the descending aorta at the level of the diaphragm, (4) the abdominal aorta at the level of the superior mesenteric artery (Figure 1). The flow sensitivity of the sequence was set to 180 cm/s (and increased if phase aliasing occurred) and the in-plane spatial resolution to 1 mm, with a 256 x 256 scanning matrix [7]. Temporal resolution was approximately 50 ms, and 30 cardiac phases were reconstructed retrospectively [7]. Breath-hold time varied between 25 to 40 seconds per measurement depending on heart rate. Imaging parameters were as follows: 2-dimensional fast spoiled gradient-echo, repetition time 6 to 7 ms, echo time 3 ms, flip angle 20°, readout bandwidth 90 kHz, slice thickness 6 mm, and 4 lines/segment. A fifth measurement was performed through the sinus of Valsalva to quantify aortic valve gradients. During each flow measurement, peripheral blood pressure was measured at the level of the brachial artery using a sphygmomanometer cuff.

Image analysis and calculations

Flow images were quantitatively analyzed using MEDIS Flow analysis software version 2.0 (MEDIS Medical Imaging Systems, Leiden, the Netherlands). Vessel areas were manually drawn for each cardiac phase (30 phases), and maximal and minimal aortic areas were selected (Figure 1). Distensibility (10^{-3} mm Hg^{-1}), defined as the relative area change for a pressure increment, was calculated by means of the following equation [9]:

$$D = (Amax - Amin) / (Amin \times [Pmax - Pmin]),$$

where D is distensibility, Amax is the maximal (systolic) aortic area (square millimeters), Amin is the minimal (diastolic) aortic area, Pmax is systolic blood pressure (mm Hg), and Pmin is diastolic blood pressure. Mean blood pressure was calculated using the following equation: mean blood pressure = ([2 x Pmin] + Pmax) / 3.

Aortic diameters (square millimeters), aortic diameters indexed for BSA (millimeter per square millimeter), and the ascending/descending aortic diameter ratio were calculated using vessel areas. Values outside the mean ± 2 SDs range from our controls were considered abnormal. Pressure gradients were calculated

using the simplified Bernoulli's equation ($\Delta P = 4 \times Vmax^2$). At the sinus of Valsalva, a maximal flow velocity of 3 m/s (gradient > 36 mm Hg) was regarded as indicating an aortic valve stenosis of importance. A coarctation of the aorta was defined to be hemodynamically important in the presence of a pressure gradient > 20 mm Hg (on the basis of the measurement of maximal flow velocity).

Statistical analysis

Analysis was performed using SPSS statistical software version 11.5 (SPSS, Inc., Chicago, Illinois). Results are expressed as mean ± SD. A p value ≤ 0.05 was considered to indicate significance unless specified otherwise. Differences in aortic diameter and aortic distensibility between patients and controls were tested using the independent Student's *t*-test and analysis of covariance (with correction for systolic and diastolic blood pressures), respectively. To deal with multiple comparisons a p value ≤ 0.0167 was considered to indicate significance when a GH dosage group was compared with the controls. Differences among dosage groups were tested using analysis of variance for diameters and analysis of covariance (with correction for systolic and diastolic blood pressure) for distensibility. Two dummy variables were designed to distinguish among dosage groups. We corrected for multiple group comparisons using Bonferroni's method. Spearman's correlation coefficients were calculated for the assessment of bivariate correlations.

Results

Patients

The 38 patients with TS included in the present study were a representative sample of the original population on the basis of voluntary participation in this MRI evaluation. One patient previously received metal implants during scoliosis surgery and was excluded from final analysis because of substantial MRI artifacts. Characteristics of patients and controls are listed in Table 1. Among subgroups, differences were found only for final height and blood pressure (Table 1). Mean plasma levels of high-density lipoproteins, low-density lipoproteins, triglycerides, apolipoprotein-A1, and apolipoprotein-B were normal and did not differ among subgroups (data not shown).

Cardiac histories showed bicuspid aortic valve in 10 patients (26%), tricuspid aortic valves in 25 patients (66%), and inconclusive valve anatomies in the remaining 3 patients (1 in each GH subgroup). Bicuspid valves were diagnosed in 3 patients in group A, 5 patients in group B, and 2 patients in group C (P = NS). In all but 1 patient, the aortic valve pressure gradient was < 35 mm Hg. One patient had severe aortic valve stenosis (Doppler peak velocity 5 to 6 m/s). One type 2 atrial septal

defect had been surgically closed. Three other patients underwent surgical repair for aortic coarctation, with a residual but stable Doppler gradient of 15 to 20 mm Hg in 1 patient.

Table 1: Characteristics of patients and controls

	Group A n = 13 (35%)	Group B n = 13 (35%)	Group C n = 11 (30%)	Total Patients n = 37 (100%)	Controls n = 27
Age (years)	20 ± 2	20 ± 2	19 ± 2	20 ± 2	21 ± 2
Height (cm)	158 ± 7*	164 ± 6*	165 ± 6*†	162 ± 7*	172 ± 7
Height SDS‡	−1.7 ± 1.2*	−0.7 ± 0.9*	−0.5 ± 1.0*†	−1.0 ± 1.2*	0.6 ± 1.1
Height gain (cm)§	12 ± 4	15 ± 4	18 ± 7	15 ± 5	–
Height gain SDS‖	1.7 ± 0.7	2.6 ± 0.8	2.0 ± 1.1	1.6 ± 0.9	–
BSA (m^2)	1.7 ± 0.2	1.8 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	1.8 ± 0.1
Systolic blood pressure (mmHg)	129 ± 17*	116 ± 8†	113 ± 12†	119 ± 15	115 ± 10
Diastolic blood pressure (mmHg)	77 ± 12*	69 ± 8	64 ± 9†	70 ± 11*	64 ± 9
Mean blood pressure (mmHg)	94 ± 13*	84 ± 7	80 ± 10†	86 ± 12*	81 ± 9
Duration GH-Therapy (years)	9 ± 1	8 ± 3	9 ± 2	9 ± 2	–
GH-discontinuation (years)	5 ± 2	5 ± 2	4 ± 2	5 ± 2	–
Estrogen-Therapy (years)	8 ± 2	7 ± 2	6 ± 2	7 ± 2	–

***** Statistically significant difference compared to controls. † Statistically significant difference compared to group A. ‡ Height for age: references healthy Dutch girls [24]. § Final height − projected adult height at start of GH-treatment (calculated using the Lyon equation) [8]. ‖ Final height SDS − height SDS at start GH therapy: references untreated Turner girls [25].

Aortic anatomy

In 5 patients, anatomic aortic coarctations were diagnosed on MRI. Of these patients, 2 had had previous surgical coarctectomies. In 1 patient, the aortic coarctation had not previously been diagnosed. Pressure gradient of importance were found in 2 of 5 patients (27 and 28 mm Hg). In a sixth patient, tapering of the descending thoracic aorta over a length of about 8 cm starting at the isthmus was observed. One patient had a right aortic arch. Peak velocity measurement at the sinus of Valsalva in the patient with known severe aortic valve stenosis was > 5.5 m/s (maximal setting) and < 3.0 m/s in all others. No pathology was found in controls.

Aortic diameters

The patient with severe aortic valve stenosis was excluded from analysis of ascending aortic diameters, and the 2 patients with aortic coarctations with gradients > 20 mm Hg were excluded from the analysis of descending aortic diameters. All 3 were

excluded from the analysis of the ascending/descending aortic diameter ratio. Aortic diameters are listed in Table 2. Absolute ascending aortic diameters were dilated (greater than the mean +2 SDs in controls) in 11 patients (30%), and diameters indexed for BSA were dilated in 18 patients (49%). Ascending/descending aortic diameter ratios were larger in patients (1.5 ± 0.3 in patients *vs.* 1.4 ± 0.1 in controls, P = 0.05). In 13 patients (35%), this ratio was abnormally large (greater than the mean + 2 SDs in controls). No differences among subgroups were found.

Table 2: Aortic diameters (mm/m²).

	Controls	Total patients	Group A	Group B	Group C
Level 1	15.2 ± 1.2 n = 27 (100%)	17.6 ± 2.6*† n = 36 (97%)	17.4 ± 2.0† n = 13 (100%)	18.2 ± 3.3*† n = 12 (92%)	16.5 ± 1.6† n = 11 (100%)
Level 2	10.9 ± 1.2 n = 27 (100%)	11.8 ± 1.9† n = 35 (95%)	12.8 ± 2.2† n = 11 (85%)	10.9 ± 1.6 n = 13 (100%)	11.7 ± 1.4 n = 11 (100%)
Level 3	9.6 ± 0.7 n = 27 (100%)	10.4 ± 1.2* n = 36 (97%)	11.2 ± 1.4*† n = 13 (100%)	9.8 ± 0.6 n = 13 (100%)	10.3 ± 1.0 n = 10 (91%)
Level 4	8.5 ± 0.6 n = 27 (100%)	8.7 ± 0.9 n = 34 (92%)	9.1 ± 1.0 n = 13 (100%)	8.5 ± 0.7 n = 10 (77%)	8.6 ± 1.0 n = 10 (91%)

* Statistically significant difference compared to controls, absolute diameters (mm²).
† Statistically significant difference compared to controls, diameters adjusted for BSA (mm/m²)

Aortic distensibility

The 2 patients with aortic coarctation were excluded from the analysis of data for the descending aorta. Distensibility measurements are listed in Table 3 and shown in Figure 2. Aortic distensibility was negatively correlated with systolic and diastolic blood pressure at levels 3 (systolic blood pressure r = −0.67, P < 0.001, diastolic blood pressure r = −0.39, P < 0.05) and 4 (systolic blood pressure r = −0.60, P < 0.001, diastolic blood pressure r = −0.35, P < 0.05). After correction for systolic and diastolic blood pressure, patients still tended to have smaller distensibility at level 3 (P = 0.07). Analysis of GH subgroups showed aortic distensibility corrected for blood pressure in group A to be smaller at level 4, with a tendency for smaller distensibility at levels 1 (P = 0.05), and 3 (P = 0.07) compared with controls. Distensibility did not show a significant correlation with time duration after the discontinuation of GH treatment at any of the levels of measurement.

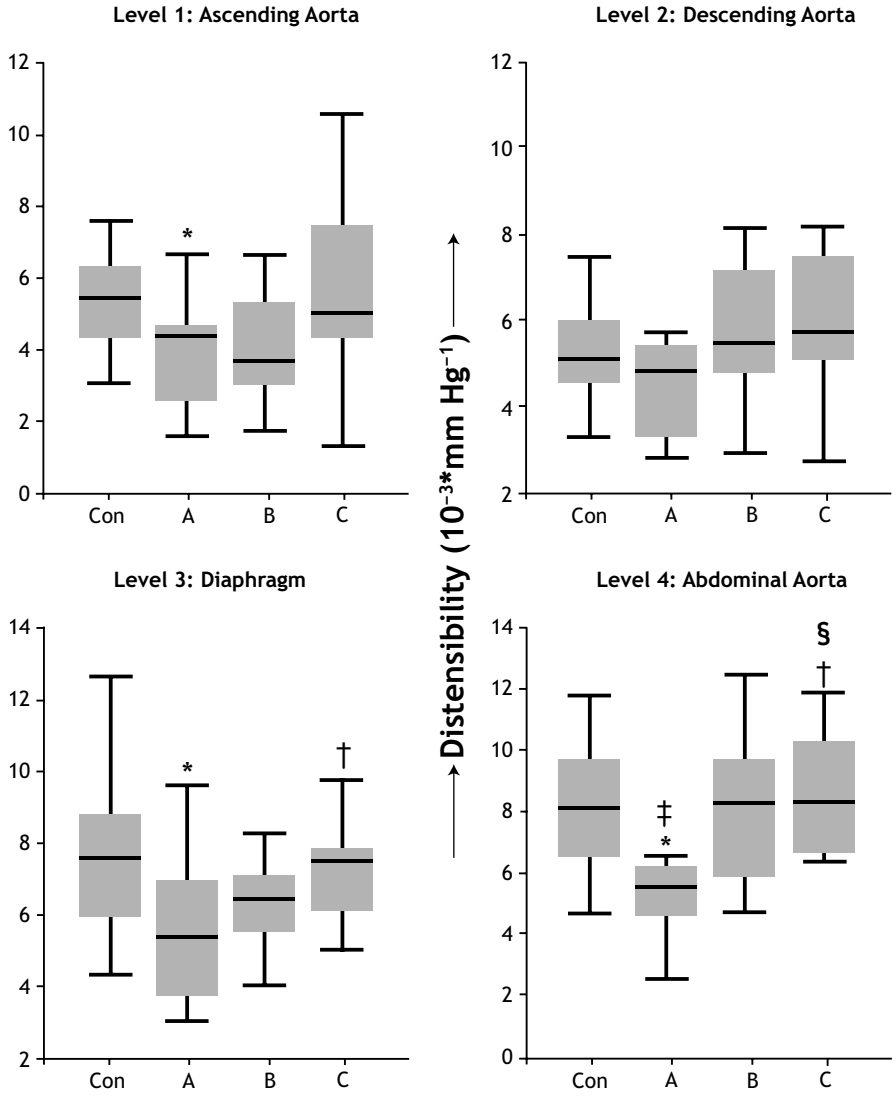

Figure 2: Aortic distensibility per level of measurement.

GH groups: Con = controls, A = 0.045 mg/kg/d, B = 0.067 mg/kg/d, C = 0.09 mg/kg/d. * Statistically significant difference compared to controls. † Statistically significant difference compared to group A . ‡ Statistically significant difference compared to controls adjusted for diastolic and systolic blood pressures. § Statistically significant difference compared to group A adjusted for diastolic and systolic blood pressures

Table 3: Aortic distensibility (10^{-3} * mm Hg^{-1}).

	Controls	Total patients	Group A	Group B	Group C
Level 1	5.3 ± 1.2 n = 27 (100%)	4.7 ± 2.3* n = 36 (97%)	4.1 ± 1.7* n = 13 (100%)	4.7 ± 2.5 n = 13 (100%)	5.5 ± 2.7 n = 10 (91%)
Level 2	5.2 ± 1.2 n = 27 (100%)	5.7 ± 2.4 n = 35 (95%)	5.0 ± 2.3 n = 11 (85%)	5.9± 2.4 n = 13 (100%)	6.3 ± 2.6 n = 11 (100%)
Level 3	7.5 ± 2.2 n = 27 (100%)	6.4 ± 1.9* n = 36 (97%)	5.4 ± 2.0* n = 13 (100%)	6.5 ± 1.5 n = 13 (100%)	7.4 ± 1.7† n = 10 (91%)
Level 4	8.4 ± 2.2 n = 27 (100%)	7.3 ± 2.5 n = 32 (86%)	5.8 ± 2.4*‡ n = 13 (100%)	8.1 ± 2.4 n = 10 (77%)	8.6 ± 1.9†§ n = 9 (82%)

Statistically significant difference: * compared to controls, † compared to group A, ‡ compared to controls adjusted for diastolic blood pressure and systolic blood pressure, § compared to group A adjusted for diastolic blood pressure and systolic blood pressure.

Discussion

The present study shows that patients with TS have larger aortic diameters (millimeter per square meter) at all thoracic levels of measurement and larger ascending/ descending aortic diameter ratios than controls. Less aortic distensibility was found at levels 1 and 3 before correction for blood pressure and a tendency (P = 0.07) at level 3 after this correction in patients compared with controls. Therefore, we conclude that the aorta in TS shows abnormal dimensions and signs of impaired wall function. Furthermore, we assessed the influence of received GH-dose on aortic distensibility. We discuss here the results corrected for blood pressure. Distensibility increased with increasing GH-dose (Figure 2), which resulted in significantly smaller distensibility in patients who received the smallest GH-dose compared with those who received the largest dose at aortic level 4. Moreover, we found group A to have less aortic distensibility compared with controls at level 4 and a tendency towards less distensibility at levels 1 (P = 0.05) and 3 (P = 0.07). No differences from controls were found in the other 2 subgroups. Our results point toward normalization of aortic distensibility with increasing GH-dose and suggest GH to have a beneficial influence on aortic wall properties in TS.

