Added Centimetres 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?

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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?

Toegevoegde centimeters en hun gevolgen

Hoe effectief en veilig is groeihormoon als behandeling voor kleine lengte in meisjes met het syndroom van Turner en in kinderen die te klein zijn geboren?

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

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Aan mijn lieve en bovenal geduldige echtgenoot

Contents

Part ‘Turner Syndrome’

CHAPTER 1

Part 'Turner Syndrome': Introduction

TABLE 1. Clinical features of Turner Syndrome

> 80% of cases		
	Growth	Retarded growth and reduced adult height
		Bone age retardation
	Ovaries	Gonadal dysgenesis
50-80% of cases		
	Face	Micrognatia
		Narrow maxilla and dental crowding
	Ears	Recurrent otitis media
		Malformation and rotation of outer ear
	Neck	Low posterior hairline (40-60% of cases)
	Skeleton	Cubitus valgus
	Liver	Elevated hepatic enzymes
20-50% of cases		
	Face	Eye anomalies (i.e. epicanthus, ptosis)
	Ears	Hearing loss (30-90% of cases)
	Neck	Webbed neck
	Chest	Broad chest with widely spaced nipples (less frequent: inverted or hypoplastic nipples)
	Skin	Multiple pigmented naevi
		Congenital lymphedema of hands and feet (25-60% of cases)
		Short fourth metacarpals
	Skeleton	Genu valgum
	Heart and vessels	Hypertension
		Cardiovascular anomalies (i.e. bicuspid aortic valve, coarctatio aortae, aortic dilatation)
	Kidneys	Renal abnormalities (i.e. rotational abnormalities, double collecting system, horseshoe kidney)
	Endocrine	Glucose intolerance
		Hypothyroidism (10-30%)
		Obesity
<20% of cases		
	Eyes	Strabismus
		Amblyopia
	Skin	Halo Naevus
	Skeleton	Congenital hip dislocation
		Scoliosis
		Osteoporosis
	Nails	Nail hypoplasia
	Endocrine	Diabetes Mellitus type 1 and 2
	Other	Gonadoblastoma
		Inflammatory bowel disease
		Colon cancer
		Neuroblastoma
		Juvenile rheumatoid arthritis
		Liver disease
		Coeliac disease

Adapted from Ranke [54], Frias [55], Saenger [56], Gravholt [57], and Brazzelli et al. [58].

Introduction

In 1938 Henry Turner described a syndrome characterised by short stature, lack of pubertal development, a webbed neck, low hair implantation, and cubitus valgus [1]. Eight years previously, a German paediatrician named Otto Ullrich had described a similar patient in a German journal [2]. In some countries, this has led the syndrome to be called the Ullrich-Turner syndrome. In 1959 Ford discovered that a 45, X karyotype caused Turner syndrome (TS) [3]. We now know that Turner syndrome is caused by either a totally or partly absent second X chromosome. The resulting haploinsufficiency of certain pseudo-autosomal genes may be an explanation for its phenotype.
Turner syndrome is characterised mainly by growth retardation, ovarian dysgenesis and organ malformations. The distribution and severity of its typical bodily features varies between individuals (Table 1). Associated organ malformations are mainly of the heart and kidneys. While 1 in 2000 live-born girls are diagnosed with TS, it is estimated that only 1% of all embryos with TS are born alive. The remaining 99% are aborted spontaneously [4, 5]. Approximately 20% of the girls with TS are diagnosed during infancy, displaying typical features such as lymphoedema of the hands and feet and a webbed neck [6]. A Danish study showed that 76% of TS cases are diagnosed before the age of five years, compared to 26% in the years before 1980 [4]. Possibly, the now more widely applied prenatal screening by maternal serum screening, nuchal translucency measurement by ultrasound, chorionic sampling, or amniocentesis is also responsible for the increase in TS cases diagnosed early. TS girls diagnosed at a later age are usually recognised by their growth retardation, absent pubertal development or in adulthood by infertility or recurrent pregnancy loss [6]. A French study showed that almost 5% of girls who visited a specialist due to growth retardation were diagnosed with TS [7].

Height and bone age
In the North-west of Europe, mean adult height of a woman with TS, without the use of growth-stimulating substances, is 1.47 m, approximately 25 cm below mean adult height in normal Dutch women [8, 9]. The development of the short stature can be divided into several stages. Firstly, intra-uterine growth is retarded, resulting in a birth length several centimetres shorter than normal. Secondly, growth in the first years of life is slightly less than normal. In the next years of childhood, height deviates more markedly from the normal height curve [10]. An absent growth spurt causes a further deviation from the normal curve. Due to the delayed closure of the epiphysial growth plates, TS women reach adult height at a later age, some even as late as at their early twenty's [10]. Previous studies showed that girls with TS are not growth hormone (GH) deficient [11-13]. It has been suggested, however, that TS girls have a diminished sensitivity for growth factors [12, 14]. Recent studies have identified a pseudoautosomal gene on the sex chromosomes, called the 'Short-stature Homeobox-containing gene' (SHOX) [15, 16]. Haploinsufficiency of this gene, which is seen in the majority of TS girls, might be responsible for

(part of) the short stature. The function of the proteins it encodes for and the pathway to causing short stature are still under investigation.
Bone maturation, expressed as bone age, appears to develop similarly to height. Up to the age puberty was supposed to start, bone age delay is approximately 1 year. Thereafter, bone age delay increases further. A possible explanation for this is the oestrogen deficiency resulting from the ovarian dysgenesis [17].
Ovarian dysgenesis
In utero the ovaries seem to develop normally in the first months. Then, without an apparent reason, most of the oocytes die before birth. This dysgenesis of the ovaries results in the appearance of the so-called streak gonads. Almost all women with TS are therefore infertile [18]. Due to the resulting oestrogen deficiency, spontaneous pubertal development is absent. Still, some girls with TS produce enough oestrogen to have spontaneous breast development, although sometimes temporary, and, in a small percentage, even menstruate. An Italian study showed that 14% of the 45,X girls and 32% of the girls with a 46, XX cell-line mosaïcisme showed signs of spontaneous puberty [19]. While spontaneous pregnancies have been described in TS, the chance of a child with a chromosomal malformation is increased [20]. Possible pseudo-autosomal genes (on the X-chromosome) thought to be responsible for oogenesis and ovarian function are the USP9X and the DIAPH2 gene, respectively [21]. Missing a second copy of these genes might cause the ovarian dysgenesis in Turner syndrome.

Diabetes Mellitus and Cardiovascular disease
Research has shown that untreated TS women have a predisposition for diabetes mellitus (DM) and cardiovascular disease (CVD) [22, 23]. It has even been reported that CVD is the main cause of their reduced life expectancy [24, 25]. In addition, risk factors for CVD, such as hyperlipidaemia, hypertension, impaired glucose tolerance, and visceral obesity (high waist/hip ratio) occur more often in TS [25-29]. This clustering of risk factors is known as metabolic syndrome or syndrome X.
It has been suggested that the impaired glucose homeostasis in TS is caused by a metabolic defect in the non-oxidative pathways of intracellular glucose metabolism [23]. Compared to age and body mass index matched normal women, however, one study demonstrated a reduced stimulated insulin response in TS women pointing to a beta-cell dysfunction as a possible cause for the impaired glucose homeostasis [30]. The frequent co-occurrence of obesity with all of the previously mentioned risk factors for CVD suggests a possible causal link. The precise aetiology of the predisposition for CVD and its risk factors, however, remains unclear [31].

Psychological development
Most girls with TS have a normal intelligence. Several girls, however, have difficulty with mathematics and spatiality. IQ tests, in these girls, showed a low performal IQ compared to the verbal IQ [32]. Psycho-socially, untreated girls with TS have been described as more immature, having a lower self-esteem, poor concentration, and being hyperactive [33, 34]. In adulthood, issues such as infertility, sexuality, and social awkwardness become issues of concern for TS women [35-37]. Studies have found that for some TS women

inadequate coping mechanisms with these issues lead to a higher than expected prevalence of depression [35].

GH and oestrogen treatment

As early as the seventies, GH was used to treat short stature in TS, then with little success. By way of dose-response studies it was found that supra-physiological dosages of GH led to better results than substitution dosages. A previous Dutch study, however, showed that after one year of GH treatment a waning effect occurred [38]. Subsequent Dutch studies in mostly older girls (>11 years) with TS showed disappointing results [39, 40]. In 1997 this led to a discontinuation of financing of GH treatment for TS in the Netherlands, until another Dutch study showed that using a higher dosage of GH in a group of older TS girls resulted in better height results [41]. To optimise GH treatment in TS, in 1989 a new Dutch dose-response study was initiated with 68 young TS girls. To reduce the waning effect, a yearly stepwise increase in GH dose was used from 4 to 6 IU/m^2/day in one group, and from 4 to 6 to 8 IU/m^2/day in another, while one group remained on a GH dose of 4 IU/m^2/day. Eight years of GH treatment resulted in a significant increase in height SD-score during childhood and adolescence in all GH dosage groups, with a modestly better result in both higher dosage groups [42]. Although GH treatment for short stature in TS is now an accepted treatment in many countries, reports on final height are inconsistent [41, 43].

Another clinical feature in most girls with TS, is the absence of spontaneous pubertal development, for which oestrogen substitution is necessary to induce puberty. Which age to start puberty induction is still an issue of debate. Several paediatricians believe oestrogen treatment should be postponed as long as possible to prevent it to stimulate the closure of the epiphysial growth plates. Both the professional experience of paediatricians and research in girls with TS, however, have shown that the psychological importance of inducing puberty at an age as close as possible to their peers [34]. In addition, in the Dutch dose-response study mentioned above, the initiation at normal pubertal age of a low dose of natural oestrogens did not interfere with the efficacy of GH treatment to normalise height during childhood and adolescence in the TS girls [44].

As one of the side effects of GH treatment is that it increases insulin levels, one of the concerns regarding long-term GH treatment is its effect on carbohydrate metabolism. Another concern would be its effect on other risk factors for cardiovascular disease, such as hyperlipidaemia and hypertension, which also occur more frequent in women with TS [45, 46]. Several studies, describing the effect of GH treatment on blood pressure or lipid levels in TS, showed either a positive effect or no effect [47-50]. A previous data analysis of the Dutch dose-response study showed that glucose levels did not change while insulin levels rose during GH treatment. Mean systolic blood pressure during GH treatment remained in the high normal range, while mean diastolic blood pressure showed a slight decrease, and lipid levels showed a favourable change [45, 51].