Previous studies have shown that mean aortic root diameter is larger in patient with TS than controls [1,3,10]. Dilation of the ascending aorta was found in 18% to 33% of patients [1,10]. Dilation and dissection most frequently (in 50% to 70% of patients), but not exclusively, occurred in the ascending aorta [2,11]. We found ascending aortic diameters to be dilated in 11 patients (30%) (greater than mean +2 SDs in controls) and even in 18 patients (49%) when diameter was indexed for BSA. In accordance with previous findings, the ascending aorta / descending aortic diameter ratio was

> 1.5 (a commonly used criterion for ascending aorta dilation) in 13 of our patients (35%) [1,4,2]. In TS, the ascending/descending aortic diameter ratio should be interpreted with some caution because of the frequently encountered abnormalities at the classic coarctation site that may influence this ratio [1,6]. Furthermore, the concurrent dilatation of the ascending and descending aorta, as we found in TS, may result in a false-normal outcome of this ratio. We conclude that mean ascending aortic diameters are dilated in 30% to 50% of patients with TS formerly treated with GH. Furthermore, we found that dilation of the aorta was not limited to the aortic root and/or ascending aorta but was present throughout the entire thoracic aorta. Abnormalities were most prominent in patients who had previously received the smallest GH dose.

The number of reports on aortic dissection in TS, especially during pregnancy, is increasing [2,6]. Postmortem findings similar to arterial cystic media degeneration seen in Marfan's syndrome have been described in TS [2,13]. Increased incidence of complications after surgery for coarctation has been reported in TS as a result of apparent friability of the aortic wall [2]. A recent study showed increased carotid-femoral pulse wave velocity, increased carotid intima-media thickness, and a greater carotid augmentation index in TS [10]. These reports support the hypothesis that connective tissue abnormalities might play a role in TS. Our findings, especially those in subgroup A, bear similarity to results found in Marfan's syndrome on aortic wall distensibility and support the theory that connective tissue abnormalities play a role in aortic disease in TS [14,15]. MRI-determined aortic distensibility has been shown to be decreased in a variety of cardiovascular diseases [7]. Our findings support the use of MRI-determined measurements for the (early) detection of aortic wall dysfunction and the quantification of intervention-related effects on wall function [7].

Aortic distensibility in normal subjects is negatively correlated with age, the progression of atherosclerosis, an abnormal lipid profile and postmenopausal state in women [16-18]. With increasing age, plasma levels of anabolic hormones, such as GH and insuline-like growth factor-I, decrease. GH replacement can reverse age-related catabolic changes [16]. In women, postmenopausal estrogen replacement therapy has been shown to reduce age-associated increases in arterial stiffness [17,18]. Patients with GH deficiencies show wall thickening of large arteries and a tendency toward decreased arterial wall compliance [19,20]. In response to GH treatment for GH deficiency, arterial wall thickness decreases and compliance increases [19,20]. Our patient subgroups did not differ in (1) age; (2) plasma levels of cholesterol, triglycerides, apolipoprotein-A1, and apolipoprotein-B; or (3) estrogen treatment protocol. Because all our patients were treated with GH, we cannot quantify the sole effect of estrogen replacement therapy on aortic wall distensibility in TS.

The elastic properties of the aorta depend on the presence, proportion and interaction of smooth muscles, collagen, and elastin proteins. This mixture of elements with different elastic properties results in a nonlinear arterial pressure-volume relation. In rats, GH was found to influence collagen metabolism and change the mixture of fibrous elements in the aortic wall [21]. In young rats, GH increased aortic extensibility [21]. In accordance with these findings, our results in TS indicate a positive effect of GH on aortic wall distensibility. Like others, we found distensibility to be negatively correlated with systolic and diastolic blood pressure [22]. We therefore present distensibility results corrected for blood pressure. The aortic pressure-diameter curve has a linear, elastin-determined and an exponential, collagen-determined part. An increase in blood pressure within the exponential part results in a decrease of aortic distensibility [23]. With aging, the curve shifts downward and to the right (age-related aortic dilation), through what pressure changes may occur in the exponential part [23]. In young subjects, like our patients, pressure changes are expected to occur in the linear part. However, the degenerative aortic wall alterations described in TS may change the age-specific behavior. There are only few data on degenerative alterations in TS, no quantitative data on progression of such alterations over time, and no data on possible changes in wall composition in TS in relation to GH treatment.

On the basis of our data, we think GH treatment has a (direct or indirect) beneficial effect on the biophysical properties of the aortic wall in TS. Further evaluation of the role of GH, arterial blood pressure, and their interaction on aortic distensibility in TS is required. Limitations of this study are that it provides a cross-sectional evaluation in a population with a small age range, thus limiting analysis of age and time effects. This study was not designed to determine the optimal GH dose leading to normal aortic function or to determine the exact mechanism in which GH affects the aortic wall.

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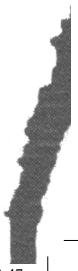
Chapter 9



General discussion

General discussion

Growth hormone (GH) treatment in short children, who are not GH-deficient, seems conflicting. It has, however, been proven to be beneficial for growth velocity and adult height in short children born small for gestational age (SGA) and children with Turner Syndrome (TS) [1-9], the two populations described in this thesis.

In the present doctoral dissertation, various aspects of GH treatment have been described that were beyond the effects of GH treatment on height. Until adult height, the short SGA children received 1 or 2 mg GH/m^2/day, and TS girls received 1.3, 2 or 2.7 mg GH/m^2/day.

Overall, this thesis focuses on the following: **1**. circulating IGF-I levels during and after GH-administration (chapters 2 and 3); **2**. quality of life shortly before and after reaching adult height (chapter 4 and 5); **3**. pubertal development in TS both near the end of GH and after termination (chapter 6); and **4**. on sequel effects of GH treatment in TS several years after end of treatment (chapter 7 and 8). In this chapter our main findings are presented, compared and discussed, followed by methodological considerations.

The effects of long-term GH treatment on free-dissociable IGF-I

GH acts on peripheral organs such as the liver to stimulate production of IGF-I, which is synthesized by the liver and, to a lesser extent, by many non-hepatic tissues. In the circulation, IGF-I is bound mainly to IGF-binding proteins (IGFBP), six classes of which have been identified (IGFBP- 1 to – 6) [10]. These binding proteins prolong plasma half-life and modulate the metabolic action of IGF-I. The major carrier protein of IGF-I in the circulation is GH-dependent IGFBP-3, which normally accounts for more than 90% of the IGF-binding [11]. Under normal circumstances, less than 1% of the total plasma IGF-I pool is considered to be present in the unbound free form, which is also the biologically active form [12,13]. This free IGF-I exchanges rapidly with the tissue compartments [10].

Many organs and tissues contain IGF-I receptors. IGF-I plays a significant role in many conditions, as it does during normal organ development. While it is also involved in tissue function and maintenance [14], IGF-I may also have a role during pathophysiological processes such as atherosclerosis and diabetes mellitus [15,16]. As GH stimulates IGF-I production, GH-treatment may add to its growth-promoting effects.

For our measurements of free IGF-I in present studies, which were performed under strictly controlled conditions, we used the DSL IRMA, which employs solid-phase antibodies directed against unbound IGF-I. It has been suggested that this assay in fact determines the sum of truly free IGF-I and readily IGFBP-dissociable IGF-I, which has been hypothesized to represent a more biologically relevant pool than free IGF-I alone [12].

We also established a normative range of values for serum free IGF-I. Serum samples were collected from healthy children who underwent minor surgery (116 girls and 211 boys, aged 0–17 years). Serum free IGF-I levels in healthy boys and girls appeared to increase with age, and were higher in prepubertal girls than in boys, which is in agreement with earlier reports [12,13,17].

We compared free IGF-I levels in sera obtained after an overnight fast with those derived in the non-fasting state, using 13 healthy adult volunteers working in our department. No significant differences were found, which was in agreement with a previous report showing that, despite a marked elevation in IGFBP-1, overnight fasting did not alter circulating free IGF-I levels [18].

Most samples were analyzed at the end of the study, several years after they had been collected. Although our experience suggests that IGF-I and IGFBP-3 in EDTA plasma or serum is very stable – the values do not change significantly even after ten years of storage at –80°C – we did not have an opportunity to examine the stability of free IGF-I after long-term storage. However, we obtained similar results with free IGF-I when we repeated the tests after a year of storage.

— SGA —

We found that untreated short SGA children, either with or without GH-deficiency (GHD), have normal free IGF-I levels. Total IGF-1 and IGFBP-3 levels, however, were significantly lower than normal, which is in line with previous reports [19-22]. As lower free IGF-I levels have been reported in untreated GHD children and adults, [12,17,23], the pathophysiology of short stature in GHD children may be different from that in SGA subjects with GHD.

Under several conditions with primary abnormalities in GH secretion (e.g. acromegaly and GHD), there is usually a close relationship between serum free IGF-I levels, total IGF-I levels and the total IGF-I/IGFBP-3 molar ratios [24]. In short SGA children, however, these levels differed from each other with regard to direction and magnitude. At baseline, total IGF-I and IGFBP-3 levels were significantly lower than controls, but free IGF-I levels were normal. Neither was there any correlation between free IGF-I SDS and total IGF-I SDS.

This discrepancy between free and total IGF-I in short SGA children may be explained by subtle alterations in the binding of IGF-I to its various binding proteins

(e.g. changes in enzymatic proteolysis of IGFBP-3 and, as a consequence, lower IGF-I binding). Another explanation may be that short stature in SGA is caused by genetic variations in the IGF-I gene or IGF-I receptor gene that alter IGF-I binding or IGF-I signaling pathway. Several polymorphisms in the IGF-I gene and its receptor have been reported in short SGA children [25, 26].

Although free IGF-I increased during GH-treatment, it remained largely within the normal range, whereas total IGF-I increased to a greater extent than free IGF-1, the mean reaching the +2 SDS. In short SGA children we did not find a relationship between total IGF-I and growth response or adult height, the major bioactive endpoint of IGF-I. In contrast, baseline free IGF-I levels predicted the efficacy of GH therapy, in terms both of short-term growth response and of adult height. When predicting growth response to GH treatment in short SGA children, free IGF-I levels should therefore be taken into account before GH-therapy starts.

- **Untreated short children born SGA, either with or without GHD, had normal free IGF-1 levels, whereas total IGF-I and IGFBP-3 were lower.**
- **In GH-treated SGA children, baseline serum free IGF-I was a better predictor for short term growth response and adult height than were total IGF-I, IGFBP-3, or total IGF-I/IGFBP-3 molar ratio.**

— Turner Syndrome —

In untreated girls with TS, serum free IGF-I levels were as low as total IGF-I and IGFBP-3. The GH-IGF axis was similar to that reported in GHD, a primary abnormality in the GH secretion, showing a close relationship between serum free IGF-I levels, total IGF-I levels, and total IGF-I/IGFBP-3 molar ratios [24].

Though mean free IGF-I levels increased during GH therapy, they stayed within the normal range. Mean total IGF-I SD-scores were higher than the free IGF-I SD-scores, and even exceeded the upper normal range. While similar observations have been reported for GH-treated children and adults with GHD, there are also conflicting reports [12,24,27,28].

Additionally, in GH treated TS girls, total IGF-I levels and the total IGF-I/IGFBP-3 molar ratio were no proper representatives of free IGF-I levels. The predictive value of serum total IGF-I and this ratio is much higher for several biological endpoints of IGF-I bioactivity in TS girls, than that of free IGF-I. Besides measuring serum total IGF-I levels, measuring serum free IGF-I levels does not seem to play an additional role in predicting growth response, adult height, or insulin sensitivity in girls with TS. In this respect it is important to emphasize that the kinetics of free IGF-I in the

circulation differ strikingly from those of total IGF-I. Under normal circumstances, free IGF-I has a very short half-life (about 14 min) compared to the much larger pools of binary and ternary complex IGFBP-bound IGF-I. GH-induced changes in plasma levels of IGFBP-1, −2, −3, and −5 may have different effects on steady-state concentrations of free-IGF-I and total IGF-I levels. A GH-dose-dependent rise in free IGF-I levels may be compensated by increased clearance of free-IGF-I from the circulation to tissue compartments that results from the very short half life of free IGF-I. Gravholt et al also suggested that higher clearance of free IGF-I from the circulation might explain the paradoxical inverse correlation between free IGF-I levels in adult TS patients and the degree of IGFBP-3 proteolysis [29]. The recently developed sensitive IGF-I kinase receptor activation assay (KIRA) [30], which enables the determination of IGF-I-receptor activating IGF-I concentrations, may help clarify the bioactive capabilities of circulating IGF-I levels.

- **The extent of low free dissociable IGF-I in untreated girls with TS is the same as that of total IGF-I and IGFBP-3 levels.**
- **In GH-treated girls with TS, total IGF-I and the total IGF-I/IGFBP-3 molar ratio have a much higher predictive value for several biological IGF-I endpoints, specifically IGF-I bioactivity, than free IGF-I levels.**

Free IGF-I in short SGA versus TS children

In both study populations, GH therapy increased serum levels of free IGF-I, total IGF-I and IGFBP-3 to a similar extent. However, girls with Turner syndrome received a higher GH dose than the SGA children. While baseline free IGF-I helped predict short-term growth response and adult height In SGA children, in Turner girls it did not help to predict bioactive outcome of IGF-I. In Turner girls, total IGF-I and the total IGF-I/IGFBP-3 molar ratio seem to have a much higher predictive value for several biological endpoints of IGF-I bioactivity, whereas such a relationship between total IGF-I levels and growth response or adult height was lacking in short SGA children.

To understand the differences in the results between the SGA and the Turner population, it is necessary to understand the basic etiology of short stature in both populations. It is widely suggested that the characteristic Turner features are caused by haploinsufficiency of specific homologous X-Y genes which escape X-inactivation. Haploinsufficiency of the SHOX gene (***s***hort stature ***ho***meobox containing gene on the ***X***-chromosome) leads to skeletal abnormalities and growth failure [31-33]. Similarly, on the basis of the phenotypical differences between patients with Leri-Weill dyschondrosteosis (haploinsufficiency of SHOX) and patients with Turner syndrome,

it has been concluded that haploinsufficiency of the SHOX gene accounts for most, but not all of the TS height deficit [34]. However, it has yet to be established which mechanisms underlie the growth failure caused by haploinsufficiency of SHOX and/or other factors.

To attain normal height, girls with TS required a higher GH dose than short SGA children (1.3 mg/m^2/day *vs.* 1 mg/m^2/d). However, while a higher dose of 2 or 2.7 mg/m^2/d was even more beneficial in Turner girls, a higher dose in short SGA children (2 mg/m^2/d) did not further increase adult height [3,4]. Turner girls attained near normal heights with supraphysiological GH and IGF-I levels, indicating that they needed higher levels of GH and IGF-I to attain a normal growth effect. This indicates that, as suggested previously, a role in the short stature of Turners syndrome may be played by lower sensitivity to growth factors [35,36], which may originate pre- or post- IGF-I or GH-receptor. A post-receptor defect within the cell seems more likely as, for an optimal response, both IGF-I and GH need to be in supraphysiological levels. Furthermore, TS is a chromosomal disorder which affects some or all of the body cells.

The GH-IGF axis in Turner girls responded as expected during GH-treatment (similarly to GHD patients). In addition, 76% of the adult height in TS could be predicted by auxological and treatment-elated parameters [4]. The lack of association between free IGF-I levels and IGF-I bioactive outcome parameters such as height can be explained by an increased shift to the tissue compartments caused by the very short half-life of free IGF-I. Total IGF-I is a better predictor in GH-treated girls with Turner syndrome, as it has a longer half-life in the circulation.

In contrast, with respect to their etiologies and to prenatal and postnatal growth disorders, the group of SGA children is a very heterogeneous group (Chapter 1). A variety of factors are therefore likely to contribute to the lack of postnatal catch-up growth – making it difficult to draw a single unequivocal conclusion. Previously, it was suggested that a role in the etiology of the prenatal and postnatal growth retardation might be played by genetic factors, such as deletions or polymorphisms in the genes encoding for growth factors or growth factor receptors. But while several polymorphisms in the IGF-I gene and its receptor have been reported in short SGA children [25,26], none has led to the exact etiology of short stature in SGA.

It has also been suggested that subtle alterations in the binding of IGF-I to its various binding proteins (eg changes in enzymatic proteolysis of IGFBP-3 and, as a consequence, reduced IGF-I binding), may lead to alteration of the ratio of total and free IGF-I, and thus bioactive IGF-I.

IGFBP-3 is not only the major carrier protein for IGF-I, it also presents IGF-I to its receptor, regulates IGF-I trafficking, and has IGF-I independent effects on cells. Besides free IGF-I, IGFBP-3 was a predictor for adult height in the GH-treated SGA

children. The affinity for IGF-I may thus be influenced by a defect in the synthesis of IGFBP-3, thereby explaining the combination of normal free IGF-I levels and low total IGF-I levels. Alternatively, it may also provide less IGF-I at its receptor, resulting in a smaller effect. However, this theory would not explain the lower levels of total IGF-I.

The various etiologies of shortness in SGA children may explain why it was possible to predict only 42% of adult height [3]. However, when baseline free IGF-I was included in the prediction model, the prediction of adult height increased to 55%, while total IGF-I and IGFBP-3 were of no predictive value. This suggests that binding of IGF-I to its binding proteins may be different in short SGA children.

The response of the GH-IGF axis in short SGA children during GH-treatment was not as predictable as in TS girls. The suggested shift of free IGF-I to the tissue compartments, as might have occurred during GH treatment in TS, could not be ruled out. Nevertheless, free IGF-I levels may play an important role in future evaluation of GH therapy in short SGA children, and possibly when further exploring the etiology of shortness in SGA.

Quality of life after long-term GH therapy

A fundamental goal of medical intervention, and thus of GH-treatment, is to improve quality of life (QoL), which is conceptualized as a multidimensional construct encompassing several domains, including physical, emotional and social well-being. In both our study populations, health-related QoL (HRQoL) was measured using standardized and validated questionnaires, of which there are two different kinds: generic questionnaires and disease-specific or disorder-specific questionnaires. A generic questionnaire allows for screening in a healthy population, and enables standardized comparisons across health conditions. However, it may not be as responsive to changes in disease- or disorder-specific symptoms, and typically does not measure specific symptoms and treatment (side) effects relevant to particular disease or disorder group. Several studies have shown that the use of a generic questionnaire for a special disorder or disease makes it possible to miss relevant QoL issues [37-40]. In contrast, specific questionnaires may be more sensitive to specific clinical changes in patients with a particular disease or disorder, and may be more informative in specific patient populations. Unfortunately, specific questionnaires are unable to provide comparisons across populations with a specific disease or disorder, or in healthy populations.

— SGA —

Using a specific self-report questionnaire (TNO-AZL-Child-QOL-Short stature (TACQOL-S)) after long-term GH-therapy, our study showed that SGA children had a better health-related QoL (HRQOL) regarding 'physical abilities', 'contact with adults' and 'body image' than untreated SGA adolescents. However, when a *generic* questionnaire such as the child health questionnaire (CHQ) was used, there were no such differences. As stated previously, if a generic questionnaire were used for a special disorder or disease, relevant QoL issues might be missed [37-40]. The items of the TACQOL-S are more sensitive for effects of short stature on QoL outcome. Our study shows that in addition to generic QoL measurements, height-specific QoL measurements are very informative in growth studies.

A generic self-report questionnaire showed that GH-treated and untreated SGA adolescents had a near-normal QoL. However, whereas the GH group had been extremely short at start and normalised during GH treatment, the untreated group had never been as short as the GH group, and had a height SDS just below the normal range for many years, and just within the normal range at the time of the QoL evaluation.

Adults were less surprised when they heard the actual age of the GH-treated adolescents than when they heard the actual age of the untreated adolescents. The adolescents in the GH-group felt that they were treated more age-appropriately by adults than those in the untreated group. Also in the GH-treated SGA population, the closer their height was to that of their parents, the happier they were with their body. SGA adolescents who were taller experienced fewer behaviorally related social problems. These data emphasize the importance of feeling equal to one's peers.

It has been suggested that psychological support in learning to cope with the psychosocial problems related to short stature would be a less invasive alternative to GH treatment. To our knowledge, however, no structural psychological program has yet been proved either to be effective or to be of practical use, whereas GH treatment in SGA has not only improved participants' adult height, but has also significantly reduced total problem behaviour, externalising behaviour and self perception [41,42]. It also has been shown that, over time, the taller children showed less problem behaviour [41].

- **The disorder-specific TACQOL-S showed that GH-treated children born SGA had a significantly better quality of life with regard to physical abilities and contact with adults, and fewer problems with body image than untreated children born SGA.**
- **After long-term GH therapy, CHQ, a generic QoL, showed that adolescents had a near-normal HRQoL.**
- **In addition to generic QoL measurements, height-specific QoL measurements should be used in growth studies.**

— Turner Syndrome —

Young TS women have a normal or even better HRQoL than normal Dutch age-matched women. This HRQoL was measured after most of them had reached a height within the normal range and after they had started bodily feminization at an age comparable to that of their peers.

As stated previously, in addition to a generic QoL instrument, a height-specific QoL instrument should be applied in growth studies to measure specific relevant QoL issues. At the time of the QoL-evaluation, the TS population, had to use 'adult' QoL-questionnaires, as the mean age was nearly 20 years. A height-specific QoL questionnaire was not available for adults. In order to assess the therapy's possible influence on the QoL of young TS women, we used an additional questionnaire containing subjective parameters. This included questions such as 'Are you satisfied with your adult height?' and 'Are you satisfied with your breast development?'. A number of HRQoL-scales were positively influenced by satisfaction with adult height and/or breast development.

Similarly, social functioning was positively influenced when physical appearance did not lead to an increase in feelings of insecurity – data which helps emphasize the importance of feeling equal to one's peers. GH and estrogen treatment brought about normal height and breast development in most of the girls [4,43], narrowing the differences with their peers. Because short stature and delayed pubertal development can have a significant negative influence on HRQoL [44,45], we hypothesize that GH treatment and age-appropriate puberty induction may normalize HRQoL in TS women.

Estrogen substitution may also have a direct, positive influence on several aspects of HRQoL. Estrogens improve motor performances and motor speed in TS [46,47], and may therefore increase the QoL item that applies to motor performances. Estrogens significantly decrease aggressive and delinquent behavior in girls with TS [48], and may therefore produce a better score on the HRQOL item relevant to aggressive emotions. All in all, we therefore hypothesize that estrogen therapy that starts at an appropriate age is beneficial for the HRQoL in TS women.

On some of the scales, the TS women showed a remarkably high HRQoL. In Chapter 5, we discussed the possibility that they might have undergone what is called 'response shift' [49], i.e. a change in their internal standards or values. This phenomenon has been reported in the elderly [50], who excluded some age-related problems or 'morbidity' when assessing their susceptibility to illness. With regard to our study, however, we cannot say whether the relatively high HRQoL scores were associated with a shift in internal standards or reflected a real health effect.

As a majority of adults with TS are infertile, the consequences of infertility on the HRQoL may become a more prominent issue in the future of the Turner women we

studied. Infertility may significantly influence the HRQoL (51-54); to determine its influence on the HRQoL of women with TS, we recommend that further research be conducted.

- **After long-term GH-treatment and puberty induction at an appropriate age, young women with TS have a normal HRQoL.**
- **The relatively high scores on some of the HRQoL-scales in TS women may be explained by an estrogen effect, or possibly by a response shift. The latter would indicate that these women had acquired a different internal reference.**
- **TS women's satisfaction with their height and/or breast development positively influences several HRQoL scales, including social functioning and physical functioning.**
- **GH and estrogen treatment may have positively influenced HRQoL in young women with TS.**

QoL in short SGA versus TS children

Both in SGA adolescents and the Turner women, the data emphasize the importance of feeling equal to one's peers. The generic questionnaires suggested that, after long term GH-treatment, both groups had a normal or near-normal QoL relative to a sample of the general population. This was after most patients in both groups had reached a height within the normal range. Additionally, using exogenous estrogen substitution, the TS women had started bodily feminization comparable to that of their peers.

A height-specific QoL questionnaire was not available for adults. Because, at the time of the QoL-evaluation, the TS population was slightly older than the SGA population, 'adult' QoL-questionnaires had to be used. The SGA population could use the specific 'child' QoL-questionnaires available. As described above, we used an additional questionnaire containing subjective parameters to assess the therapy's possible influence on the QoL of young TS women.

We cannot conclude that HRQoL had *improved*. This is due to the lack of baseline data: the GH-trials in SGA started in 1991, and those in TS in 1989, when the importance of HRQol as an important outcome measure was less recognized than it is today. The questionnaires used in both studies were developed only during early and mid-nineties, thus after the start of the GH-trials. Although there was an untreated parallel SGA group, none of the studies had included a randomized control group for ethical reasons.

Another difficulty is that the literature contains very few data on HRQoL in untreated SGA adolescents or TS women. Very recently, Carel *et al* described the health-related quality of life (HRQoL) in GH-treated TS women [55]. While they found normal HRQoL in TS without any significant influence of GH treatment related variables, such as adult height or estimated height gain, our own data showed that height gain had a significant influence on 'daily activities' and 'role limitations due to physical health problems'. Our own study showed that satisfaction with height and/ or breast development had a significant positive influence on several scales of the HRQoL. Very recently, it was reported that 18 months of GH treatment improves QoL in acquired or idiopathic GHD children [56].

It should be emphasized that SGA and TS affect more than height alone. While SGA has also been associated with lower intelligence, poor academic performance, lower social competence and behavioral problems [57-59], girls with TS have been described as more immature, having lower self-esteem, and being hyperactive [48,60,61]. As well the positive effect that GH-induced height gain may have on HRQoL, GH may influence other mechanisms contributing to the HRQoL. Recently, our group showed that, after long-term GH treatment, SGA children had less problem behavior and greater self-esteem than at start of GH treatment [62]. Over time, taller children had less problem behavior.

With regard to the TS women, the lack of longitudinal data makes proof impossible. However, it is likely that GH and estrogen treatment improved psychosocial functioning, as discussed previously [63]. Also in children with GH-deficiency (GHD) and idiopathic short stature (ISS), positive behavioral changes were reported during GH treatment [42]. This indicates that GH-treatment may improve several aspects in short children, not only height. GH may improve QoL in children or adolescents by mechanisms unrelated to growth.

GH-therapy has also been reported to improve QoL in GHD *adults* [64-67], thus supporting our hypothesis that GH is capable of improving aspects of QoL besides height. It being well established that the placebo effect in GH-controlled trials lasts only three months after start of GH treatment, this is unlikely to be a placebo effect [68,69].

Although – as stated above – TS women may have undergone what is called 'response shift' [49] (i.e. a change in internal standards or values), we could not find any evidence of an internal shift in the SGA population studied, and therefore think it played no role in GH-treated SGA adolescents. There is nonetheless a slight possibility that the shorter adolescents experienced a response shift. This might explain the nearly normal QoL in the untreated SGA group, which was significantly shorter than in the GH-treated group. Further follow-up will show whether GH treatment also has an effect on their life achievements.

Previously we discussed the importance of being equal to one's peers, something which is also reflected in the development of puberty. In TS women, puberty was induced at an appropriate age. Because in the SGA children in our group, start, progression, and duration of puberty were comparable to those in the normal Dutch population [70], the QoL outcome was not influenced by a delayed or precocious puberty in GH treated SGA children.

- **GH may improve QoL through mechanims unrelated to growth.**
- **Future research will show whether GH treatment also affects life achievement.**

Pubertal development in TS

The main objective of estrogen therapy in girls with TS is to induce a sufficient degree of puberty and feminization as physiologically as possible without sacrificing adult height. As previously shown in chapter 5, TS women who were satisfied with height and/or breast development have a better QoL in social and physical functioning. We showed that, using a very low dose of oral estrogen therapy, the majority of Turner women had normal breast development, albeit with a two-year delay. As it does not affect adult height, the low dose makes it possible to start this estrogen regimen during GH therapy at an age-appropriate time [4,71]. A recent study reports that the administration of GH with a very low dose of estrogens (0.2 mg estradiol per month i.m.) at the age of 12 years enhanced height velocity more than GH alone, while also preserving height potential [71]. In one third of the subjects, however, this dosage did not bring bodily feminization. It may thus be possible to start estrogen at an even lower age without sacrificing adult height, and to start bodily feminization at an appropriate age. For estrogen therapy to have the greatest benefit on adult height and bodily feminization, not to mention other positive influences of estrogens, the optimal dosage and age of initiation should be established in further research.

Estrogen action depends on the presence of the estrogen receptors (ERs). Research in hypophysectomized and oophorectomized rats has demonstrated that GH treatment increases the expression of ERs in breast tissue [72]. Although the mechanism underlying stunted growth in TS is not well understood, despite normal pretreatment GH levels, GH treatment improves growth velocity. Theoretically, the GH therapy also may have increased the amount of ERs in the breast tissue of the TS girls, thereby increasing the sensitivity of the breast tissue to estrogen treatment, which led in turn to normal breast development using a low estrogen dose.

In our Turner population, uterine volume was smaller (24.9, range 4.4–57.9 ml) than that reported in normal women of the same age (61 ml, range 37–130 ml) [73]. Uterine volume, length and shape were comparable to those in normal girls who had reached breast stage B5, and/or were aged 14–16 years. This underdevelopment of the uterus in the young TS women may be explained by the estrogen dose. If so – due to the association reported between a higher estrogen dose and greater uterine length, and also to the uterine length reported to be positively associated with uterine shape – a higher estrogen dose might lead to normal uterine dimensions in adulthood [74]. Alternatively, as the induction of puberty started two years after physiological pubertal onset, it is also possible that uterine development was delayed. Yet again, remaining subnormal uterine dimensions may be explained by other factors related to TS.

Young women with GH-deficiency (GHD) also had a smaller uterus than normal women, despite GH therapy [75]. In GHD women of similar age, the uterus length was nearly comparable tot that of our TS women (67mm in GHD, range 47–84mm versus 60mm in TS, range 25–87mm respectively). This suggests that the growth and development of the uterus is possibly due to the influence not only of estrogens, but also of other factors, such as GH or IGF-I, which may act directly or indirectly by the expression of ER. To confirm this theory, however, further research will be required.

Pubic hair developed much as normal, and in most girls had started to do so before estrogen treatment was initiated. This indicates that, despite the absence of ovarian androgen production, adrenarche in girls with TS proceeds normally, which is in agreement with earlier reports [76].

Turner women produce neither ovarian estrogen nor ovarian androgen. Studies in postmenopausal women have demonstrated that a combination of estrogen and androgen therapy increased well-being while providing beneficial effects on bone, lipids, body composition, sexual functioning, and quality of life [77-80]. The effect of the estrogen/androgen therapy was greater than the effect of estrogen therapy alone [77]. In TS women, a combination of estrogen/androgen therapy may also be beneficial. Indirectly, this may also be suggested by Ross *et al*, who reported improved performance of the working memory in TS girls treated with the androgen oxandrolone over two years [81]. The benefits of androgen therapy in women with TS should be clarified in future research.

Although levels of the serum estradiol (E2) at the lowest oral estrogen dose were comparable with normal E2 concentrations in late pubertal and adult serum [82], it is evident from the lack of an increase in serum SHBG and the remaining high gonadotropin levels that the overall serum E2 effect was low. The lowest E2 (5 µg/kg/d) dose suppresses LH and FSH. However, at the double E2 dose (10 µg/kg/d), also providing double serum E2 levels, LH and FSH levels increased. This effect may

be a result of changed sensitivity of the E2 feedback, possibly due to an increased age or to a higher tolerance. The LH and FSH levels in postmenopausal women receiving 2 mg estradiol per day were comparable [83] or lower [84] than the levels reported in our study at the adult dose of 1 or 2 mg/d estradiol.

Finally, the route of administration may be important for optimal replacement therapy. Although oral administration has its advantages, the intestinal and hepatic first pass metabolism causes the bioavailability of oral estrogens to fluctuate. To avoid the first pass effect, transdermal patches containing 17ß-estradiol can be used to mimick spontaneous pubertal hormonal changes, inducing normal puberty [85,86] and avoiding the excessively high levels of estrone that are seen after oral administration of estrogens. While oral forms of administration cause estrone to accumulate in the body, continuous wearing of the transdermal estradiol patches for three weeks did not result in any accumulation of estradiol or estradiol metabolites [87].

It has been reported that, when percutaneous gel is used to induce puberty in girls with TS, secondary sexual characteristics and uterine growth proceed gradually, mimicking natural puberty [88]. However, the results of gel on growth velocity were unclear. Future research should evaluate whether transdermal patches and/or percutaneous gel are the optimal oestrogen treatment for inducing puberty in girls with TS at an age similar to that in girls with spontaneous puberty, and without negative impact on final height.