One of the reasons why GH treatment is given to TS girls is its assumed effect on their psychosocial development. Several studies have shown that treatment with oestrogens and/or GH treatment improved self-perception and behavioural problems [34, 52, 53].

Research questions and aims of the studies

Previous data analyses have shown a positive effect of GH treatment on height gain in TS during childhood and adolescence. We still, however, did not know whether GH treatment would actually lead to a normalisation of final height. In addition, to optimise individual treatment with GH in future, we wanted to know which parameters were important to consider before initiating treatment. Possible parameters to include were GH dose and age to start treatment. Another question we wanted to answer was what is the effect on adult height of initiating oestrogen treatment at a pubertal age. Regarding side effects, we asked ourselves what the effect would be of discontinuation of GH treatment after long-term GH treatment on carbohydrate metabolism and risk factors for CVD.
Regarding psychosocial development during long-term GH treatment, we wanted to know what the psychosocial functioning was after reaching adult height and, in comparison with previous studies, whether GH treatment and oestrogen treatment might have had an influence.
To answer these research questions, the following aims were used:

1. Evaluation of the effect of long-term GH treatment on adult height in 60 girls with TS treated in a randomised dose-response trial comparing 3 dosage schedules.
2. Assessment of the effect of low dose oestrogen treatment begun at a relatively young age on adult height in girls with TS during long-term GH treatment.
3. Evaluation of several parameters to predict adult height SD-score to aid individual treatment with GH in girls with TS during long-term GH treatment.
4. Investigation of carbohydrate metabolism in girls with TS after discontinuation of long-term GH treatment in a dose up to 8 IU/m^2/day (~ 0.090 mg/kg/day).
5. Investigation of several factors that may predict development of CVD, such as blood pressure, body mass index and blood lipid levels in TS women after discontinuation of GH treatment.
6. Evaluation of psychosocial functioning in a group of 50 TS women with adult height after long-term GH treatment.

Outline of the thesis

In this thesis results are presented of a randomised multi-centre dose-response GH trial evaluating the efficacy, safety and psychosocial effect of long-term GH treatment in girls with TS. The TS trial included the first participants in 1989.
Chapter 2 describes the effect of long-term GH treatment and of low dose oestrogen treatment begun at a relatively young age on adult height in 60 girls with TS treated in a randomised dose-response trial comparing 3 dosage schedules. In addition, a prediction model on adult height SD-score is presented. Chapter 3 describes carbohydrate metabolism and several risk factors for CVD in girls with TS after discontinuation of long-term GH treatment. Chapter 4 describes psychosocial functioning in a group of 50 TS

women with adult height after long-term GH treatment. Chapter 5 discusses the results in relation to other literature and the implications of the results. In addition, it concludes and gives recommendations for future research. Chapter 6 summarises the TS part of the thesis in English and Dutch.

Previous dissertations

In his thesis entitled "Growth hormone treatment modalities in girls with Turner syndrome", Rotterdam 1996, Arne van Teunenbroek described the four-year results of the TS trial. His successor, Theo C.J. Sas, investigated seven-year data of the TS trial. His thesis was entitled "Long-term growth hormone treatment in two growth disorders", Rotterdam 1999.

CHAPTER 2

Part 'Turner Syndrome': Final height in girls with Turner syndrome after long-term GH treatment in three dosages and low dose oestrogens

Final height in girls with Turner syndrome after long-term GH treatment in three dosages and low dose oestrogens

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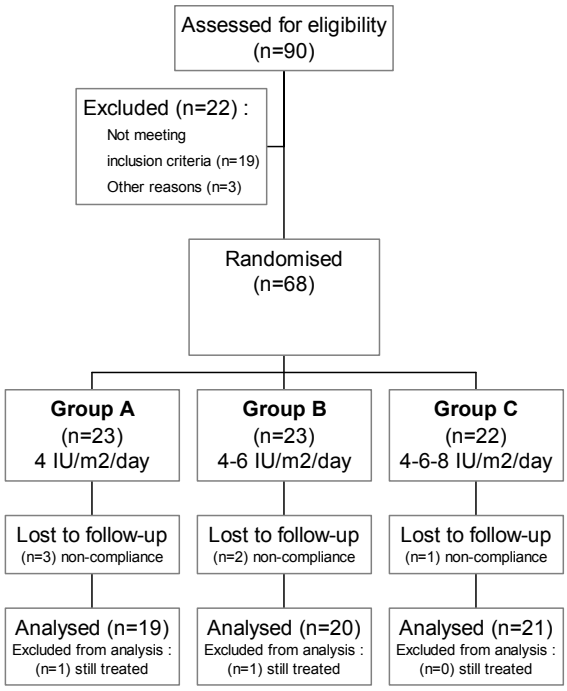
Abstract

Although growth hormone (GH) treatment for short stature in Turner syndrome is an accepted treatment in many countries, which GH dosage to use and which age to start puberty induction are issues of debate. This study shows final height (FH) in 60 girls with Turner syndrome treated in a randomised dose-response trial, combining GH Treatment with low dose oestrogens at a relatively young age.
Girls were randomly assigned to group A (4 IU/m^2/day ~ 0.045 mg/kg/day), group B (1st yr: 4 IU/m^2/d, thereafter 6 IU/m^2/d), or group C (1st yr 4 IU/m^2/d, 2nd yr 6 IU/m^2/d, thereafter 8 IU/m^2/d). After a minimum of 4 years of GH treatment, at a mean age of 12.7 (0.7) years, low dose micronised 17 β-oestradiol was given orally. After a mean duration of GH treatment of 8.6 (1.9) years, final height (FH) was reached at a mean age of 15.8 (0.9) years. FH, expressed in cm or SD-score, was 157.6 (6.5) or -1.6 (1.0) in group A, 162.9 (6.1) or -0.7 (1.0) in group B, and 163.6 (6.0) or –0.6 (1.0) in group C. The difference in FH in cm, corrected for height SD-score and age at start was significant between groups A and B (regression coefficient 4.1; 95% CI: 1.4, 6.9; $P < 0.01$), and groups A and C (coefficient 5.0; 95% CI: 2.3, 7.7; $P < 0.001$), but not between groups B and C (coefficient 0.9; 95% CI: -1.8, 3.6). Fifty of the 60 girls (83%) had reached a normal FH (FH SD-score > -2). After starting oestrogen treatment, the decrease in height velocity (HV) changed significantly to a stable HV, without affecting bone maturation (ΔBA/ΔCA). The following variables contributed significantly in predicting FH SD-score: GH dose, height SD-score (ref. normal girls) and CA at start, and HV in first year of GH treatment. GH treatment was well tolerated.
In conclusion, GH treatment leads to a normalisation of final height in most girls, even when puberty is induced at a normal pubertal age. The optimal GH dosage to use depends on height and age at start of treatment, and first year height velocity.

FIGURE 1. Flow diagram of the progress through the phases of the trial



Introduction

The most common clinical characteristic of Turner syndrome (TS) is short stature. The reason for the short stature is still under investigation. A recent study has shown that the aetiology of the growth retardation possibly lies in the haploinsufficiency of the SHOX gene [15]. Although girls with TS are not growth hormone (GH) deficient [12], subnormal levels of GH and IGF-I have been reported [11, 42]. It has been postulated that a diminished sensitivity for growth factors might explain their growth retardation [14, 59]. Nevertheless, GH treatment in a supra-physiological dosage has been shown to accelerate growth [42, 60]. Another clinical feature, in most girls with TS, is the absence of spontaneous pubertal development, for which oestrogen substitution is necessary. Although GH treatment for short stature in TS is now an accepted treatment in many countries, reports on final height are inconsistent [41, 43], and which dosage to use and which age to start puberty induction are issues of debate.
Previously, we have demonstrated that long-term GH treatment in TS leads to normalisation of height [42, 61]. This study shows final height results in 60 girls with TS treated in a randomised dose-response trial comparing 3 dosage schedules. In addition, we show the effect of low dose oestrogen treatment begun at a relatively young age. Thereby, we have constructed a prediction model for final height SD-score to aid individual treatment.

Patients/Methods

Study Subjects
Sixty-eight previously untreated girls with TS were enrolled from 8 academic and 3 major non-academic paediatric departments in The Netherlands in an open randomised multi-centre GH dose-response study. Six girls dropped out of the study because of non-compliance and were lost to follow-up. Two girls were still treated with GH at time of analysis (24th January 2002) and had not yet reached final height, leaving 60 girls for analysis of final height (FH) in this study (Figure 1). As the 8 girls not used in the analysis (either lost to follow-up or have not yet reached FH) were normally distributed over the randomization groups (4/3/1) and the baseline clinical data showed no significant difference compared to the 60 girls with FH, selection bias was unlikely.
The diagnosis was confirmed by lymphocyte chromosomal analysis. Three of the 68 girls had a prenatal diagnosis. Inclusion criteria were: a chronological age (CA) between 2 and 11 yr, height below the 50th percentile for healthy Dutch girls [62], and normal thyroid function. Exclusion criteria were: associated endocrine and / or metabolic disorders, growth failure caused by other disorders or emotional deprivation, hydrocephalus, previous use of drugs which could interfere with growth hormone treatment, and spontaneous puberty [63]. Written informed consent was obtained from the girls and their parents or custodians. The study protocol was approved by the ethics committee of each participating centre.

Study Design
At start of the study, a total of 15 patients per dosage group was calculated to be necessary to discover a true mean difference in height velocity of 1.0 cm/year between dosage groups after two treatment years with a probability of 80% (based on a 2-sided t-test for paired observations). Based on this calculation, 68 girls were included from November 1989 until October 1990 in the study to evaluate the effect of augmentation of GH dosage on height velocity and final height. Sixty-eight girls were randomly assigned to three groups in blocks of 2, 4, or 6 (randomly chosen) in 4 strata defined by age and height SD-score at start. The sequence was concealed in envelopes until treatment was assigned. The treatment regiments were:

A (n=23)	receiving 4 IU/m^2 body surface/day (~ 0.045 mg/kg/day),
B (n=23)	receiving 4 IU/m^2/day in the first year, followed by 6 IU/m^2/day (~ 0.0675 mg/kg/day)
C (n=22)	receiving 4 IU/m^2/day in the first year, 6 IU/m^2/day in the second year, and thereafter 8 IU/m^2/day (~ 0.090 mg/kg/day).