- **In girls with TS, a low dose of 17ß-estradiol can be used for puberty induction at an appropriate age. Breast development in these girls is then comparable to that in normal Dutch girls.**
- **Serum hormone levels do not provide additional information for evaluating the development of the puberty induced.**
- **Uterine dimensions at the age of nearly 20 years were comparable to those in a normally matured girl at the age of 15 years.**
- **It remains unclear whether subnormal uterine dimensions in young Turner women are related to the timing or dosage of the estrogen therapy, or to factors related to Turner syndrome.**

Safety aspects several years after long-term GH treatment in TS

Insulin and glucose metabolism

To estimate insulin sensitivity and β-cell function, we used the Homeostasis model (HOMA in %), a structural model of glucose/insulin interaction, with mathematical equations describing the functioning of the major effector organs [89-91]. During GH therapy, insulin sensitivity was reduced and fasting insulin and ß-cell function were increased. There was no difference between the three GH dosage groups. At short-term follow-up, which was six months after ending long-term GH treatment, insulin and glucose metabolism were unchanged. Nearly five years after GH-discontinuation, parameters of insulin sensitivity, ß-cell function and fasting insulin levels were still unchanged relative to levels measured four years after start of GH-treatment and measured at short-term follow-up. Insulin sensitivity remained constant at approximately 75–85%, and ß-cell function at approximately 150–160%.

In untreated young TS women, a higher prevalence of insulin resistance and impaired glucose tolerance has been reported [92]; more recently it was also suggested that haploinsufficiency for one or more unknown X-chromosomal genes may be responsible for a finally impaired ß-cell function in TS [93]. As several studies have shown a positive correlation between insulin levels and BMI in normal children and adults, the higher insulin levels after GH may also result from increased BMI [94,95]. This possible explanation is supported by the positive association of BMI with ß-cell function and with fasting insulin levels found several years after GH therapy. Very recently, however, it has been suggested that impaired peripheral and hepatic insulin sensitivity in TS is caused by an intrinsic defect independent of fat mass that is related to the karyotype in TS [96].

Salgin et al recently presented data on insulin and glucose levels in untreated TS women aged 29.9 (16.5–46.6), whose insulin sensitivity was 103 (78.6)%. This value is not significantly different from the 81.6 (46.5)% in our GH-treated TS women aged 20.0 (2.1) years [96]. Despite this, and despite the lack of an association with GH dosage or GH duration, a possible effect of the GH therapy itself cannot be completely ruled out. The findings that insulin sensitivity had not changed nearly five years after the end of GH treatment may suggest a partly irreversible or a very slowly reversible phenomenon. In a large epidemiological study on GH-treated children and adolescents (n = 23 333), the incidence of type 1 diabetes mellitus was not affected [97]. There was, however, a more than expected increased incidence of type 2 diabetes mellitus. The authors assigned this finding to an acceleration of the disorder in predisposed individuals [97].

Mean fasting glucose levels increased significantly from six months after GH discontinuation to nearly five years after end of GH treatment. However, the

increment was small, and glucose levels were all within the normal range. Future research should clarify whether these glucose levels continue to increase, and whether impaired glucose tolerance will be reached. In addition, future research should clarify whether this impaired insulin sensitivity is the result of TS, increased BMI, the long-term GH-therapy itself, or a combination of these factors. Since insulin sensitivity can be affected by TS and a high BMI, the sole influence of long-term GH treatment is difficult to assess. Follow-up of other long-term GH-treated populations, such as children who were born small for gestational age, may help provide an answer.

- **Nearly five years after end of GH treatment in TS, insulin sensitivity was decreased (75–85%), and ß-cell function was increased (150–160%).**
- **Insulin sensitivity in untreated women aged 29.9 (16.5–46.4) with TS was similar to that in our GH treated TS women, aged 20.0 (2.1): this is 103 (78)% *vs*. 75–85%.**
- **Nearly five years after end of GH treatment in TS, glucose levels were slightly higher than six months after end of treatment, but within the normal range.**

Blood pressure (BP)

Although GH therapy lowered diastolic BP, nearly five years after end of GH treatment it was similar to its pre-treatment levels. Systolic BP did not change during GH treatment, and was not influenced by GH dose or duration. Nearly five years after end of GH treatment, however, systolic BP was higher than pre-treatment levels. Untreated TS women aged 35.7 (9.3) years, and thus approximately 15 years older than our TS population, had a higher systolic and diastolic BP than our group of GH treated TS women [98].

One may conclude that GH treatment overall had a positive effect on diastolic BP, especially in the light of the fact that, even several years after the discontinuation of hormone treatment, there was a continued association between BP and the dosage of GH: diastolic BP was lower in patients whose dose has been higher, also after adjusting for the BP at start. The lack of significant difference between the GH dosage groups may be explained by a non-dose-response effect of GH on diastolic BP. The increased BMI at LT-fu did explain the increased blood pressure. In adult GH-deficient patients it has also been reported that GH therapy tended to reduce systolic and diastolic BP [99].

- **Nearly five years after end of GH treatment, diastolic BP had increased, despite a decrease during GH-therapy.**
- **Systolic BP did not change during GH therapy. The GH dose or duration was of no influence on systolic BP.**
- **Nearly five years after end of GH treatment, systolic BP was higher than pre-treatment values, and diastolic BP was similar to pre-treatment values.**

Serum lipids

We measured fasting serum lipid levels at baseline, 4 years after start of GH therapy, six months after end of GH treatment (short-term follow-up), and a mean of 4.8 (1.9) years after end of GH treatment (long-term follow-up). Several years after end of GH treatment, serum total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) had all increased relative not only to the values 4 year after start of GH treatment, but also to the values six months after end of GH treatment. After start of GH therapy, the atherogenic index (AI = TC/HDL) had decreased, and remained constant for several years after end of GH treatment.

GH treatment thus has a positive effect in girls and young women with TS on serum lipids, adding to the positive effect on serum lipids described after discontinuation of treatment in GH-deficient patients [100]. A similar trend has also been observed in GH-treated short children born small for gestational age (SGA) [101].

- **Nearly five years after end of GH treatment, serum HDL had further increased, and, relative to the level six months after end of GH treatment, atherogenic index was constant. However, TC and LDL had also increased.**

Body mass index (BMI)

BMI increased gradually during and after GH-discontinuation. At an average age of 20 years, fewer than 40% of the TS women had a normal BMI (below 25 kg/m^2). In a large study in the USA among GH-treated TS (n = 2468) [102], BMI SDS increased with age, starting approximately at age nine. GH did not influence the magnitude of the increase.

- **Nearly five years after end of long-term GH-therapy, the mean BMI in the women with TS was 26.5 (5.1) kg/m^2. Of the TS women aged 20.0 (2.1) after long-term GH therapy, 38.5% had a BMI between 20−25 kg/m^2, 46% were obese (BMI 25−30 kg/m^2), and 15.5% were morbid obese (> 30 kg/m^2). GH did not influence the magnitude of the increase in BMI.**

Aortic dimensions

Using MRI-techniques several years after end of GH treatment, young women with TS had larger mean aortic diameters (mm/m^2) at all thoracic levels measured, and a larger mean ascending/descending aorta diameter-ratio than controls matched for age and BMI. The advantage of the ascending/descending aorta diameter ratio is that it can be adjusted for intrinsic variation. A ratio lower than 1.5 is assumed to be normal [103-105]. In our study, this ratio was higher than +2 SD-score − based on the matched controls − in 27% of the cases aged 19.9 years, although a very recent study in 18-year-old GH-treated TS women [106] reported no increase in the ratio of ascending/ descending aorta. It should nonetheless be remembered that concurrent dilatation of the ascending and descending aorta, as we found in our TS population, may result in a false normal outcome of this ratio.

In almost 30% of cases, the diameter of the ascending aorta was higher than +2 SDS of the matched controls. After adjustment for BSA this figure was even 49%. The abnormality was most prominent in patients who had received the lowest GH dose (1.3 mg/m^2/d). Previous studies using MRI reported ascending aorta diameters to be dilated in a substantial percentage of patients (18−33%), 31% of the studied population was treated with GH for an average of 4 years [103,105].

One rare but potentially devastating result of aortic dilatation is aortic dissection, which has been reported several times in women with TS [107,108]. An obvious reason for this finding is that the factors known to predispose for aortic dissection are seen more frequently in TS than in normal women; these include hypertension, bicuspid aortic valve, and cardiac malformations [109,110]. It has also been suggested that the higher risk for arterial dilation and dissection in women with TS may be explained by a connective tissue defect [108,110].

In our study, we found that dilation of the aorta was not limited to the aortic root or ascending aorta, but was present throughout the entire thoracic aorta, and, although differences between the GH dosage was lacking, was more prominent in patients who had received the lowest GH dose. A very recent study in 18-year-old GH-treated TS women reported no differences in aortic diameter between GH-treated and untreated women [106], concluding that GH does not produce an adverse effect upon aortic structure. In the study in question, however, the average duration of the

GH treatment was five years; in ours it was nearly 9 years. One can thus conclude that GH therapy does not have an adverse effect on aortic structure, but may even have protective effects on aortic wall properties. These results may be confirmed in future studies that include larger GH-treated study groups or MRI measurements made at a higher age.

- **Ascending and descending aorta diameter was larger in young TS women than in controls matched for age and BMI. The ascending aortic diameter was dilated in 30–50% of the women with TS. Dilatation of the aorta was not just limited to the ascending part, but was present throughout the entire thoracic aorta.**
- **GH therapy does not have an adverse effect on aortic structure. On the contrary, it may even have beneficial effect, as the abnormalities seemed to be more prominent in patients who had received the lowest GH-dose.**

Aortic distensibility

Impaired aortic wall function may alter aortic distensibility. Several years after end of GH treatment, we found lower aortic wall distensibility in TS women than in age- and BMI-matched controls. Distensibility was higher in the patients who had received the highest GH dose than in those who had received the lowest. At the ascending aorta and abdominal descending aorta, the difference between the group with the highest dose and that with the lowest was significant; at the level of diaphragm, it was nearly significant (P = 0.07).

Aortic distensibility in normal subjects is negatively correlated with age, progression of atherosclerosis and an abnormal lipid profile [111]. Between GH-dosage groups there were no differences with regard to age or serum lipid levels, which do not therefore explain the differences in distensibility between the GH-dosage groups. Levels of GH and IGF-I, which are both anabolic hormones, decrease with increasing age. GH replacement can reverse age-related catabolic changes [111]. Patients with GH-deficiency (GHD) have been reported to have wall thickening in large arteries and a tendency towards a lower arterial-wall compliance than in controls [112,113]. GH treatment in GHD patients decreased the arterial wall thickness and increased wall compliance [112,113], supporting our finding of a possible positive GH-effect on aortic wall distensibility in TS.

Ironically, there are similar aortic abnormalities in patients with TS, which is associated with short stature, and patients with Marfan syndrome, which is associated with tall stature. Not only do both have a higher risk of enlargement of the ascending aorta, both are reported to have a higher risk for aortic dissection.

Similarly, medial necrosis has also been reported in both [108,110]. Lower aortic distensibility has also been found in patients with Marfan syndrome [114]. As the underlying pathophysiology of aortic disease in Marfan syndrome is primarily the result of the expression of a mutant form of the protein fibrillin, it may be possible that the underlying pathophysiology of aortic disease in TS is also a connective-tissue disease. However, such an etiology has not been identified in TS, making it too early to transfer to TS knowledge and guidelines relating to Marfan syndrome. For example, in Marfan syndrome the aortic root dimensions progress with age [115]. In TS, the likelihood of such a progression is not yet known. Another example in Marfan syndrome is that beta-blocker therapy reduces blood pressure and increased aortic distensibility [116]; in TS, however, the role of beta-blocker therapy in preventing aortic disease – especially during pregnancy – remains to be investigated.

- **The aorta in young women with TS showed decreased aortic wall distensibility, which is a sign of impaired aortic wall function.**
- **In the group with the lowest GH-dose, aortic distensibility was significantly lower than in matched controls, whereas both other GH-dosage groups showed no significant differences relative to matched controls. In women with TS, GH seemed to have a beneficial influence on aortic wall properties.**

Body proportions

Several years after discontinuation of GH therapy in TS, we found a moderate improvement with regard to the disproportion between height and sitting height (SH) that had existed before treatment, and an increase in the disproportion between height and foot length relative to baseline data. The disproportion between SH and height nearly normalized, whereas disproportion between hand, biacromial diameter (Biac), biiliacal diameter (Biil), and height remained unchanged relative to baseline. Untreated adult TS women, however, appear to have a larger disproportion between height, Biac and Biil [98]. GH therapy possibly prevented the disproportion from getting worse. Although there was also a disproportion between the height and foot length in untreated adult women [98], the foot length was associated with the GH-dosage used.

- **GH therapy normalized the pre-existing disproportion between height and sitting height.**
- **GH may have prevented worsening of the disproportion between height and biacromial diameter, and biiliacal diameter.**
- **The increase in the disproportion between height and foot length was due partly to the natural development of TS and partly to GH-dosage.**

Methodological considerations

Strength of the studies

The design of the present research has several strengths.

First, subject recruitment in both studies was restricted to well-defined criteria. For the SGA study, the inclusion criteria were applied by one and the same person. For the TS study, the TS diagnosis was confirmed by lymphocyte chromosomal analyses, and, once again, the inclusion criteria were applied by the same person.

Second, both studies were multicenter studies that took place in the Netherlands and included academic hospitals. Over the years, all measurements were carried out by the same five researchers (Arne van Teunenbroek (TS), Wouter de Waal (SGA), Theo Sas, Yvonne van Pareren, and Ellen Bannink) and the same research nurses (Anne van der Wiel (TS), Ingrid van Slobbe (TS), and Janneke van Nieuwkasteele (SGA)). This consistency lessened the risk of inter-individual bias, and may have stimulated compliance with the therapy.

Third, over the years most of the laboratory measurements were performed in the same laboratory. With regard to the IGF-I and IGFBP measurements, the reference values for healthy children were also determined in the same laboratory [117,118], thereby reducing the variability between different laboratories.

Fourth, both studies were randomized prospective long-term longitudinal studies, not 'pseudo-longitudinal' studies. Pseudo-longitudinal describes changes over time on the basis of data seen only once per subject, such as that used in studies to derive cross-sectional growth charts for children [119].

Weaknesses of the studies

Firstly, due to ethical considerations, neither study – SGA or TS – used a control group of untreated children, though a randomized untreated control group might have been of additional value in the studies on quality of life (QoL). The lack of such a control group was of less importance showing a GH-effect in the other parameters measured, as different GH dosages were used, and the development of the different

measurements after GH discontinuation could be evaluated. Regarding the TS study, however, it is difficult to establish the long-term effects of the GH therapy: because such therapy has been an accepted indication in Turner syndrome for many years, there are no representative data in an untreated group of young women with TS.

Secondly, the SGA study sample might be too various with respect to underlying pathology, and therefore to accompanying pathology. Short SGA is a diagnosis based on auxological parameters. The underlying pathophysiology is unknown; genetic factors and environmental (intrauterine) factors may both be related to the underlying cause [120,121]. The natural development of certain aspects may therefore differ within the group. Future research will reveal the underlying pathophysiology of shortness in SGA, possibly bringing greater understanding of the pathology related to the SGA syndrome, and possibly leading to a more specific and thus successful treatment of the health problems related to being born SGA.

While the pathology underlying TS has been identified – i.e. the total or partial absence of one of the X-chromosomes in all or some of the body cells – the underlying pathophysiology of Turner features (such as short stature, impaired lymphatic development, and the increased risk of developing diabetes mellitus and cardiovascular diseases) is not yet known. There are genotypic or phenotypic differences that may influence the growth response and other effects of GH treatment.

A final weakness may have been the number of children in the present studies. As the QoL and long-term follow-up studies were added to the original GH-trial, not all patients were motivated to participate. Although there were no indications of selection bias per individual study, this could not be ruled out completely.

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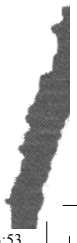
Chapter 10



What have we learned from all these years of GH therapy?

What have we learned from all these years of GH therapy?

Around the world, issues for debate are still being raised by long-term GH therapy in populations who are not GH-deficient. After more than 15 years of experience with GH therapy in short children born SGA and in girls with Turner syndrome, the time has come to sum up the results of these two long-term studies. The following chapter summarizes the most important aspects of all results of both GH-trials, evaluates the benefits and costs of the GH treatment, and ends by answering the question "Was it – and is it – all worthwhile?"

SGA	
	Name author/ researcher
Effect of GH treatment in short children born SGA	
Growth and adult height	
– After two years of GH treatment, most patients grew to within the normal range. – After five years of GH treatment, most children grew along their target height range, and the difference in growth-response of short children born SGA between those receiving 2 mg/m^2/day and those receiving 1 mg/m^2/day was small, and statistically significant only in the children who remained prepubertal during the study. – In most children, a dose of 1 mg proved to be as effective as the higher dose of 2 mg/m^2/day. Only those with an extremely short stature may thus need a higher GH dose to normalize height. – Long-term continuous GH treatment in short children born SGA led to a normalization of height in childhood and a normal adult height in 85% of the children, though 98% attained an adult height within their target range. – Thus, **in conclusion**, GH treatment leads to normal height during childhood and adulthood in short children born SGA.	WJ de Waal [1] TCJ Sas [2] YK van Pareren [3]
Predictors of adult height	
– Adult height SD-score = 0.02 + 0.29 x TH SD-score + 0.42 x height SD-score at start + 0.20 x (calendar age – bone age) at start + 0.07 x GH dose. – **42%** of the variance in adult height SD-score could be explained. – In GH-treated SGA children, baseline serum-free IGF-I was a better predictor for short-term growth response and adult height than were total IGF-I, IGFBP-3, or total IGF-I/IGFBP-3 molar ratio. It increases the predicted variance of adult height up to **55%**.	YK van Pareren [3] EMN Bannink (present thesis)
Additional effects of GH treatment	
Bone maturation – Five-year of GH showed, during the first 2 years of GH, a GH dose-independent acceleration of bone maturation that was not progressive during further GH treatment. This appeared to be influenced by the natural bone age development in these children at the age of 7 to 10 years.	WJ de Waal [1]

Cardiovascular risk factors – GH treatment in short children born SGA did not negatively influence glucose levels, even with dosage up to 2 mg/m^2/day. – GH treatment induced higher insulin levels, indicating more insulin resistance, which normalized after end of GH treatment. – Long-term GH treatment had no adverse effects on body composition, blood pressure, and serum lipids. – The beneficial effect of GH on blood pressure, systolic and diastolic, remained after discontinuation of GH. – Although most children had normal serum lipid levels, we found an indication for clustering of risk factors for DM type 2 and cardiovascular disease in adolescents with relatively higher BMI.	TCJ Sas [2] YK van Pareren [3]
Body proportions – Body proportions in short SGA children were not negatively influenced by six years GH treatment with 1 or 2 mg/m^2/day.	TCJ Sas [2]
Psychosocial functioning – Over 8 yrs of GH treatment, test scores for IQ and psychosocial functioning improved for most children, starting significantly below average and becoming similar to those of Dutch peers. Similarly, the taller a child became over time, the less problem behavior he or she showed.	YK van Pareren [3]
Puberty – GH treatment did not affect serum DHEAS levels. – The age at onset of puberty of GH-treated children born SGA was comparable with that of normally statured AGA children, regardless of treatment with 1 or 2 mg/m^2/day. – Age at menarche and progression of puberty in girls were comparable with those in the normal population, regardless of treatment with 1 or 2 mg/m^2/day. – Duration of puberty and the pubertal height gain were not significantly different between the GH dosage groups.	VH Boonstra [4]
Quality of life – Using the disorder-specific TACOL-S, GH-treated children born SGA had a significantly better quality of life in terms of physical abilities and contact with adults, and fewer problems with body image than untreated children born SGA. A generic QoL showed that SGA adolescents had a near-normal HRQoL after long-term GH therapy. GH may have improved QoL through mechanisms unrelated to growth.	EMN Bannink (present thesis)
Short children born SGA related results	
Endogenous GH production – In 50–60% of the short-statured children born SGA, there were signs of GH insufficiency, and 25% showed low GH peaks during GH-provocation tests.	WJ de Waal [1]
IGF-I polymorphism – There was an association between a polymorphism of the IGF-I gene and low-serum IGF-I levels in a group of short children born SGA. Genetically determined low-serum IGF-I levels may cause not only a reduction in birth length, weight and head-circumference, but also persistent short stature and small head-circumference during childhood and adulthood (proportionate small). – The IGF-I gene thus may be one of the links between low birth weight and an increased risk of adult disease.	NJT Arends [5]

Puberty – Children born SGA had neither a disturbed adrenarche nor a higher incidence of premature pubarche. – Prepubertal short SGA girls had normal serum AMH levels, indicating a normal follicle pool size. Short adolescent SGA girls did not have a relatively small ovarian follicle pool. – Compared to AGA young men, young men born SGA, with or without short stature, had normal serum inhibin B, LH, FSH and testosterone levels. Serum AMH levels were significantly higher in young men born SGA. This indicates that small size at birth, which might reflect fetal growth restriction, does not reduce the number of Sertoli cells, and has no effect on testicular function in men born SGA.	VH Boonstra [4]
Growth factors – Untreated short children born SGA, either with or without GHD, had normal free IGF-1 levels, whereas total IGF-I and IGFBP-3 were lower.	EMN Bannink (present thesis)

Short SGA and GH treatment: "Was it – and is it – all worthwhile?"

Eighty-five percent of the GH-treated SGA children attained normal adult height, and 98% attained a height within their target height range. This represents a height gain of 11–13 cm in girls and 12–14 cm in boys. In up to twelve years of treatment, GH treatment had no major adverse effects. However, fasting insulin levels and total insulin production after glucose loading rose significantly during GH treatment, and although the decreased insulin sensitivity induced during GH treatment disappeared after the end of GH treatment, the long-term consequences of decreased insulin sensitivity induced during childhood should still be investigated.

On the basis of our studies we recommend GH treatment in short SGA children, who are otherwise at increased risk for short stature as adults. GH treatment not only improves adult height, it also improves blood pressure and serum lipids, as well as IQ, psychosocial functioning, and certain domains of these children's quality of life. It does not have any influence – either negative or positive – on start of puberty or pubertal progression.

In other words, although GH treatment has several positive effects beyond its positive influence on height in short children born SGA, the long-term sustainability of these effects remains to be investigated. Future studies have to clarify whether long-term GH treatment during childhood reduces development of adult diseases in patients born SGA. Follow-up will also show whether, in adulthood, these GH treated patients go on to attain relatively greater personal, vocational and overall life achievements at the long term.

Turner Syndrome	
	Name author/ researcher
Effects of GH treatment in girls with Turner Syndrome	
Growth and adult height	
– GH treatment in girls with TS led to a normalization of height during childhood, with 83% of the girls achieving a normal adult height, and 63% gaining an adult height within their target height range. – A GH dose of 1.3 mg/m^2/day led to a height gain of 11.9 (3.6) cm; a dose of 2 mg/m^2/day led to a height gain of 15.7 (3.5) cm; and a dose of 2.7 mg/m^2/day led to a height gain of 16.9 (5.2) cm. The TS women who used a GH dose of 2 or 2.7 mg/m^2/day were significantly taller than the ones who used 1.3 mg/m^2/day. Between the two highest GH dosages given there was no significant difference in adult height. – Giving injections more than once a day did not increase the effect of GH on adult height. – Low doses of estrogens at a normal pubertal age did not interfere with the capability of GH treatment to normalize adult height in girls with TS	TCJ Sas [6] YK van Pareren []
Predictors of adult height	
– Adult height SD-score = −2.29 + 0.8 x Height SD-score at start + 0.81 x group with highest GH dosage (2.7 mg/m^2/d; yes = 1/no = 0) + 0.68 x group with intermediate dose (2.0 mg/m^2/d; yes = 1/no = 0) + 0.24 x Height velocity in 1st year (cm) + 0.087 x calendar age at start (yr). – **76%** of the variance in adult height SD-score could be explained. – The optimal dose depends on height and age at start of treatment, and first year height velocity. – If GH is started at an early age, adult height will not be affected by early initiation of estrogen. – In GH-treated girls with TS, total IGF-I and the total IGF-I/IGFBP-3 molar ratio seem to have a much higher predictive value for several biological IGF-I endpoints, including height, and therefore for IGF-I bioactivity, than free IGF-I levels have.	TCJ Sas [6] YK van Pareren [3] EMN Bannink (present study)
Additional effects of GH treatment	
Bone maturation – Bone maturation during GH treatment was positively related to the degree of bone age retardation at start of study and negatively related to baseline calendar age.	A van Teunenbroek [7]

Bone mineral density (BMD) – Most girls had a normal BMD during childhood. – During GH treatment, BMD increased significantly. After end of GH treatment and the increase of estrogen dosage to adult levels, the BMD reached adult values.	TCJ Sas [6]
Growth factor levels – In agreement with other studies, our studies show a decrease in GHBP-levels. However, the GHBP-levels were not dose or administration-frequency dependent. – Free IGF-I levels remained mainly within the normal range, while total IGF-I levels rose to the upper normal range, and even exceeded it.	A van Teunenbroek [] TCJ Sas [6] EMN Bannink (present thesis)
Cardiovascular risk factors – Although long-term GH treatment with GH dosages up to 2.7 mg/m^2/day had no adverse effect on glucose metabolism, it induced hyperinsulinaemia, thereby indicating relative insulin resistance. Six months after end of GH treatment, insulin levels decreased to values close to or equal to pretreatment values. – Several years after end of GH treatment, glucose metabolism remained low in young women with TS, leading to lower insulin sensitivity (75–85%) and higher ß-cell function (150–160%). In untreated TS women aged 29.9 (16.5–46.4) years, insulin sensitivity was not significantly different from that in our GH-treated TS women, aged 20.0 (2.1). – Several years after discontinuation of GH, glucose levels were slightly higher than they were six months after discontinuation. – During GH treatment, the lipid profiles changed in a more cardioprotective direction, with a significant reduction in the ratio of total cholesterol to HDL cholesterol, i.e. the atherogenic index (AI). – Six months after end of GH treatment, lipid levels were more beneficial regarding the development of cardiovascular disease than in a normal control group, suggesting a positive effect of the GH and estrogen treatment in TS. – Several years after end of GH treatment, serum TC, LDL, and HDL were higher than they had been six months after GH-discontinuation. The AI remained constant. – The decrease in diastolic blood pressure during and six months after end of GH treatment suggests a positive effect of the GH and estrogen treatment in TS. – Several years after end of GH treatment, systolic and diastolic BP were higher than at six months after end of GH treatment. – Several years after end of GH treatment, systolic BP was higher than pre-treatment values, and diastolic BP was similar to pre-treatment values.	A van Teunenbroek [7] TCJ Sas [6] YK van Pareren [3] EMN Bannink (present thesis)

Effects on the heart – GH treatment using up to 2.7 mg/m^2/day for up to 7 years caused no adverse cardiovascular effects. The structure of the heart muscle increased proportionally with general development. LV activity, heart axis and BP showed an age-dependent development, with values well within the normal range. – GH therapy does not have an adverse effect on aortic structure. On the contrary, it may even have a beneficial effect, as the abnormalities seemed to be more prominent in patients who had received the lowest GH dose. However, there were no significant differences between GH dosage groups. – In the group with the lowest GH dose, aortic distensibility was significantly lower than in matched controls, whereas both other GH dosage groups showed no significant differences from matched controls. GH seemed to have a beneficial influence on aortic wall properties in women with TS.	A van Teunenbroek [7] TCJ Sas [6] EMN Bannink (present thesis)
Body proportions – GH therapy normalized the earlier disproportion between height and sitting height, and possibly preventing worsening of the disproportion between height, biacromial diameter and biiliacal diameter. – The increased disproportion between height and foot length was due partly to the natural development of TS, and was also influenced partly by the GH dosage used. The development of large feet can play a role in a girl's decision to discontinue GH treatment in the last phase of growth.	A van Teunenbroek [7] TCJ Sas [6] EMN Bannink (present thesis)
Psychosocial functioning – Even after normalization of height and puberty induction at a normal pubertal age, some TS women still experienced psychosocial problems. Although it is likely that GH and estrogen treatment improved psychosocial functioning, it did not normalize it for some women.	YK van Pareren [3]
Puberty – In girls with TS, the use of a low dose of 17ß-estradiol to induce puberty at an appropriate age resulted in breast development comparable to that in normal Dutch girls.	EMN Bannink (present thesis)
Quality of life – Young women with TS had a normal HRQoL after long-term GH-treatment and puberty induction at an appropriate age. – The relatively high scores on some of the HRQoL-scales in TS women can be explained by an estrogen-effect or by a possible response shift, indicating that they might have acquired a different internal reference. – In the TS woman, personal satisfaction with their height and/or breast development positively influenced several HRQoL scales, including social functioning and physical functioning. – GH and estrogen treatment may have positively influenced HRQoL in young women with TS. – GH may improve QoL through mechanisms unrelated to growth.	EMN Bannink (present thesis)

Body mass index (BMI) – Nearly 5 years after the end of long-term GH therapy at an age of 20.0 (2.1) years, the mean BMI was 26.5 (5.1) kg/m^2 in the women with TS; 38.5% had a BMI between 20 – 25 kg/m^2, 46% were obese (BMI 25–30 kg/m^2), and 15.5% were morbid obese (> 30 kg/m^2). GH did not influence the magnitude of the increase in BMI.	EMN Bannink (present thesis)
Turner syndrome related results	
TS Prediction models – In girls with TS, the Turner-specific adult height prediction methods were valuable additions to existing methods for predicting adult height.	A van Teunenbroek [7]
Cardiovascular risk factors – Together with the predisposition in TS for insulin resistance, the positive relationship between insulin and BMI should prompt clinicians to do their utmost to prevent further weight-gain in girls and women with TS.	YK van Pareren [3]
Body proportions – Relative to their height, untreated girls with TS had a relatively large trunk, relatively large hands and feet, and relatively broad shoulders and pelvis.	TCJ Sas [6] EMN Bannink (present thesis)
Puberty – Serum hormone levels do not provide additional information for evaluating the development of the puberty induced. – Uterine dimensions at the age of nearly 20 years were comparable to those in a normally matured girl at the age of 15. – It remains unclear whether subnormal uterine dimensions in young Turner women are related to the estrogen dosage administered, or to factors related to Turner's syndrome.	EMN Bannink (present thesis)
Growth factors – Untreated girls with TS had lower levels of free dissociable IGF-I, to an extent similar to the decrease in total IGF-I and IGFBP-3.	EMN Bannink (present thesis)
Aortic dimensions and distensibility – Ascending and descending aorta diameter is larger in young TS women than in controls matched for age and BMI. The ascending aortic diameter is dilated in 30–50% of the women with TS. Dilation of the aorta is not just limited to the ascending part, but is present throughout the entire thoracic aorta. – The aorta in young women with TS showed decreased aortic wall distensibility, which is a sign of impaired wall function.	EMN Bannink (present thesis)

Turner syndrome and GH treatment: "Was it – and is it – all worthwhile?"

Eighty-three percent of the GH-treated Turner girls attained normal adult height, and 63% gained a height within their target height range – overall, a height gain of between 12 and 17 cm.

The Turner study also showed that GH treatment lasting up to thirteen years produced no major adverse effects. However, while dosages during treatment of up to 2.7 mg/m^2/day produced no adverse effect on glucose metabolism, they did induce increased serum insulin levels, indicating relative insulin resistance. Nearly five years after end of GH treatment, insulin metabolism remained affected in young women with TS, leading to lower insulin sensitivity (75–85%) and a higher β-cell function (150–160%). Nonetheless, insulin sensitivity at age 20 (2.1) was not significantly different from that in untreated TS women aged 29.9 (16.5–46.4) years (81.5 (46.5)% *vs.* 103.2 (78.6)%). Similarly, the disproportion between height and foot length was increased, partly due to the natural development of TS, but also influenced by the GH dosage used.

On the basis of our studies we therefore recommend GH treatment in TS girls, as it has been proven to be effective, and to have several positive additional effects besides height improvement.

GH treatment has a positive effect on blood pressure, bone mineral density, and serum lipids. And although, nearly 5 years after end of GH treatment, the effect on blood pressure had disappeared, that on lipid levels remained. GH treatment also seemed to have had a positive effect on quality of life, aortic distensibility and structure, and the disproportion between height and sitting height. Furthermore, it did not seem to influence BMI or left ventricular mass. Additionally, we found that GH and estrogen treatment are likely to have improved psychosocial functioning. In other words, GH treatment in TS girls has several positive effects beyond that on height.