Biosynthetic human GH (Norditropin, Novo Nordisk A/S, Bagsvaerd, Denmark) was given subcutaneously once daily at bedtime using a pen injection system. Every 3 months the total GH dose was adjusted to the calculated body surface. According to the study protocol, the GH treatment was discontinued when height velocity was < 1 cm over 6 months, or on the decision of the patient due to satisfaction with achieved height. In the first 4 years of GH treatment, no oestrogen for pubertal induction was given to the girls. After 4 yr of GH treatment, oestrogen treatment was started at the yearly visit after reaching the age of 12 yrs. In the girls who became 12 years during the first 4 yrs of GH treatment, oestrogen treatment was started at 4 years of GH treatment. Five µg 17β-estradiol/kg body weight/day (~ 0.05 µg ethinyl oestradiol/kg/day), orally, was given in the first 2 years, 7.5 µg /kg/d in the third year and 10 µg/kg/d thereafter (Tablets containing 0.1 mg micronised 17β-estradiol were supplied for the study by Novo Nordisk A/S, Bagsvaerd, Denmark). Cyclic progesterone therapy (Duphaston® 5 mg/d during the first 14 d of the month) was added after 2 years of oestrogen therapy. If puberty had developed spontaneously (Tanner breast stage ≥ 2) before start of oestrogen, no exogenous oestrogen was given. Height was measured in 8 academic and 3 major non-academic paediatric departments at baseline and subsequently every 3 months in the same department using a Harpenden stadiometer by 3 observers (A van Teunenbroek '89-'95, T Sas '95-'98, and Y van Pareren '98-'01). The mean of 4 measurements was used for analysis. Final height (FH) was defined as the most recent available height after discontinuation of GH treatment (mean 0.5 (0.2) years after discontinuation of GH treatment). Height was expressed as standard deviation score (SD-score) using the references for healthy normal Dutch girls (ref. normal girls) [9] or the references for North European untreated girls with TS (ref. TS) [8]. Height velocity (HV) per year was defined as the increase in height in cm per year. HV SD-score was calculated using reference values for HV in North European untreated girls with TS [64]. Target

height (TH) was adapted from Dutch reference data with addition of 4.5 cm for secular trend: TH = ½ x (H^{mother} + H^{father} - 13 cm) + 4.5 cm [9]. TH range was defined as the TH ± 1.3 SD and TH was expressed as SD-score [9, 65]. During GH treatment pubertal stages were assessed according to Tanner [63]. Bone age (BA) was determined by the same 3 observers according to the Tanner & Whitehouse radius, ulna, short-bones score [66]. Bone maturation was expressed as the ratio of the change in BA to the change in CA (Δ BA/Δ CA). Adult height without GH treatment was calculated for each girl with the modified projected adult height method (mPAH), using the equation of Lyon, adapted to North European untreated girls with TS [8, 67, 68]. To assess the gain in FH, FH was compared with the mPAH at start of GH treatment.

Biochemical parameters and hormone assays
Blood samples were taken at start of the study and subsequently every year, and 6 months after discontinuation of GH treatment for determination of the glycosylated haemoglobin, leukocytes, haemoglobin, creatinine, aspartate aminotransferase (ASAT), alanine aminotransferate (ALAT), Alkaline Phosphatase (AP), free thyroxin (T4), and thyroid-stimulating hormone (TSH) levels. Plasma insulin-like growth factor (IGF-I) levels were determined at start, at 6, 18, 30, and at 48 months, thereafter at every year visit until discontinuation of GH treatment, and 6 months after discontinuation of GH treatment. After centrifugation, all samples were frozen (-200C) until assayed. All measurements of IGF-I were performed in one laboratory by radioimmunoassay (RIA), as described previously [69] and were transformed to SD-scores using reference levels for healthy children determined in the same laboratory [70].

Statistical Analysis
Results were expressed as mean (SD), unless indicated otherwise. Differences between the dosage groups were tested by linear regression analysis with the variables age and height SD-score (ref. normal girls) at start of treatment and two dummy variables for dosage group. Differences in time between continuous variables were compared by paired 2-sided t-test for the whole group unless otherwise specified. A stepwise forward linear regression analysis was used to construct a prediction model for FH SD-score and gain in height (FH – mPAH in cm). The following potential predictor variables were used: bone age (BA), chronological age (CA), height SD-score (ref. normal girls), and IGF-I SD-score at start, GH dosage group, target height SD-score, karyotype (45,X or other), first year increase in alkaline phosphatase and in HV (in cm), and first 18 months increase in IGF-I SD-score. Only the variables with a P-value < 0.05 were kept in the model. Subsequently, the squares of the remaining variables were tested for significance, after which the variables were tested for possible interactions. All correlations were partial correlations, adjusted for GH dosage. A P-value < 0.05 was considered significant. All calculations were performed by SPSS version 9.0.

TABLE 1. Mean (SD) baseline clinical data

	Group A	Group B	Group C
Number of girls	19	20	21
Chronological age (yr)	6.5 (1.9)	6.9 (2.3)	6.5 (2.4)
Bone age (yr)	5.9 (2.1)	6.2 (2.5)	5.8 (2.4)
Height SD-score	-2.9 (0.9)	-2.7 (0.9)	-2.7 (1.0)
Height SD-score	0.0 (1.1)	0.3 (0.9)	0.2 (1.1)
Maximal GH response (ATT)* (mU/L)	18.5 [4-67]	16.5 [5-74]	21.3 [3-66]
Modified projected adult height (cm)	145.7 (5.7)	147.2 (4.9)	146.6 (5.6)
Target height (cm)	170.4 (6.6)	171.3 (6.0)	170.5 (5.6)
Karyotype: 45,X	16 (84%)	19 (95%)	16 (76%)
Karyotype: other	3 (16%)	1 (5%)	5 (24%)

GH = growth hormone, ATT = arginine tolerance test. * Geometric mean [range]

TABLE 2. Mean (SD) height data of 60 girls with Turner syndrome after long-term GH treatment

	Group A (N = 19)	Group B (N = 20)	Group C (N = 21)
Final height (cm)	157.6 (6.5)	162.9 (6.1)*	163.6 (6.0)**
[Range]	[143.1, 172.1]	[152.4, 176.2]	[153.3, 172.4]
Final height SD-score	-1.6 (1.0)	-0.7 (1.0)*	-0.6 (1.0)**
(ref. normal Dutch girls)	[-3.8, 0.5]	[-2.6, 1.1]	[-2.1, 0.9]
Height gain (cm)	11.9 (3.6)	15.7 (3.5)*	16.9 (5.2)**
(FH minus mPAH)	[2.8, 17.8]	[8.1, 20.4]	[7.2, 28.7]
Δ Height SD-score	1.2 (0.6)	1.9 (0.5)**	2.1 (0.8)**
(from start until FH)	[-.2, 2.0]	[0.9, 2.6]	[0.3, 3.6]
GH duration (yr)	8.9 (1.4)	8.3 (2.1)	8.7 (2.0)
	[6.5, 11.1]	[5.5, 12.0]	[5.3, 11.5]

FH = final height, mPAH = modified projected adult height. Linear regression analysis with correction for age and height SD-score at start (vs group A):
* P < 0.01 ** P < 0.001

Results

In Table 1 the pre-treatment clinical data of the 60 girls with final height are shown. Baseline clinical data were similar for the three GH dosage groups. After a mean duration of GH treatment of 8.6 (1.9) years, final height was reached at a mean age of 15.8 (0.9) years, and with a bone age of 15.5 (0.6) years. Forty-eight of the 60 girls had GH treatment duration of 7 years or more.
Final height (FH) in cm was 157.6 (6.5) in group A, 162.9 (6.1) in group B, and 163.6 (6.0) in group C (Table 2). When translated to SD-score, using references for normal girls, FH was -1.6 (1.0) for group A, -0.7 (1.0) for group B, and –0.6 (1.0) for group C (Figure 2). The difference in FH in cm, corrected for height SD-score and age at start was significant between groups A and B (regression coefficient 4.1; 95% CI: 1.4, 6.9; $P < 0.01$), and groups A and C (coefficient 5.0; 95% CI: 2.3, 7.7; $P < 0.001$), but not between groups B and C (coefficient 0.9; 95% CI: -1.8, 3.6). Fifty of the 60 girls (83%) had reached a normal FH (FH SD-score > -2). Thirty-eight of the 60 girls (63%) reached a FH within their TH range.
The mean gain in final height in cm (FH - mPAH) in group A was 11.9 (3.6) cm, being significantly lower compared to 15.7 (3.5) cm in group B (regression coefficient 4.2; 95% CI: 1.5, 6.9; $P < 0.01$) and compared to 16.9 (5.2) cm in group C (coefficient 5.2; 95% CI: 2.6, 7.8; $P < 0.001$), but the height gain in group B was not significantly different to group C (coefficient 1.0; 95% CI: -1.6, 3.6; $P = 0.44$) (Figure 3). Similarly, the mean increase in SD-score from start of GH treatment until FH in groups B and C was significantly higher compared to group A (coefficient 0.7; 95% CI: 0.31, 1.11; $P < 0.001$), but the increase in group B was comparable to group C (coefficient 0.12; 95% CI: -0.27, 0.5; $P = 0.5$) (Table 2).

Oestrogen effect
Oestrogen treatment was started at a mean age of 12.7 (0.7) years. Tanner breast stage 2 was reached at a mean age of 12.9 (0.6) and stage 4 at a mean age of 14.8 (1.1). Height velocity (HV) before and after initiation of oestrogen treatment is depicted in Figure 4. To homogenize the group for age, only the girls who started oestrogen treatment at age 12 were analyzed (n=47). HV in the year after initiation of oestrogen treatment compared with the HV in the previous year showed no significant difference (HV0yr vs HV1yr). The downward trend in HV before initiation of oestrogen treatment, however, changed significantly to a stabile HV after initiation (Δ HV-1yr-0yr vs Δ HV0-1yr; $P < 0.05$). Bone maturation (Δ BA/Δ CA) in the year before and in the year after initiation of oestrogen treatment was not significantly different (t=0 vs t=1 yr; Figure 4). GH dosage, GH duration before start of oestrogen, and height at puberty had no significant effect on the differences (between before and after initiation of oestrogen) in HV, in the change in HV, or in bone maturation.

FIGURE 2. Height SD-score for chronological age (ref. normal Dutch girls) during GH treatment for group A (white bars), group B (gray bars), and group C (black bars), respectively

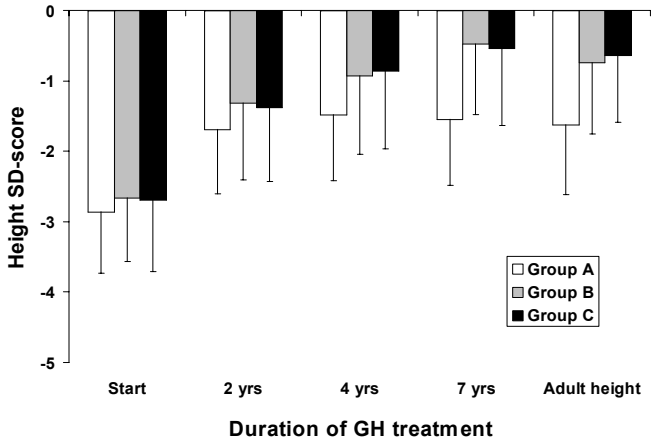


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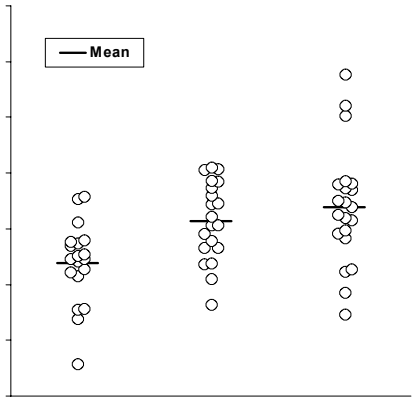
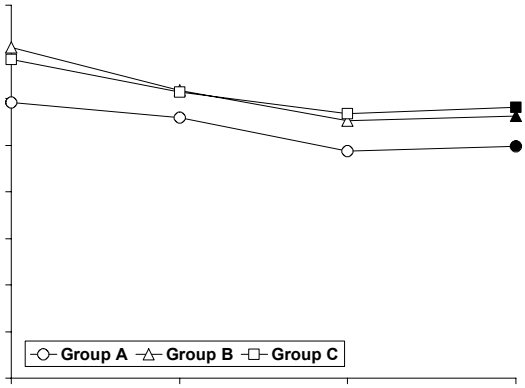
FIGURE 3. Height gain (difference in cm between final height and modified projected adult height) for group A (n=19), group B (n=20), and group C (n=21), respectively.

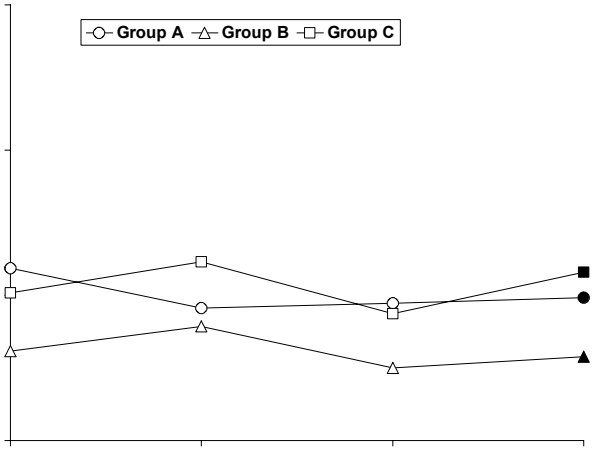


Prediction model for final height SD-score and gain in height
A stepwise forward linear regression analysis resulted in a model using the predictor variables GH dose, height SD-score (ref. normal girls) and CA at start of treatment, and HV in first year of GH treatment accounting for 75.6% of the variation in FH SD-score (residual SD 0.55). Table 3 shows the coefficients of the linear regression model. Variables, which showed a non-significant effect on FH SD-score were IGF-I SD-score and BA at start, increase in IGF-I SD-score in first 18 months and increase in alkaline phosphatase in first year, TH SD-score, and karyotype (45,X yes or no). The model equation was: FH SD-score = -2.29 + 0.80 x Height SD-score at start + 0.81 x Group C (yes=1/no=0) + 0.68 x Group B (yes=1/no=0) + 0.24 x HV in 1st year (cm) + 0.087 x CA at start (yr). To explore the effect of CA at start on FH SD-score, a partial correlation was done, controlling for GH dosage. The result was a significant negative correlation between FH SD-score and CA ($r = -0.30$, $P < 0.05$).
When using the same predictor variables in a stepwise forward regression analysis to predict height gain (FH – mPAH in cm), the following model was obtained, after substitution of HV in 1st year in cm by HV SD-score (ref. TS) and age at start, and the addition of GH peak during ATT: height gain (cm) = 10.95 (SE:2.58) + 1.15 (0.47) x HV SD-score in 1st year + 4.01 (1.18) x Group B (y/n) + 5.55 (1.16) Group C (y/n) – 1.57 (0.59) x Height SD-score at start – 1.04 (0.28) x CA at start (yr) – 0.083 (0.032) x GH peak (mU/L). The model explained 45.6% of the variation in height gain (residual SD 3.6 cm).

IGF-I levels
Mean plasma IGF-I SD-score before, during and 6 months after discontinuation of GH treatment is shown in Figure 5. Mean plasma IGF-I SD-score increased significantly from start of GH treatment until 7 years of GH treatment ($P < 0.0001$ for whole group), from -2.3 (1.5) to 1.9 (0.8) for group A, from –1.4 (1.2) to 2.5 (0.9) for group B, and from –1.9 (1.4) to 2.7 (0.9) for group C. Although the increase from start until 7 years of GH treatment was not significantly different between groups A, B and C, we did find significantly higher IGF-I SD-score at 7 years of GH treatment in group C compared to group A ($P < 0.05$). After discontinuation of GH treatment IGF-I SD-score decreased significantly ($P < 0.0001$ for whole group) to a mean SD-score of –0.6 (1.0) for group A, -0.1 (0.7) for group B, and –0.1 (0.9) for group C, the decrease being not significantly different between groups. IGF-I SD-score 6 months after discontinuation of GH treatment was not significantly different from zero (= mean IGF-I level for same age and sex reference population) for groups B and C, but lower than zero for group A ($P < 0.05$).

FIGURE 4. Height velocity and bone maturation before and after initiation of low dose oestrogen (E2) treatment for group A (circle), group B (triangle), and group C (square) in girls who initiated oestrogen treatment at age 12 years (n=47). The black signs indicate the height velocity and bone maturation after initiation of oestrogen treatment.





Safety
No adverse events were detected that were considered to be GH related. Treatment was well tolerated. A previously published report, using the same study group to describe glucose tolerance during long-term GH treatment, showed no adverse effects on glucose levels [51]. Furthermore, no significant differences in glucose and insulin levels between the GH dosage groups during long-term GH treatment were found [51]. With the exception of two girls, glycosylated haemoglobin levels, but also leukocytes, haemoglobin, creatinine, ASAT, ALAT, alkaline phosphatase, free T4, and TSH levels remained within the normal range and none of the children developed diabetes mellitus. Two girls had abnormal laboratory findings: one girl developed autoimmune hypothyroidism and was treated with thyroid hormone during the study, another girl developed elevated levels of hepatic enzymes and hepatomegaly resulting from hepatic glandular malformations.

Discussion

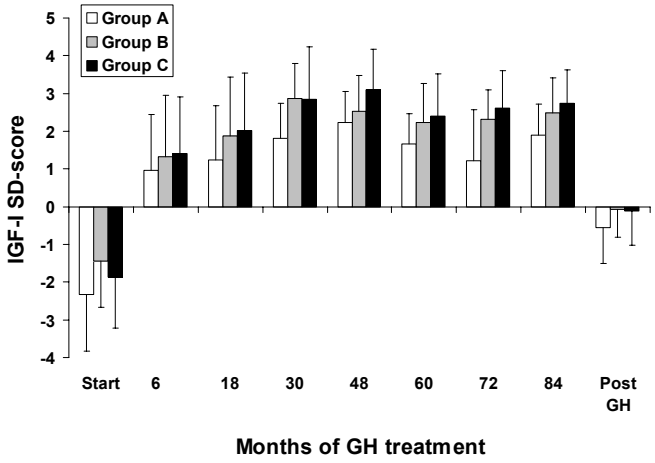
In this paper we present final height results in 60 girls with Turner syndrome after long-term GH treatment in three dosages. We show that when puberty is induced at a normal age, a GH dosage of 4 IU/m^2/day (group A), in Turner Syndrome (TS), leads to a mean final height (FH) of 157.6 cm, which is equal to an SD-score of -1.6 when using references for normal Dutch girls, whereas using 6 IU/m^2/day (group B), leads to a significantly higher final height of 162.9 cm (SD-score -0.7). Administration of an even higher dosage of GH (8 IU/m^2/day; group C) did not lead to a significant increase in attained final height compared to group B. As a result, 83% of the girls with TS reached a normal final height (FH SD-score above -2) and 63% reached a final height within their target height range (TH ± 1.3 SD).
Our study shows that, when GH was started at a mean age of 6.6 years, the mean gain in FH (estimated by subtracting Lyon's predicted adult height, adjusted for Dutch girls with TS (mPAH), from attained FH) varied between 11.9 cm in group A to 16.9 cm in group C. The reason why previous studies reported a considerably lower gain in final height probably lies in the fact that they started GH treatment at an older age [43, 71, 72]. This is confirmed by the finding that other studies, starting GH treatment at a younger mean age, also showed a greater gain in final height [60, 73, 74]. In addition, in our study, we found that in a regression model, containing height velocity SD-score in the first year, height SD-score at start, GH peak, GH dosage, and a lower age at start predicted a greater gain in final height. Another factor that predicted FH gain was the dosage of GH. Our study showed that using a dosage of 6 or 8 IU/m^2/day instead of 4 significantly increased gain in FH. Using a dose of 8 IU/m^2/day, however, showed no advantage over 6 IU/m^2/day for gain in FH. Our results are confirmed by two non-randomised studies showing a dose-dependent increase in final height gain [75, 76].