We should also stress the beneficial effects of additional appropriate-age-started estrogen substitution, such as those on quality of life, and probably on cardiovascular risk factors. Future studies should clarify whether the combination of long-term GH treatment with the age-appropriate start of estrogen treatment helps create a greater likelihood of better personal, vocational and overall life achievements, and also whether it reduces earlier cardiovascular risks in TS. If possible, these studies should also explore the decreased insulin sensitivity brought about by the long-term GH treatment or inherent to TS.

Recommendations for future research

– Small for gestational age –

SGA is a description of size at birth, not of a disease, syndrome or disorder. In this population, the heterogeneity of the underlying pathology can be broad and varied, with environmental (intrauterine), metabolic and genetic factors being related to the underlying cause [8,9]. The natural development of certain aspects may therefore differ between subgroups within the SGA-population. Future research that reveals the mechanisms underlying shortness in SGA may lead to understanding of the pathology related to the SGA syndrome. It may also provide better, more specific treatments for the health problems related to being born SGA, and improve prediction of positive and negative reactions to GH.

Among the mechanisms on which research might focus are the IGF-I gene, IGF-I receptor gene, and the IGFBP-3 gene, as they are all involved in the potency of IGF-I, which is involved in prenatal and postnatal growth. IGF-I is also involved in longevity [10], low IGF-I levels being positively associated with degenerative diseases such as cardiovascular diseases, atherosclerosis and diabetes, which are also involved in the SGA syndrome.

Another aspect that needs to be clarified is whether prematurity influences the development of the risk factors faced by children born SGA. After birth, most babies first lose weight, before they start growing in length and weight, while the intrauterine process of growing would have continued. Further research should therefore establish 1.) whether children born SGA preterm are more at risk of shortness and of developing adult diseases than those born SGA at term, 2.) whether preterm children born SGA who are AGA by the time they are at term face similar risks of shortness and of developing adult diseases as those born SGA at term; and 3.) whether preterm children born AGA who are SGA when they are at term are also at risk of shortness at adulthood and of developing adult diseases.

While long-term GH therapy has been proved to be effective, long-term follow-up studies need to confirm the beneficial effects of this treatment during childhood relative to adult diseases such as hypertension, diabetes mellitus type II, and cardiovascular disease.

This study showed that, six months after end of GH treatment, GH-induced relative insulin resistance had returned to levels similar to those in peers. These results should now be confirmed several years after end of GH treatment. Similarly, follow-up studies should investigate the long-term consequences of GH-induced decreased insulin sensitivity during childhood.

GH increased IGF-I levels as high as +2 SDS; in adulthood, high IGF-I levels are associated with cancer risk [11-14]. The studies in question were conducted in subjects who were at an age at which the preexisting risk for cancer is much higher than it is during childhood. It remains to be seen whether high IGF-I levels during childhood also cause a higher cancer risk in adulthood. Until causality can be ruled out, this group of GH-treated SGA adults should be monitored in follow-up settings.

GH seems to have a positive influence on QoL measured at adolescence. These data should now be confirmed in a longitudinal trial. Longer-term follow-up would show whether or not GH-treated SGA patients' life achievements (i.e. personal and vocational achievements) have improved more than those of untreated short SGA patients.

– Turner syndrome –

Long-term GH treatment has been proved to be effective, and to have several favorable aspects in TS. In addition, age-appropriate low-dose estrogen substitution has proved effective in inducing puberty without sacrificing adult height. Furthermore, estrogens have additional beneficial effects beyond starting feminization. Investigation should nonetheless take place with regard to uncertainties about the combination of long-term GH treatment and low-dose estrogen treatment.

Nearly five years after end of GH treatment, glucose metabolism in young women with TS remains low, leading to lower insulin sensitivity (75–85%) and higher β-cell function (150–160%). Salgin *et al*, as discussed earlier, recently presented data on insulin and glucose levels in untreated TS women aged 29.9 (16.5–46.6) years, showing an insulin sensitivity of 103 (78.6)%, which is not significantly different from the 81.6 (46.5)% in our GH-treated TS women aged 20.0 (2.1) years [15].

Despite a significant difference between insulin sensitivity of the GH treated and the untreated TS women, and despite the lack of an association of insulin sensitivity or ß-cell function with GH dosage or GH-duration, it cannot be ruled out that GH therapy itself had an effect on glucose metabolism. Although our group previously suggested that the higher post-GH levels might be the result of higher BMI, it was suggested very recently that impaired peripheral and hepatic insulin sensitivity in TS may be caused by an intrinsic defect that is independent of fat mass but related to the karyotype in TS [15]. It has also been suggested that a beta-cell dysfunction causes a higher prevalence of diabetes mellitus in TS women [16]. More definitive answers may be provided by studies on the etiology of glucose metabolism in women with TS; these may show whether this lower insulin sensitivity is related to GH, to TS syndrome (including increased BMI), or to a combination, and whether the disturbances in glucose metabolism are karyotype related. Answers may also be provided by follow-

up of other long-term GH-treated populations, such as children who were born small for gestational age.

Nearly five years after end of GH treatment, positive effects on lipid levels were still measurable, whereas positive effects on blood pressure had disappeared. TS women are predisposed to hypertension, though this is of unknown etiology. Whether GH and estrogen treatment provided protection for cardiovascular diseases in TS women on the longer term, remains to be investigated. Future research in TS should therefore include the etiology of hypertension, the need for treating hypertension, and the proneness to cardiovascular diseases in TS.

Women with TS are at a relatively high risk of aortic dilatation and dissection, though no cause for this has as yet been established. A similarly high risk of aortic dilatation and dissection is found in Marfan syndrome, a syndrome involving tall stature. Nearly five years after end of GH treatment, we found dilatation of the ascending aorta in 30–50% of women with TS; rather than being restricted to the ascending part, this was present throughout the entire thoracic aorta. In young women with TS, we also found relatively low distensibilities of the aortic wall, a sign of impaired wall function.

While it has been suggested that the evaluation and treatment of TS should be similar to that used in people with Marfan syndrome, great care should be taken in extrapolating any knowledge of Marfan syndrome to TS, of which etiology may be of an entirely different order. In Marfan syndrome, aortic dilatation increases with aging; in TS it is not yet known whether there is any progression of the aortic dimensions and distensibility. Follow-up of groups of TS women, preferably larger groups, should therefore be performed for monitoring aortic abnormalities, including aortic distensibility, as this may have consequences for treatment of e.g. hypertension. It should also be investigated whether beta-blocker therapy benefits TS women, also those without obvious signs of hypertension.

The finding that GH therapy had no adverse effect on aortic structure or distensibility – even seeming to have protective effects on aortic wall properties – should be examined in future studies which expressly include a larger GH-treated study group with longitudinal measurements, or MRI measurements at a higher age.

During GH treatment, IGF-I levels increase as high as +2 SDS. High IGF-I levels at an adult age are associated with cancer risk [11-14]. All these studies were performed at an age when the preexisting risk for cancer is much higher than during childhood. As stated above under the recommendations for future research in SGA, it remains to be investigated whether high IGF-I levels during childhood also increase cancer risk in adulthood. Until causality can be ruled out, this group of GH-treated TS women should be monitored in follow-up settings. With regard to our previous discussion on the possibility of an increased shift of free IGF-I to the tissue compartments (see

chapter 3), it would be interesting to determine IGF-I concentrations activating IGF-I-receptor. As the recently developed sensitive IGF-I kinase receptor activation assay (KIRA) [17] is able to do this, it might be helpful in clarifying the bioactive capabilities of circulating IGF-I levels.

During GH treatment, estrogen treatment was started at a low dose, without sacrificing adult height [18,19]. We showed that this dose was sufficient for a feminization process comparable to that of normal peers, albeit with a two-year delay. Although a recently published study reports that GH with an even lower dose of estrogens enhanced height velocity while preserving height potential [19], the dosage in question failed to bring about bodily feminization in one third of the subjects. This nonetheless implies that it may be possible to start estrogen therapy at an even lower age without sacrificing adult height, and to start bodily feminization at an appropriate age. Future research is thus needed to establish the dosage and initiatial age of which estrogen therapy might most benefit adult height and bodily feminization. As well as establishing other potentially positive influences of estrogens, such research might determine whether 1.) transdermal patches and/or percutaneous gel are the best oestrogen treatment for inducing puberty in TS girls at an age similar to that of girls with spontaneous puberty without negative impact on adult height, and 2.) whether these methods are preferable to oral estrogens.

When QoL is measured at young adulthood, it seems to have been positively influenced by GH. These data should also be confirmed in a longitudinal trial. Follow-up will show whether or not personal, vocational and overall life achievements in GH-treated Turner women have improved.

As a majority of adults with TS are infertile, the consequences of infertility on the HRQoL may become a more prominent issue in the future of the Turner women we studied. Infertility may significantly influence the HRQoL [20-23]; to determine its influence on the HRQoL of women with TS, we recommend that further research be conducted.

The progression booked in possibilities for oocyte donation and cryopreservation are promising. Human ovarian tissue containing immature primordial follicles has been successfully cryopreserved. A possible future prospect is cryopreservation of ovarian tissue containing immature follicles before the onset of early menopause, but methods of replantation and in-vitro maturation still need to be developed [24,25].

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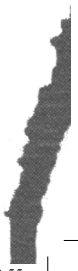
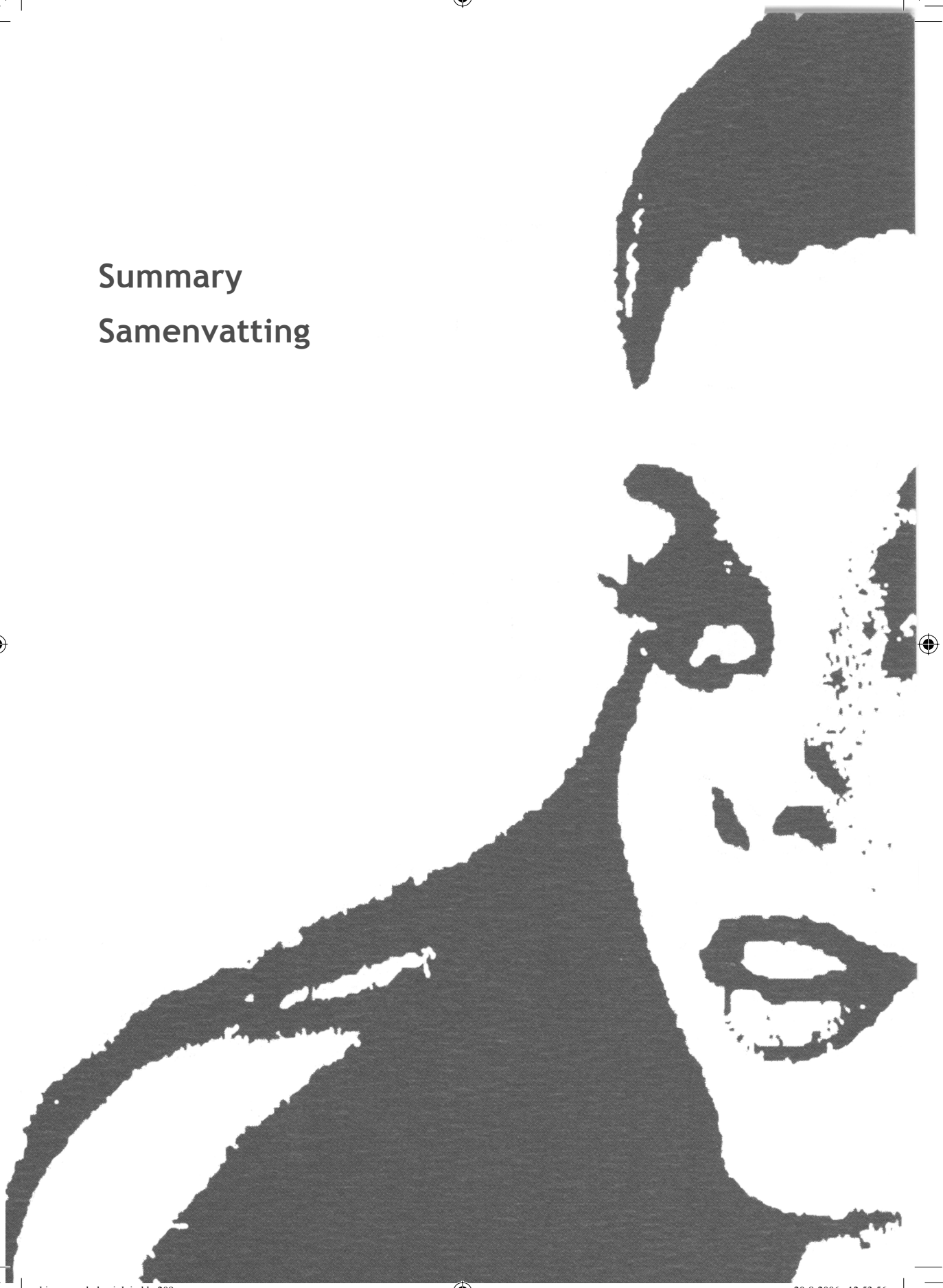
Chapter 11



Summary

Samenvatting



Summary

By definition, 2.3% of the children is born Small for Gestational Age (SGA), which is a length and/or height < −2 standard deviation (SD). Most children born SGA show catch-up growth during the first years of life. Approximately 10% of them remain short with a height below the normal range. Previously, this study showed that long-term growth hormone (GH) treatment in short SGA children resulted in an adult height within the normal range in 85%, and in 98% a height within the target height range (i.e. the midparental height). The mean gain in height is 11−13 cm in girls and 12−14 cm in boys.

In Turner syndrome (TS), one of the main characteristics is short stature. The mean adult height of women with TS is approximately 20 cm below normal, which is about 148 cm in the Netherlands. GH treatment in girls with TS resulted in an adult height within the normal range in 83%, and in 63% a height within the target height range. The mean height gain is between 12 and 17 cm. Another major characteristic of TS is gonadal dysgenesis, leading to ovarian failure in 80−100% of the Turner patients. Most girls do not enter puberty spontaneously due to a lack of estrogen production. Therefore, puberty is induced by administration of exogenous estrogens. Route, timing, dose, and form of estrogen therapy are still issues of debate.

These studies investigated different aspects of long-term GH treatment in children born SGA (n = 72) and girls with TS (n = 65). The effects of long-term GH treatment on the IGF-system, especially the free fraction of IGF-I (*chapter 2 and 3*), and the quality of life after ending GH treatment (*chapter 4 and 5*) are discussed. In TS girls we described the pubertal development and uterine dimensions, after puberty has been induced starting with a low estrogen dose during GH treatment, at an appropriate age (*Chapter 6*). Several years after the end of GH treatment, we measured serum lipids, insulin and glucose metabolism, body mass index (BMI) and body proportions in 39 TS girls (*chapter 7*). At the same time, we also investigated aortic dimensions and aortic distensibilities, representing aorta wall function (*chapter 8*).

Chapter 1 provides a general overview of the literature regarding SGA and TS. In this chapter definitions, etiology, prevalence and clinical characteristics of these two patient populations are described. In addition, it gives a summary of the previously reported results of the GH treatment regarding the growth effects, safety aspects, and other possible GH effects. In the girls with TS, the puberty induction is discussed. At the end of this chapter, a time-frame is given of these two long-term GH studies (SGA study started in 1991 and the TS study started in 1989), and an overview of the different doctoral dissertations resulting from these studies. Finally, the objectives of the different studies presented in this dissertation are described.

Serum IGF-I levels

Insulin-like-growth-factor-I (IGF-I) plays an important role in both pre- and postnatal growth and development and its serum levels are regulated by both metabolic and genetic factors; GH is one of the main regulators of IGF-I. In the circulation, IGF-I is bound mainly to IGF-binding proteins (IGFBP), of which six classes have been identified (IGFBP- 1 to – 6). The major carrier protein of circulating IGF-I is IGFBP-3, which normally accounts for more than 90% of the IGF-I binding. Under normal circumstances, less than 1% of the total plasma IGF-I pool is present in the unbound free form, which is considered to be the biologically active form.