TABLE 3. Variables in prediction model for Final height SD-score (N = 60)

Independent variable	Regression Coefficient	SE	P-value
Height SD-score at start *	0.80	0.09	< 0.0001
Group C #	0.81	0.18	< 0.0001
Group B #	0.68	0.18	< 0.001
First year height velocity**	0.24	0.06	< 0.01
Age at start	0.087	0.04	< 0.05

Regression equation: FH SD-score = -2.29 + 0.80 x Height SD-score at start + 0.81 x Group C (yes=1/no=0) + 0.68 x Group B (yes=1/no=0) + 0.24 x HV in 1st year + 0.087 x CA at start.
* Using references for normal girls, # Group C 1=yes/0=no, Group B 1=yes/0=no, ** In first year of GH treatment (cm/yr).

FIGURE 5. IGF-I SD-score during GH treatment for group A (white bars), group B (gray bars), and group C (black bars), respectively.



Oestrogen treatment, in our study, was initiated from the age of 12 years to mimic normal pubertal development as much as possible. The result was a breast development about 2 years later than the 50th percentile in normal Dutch girls [9]. In other studies, most girls start at a later age [71, 76], some of which even start oestrogen treatment at 15-16 years [60, 73]. In our opinion, however, based on our professional experience and research [34], it is important for the psychological well being in girls with TS that puberty is induced at an age as close as possible to their peers.

In addition, we show that starting a low dose of natural oestrogen at a relatively young age did not have a negative effect on height velocity (HV) and on bone maturation, and therefore possibly on height gain. Other studies, in contrast, found a decrease in height velocity and an increase in bone maturation leading to a decrease in height gain [73, 77]. The reason why our results are in conflict with these studies, however, might be that a higher dose of oestrogen was used compared to our study. Another possible explanation might be that oestrogen treatment was started after at least four years of GH treatment. In these four years height SD-score for the whole group increased from – 2.7 at start of GH therapy to – 0. 9 at start of oestrogen treatment, showing that most of the catch-up had already occurred before oestrogen treatment was initiated. Confirming this explanation, Reiter et al. found that a longer duration of oestrogen-free GH treatment strongly predicted a greater gain in near final height [74]. Our results therefore suggest that if GH is started at a young age final height will not be affected by early initiation of oestrogen.

When analyzing the factors most likely to influence FH SD-score, we found that a model containing height SD-score at start using references for normal girls, GH dosage, first year height velocity (in cm), and age at start (in years) explained 76 percent of the variation in FH SD-score outcome (Table 3). To keep the model accessible for all clinicians peak GH level during ATT, which is often not available in clinical practice, was not tested as a potential predictor and HV was expressed in centimetres. The model can be used to decide which dosage to use by filling in the different variables. For example, a girl with TS, with a height SD-score at start of GH treatment of –3, a HV of 10 cm/yr after 1 yr of GH treatment (1 year conventional dose of 4 IU/m^2/d), and an age at start of 6 years, would attain a final height SD-score of –1.5 when the GH dose is not increased, and a FH SD-score of –0.8 or –0.7 when the GH dose is increased by 50 or 100 percent. Illustrating the effect of height SD-score at start, in a second example, a similar girl with a height SD-score at start of –4 would attain a FH SD-score of –2.6, -1.9, and –1.7, respectively. In a third example, using the same characteristics as in the first example, changing the HV in the first year to 8 cm/yr would result in a FH SD-score of –2.2, -1.6, and –1.4, respectively. As a forth and last example, when the girl is 10 years at start, with a lower 1st year HV of 8.5 cm/yr (mean 1st year HV in our study for that age, as 1st year HV decreases with age), and a similar height SD-score at start, she would attain a FH SD-score of –1.8, -1.1, and –1, respectively. Depending on one's goal, for instance achieving a normal final height or reaching the target height range, the GH dosage could be adjusted accordingly.

Reasons why one might choose to increase the GH dosage are a low height SD-score at start, and/or a low HV in the first year. Examples two and three show that these variables might lead to a lower FH SD-score when using the conventional GH dose. Another reason for increasing the GH dosage is an older age at start, shown in a separate correlation-analysis between age at start and FH SD-score. In the model, however, we found a positive correlation between age at start and FH SD-score. This finding results from the adjustment for the other variables in the model, since both first year HV and height SD-score at start are negatively correlated with age at start ($r = -0.54$, $r = -0.41$, respectively), In other words, older girls, due to their age, have a lower first year HV and height SD-score at start. Therefore, to explore the effect of age at start in the model, not only age at start, but also first year HV and height SD-score should be taken into account. Example four illustrates the relationship, showing a lower predicted FH SD-score compared to the first example. A higher GH dosage, however, not only leads to a 50-100% increase in cost, but also leads to a higher IGF-I SD-score and the long-term effects of high IGF-I levels remain to be investigated [78, 79]. In addition, we would like to emphasize that, although the model has a high prediction percentage, it does leave 24 percent to be explained by unknown factors. In addition, the predicted FH SD-score has a large prediction interval (residual SD of 0.54).

In conclusion, GH treatment leads to a normalisation of final height in most girls, even when puberty is induced at a relatively normal pubertal age. The optimal dosage to use depends on height and age at start of treatment, and first year height velocity, although the very long-term safety of using a higher GH dosage remains to be investigated.

CHAPTER 3

Part 'Turner Syndrome': Effect of discontinuation of long-term growth hormone treatment on carbohydrate metabolism and risk factors for cardiovascular disease in girls with Turner syndrome

Effect of discontinuation of long-term growth hormone treatment on carbohydrate metabolism and risk factors for cardiovascular disease in girls with Turner syndrome

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Abstract

GH treatment increases insulin levels, in girls with Turner syndrome (TS) who are already predisposed to develop diabetes mellitus and other risk factors for developing cardiovascular disease (CVD). Therefore, in the present study, we investigated carbohydrate metabolism and several other risk factors that may predict development of CVD in girls with TS after discontinuation of long-term GH treatment. Fifty-six girls, participating in a randomised dose-response study, were examined, before, during, and 6 months after discontinuing long-term GH treatment in a dosage of 4 IU/m^2/d (~ 0.045 mg/kg/d), 6 IU/m^2/d, or 8 IU/m^2/d. After a minimum of 4 years of GH treatment low dose micronised 17 β-estradiol was given orally. Mean (SD) age at 6 months after discontinuation of GH Treatment was 15.8 (0.9) years. Mean duration of GH treatment was 8.8 (1.7) years. Six months after discontinuation of GH treatment, fasting glucose levels decreased and returned to pre-treatment levels. The AUC for glucose decreased to levels even lower than pre-treatment ($P < 0.001$). Fasting insulin levels and the AUC for insulin decreased to levels just above pre-treatment ($P < 0.001$ for both), although being not significantly different to the control group. No dose-dependent differences between GH dosage groups were found. At 6 months after discontinuation impaired glucose tolerance (IGT) was present in 1/53 girls (2%) and none of the girls developed diabetes mellitus type 1 or 2. Compared to pre-treatment, BMI SD-score had increased ($P < 0.001$), and systolic and diastolic BP SD-score had decreased significantly at 6 months after discontinuation of GH treatment ($P < 0.001$ for both), although remaining above zero ($P < 0.001$, $P < 0.05$, $P < 0.005$, respectively). Compared to pre-treatment, TC did not change after discontinuation of GH treatment, while the atherogenic index (AI: TC/HDL-c) and LDL-c had decreased and both HDL-c and triglyceride levels increased ($P < 0.001$ for AI,

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LDL-c and HDL-c, $P < 0.05$ for triglyceride). Compared to the control group, AI, serum TC, and LDL-c levels were significantly lower ($P < 0.001$ for all), whereas HDL-c levels were significantly higher ($P < 0.05$).
In conclusion, after discontinuation of long-term GH treatment in girls with TS, the GH induced insulin resistance disappeared, blood pressure decreased but remained higher than in the normal population, and lipid levels and the atherogenic index changed to more cardio-protective values.

Introduction

One of the main clinical features of Turner syndrome (TS) is short stature. Although girls with TS are not growth hormone (GH) deficient [12], GH treatment has been proven to lead to a considerable height gain in girls with TS in whom treatment with GH was started at a young age and were treated with supra-physiological dosages [42, 75]. However, as GH treatment increases insulin levels, several authors have expressed their concern regarding long-term effect of GH treatment in children with a predisposition for diabetes mellitus (DM) [45, 46].
Besides DM, girls with TS are also predisposed to develop cardiovascular disease (CVD). It has even been reported that CVD is the main cause of their reduced life expectancy [24, 25]. In addition, risk factors for CVD, such as hyperlipidaemia, hypertension and insulin resistance, occur more often in TS [25-28].
In the present study, we investigate carbohydrate metabolism in girls with TS after discontinuation of long-term GH treatment in a dose up to 8 IU/m^2/day (~ 0.090 mg/kg/day). Furthermore, we investigate several factors that may predict development of CVD, such as blood pressure, body mass index and blood lipid levels.

Subjects and Methods

Study group and treatment regimens
The study group comprised 56 girls with Turner syndrome (TS) who were examined 6 months after discontinuation of GH treatment. Fifty-four children had an oral glucose tolerance test (OGTT) at 6 months after discontinuation of GH. All girls had been part of a multi-centre GH dose-response study in The Netherlands, in which 68 girls were included. Inclusion criteria of the dose-response trial were described previously [51]. In short: a chronological age between 2 and 11 yr, height below the 50th percentile for healthy Dutch girls, and a normal thyroid function. Of the original 68 girls, 6 girls were not examined at 6 months after discontinuation of GH treatment, and 6 girls were still treated with GH. Written informed consent was obtained from the girls and their parents or custodians. The study protocol was approved by the ethics committee of each participating centre.
After stratification for chronological age and height SD-score for chronological age, girls were randomly assigned to group A (4 IU/m^2/day ~ 0.045 mg/kg/d), group B (1st yr: 4 IU/m^2/d, thereafter 6 IU/m^2/d), or group C (1st yr: 4 IU/m^2/d, 2nd yr: 6 IU/m^2/d, thereafter 8 IU/m^2/d). Biosynthetic human GH (Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily. GH treatment was discontinued when height

velocity was < 1 cm/ 6 months or when satisfied with their attained height. After a minimum of 4 yr of GH treatment, micronised 17 β-estradiol was given orally to the girls of 12.0 yr and older (5 μ g/kg body weight/day in the first 2 yrs, 7.5 μ g/kg/d in the 3rd yr, and thereafter 10 μ g/kg/d). After 2 years of oestrogen treatment a progestagen was added (5 mg Duphaston®). The oestrogen dose was gradually increased to adult level (2 mg) after discontinuation of GH treatment. Five of the 56 girls had a repaired coarctation without a residual gradient, left ventricle hypertrophy or hypertension, 17 girls had a non-stenotic abnormal aortic valve, and none of the girls had a renal malformation that could influence blood pressure. One of the 56 girls, after repair of multiple congenital cardiac malformations, had a remaining left ventricle hypertrophy, which could explain her higher blood pressure during and after discontinuation of GH treatment.