During GH treatment, total IGF-I levels rise to high-normal and supraphysiological levels. IGFBP-3 also increases during GH therapy, however, less markedly. No reports are available on free IGF-I levels during GH treatment are available, nor on the contribution of free IGF-I in predicting growth response.

To interpret the measured free IGF-I levels, we established normative range values for circulating free IGF-I in collected serum samples of healthy children aged 0–17 years (116 girls and 211 boys).

Chapter 2 describes the effects of long-term GH treatment on free IGF-I, total IGF-I, IGFBP-3 levels and the molar ratio of total IGF-I/IGFBP-3 in the GH treated SGA population. Furthermore, we evaluated whether measuring free IGF-I levels adds value in evaluating the GH treatment, by means of its (relative) contribution in predicting of first year growth response and/or adult height. Free IGF-I, total IGF-I, IGFBP-3 and the molar ratio of total IGF-I/IGFBP-3 were determined at baseline, after 1 and 5 years of GH treatment, at the end of GH treatment, and 6 months after the end of GH treatment.

Untreated SGA children showed a normal mean free IGF-I SDS (–0.2 (1.2) SDS), not related to total IGF-I. During GH therapy, free IGF-I increased significantly, but remained within the normal range (1.6 (0.7) SDS). In contrast, untreated SGA children showed reduced levels of total IGF-I and IGFBP-3 (–1 SDS). During GH therapy, total IGF-I rised in a larger extent than free IGF-I did (up to 2.0 (0.8) SDS). Under normal circumstances, total and free IGF-I are closely related. However, in small SGA children there was no correlation between total IGF-I and free IGF-I. In chapter 2 this discrepancy is discussed. Multiple regression analysis showed a negative correlation between baseline free IGF-I SDS and one-yr growth response and adult height, also after adjusting for baseline total IGF-I SDS. Total IGF-I, IGFBP-3, or their ratio showed no correlations with growth response. These data showed that free IGF-I was a better predictor for short-term growth response as well as for adult height in GH treated SGA children than were total IGF-I, IGFBP-3, or their ratio. This suggests a possible role for free IGF-I measurement in predicting the effect of GH therapy in short SGA children.

Chapter 3 describes the effects of the long-term GH treatment on free IGF-I, total IGF-I, IGFBP-1, 2, 3 levels and the molar ratio of total IGF-I/IGFBP-3 in GH treated girls with TS. Furthermore, we verified relationships between free IGF-I levels and total IGF-I, IGFBP-1, 2 and 3. Additionally, we examined whether free IGF-I, total IGF-I, IGFBP-3 or the total IGF-I/IGFBP-3 molar ratio were related to the IGF-I bioactivity outcome parameters, such as adult height, height gain, glucose- and insulin levels, and aorta wall distensibility. Free IGF-I, total IGF-I, IGFBP-3, −2, and −1, and the molar ratio of total IGF-I/IGFBP-3 were determined at baseline, after 1, 2, 3 and 5 years of GH treatment, before the start of estrogen therapy, during the final year of GH treatment, 6 months after and 4.8 (2.0) years after end of GH treatment.

Untreated girls with TS showed free IGF-I levels which were comparable to total IGF-I levels (approximately −1 SDS). During GH treatment, mean free IGF-I levels remained < +2 SDS, whereas mean total IGF-I and total IGF-I/IGFBP-3 ratio were > +2 SDS. There were no differences in free IGF-I levels between the three GH groups, whereas total IGF-I and ratio levels were significantly higher in the highest GH group. Total IGF-I levels or the ratio total IGF-I/IGFB-3 levels did not accurately represent free IGF-I levels. Total IGF-I and the ratio predicted the IGF-I bioactivity *better* then the free fraction of IGF-I did. In chapter 3 we discussed that these results may be explained by an increased shift of free IGF-I from the circulation to the tissues compartments. This theory needs to be explored further in vivo and in vitro studies.

Quality of life

Improving or maintaining quality of life (QoL) is one of the main goals of a medical intervention, such as GH treatment. QoL can be measured using standardized and validated questionnaires, encompassing several domains, including physical, emotional en social well-being. Two different kinds of QoL questionnaires can be distinguished: a generic and a disease- or disorder specific questionnaire. A generic questionnaire allows for screening in a healthy population, and enables standardized comparisons across health conditions. However, it may not be as responsive to changes in disease- or disorder specific symptoms. The disease/disorder specific questionnaire may be more sensitive to specific changes in patients with a particular disease or disorder. Unfortunately, specific questionnaires are unable to provide comparisons across populations, or comparison with a healthy population.

Chapter 4 describes the QoL in the SGA children with a mean age of nearly 16 years, after almost 10 years of GH treatment. We used a generic questionnaire, the 'Child Health Questionnaire' (CHQ), and a specific questionnaire, specially developed for a population with short stature, the 'Short stature QoL questionnaire' or TACQOL-S. Two groups of adolescents born SGA without spontaneous catch-up growth were

compared: a GH-treated group (n=44) and an untreated group (n=28). At start of the GH treatment, the GH-group had a mean height of −3.1 SDS, whereas the mean height of the untreated group was −2.3 SDS. At time of the QoL evaluation, mean height of the GH group was -0.6 SDS, and of the untreated group −1.8 SDS.

The GH group reported a better QoL than the untreated group on the domains 'physical functioning' and 'contact with adults', measured with the disorder specific questionnaire. Additionally, also fewer problems with body image were reported in the GH group. The 'effect sizes' of the differences in QoL outcomes of the GH group versus the untreated group were large, but did not reach statistical significance. 'Effect size' contributes to an understanding of the differences between groups. 'Effect size' is a tool for measuring the (clinical) effect of the difference; a statistical significant difference might not be synonymous with what is of clinical importance. We ended up with the advise to use, in addition to a generic questionnaire, a height-specific questionnaire for measuring the influence of GH treatment on QoL.

Chapter 5 describes the QoL in 49 young women with TS girls after long-term GH therapy and induced puberty at an appropriate age. They were 19½ years of age, and had a height gain of 1.7 SDS, resulting in an adult height of −1.2 SDS compared to the normal Dutch population. Additionally, we analyzed whether QoL was influenced by auxological parameters, pubertal development, or subjective parameters. Puberty onset was started before the age of 13 using low dose of oral estrogens. Two different adult generic questionnaires were used: the SF36 and the TAAQOL. An additional questionnaire provided information on socio-economic status, living situation, and subjective parameters, such as their experience with having typical Turner features, the GH treatment and the puberty induction. For example, the following questions were asked: "Do you think that other people can see that you have Turner syndrome?" If answered 'yes', "does that bother you?", "Are you satisfied with your adult height/ breast development?".

Young women with TS who reached normal height and had age-appropriate pubertal development showed normal QoL. Remarkably, the TS women had higher QoL scores on some of the QoL domains, which might be explained by an estrogen effect or by a possible response shift, indicating a different internal reference in women with TS. Satisfaction with height and breast development had a positive influence on several QoL scales, such as social and physical functioning. We hypothesized that GH and estrogen treatment positively influenced QoL in young women with TS.

Puberty development

Chapter 6 describes the pubertal development in 56 girls with TS, using a low dose of 17β-estradiol orally, starting during GH treatment at an appropriate age of 12 years. The pubertal development is described and compared to that of normal Dutch peers. Previously, our study demonstrated that estrogens in a low dose did not negatively influence height velocity or adult height. Additionally, it was determined whether serum hormone levels, such as estradiol, estone, LH, FSH, SHBG, contribute to the evaluation of pubertal progression. In 39 women with TS, the uterus dimensions were measured by ultrasound techniques, at an age of nearly 20 years, using an adult oral estrogen dose.

The breast development and pubic hair development were comparable to that of normal Dutch girls, however the breast development was 2 years delayed. In a clinical setting, serum hormone levels provided no additional information for evaluating pubertal progression. After age-appropriate pubertal induction, uterine dimensions in women aged nearly 20 were subnormal, and comparable to those of normal girls aged 15 years with breast stage B4/5. It remains unclear whether this was related to E2-dosage or duration, or factors related to TS.

Safety aspects several years after the end of long-term GH treatment in TS

Chapter 7 describes several safety aspects nearly 5 years after the end of the GH treatment in 39 TS women, aged 20 years. The mean GH duration was nearly 9 years. The described measurements are: 'Body mass index' (BMI), fasting glucose and insulin levels, serum lipids, blood pressure, and body proportions. These were compared with levels before and during GH treatment, correlations were made, and possible GH effects were examined. Additionally comparisons were made with normal women, and, if possible, with untreated TS women.

Nearly 5 years after the end of the GH treatment, insulin sensitivity remained lower; β-cell function and fasting insulin levels remained higher than pre-treatment. The β-cell function was positively influenced only by BMI. The diastolic blood pressure was inversely related to GH dose, indicating a higher GH dose was related to a lower diastolic blood pressure. The GH dose or duration had no influence on the systolic blood pressure. Serum total cholesterol (TC), LDL and HDL had further increased compared to 6 months after GH, resulting in higher TC, but also higher HDL levels compared to controls. AI (TC/HDL) remained constant, which was lower than the AI in controls. The BMI increased gradually during and after GH-therapy. During GH treatment, the shape value sitting height decreased, and therefore improved, the shape value foot length increased, and therefore worsened, and shape values of hand length, biacromial- and biiliacal diameter remained constant.

We conclude that, as well as height gain, GH treatment brings women with TS benefits regarding blood pressure, serum lipids, and some body proportions. Nearly 5 years after GH the favorable effects of GH on serum lipids was still noticeable, but waning. The effect on diastolic blood pressure had disappeared, as it had returned to pre-treatment levels. The lower GH-induced insulin sensitivity, however, remained unchanged, possibly due to having TS.

Chapter 8 describes aorta dimensions and biophysical properties of the aorta by measuring the aortic size and wall distensibility in 38 TS women at an age of nearly 20 years, after long-term GH treatment. The results were compared to the aortic measurements of 27 age, sex and BMI matched controls. All underwent 'Magnetic Resonance Imaging', MRI, to determine aortic dimensions and distensibility at four predefined levels: 1) in the ascending aorta at the level of the pulmonary artery bifurcation, 2) in the descending aorta at the level of the pulmonary artery bifurcation, 3) at the levels of the diaphragm, 4) the abdominal aorta at the levels of the superior mesenteric artery.

The TS women had dilated aortas, with a larger aortic diameter at all but level 4, the abdominal aorta, compared to the matched controls. In the TS women there were signs of impaired aortic wall distensibility. The severity of abnormalities appeared to be related to the GH dose, with a beneficial effect of a larger GH dose on the abnormalities.

Chapter 9 discusses the results of the various studies in the context of the literature and the clinical implications.

Chapter 10 provides an overview of all the results of these two long-term GH trials, starting in 1991 (SGA study) and in 1989 (Turner study). Additionally, the question arises: "Was it – and is it – all worthwhile?", and will be discussed. The chapter ends with the strengths and the limitations of the studies and suggestions for future research.

Samenvatting

Per definitie wordt 2,3% van de kinderen te klein geboren (onder de –2 standaard deviatie). De meeste kinderen die te klein waren bij de geboorte voor de zwangerschapsduur (small for gestational age, SGA), halen deze groeiachterstand in gedurende de eerste jaren na de geboorte. Ongeveer 10% behoudt echter een kleine lengte. Eerdere onderzoeksresultaten uit deze langlopende studie hebben aangetoond dat groeihormoon (GH) behandeling bij SGA kinderen met en persisterend kleine lengte in een meerderheid leidt tot normalisatie van de lengte tijdens de kinderjaren en een verbeterde volwassen lengte. Uiteindelijke zal 85% een normale volwassen eindlengte bereiken en 98% een volwassen lengte binnen de 'target height range', dat willen zeggen binnen de gemiddelde ouderlengte. Meisjes hebben een lengtewinst van 11-13 cm en jongens van 12-14 cm.

Bij meisjes met het syndroom van Turner is kleine lengte één van de opvallendste kenmerken. Zonder GH behandeling behalen zij een volwassen lengte die ongeveer 20 cm onder het gemiddelde ligt, in Nederland is dat ongeveer 148 cm. Met GH behandeling wordt bij een meerderheid van de meisjes met Turner syndroom (TS) een normale lengte tijdens de kinderjaren en een verbeterde volwassen lengte behaald. Uiteindelijk bereikt 83% van de TS meisjes een normale volwassen lengte en 63% een volwassen lengte binnen de 'target height range'. De totale lengtewinst ligt gemiddeld tussen de 12 en 17 cm. Een ander opvallend kenmerk bij de meeste meisjes met TS is de afwezigheid van spontane puberteit als gevolg van de afwezigheid van functionerende ovaria, welke de oestrogenen produceren. De puberteit wordt bij de meeste TS meisjes geïnduceerd met behulp van exogene eostrogeentherapie. De route, tijdstip van starten, dosis, en de vorm zijn nog immer een punt van discussie.

De studies beschreven in dit proefschrift onderzochten verschillende aspecten van langdurig GH behandeling bij SGA kinderen met een persisterende kleine lengte (n = 72) en meisjes met TS (n = 65). De invloed van de langdurige GH behandeling op het IGF-systeem, met name de vrije fractie van het IGF-I (*hoofdstuk 2 en 3*), en de kwaliteit van leven na de GH behandeling (*hoofdstuk 4 en 5*) worden besproken en bediscussieerd. Bij de meisjes met TS wordt de puberteit beschreven, die geïnduceerd werd met een lage dosis oestrogenen, waarmee werd gestart tijdens de GH behandeling op een adequate leeftijd. Daarnaast worden uterusdimensies onderzocht (*hoofdstuk 6*). Een aantal jaren na het beëindigen van de GH behandeling werden bij 39 TS meisjes de lipiden-, insuline- en glucosehuishouding, body mass index (BMI) en lichaamsverhoudingen onderzocht (*hoofdstuk 7*). Ook werd een aantal jaren na het beëindigen van de GH behandeling gekeken naar de aorta, de aorta breedte en de elasticiteit van de aortawand in deze patiënten groep (*hoofdstuk 8*).

Hoofdstuk 1 geeft een overzicht van de literatuur betreffende SGA en TS. Hierin worden definities, etiologie, prevalenties en klinische kenmerken beschreven van deze twee patiënten populaties. Ook worden de resultaten van de GH behandeling, de veiligheidsaspecten, en andere mogelijke GH effecten beschreven. Bij de meisjes met het syndroom van Turner wordt de puberteitsinductie besproken. Tenslotte wordt er een overzicht gegeven van de eerdere onderzoeksresultaten van deze twee langlopende studies (SGA studie gestart in 1991 en de TS studie gestart in 1989), de daaruit resulterende proefschriften, en de doelstellingen van de verschillende studies die gepresenteerd worden in dit proefschrift.

Serum IGF-I waarden

De insuline-achtige groei-factor-I (insulin-like growth factor, IGF-I) speelt een belangerijke rol bij groei en ontwikkeling, zowel voor als na de geboorte. De waarden van IGF-I in het bloed worden gereguleerd door metabole en genetische factoren, waarbij groei hormoon (GH) een van de belangrijkste regulatoren is. In de circulatie is IGF-I voornamelijk gebonden aan eiwitten, de zogenaamde IGF-binding proteïnen (BP), waarvan 6 klassen zijn geïdentificeerd (IGFBP1 t/m 6). IGFBP-3 is het belangrijkste bindingseiwit en bindt ongeveer 90% van het circulerende IGF-I. Onder normale omstandigheden is minder dan 1% van het totaal IGF-I ongebonden in de circulatie, het zgn vrij IGF-I. Het vrij IGF-I wordt verondersteld de biologisch actieve vorm te zijn van IGF-I.

Tijdens GH behandeling stijgen de totale IGF-I waarden tot hoog-normale en suprafysiologische waarden. Ook de waarden van IGFBP-3 stijgen onder invloed van GH. Het is nog niet eerder onderzocht wat de invloed is van GH behandeling op de vrije fractie van IGF-I, en of het vrij IGF-I een voorspellende waarde zou kunnen hebben bij het evalueren van de GH behandeling.

Om de gemeten vrij IGF-I waarden te kunnen interpreteren, hebben wij referentie waarden gemaakt in de leeftijdsrange van 0-17 jaar mbv serum samples van gezonde kinderen (116 meisjes en 211 jongens).