Study protocol
At start of GH treatment (pre-treatment) and every 3 months after the start of GH treatment, all girls were seen at their local hospital for a physical examination. All underwent an oral glucose tolerance test (OGTT) after overnight fasting (in the three previous days 100 g of carbohydrate (FantomaltR); oral glucose load of 1.75 g glucose /kg body weight, maximum of 50 g) at pre-treatment, after 4 yr of GH treatment, and 6 months after discontinuation of GH treatment. Blood samples were analyzed at 0, 30, 60, 90, 120, 150, and 180 min for plasma glucose and insulin levels. In addition, the following variables were described: (1) Impaired glucose tolerance (IGT) was defined according to The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [80]: the 2-hour glucose level > 7.8 mmol/l (140 mg/dl) and < 11.1 mmol/l (200 mg/dl). (2) The 3-hour area under the curve for time-concentration (AUC) for glucose and insulin was calculated using the trapezoidal rule. (3) The ratio insulin/glucose at 30 minutes and the ratio at 120 minutes were calculated as an index for relative insulin resistance. Results were compared with the data of 24 normal adolescent girls aged 14.7 (0.98) years, selected on the basis of post-pubertal stage (Tanner breast stage 5) as described by Potau et al. (control group) [81]. Height and body mass index (BMI: kg BW/(height)^2) was expressed as SD-score for sex and chronological age [8, 62]. Systolic and diastolic BP was determined four times with a single Dynamap Critikon 1846SX in sitting position using a cuff size corresponding to the size of arm. Blood pressure (BP) was expressed as SD-score, using age and sex specific reference values [82]. A child was considered normotensive if BP was below the 90th percentile. Additional blood samples were taken at start of the study and subsequently every year for determination of glycosylated hemoglobin (HbA1c) levels. Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) levels were determined after overnight fasting at start, at 4 years of GH treatment, and at 6 months after discontinuation of GH treatment. The atherogenic index was calculated as the ratio of TC to HDL cholesterol. TC, HDL-c, and LDL-c levels, and atherogenic index were compared with a Dutch control group of the same age and sex [83]. After centrifugation, all samples were frozen (-20ºC) until assayed.

TABLE 1. Pre-treatment variables

		Group A	Group B	Group C
Number of girls		19	17	20
Age at start of GH treatment		6.5 (1.9)	7.5 (1.9)	6.5 (2.4)
Height SD-score* (Normal girls)		-2.8 (0.9)	-2.7 (0.8)	-2.6 (1.0)
Height SD-score* (TS girls)		0.01 (1.1)	0.2 (0.9)	0.19 (1.1)
Karyotype**	45,X	16	16	15
	other	3	1	5

Data expressed as mean (SD). * Height SD-score for sex and chronological age in normal girls 62 and in girls with Turner syndrome (TS) 8 at start of GH treatment.** Number of girls.

TABLE 2. Carbohydrate data before, during, and after long-term GH treatment

	GH	Group A (n = 19)	Group B (n = 16)	Group C (n = 19)	Whole group (n = 54)
Fasting glucose* (mmol/l)	Start	4.4 (0.5)	4.6 (0.4)	4.6 (0.8)	4.5 (0.6)
	4 yrs	4.8 (0.5)	4.7 (0.6)	5.1 (0.9)	4.9 (0.7)$^{\#}$
	Post	4.4 (0.5)	4.7 (0.4)	4.6 (0.7)	4.5 (0.5)$^{\$}$
AUC glucose* (mmol/l x 180 min)	Start	1072 (184)	1118 (172)	1096 (181)	1095 (177)
	4 yrs	1072 (122)	1154 (143)	1126 (188)	1116 (155)
	Post	953 (111)	975 (132)	962 (134)	963 (124)$^{\#\#\$\$}$
Fasting insulin** (mU/l)	Start	4 (8)	4 (10)	5 (13)	4.5 (2.2)
	4 yrs	12 (23)	16 (38)	16 (45)	14.2 (3.3)$^{\#\#}$
	Post	11 (19)	11 (18)	11 (22)	10.7 (2.9)$^{\#\#\$}$
AUC insulin* (mU/l x 180 min)	Start	3863 (2411)	4858 (3284)	3941 (1626)	4205 (2482)
	4 yrs	7798 (3355)	14369 (15123)	9733 (3385)	10533 (9094)$^{\#\#}$
	Post	6553 (2468)	5837 (3151)	5987 (2867)	6136 (2791)$^{\#\#\$}$
Ratio ins/glu** 30 min	Start	3.5 (10.7)	4.6 (10.2)	3.8 (6.6)	3.9 (7.4)
	4 yrs	7.4 (13.8)	11.8 (37.6)	9.8 (20.6)	9.4 (21.2)$^{\#\#}$
	Post	7.8 (20.0)	7.1 (23.0)	6.5 (11.3)	7.1 (17.9)$^{\#\#\$}$
Ratio ins/glu** 120 min	Start	2.4 (7.5)	3.6 (9.6)	3.3 (6.4)	3.0 (7.5)
	4 yrs	6.5 (13.2)	8.7 (16.5)	7.6 (13.8)	7.5 (13.5)$^{\#\#}$
	Post	5.6 (8.3)	3.8 (6.1)	4.8 (10.5)	4.7 (9.6)$^{\#\#\$\$}$
HbA1c* (% Hb)	Start	4.8 (0.5)	4.9 (0.5)	4.8 (0.5)	4.9 (0.5)
	7 yrs	4.6 (0.5)	4.6 (0.7)	4.6 (0.4)	4.6 (0.5)$^{\#\#}$
	Post	4.3 (0.5)	4.3 (0.5)	4.3 (0.5)	4.3 (0.5)$^{\#\#\$\$}$

Data expressed as: * mean (SD). ** geometric mean (90th percentile). AUC = area under the curve calculated with the trapezoid rule. Paired t-test for whole group: $^{\#\#}P < 0.001$ (vs start); $^{\$}P < 0.01$, $^{\$\$}P < 0.001$ (vs 4 yrs).

Assays
The plasma glucose level was measured at the local hospital laboratories and plasma insulin was determined in one laboratory by radioimmunoassay (RIA) (Medgenix, Fleurus, Belgium) as described previously. Control samples were measured by a comparable RIA (R2 = 0.988; y = 0.397 + 0.925 * x) [81]. HbA1c levels and lipid levels were measured in one laboratory as described elsewhere [45, 51]. Lipid levels for the control group were measured by the same assays in the same laboratory [83]. All blood sample measurements were performed in the same laboratories during the whole study period.

Statistical analyses
Results were expressed as mean (SD), unless indicated otherwise. For continuous variables with a skewed distribution a log-transformation was used. Differences between the dosage groups were tested by linear regression analysis with the variables age at start and two dummy variables for dosage group. Differences in time between continuous variables were compared by paired 2-sided t-test for the whole group. To test whether variables expressed in SD-score were different from zero a one-sample t-test was performed. Differences between the whole TS group and the control group for the carbohydrate variables were tested by 2-sided independent sample t-test. All correlations were partial correlations, adjusted for GH dosage. A P-value < 0.05 was considered significant. All calculations were performed by SPSS version 9.0.

Results

In Table 1 pre-treatment characteristics of the 56 children are shown. All GH dosage groups had similar pre-treatment characteristics. Mean (SD) age at 6 months after discontinuation of GH treatment was 15.8 (0.9) years. Mean duration of GH treatment was 8.8 (1.7) years. Fourty-four of the 56 girls were treated with GH for 7 years or longer.
Six months after discontinuation of GH treatment fasting glucose levels for the whole group had decreased significantly compared to 4 years of GH treatment ($P < 0.01$), after a significant increase from pre-treatment to 4 years of GH treatment ($P < 0.01$), and returned to pre-treatment levels (Table 2). Mean glucose levels during OGTT are depicted for groups A, B and C in Figure 1. The 180-minute AUC for glucose at 6 months after discontinuation of GH treatment for the whole group decreased to levels even lower than pre-treatment ($P < 0.001$), after a small non-significant rise from pre-treatment to 4 years of GH treatment. Fasting glucose levels and the 180-minute AUC for glucose for the whole group were not significantly different between the GH dosage groups (Table 2). No significant differences were found between the whole TS group at 6 months after discontinuation of GH treatment and the control group in fasting glucose or 120 minute AUC for glucose. As the control group had a 120-minute OGTT the 120-minute AUC in the TS group was used to compare data.
Mean insulin levels during OGTT are depicted in Figure 2 for groups A, B and C.

FIGURE 1. Mean glucose levels during OGTT for group A, group B and for group C, before treatment (black circles), at 4 years of GH treatment (black diamonds), at 6 months after discontinuation of treatment (white triangles), and for the control group (white squares).

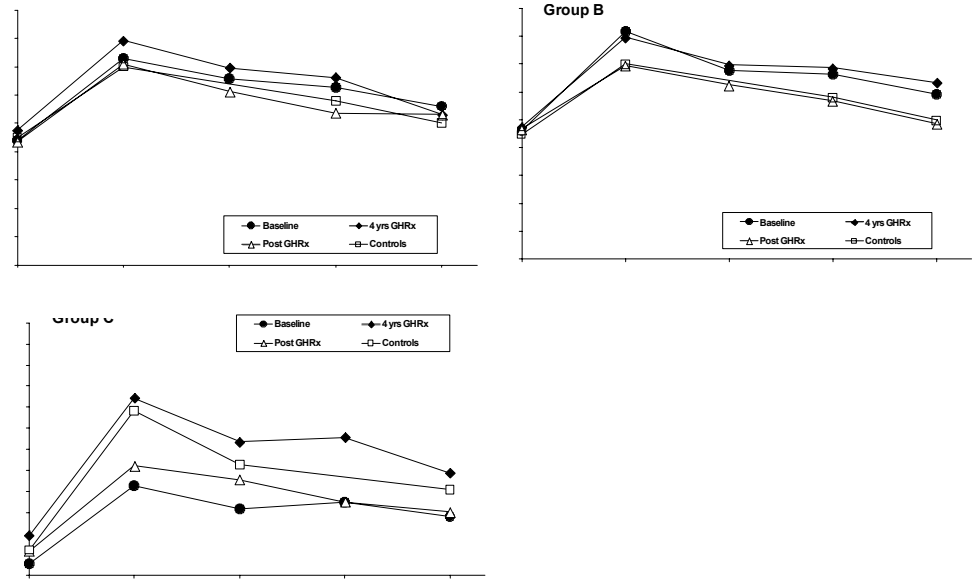
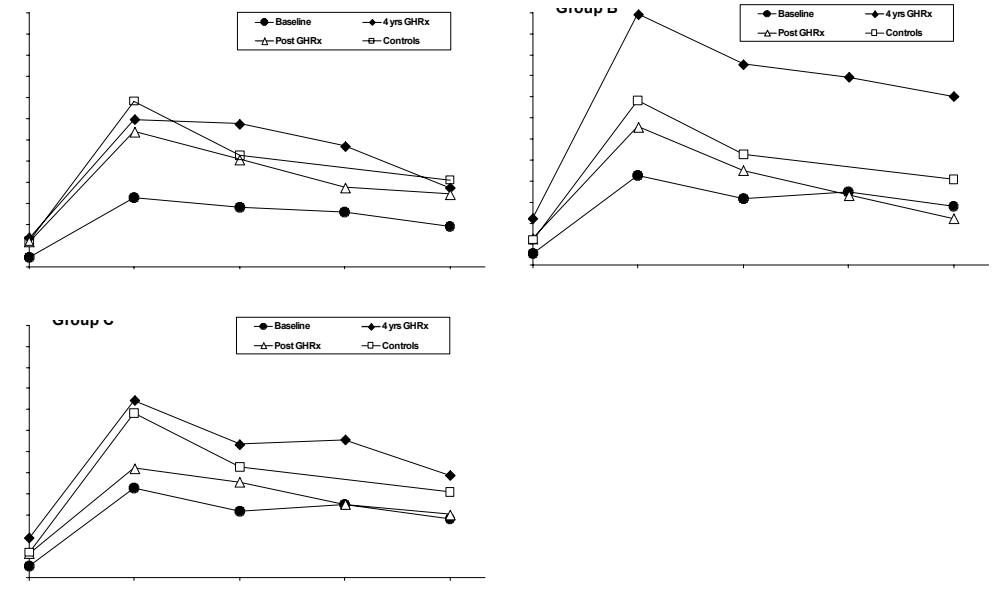


FIGURE 2. Mean insulin levels during OGTT for group A, group B and for group C, before treatment *(black circles)*, at 4 years of GH treatment *(black diamonds)*, at 6 months after discontinuation of treatment *(white triangles)*, and for the control group *(white squares)*.



Fasting insulin levels (Table 2) and the AUC for insulin at 6 months after discontinuation of GH treatment for the whole group had significantly decreased compared to 4 years of GH treatment ($P < 0.01$ after log transformation, and $P < 0.001$, respectively), after a significant rise from pre-treatment to 4 years of GH treatment ($P < 0.001$), but both remained increased compared to pre-treatment levels ($P < 0.001$ for both) (Table 2). No significant differences between GH dosage groups were found for change in time for fasting insulin levels and change in time for AUC for insulin. Compared to the control group, the AUC for insulin at 6 months after discontinuation of GH treatment for the whole TS group showed no significant difference.

The ratio for insulin to glucose at 30' (30' ratio) and 120' (120' ratio) for the whole group at 6 months after discontinuation of GH treatment decreased significantly compared to 4 years of GH treatment (both variables tested after log transformation; 30' ratio: $P < 0.01$; 120' ratio: $P < 0.001$), after an increase in both ratio's from pre-treatment to 4 years of GH treatment ($P < 0.001$ for both) (Table 2). Both the 30' ratio and 120' ratio at 6 months after discontinuation of GH treatment remained above pre-treatment values ($P < 0.001$ for both). No significant differences between GH dosage groups were found for change in time for both ratio's.

At 6 months after discontinuation of GH treatment impaired glucose tolerance (IGT) was present in 1/53 girls (2%). The IGT in this girl was not present before or during GH treatment. None of the girls developed diabetes mellitus type 1 or 2.

The HbA1c values for the whole group at 6 months after discontinuation of GH treatment had significantly decreased compared to 7 years of GH treatment ($P < 0.001$), while showing no significant differences between GH dosage groups. Throughout the years, all individual HbA1c levels remained within normal range.

From pre-treatment to 7 years of GH treatment, BMI SD-score for the whole group had increased significantly (from –0.02 (0.88) to 0.90 (0.92), $P < 0.001$). Compared to the mean BMI for the reference population (zero SD-score), BMI SD-score was not significantly different at pre-treatment, but increased to values significantly above zero ($P < 0.001$) at 7 years of GH treatment. At 6 months after discontinuation of GH treatment, BMI SD-score had continued to increase slightly, compared to 7-year values (1.13 (0.97), $P < 0.01$). BMI SD-score at 6 months after discontinuation of GH treatment and the change in time for BMI SD-score was not significantly different between GH dosage groups.

From pre-treatment to 7 years of GH treatment, systolic BP for the whole group did not change significantly, while diastolic BP showed a small decrease ($P < 0.01$), both remaining significantly higher than zero ($P < 0.001$, $P < 0.05$) (Figure 3). At 6 months after discontinuation of GH treatment systolic BP SD-score had decreased significantly compared to 7 years of GH treatment ($P < 0.05$), while the decrease in diastolic BP SD-score did not reach significance. Compared to pre-treatment, both systolic and diastolic BP SD-scores had decreased significantly at 6 months after discontinuation of GH treatment ($P < 0.001$ for both), although BP SD-scores after discontinuation of GH treatment remained significantly higher than zero ($P < 0.05$ for both).

FIGURE 3. Mean (SD) systolic blood pressure (BP) SD-score and diastolic BP SD-score using age-matched reference values, before GH treatment, at 2, 4, 7 years of GH treatment, and at 6 months after discontinuation of treatment for group A *(white bars)*, group B *(gray bars),* and for group C *(black bars)*.

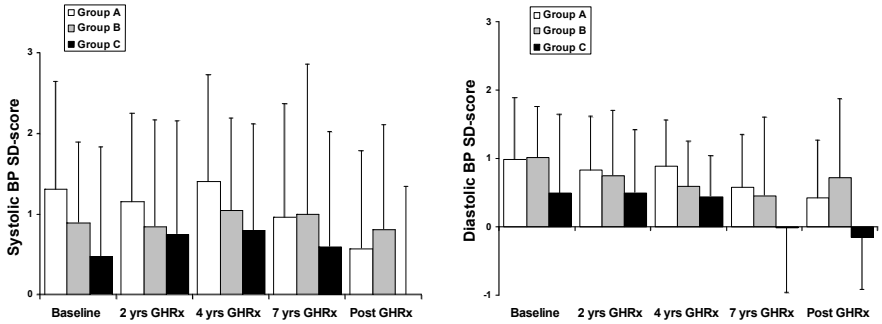


TABLE 3. Lipid levels before, during, and after GH treatment

	GH	Group A n = 19	Group B n = 17	Group C n = 20	Whole group n = 56	Controls*** n = 703
TC* (mmol/l)	Start	4.0 (0.7)	4.3 (0.8)	4.5 (0.9)	4.3 (0.8)	
	4 yrs	3.8 (0.7)	4.1 (0.8)	4.0 (0.7)	4.0 (0.7)$^{\#\#}$	
	Post	4.1 (0.8)	4.5 (0.7)	4.1 (0.6)§	4.2 (0.7)$^{\#\#}$	4.7 (0.7)$^{\&\&}$
HDL-c* (mmol/l)	Start	0.6 (0.1)	0.7 (0.1)	0.9 (0.2)	0.7 (0.2)	
	4 yrs	1.0 (0.3)	1.0 (0.2)	1.2 (0.3)	1.1 (0.3)$^{\#\#}$	
	Post	1.3 (0.3)	1.4 (0.3)	1.5 (0.4)	1.4 (0.3)$^{\#\#\$\$\$}$	1.3 (0.3)$^{\&}$
LDL-c* (mmol/l)	Start	2.6 (0.7)	2.9 (0.9)	2.9 (1.0)	2.8 (0.9)	
	4 yrs	1.9 (0.7)	2.1 (0.6)	2.2 (0.7)	2.1 (0.7)$^{\#\#}$	
	Post	2.1 (0.6)	2.5 (0.6)	2.2 (0.6)$^{@}$	2.3 (0.6)$^{\#\#\$\$}$	2.9 (0.7)$^{\&\&}$
Trigl** (mmol/l)	Start	1.0 (1.5)	0.9 (1.5)	0.9 (2.1)	0.9 (1.6)	
	4 yrs	1.3 (3.2)	1.2 (2.6)	1.2 (2.2)	1.2 (2.5)$^{\#\#}$	
	Post	1.3 (2.8)	1.0 (1.7)	0.9 (1.7)	1.1 (1.9)$^{\#\$}$	
Atherogenic index**	Start	7.1 (8.6)	6.4 (8.1)	4.9 (6.8)	6.0 (8.2)	
	4 yrs	4.0 (5.6)	4.0 (5.6)	3.2 (4.6)	3.7 (5.5)$^{\#\#}$	
	Post	3.2 (4.0)	3.2 (4.3)	2.8 (3.8)	3.0 (4.0)$^{\#\#\$\$\$}$	3.6 (4.8)$^{\&\&}$

Data expressed as: * mean (SD). ** geometric mean (90th percentile). TC = total cholesterol, HDL-c = high-density lipoprotein-cholesterol, LDL-c = low-density lipoprotein-cholesterol, Trigl = triglycerides, Atherogenic index = TC/HDL-c. *** Dutch control group of same age and sex [83].
Paired t-test for change in time for whole group: $^{\#}P < 0.05$, $^{\#\#}P < 0.001$ (vs start); $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ (vs 4 yrs);
Linear regression analysis for change from 4 yrs of GH: $^{@}P < 0.05$ (group C vs group A and B, corrected for age at start); for change from start: $^{\S}P < 0.01$ (group C vs group A and B, corrected for age at start); for control group vs whole TS group: $^{\&}P < 0.05$, $^{\&\&}P < 0.001$ (corrected for dosage group and age).