Hoofdstuk 2 beschrijft de effecten van langdurige GH behandeling op vrij IGF-I, totaal IGF-I, IGFBP-3 en de molaire ratio van totaal IGF-I/ IGFBP-3 in de GH behandelde SGA populatie. Daarbij wordt ook onderzocht of het meten van vrij IGF-I toegevoegde waarde zou kunnen hebben in het evalueren van de GH behandeling. Dit is uitgevoerd door het vrij IGF-I, totaal IGF-I, IGFBP-3 en de molaire ratio van totaal IGF-I/ IGFBP-3 te meten op de volgende tijdstippen: voor start van de GH behandeling (baseline), na 1 en 5 jaar GH behandeling, bij het beëindigen van de GH behandeling, en een half jaar na het beëindigen van de GH behandeling.

Kleine SGA kinderen zonder GH behandeling hebben normale vrij IGF-I spiegels. Tijdens GH behandeling stijgen de vrij IGF-I waarden, maar blijven binnen de normale range. Totaal IGF-I en IGFBP-3 zijn verlaagd in onbehandelde kleine SGA kinderen. Tijdens GH behandeling stijgt het totaal IGF-I méér dan het vrije IGF-I. Onder normale omstandigheden is er een sterke relatie tussen totaal IGF-I en vrij IGF-I, echter bij kleine SGA kinderen was er geen relatie aantoonbaar. In hoofdstuk 2 wordt over deze gevonden discepantie gediscussieerd. Een belangerijke bevinding van onze resultaten is dat baseline vrij IGF-I een betere voorspeller was voor de 5 jaars groei respons op GH behandeling en de volwassen lengte, dan totaal IGF-I, IGFBP-3 of de totaal IGF-I/ IGFBP-3 ratio Dit suggereert een mogelijke relevante bijdrage voor de vrij IGF-I meting bij het voorspellen van het effect van de GH behandeling bij kleine SGA kinderen.

Hoofdstuk 3 beschrijft de effecten van de langdurige GH behandeling op vrij IGF-I, totaal IGF-I, IGFBP-1, 2, 3 en de molaire ratio van totaal IGF-I/IGFBP-3 bij de GH behandelde meisjes met TS. Daarbij worden ook de relaties onderzocht tussen de IGF-I bioactiviteit en vrij IGF-I, totaal IGF-I, IGFBP-3 of de totaal IGF-I/IGFBP-3 ratio, en welke van deze metingen de IGF-I bioactiviteit het beste voorspelt. Dit wordt gemeten aan de hand van aantal parameters zoals volwassen lengte, lengte winst, glucose- en insuline waarden, en aortawand elasticiteit. Dit is uitgevoerd door het vrij IGF-I, totaal IGF-I, IGFBP-1, 2, 3 en de molaire ratio van totaal IGF-I/IGFBP-3 te meten op de volgende tijdstippen: voor start van de GH behandeling (baseline), na 1, 2, 3 en 5 jaar GH behandeling, voor het starten van de oestrogeen behandeling, bij het beëindigen van de GH behandeling, en een half jaar na het beëindigen van de GH behandeling. Vervolgens zijn de relaties gelegd dmv correlaties en multipele regressie analyses.

De meisjes met TS zonder GH behandeling hebben vrij IGF-I spiegels die vergelijkbaar zijn met totaal IGF-I spiegels (rond de −1 SDS). Tijdens GH behandeling stijgen de vrij IGF-I waarden, maar blijven gemiddeld binnen de normale range, terwijl de waarden van totaal IGF-I en van de totaal IGF-I/IGFBP-3 ratio boven de normale range van +2 SDS stijgt. De waarden van totaal IGF-I of de ratio hebben geen goede correlatie met de waarden van vrij IGF-I. Totaal IGF-I en de ratio voorspellen de IGF-I bioactiviteit *beter* dan de vrije fractie van IGF-I. In hoofdstuk 3 wordt bediscusseerd dat deze bevindingen mogelijk verklaard kunnen worden door een verhoogde shift van het vrije IGF-I vanuit de circulatie naar de weefsels. Deze theorie zal in de toekomst verder onderzocht moeten worden.

Kwaliteit van leven

Het verbeteren of handhaven van kwaliteit van leven (KvL) is een van de hoofddoelen van een medische interventie, inclusief GH behandeling. KvL wordt gemeten aan de hand van gestandaardiseerde en gevalideerde vragenlijsten die verschillende domeinen omvatten zoals fysiek, emotioneel en sociaal welbevinden. Er worden 2 typen KvL vragenlijsten onderscheiden: een generieke en een ziekte/aandoening specifieke vragenlijst. De generieke vragenlijst maakt het mogelijk om te screenen in een normale gezonde populatie, waardoor verschillende patiëntenpopulaties vergeleken kunnen worden met de normale populatie. Deze vragenlijst is echter minder gevoelig voor ziekte/ aandoening specifieke symptomen. De ziekte/ aandoening specifieke vragenlijst is meer gevoelig voor specifieke vernaderingen bij patiënten met een bepaalde ziekte of aandoening. Helaas kunnen deze KvL uitkomsten niet vergeleken worden met de gezonde populatie.

Hoofdstuk 4 beschrijft de KvL van de SGA populatie met een gemiddelde leeftijd van bijna 16 jaar, na bijna 10 jaar GH behandeling. Hierbij is een generieke vragenlijst, de 'Child Health Questionnaire', gebruikt en een vragenlijst specifiek ontwikkeld voor een populatie met kleine lengte, de zgn 'kleine lengte KvL vragenlijst'. Er worden 2 groepen kinderen SGA geboren met elkaar vergeleken: een groep SGA kinderen met (n = 44) en zonder (n = 28) GH behandeling. De GH behandelde SGA kinderen hadden bij het starten van de GH behandeling een gemiddelde lengte van −3.1 SDS, terwijl de onbehandele SGA geboren kinderen een lengte hadden van −2.3 SDS. Ten tijde van het meten van de KvL had de GH behandelde groep een lengte van −0.6 SDS, terwijl de onbehandelde groep een lengte had van −1.8 SDS.

Onze resultaten laten zien dat de GH behandelde groep een betere KvL had op de domeinen 'fysieke capaciteiten' en 'contact met volwassenen', gemeten met de aandoening specifieke vragenlijst. Ook werden er minder problemen gerapporteerd met betrekking tot lichaamsbeeld. Ondanks het ontbreken van significantie, waren de 'effect sizes' van de verschillen in KvL uitkomsten van de GH groep versus de onbehandelde groep groot. 'Effect size' is een maat voor het meten van het (klinisch) effect van het verschil, bv een heel klein verschil kan significant zijn, maar klinisch niet van belang, zoals bv een bloeddrukverschil van 2 mmHg, en vice versa. Als laatste pleiten wij in dit hoofdstuk ervoor om naast een generieke KvL vragenlijst, een lengte-specifieke KvL vragenlijst af te nemen voor het meten van het effect van GH op de KvL.

Hoofdstuk 5 beschrijft de KvL van 49 GH behandelde TS meisjes na het behalen van hun volwassen lengte. Zij hadden een gemiddelde leeftijd van 19½ jaar, een volwassen lengte van −1.2 SDS vergeleken met de normale bevolking, en een lengtewinst van

1.7 SDS. De gemiddelde leeftijd waarop de puberteit begon was onder de 13 jaar. Hierbij zijn 2 verschillende volwassen generieke vragenlijsten gebruikt: de SF36 en de TAAQOL. Daarnaast is er een vragenlijst toegevoegd die aanvullende informatie geeft over oa socio-economische status, woonsituatie, en vragen betreffende subjectieve parameters, zoals uiterlijke kenmerken, de GH behandeling en de puberteitsinductie. Er werden bv vragen gesteld, zoals "Kun je aan jouw uiterlijk zien dat je het syndroom van Turner hebt? Zo ja, waaraan en vindt je dat vervelend?", "Ben je tevreden met je lengte/ borsten".

Onze resultaten laten zien dat jonge vrouwen met TS een normale KvL hebben na langdurig te zijn behandeld met GH en een op adequate leeftijd geïnduceerde puberteit. Op een aantal domeinen scoren de TS vrouwen een opvallend hoge KvL, soms zelfs beter dan gezonde leeftijdsgenoten. Deze opvallend goede KvL uitkomsten kunnen mogelijk verklaard worden door een oestrogeen effect of door een mogelijke 'response-shift', dwz een veranderde interne referentie. De TS vrouwen die tevreden waren met hun behaalde lengte en de borstontwikkeling hadden een betere KvL op bepaalde domeinen dan de TS vrouwen die dat niet waren, dit was oa op sociaal en fysiek functioneren. Wij concluderen dat GH en oestrogeen behandeling mogelijk een positieve invloed hebben op de KvL in jonge vrouwen met TS.

Puberteitsontwikkeling

Hoofdstuk 6 beschrijft de puberteitsontwikkeling in 56 meisjes met TS, waarbij met een lage dosis oestrogenen begonnen is tijdens de GH behandeling op een leeftijd van 12 jaar, welke vervolgens langzaam werd opgehoogd. De puberteitsontwikkeling werd vergeleken met de puberteitsontwikkeling van nomale Nederlandse leeftijdsgenoten. In een eerdere studie is al aangetoond dat de dosis oestrogenen gebruikt in deze studie, geen negatieve invloed had op de groeisnelheid en de volwassen lengte. Daarnaast werden verschillende hormoonspiegels, oa oestradiol, oestron, LH, FSH, SHBG, geëvalueerd in het kader van de puberteitsprogressie. In 39 vrouwen met TS werden de uterus dimensies gemeten op een leeftijd van bijna 20 jaar, bij een volwassen dosering oestrogenen.

De borstontwikkeling en schaamhaarontwikkeling waren vergelijkbaar met normale Nederlandse meisjes, echter de verschillende borstontwikkelingstadia werden 2 jaar later bereikt. Gemeten hormoonspiegels in het bloed habben geen aanvullende klinische waarde bij de evaluatie van de puberteitsprogressie. Na een leeftijds adequate inductie van de puberteit, waren de uterus dimensies bij TS vrouwen op de leeftijd van bijna 20 jaar subnormaal, en vergelijkbaar met die van normale gerijpte meisjes op een leeftijd van 15 jaar. Het is onduidelijk of dit gerelateerd is aan de oestrageen dosis of –duur, of aan factoren die gerelateerd zijn aan het hebben van TS.

Veiligheidsaspecten enkele jaren na het beëindigen van de GH behandeling in TS

Hoofdstuk 7 beschrijft lange termijnseffecten na het beëindigen van de GH behandeling. Dit werd gedaan dmv verschillende metingen in 39 TS vrouwen, bijna 5 jaar na het beëindigen van de GH behandeling. De TS vrouwen waren bijna 20 jaar. De gemiddelde GH behandelduur was bijna 9 jaar. De metingen die verricht waren zijn: 'body mass index' (BMI), nuchtere glucose en insuline, serum lipiden, bloeddruk, en lichaamsverhoudingen. Vervolgens werden deze vergeleken met waarden van vóór en tijdens de GH behandeling, werden er verbanden gelegd, en werd onderzocht of GH een effect heeft gehad op de betreffende metingen. Ook werden de uitkomsten van de metingen vergeleken met normaal en, indien mogelijk, met onbehandelde TS vrouwen.

Bijna 5 jaar na het beëindigen van de GH behandeling bleef de insulinegevoeligheid in TS vrouwen lager dan vóór GH behandeling, de β-cel functie en nuchtere insuline waarden bleven hoger dan vóór GH behandeling. De β-cel functie werd alleen beïnvloedt door de BMI. De diastolische bloeddruk werd negatief beïnvloedt door de GH dosis, dwz een hogere GH dosis was gerelateerd aan een lagere diastolische bloeddruk. De GH dosis of duur had geen invloed op de systolische bloeddruk. De serum lipiden totaal cholesterol, HDL en LDL waren na het stoppen van de GH behandeling verder doorgestegen. Echter de atherogene index (totaal cholesterol/ HDL) was constant gebleven, welke lager was dan die van contoles. De BMI is gedurende en na de GH behandeling langzaam gestegen. Gedurende de GH behandeling was de zithoogte kleiner geworden en dus verbeterd, de voetlengte relatief groter geworden en dus verslechterd, en de handlengte, biacromiale- en biiliacale diameter waren relatief constant gebleven.

Wij concluderen dat GH behandeling in meisjes met TS, naast de lengte, bijkomende positieve effecten heeft op de bloeddruk, serum lipiden en lichaamsverhoudingen, met de uitzondering van de voetlengte. Bijna 5 jaar na het beëindigen van de GH behandeling is het voordelige effect van de GH behandeling op serum lipiden nog zichtbaar. De verlaagde insuline gevoeligheid echter, was onveranderd, mogelijk passend bij het hebben van TS.

Hoofdstuk 8 beschrijft aorta dimensies en biofysische eigenschappen van de aorta aan de hand van de aorta compliantie (elasticiteit) in 38 TS vrouwen op een leeftijd van bijna 20 jaar, na langdurige GH behandeling. Dit werd gedaan dmv 'magnetic resonance imaging', oftwel MRI onderzoek. De uitkomsten werden vergeleken met 27 leeftijd, sexe en BMI gematchte controles. De diameter en de elasticiteit van de aorta werd op 4 plaatsen in de aorta gemeten, wetende: 1) in het opstijgende deel ter hoogte van de bifurcatie van de a. pulmonalis, 2) in het dalende deel ter hoogte

van de bifurcatie van de a. pulmonalis, 3) ter hoogte van het diafragma, en 4) in het abdominale deel van de aorta ter hoogte van de a. mesenterica superior.

De vrouwen met TS hadden gedilateerde aortas met een grotere aortadiameter op alle nivo's, muv nivo 4, het abdominale deel van de aorta. De TS vrouwen hadden ook aanwijzingen voor een verminderde aortawandfunctie, gemeten aan de hand van de aorta elasticiteit. De ernst van de afwijkingen lijken gerelateerd te zijn aan de GH dosis, waarbij een hogere dosis een positief effect heeft op de afwijkingen.

Hoofdstuk 9 bespreekt de resultaten van de diverse studies in relatie met de literatuur en bediscussieert de klinische implicaties.

Hoofdstuk 10 geeft een overzicht van alle onderzoeksresultaten voortkomend uit deze twee langlopende GH-studies, begonnen in 1991 (SGA studie) en 1989 (Turner studie). Vervolgens komt de vraagstelling aan bod: "Was het – en is het – het allemaal waard?". Tevens worden de sterke kanten en de beperkingen van de studies besproken en worden er suggesties gegeven voor toekomstig onderzoek.

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Curriculum Vitae

Dankwoord

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I love you a lot lot.....Let's enjoy

Curriculum Vitae of Ellen Manon Noëlle Bannink

Ellen Manon Noëlle Bannink was born on 25th December 1972 in Uden, in the south of the Netherlands and moved to Neede, in the east of the Netherlands, at the age of 4 years. After finishing high school (VWO) in 1991 (RSG Lochem), she began to study Human Movement Science at the Vrije University (VU) in Amsterdam. After successfully passing the first 2 years of Human Movement Science, Ellen was able to start her medical study in 1993, also at the VU. During Ellen's medical studies, she spent 4 months of 1997 at the Garvan Institute in Sydney, Australia where she participated in a research project on serum growth hormone binding proteins, under supervision of Prof. Dr. K.Y. Ho in Sydney and Prof. Dr. E.A. van der Veen in Amsterdam. When returning from Australia, she continued her training and began her internship at the VU Medical Centre. When Ellen successfully completed her training in 1999, she returned to the southern hemisphere for a five month travel adventure through Australia and Indonesia. Upon returning home to the Netherlands, Ellen worked for 3 months as a school-physician for local public health care services and then took up as a pediatric resident at Hospital Eemland, Amersfoort from 2000 to 2001. In August 2001, Ellen began her research project as presented in this thesis at the Department of Pediatrics, Subdivision of Endocrinology, Erasmus MC – Sophia Children's Hospital in Rotterdam, under the supervision of Dr. S.M.P.F. de Muinck Keizer-Schrama, Prof. Dr. A.C.S.Hokken-Koelega, and Prof. Dr. S.L.S. Drop. In October 2005, she started her clinical pediatric trainingship at the VU Medical Centre (Head: Prof. Dr. W.P.F. Fetter) and was posted to the St. Lucas Andreas Hospital (Head: Dr. B.H.M. Wolf) in Amsterdam. Ellen lives in Amstelveen with her Australian husband Michael Kerlin. They are expecting their first child in December 2006.