BP SD-scores at 6 months after discontinuation of GH treatment was not significantly different between dosage groups except for diastolic BP SD-score between groups B and C (lower in group C: $P < 0.05$). The changes in time for BP SD-scores, however, were not significantly different between GH dosage groups. At pre-treatment 19/53 (36%) of the TS girls had a systolic and/or diastolic blood pressure above +1.3 SD-score (~ 90th percentile for same age and sex), at 4 years 23/55 (42%), at 7 years of GH treatment 19/44 (43%), and after discontinuation of GH treatment 14/53 (26%). Eleven of the 14 girls who had a BP above the +1.3 SD-score after discontinuation of GH treatment also had a BP above the +1.3 SD-score at pre-treatment, and/or at 4 years and/or at 7 years of GH treatment.
Serum TC and LDL-c, levels after 4 years of GH treatment for the whole group decreased significantly compared to pre-treatment ($P < 0.001$ for all), while HDL-c and triglyceride levels had increased ($P < 0.001$ for both) (Table 3). After discontinuation of GH treatment, TC, LDL-c, but also HDL-c levels had increased significantly compared to 4-year levels ($P < 0.001$, $P < 0.001$, and $P < 0.01$, respectively), whereas triglyceride levels decreased significantly ($P < 0.05$). Compared to pre-treatment, TC did not change at 6 months after discontinuation of GH treatment, LDL-c had decreased and both HDL-c and triglyceride levels increased ($P < 0.001$ for LDL-c and HDL-c, $P < 0.05$ for triglyceride). Discontinuation of GH treatment resulted in a decrease in atherogenic index (TC/HDL-c), compared to 4-year values ($P < 0.001$), but also compared to pre-treatment ($P < 0.001$). The changes in serum lipid levels were not significantly different between the GH dosage groups, except for the change in time for group C (decrease in stead of increase) in TC from pre-treatment to 6 months after discontinuation of GH treatment (group C versus group A or B: $P < 0.01$ for both) and a smaller increase in LDL-c from 4 years of GH treatment to 6 months after discontinuation of treatment (group C versus group A or B: $P < 0.05$). Compared to the control group, serum TC and LDL-c levels were significantly lower in the whole TS group at 6 months after discontinuation of GH treatment ($P < 0.001$ for both), whereas HDL-c levels were significantly higher ($P < 0.05$). Furthermore, the atherogenic index was significantly lower at 6 months after GH treatment, compared to the control group (after log transformation: $P < 0.001$).
A significant correlation was found for the whole group when fasting insulin levels (log transformed) and the AUC for insulin was correlated with BMI SD-score at 6 months after discontinuation of GH treatment, after correction for GH dosage group ($r = 0.58$, $P < 0.001$; $r = 0.35$, $P < 0.05$, respectively). No correlation was found for the whole group between the 30' ratio, 120' ratio, systolic or diastolic BP SD-score, TC, atherogenic index, or triglyceride levels and BMI SD-score at 6 months after discontinuation of GH treatment. The atherogenic index at 6 months after discontinuation of GH treatment, after correction for GH dosage, did not correlate with fasting insulin levels, AUC for insulin, systolic or diastolic BP SD-score at 6 months after discontinuation of GH treatment.

Discussion

In this article we describe the effect of discontinuation of long-term GH treatment on glucose and insulin levels, body mass index, blood pressure,

and serum lipid levels in girls with Turner syndrome. We show that both fasting and stimulated insulin levels, after an increase during GH treatment, returned to normal after discontinuation. In addition, we show that after discontinuation of GH treatment both systolic and diastolic blood pressure, and the atherogenic index (TC/HDL-c) had fallen.
Fasting glucose levels increased during GH treatment and decreased after discontinuation of GH treatment, while stimulated glucose levels showed no change during GH treatment and decreased after its discontinuation. Furthermore, after ending GH treatment, insulin levels and the indices for relative insulin resistance (30' and 120' ratio's for insulin / glucose) fell, but only to a point above pre-treatment levels. Similar results have been found previously in children with idiopathic short stature and in girls with TS after discontinuation of GH treatment [84, 85]. Moreover, we show that stimulated insulin levels after discontinuation were comparable to normal post-pubertal girls. Several studies have shown that insulin sensitivity decreased during puberty, resulting in an increase in stimulated insulin levels [81, 86, 87]. Subsequently, in post-puberty, although insulin levels were decreasing, they were still at a higher level than before puberty [86]. These results might therefore imply that the reason why insulin levels and the indices for relative insulin resistance did not return to pre-treatment positions was that our study group was in its post-pubertal stage. Another explanation for the higher insulin levels and indices for relative insulin resistance after discontinuing GH might be the increase in body mass index (BMI) SD-score we found in our study group after discontinuation of GH treatment. Previous studies have shown a positive correlation between insulin levels and BMI in normal children and adults [88, 89]. Supporting this explanation, in our study, we found a positive correlation between BMI SD-score and fasting and stimulated insulin levels after discontinuation of GH treatment. Several reports, however, have shown an increased prevalence of insulin resistance and IGT in untreated women with Turner syndrome [22, 23]. Therefore, the higher insulin levels might also be a result of having Turner syndrome. The prevalence of impaired glucose tolerance in our study, however, was low (1 girl).
After discontinuation of GH treatment we found a decrease in blood pressure SD-scores, compared to pre-treatment. As this decrease has been corrected for age, it is unlikely that age could explain this decrease. A possible explanation might be the initiation of oestrogen treatment. Confirming this explanation, Gravholt et al. showed that the start of supplementation of natural oestrogens in combination with progestagens in adult women with Turner syndrome decreased ambulatory blood pressure [27]. In another study on adult Turner syndrome women, however, no change in blood pressure was found after hormone replacement therapy was initiated [90]. Another possible explanation might be a positive effect of GH treatment on blood pressure, which has been postulated to occur in children born small for gestational age [91]. Blood pressure after discontinuation of GH treatment, however, remained slightly higher than in girls matched for age. The reason why possibly lies in the fact that having Turner syndrome is a predisposition for hypertension [25]. The aetiology of the predisposition, however, remains unclear [31]. As we found a decrease in blood pressure compared to pre-treatment, it is unlikely that GH treatment was responsible for the higher blood pressure.

During the first 4 years of GH treatment we show a decrease in atherogenic index, TC, LDL-c, and an increase in HDL-c and triglyceride. Previous studies, studying lipid levels during GH treatment in Turner syndrome, showed either similar results during GH treatment [48, 49] or no effect [50]. After discontinuation of GH treatment, compared to 4-year lipid levels, TC and LDL-c levels had increased compared to the decrease we found during treatment, while triglyceride levels had decreased slightly after an increase during GH treatment. Similar results were found in reports on the effect of discontinuation of GH in GH deficient adolescents [92, 93], thus possibly implying a GH-effect. HDL-c levels after discontinuation of GH treatment, however, showed a further increase. A possible explanation for this could be the induction of puberty with natural oestrogens and dydrogestagen, which has been shown to lead to an increase in HDL-c [94]. A second explanation for the increase in TC, LDL-c and HDL-c after discontinuing GH might be the age-effect as it has been established that after puberty, TC, LDL-c and HDL-c increase with age [95, 96]. Interestingly, when we compared lipid levels after discontinuation of GH treatment, to a normal control group of similar age, we found that TC, LDL-c and AI levels were lower and HDL-c levels were higher in our study group. This indicated that, while previous reports on lipid levels in untreated girls and women with TS have shown conflicting results regarding the prevalence of dyslipidemia [26, 28, 29, 97], in our study group after long-term GH treatment, we found no evidence of dyslipidemia.

As several studies have found that women with TS are predisposed to develop cardiovascular disease (CVD) [24, 25], and several risk factors for CVD, such as high blood pressure, dyslipidemia, and abdominal obesity, have been found to be more prevalent in TS, we analyzed our data for clustering of these CVD risk factors by way of correlations. Although, after discontinuation of GH treatment, we found a positive correlation between BMI SD-score and insulin levels, we could not detect any other correlations between the risk factors. In contrast, in a study on the effect of discontinuation of GH treatment in children of similar age, but born small for gestational age, a positive correlation between the atherogenic index, BMI SD-score, systolic and diastolic blood pressure SD-score, and fasting insulin was found [98]. The lack of correlation between the cardiovascular risk factors in our group with Turner syndrome, however, suggested that in this group no clustering of risk factors was present. As a clustering of risk factors potentially increases the risk for cardiovascular disease [99], not only follow-up of all risk factors, but also evaluation of clustering should take place in the future. While we did not find evidence of clustering, we did find a positive correlation between insulin resistance and BMI. Although this relation is also found in normal children [89, 100], insulin resistance does predispose for the development of diabetes mellitus type 2. We would therefore urge clinicians to do their utmost to prevent further weight gain in girls and women with TS.

In conclusion, after discontinuation of long-term GH treatment in girls with TS the GH induced insulin resistance disappeared. Blood pressure decreased both during and after discontinuation of GH treatment but remained higher than in the normal population, while lipid levels and the atherogenic index after discontinuation of GH treatment, were more beneficial regarding the development of cardiovascular disease than in a normal control group.

CHAPTER 4

Part 'Turner Syndrome': Psychosocial functioning after discontinuation of long-term growth hormone treatment in girls with Turner syndrome

Psychosocial functioning after discontinuation of long-term growth hormone treatment in girls with Turner syndrome

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Abstract

It is common practice in the case of Turner syndrome (TS) to treat short stature with GH treatment and to induce puberty with oestrogens at an age as close to normal puberty as possible. This approach in most cases leads to a height in the normal range in childhood, adolescence, and adulthood in TS. Little data is available, however, on its effect on psychosocial functioning. In the present study, we evaluated psychosocial functioning in a group of 50 women with TS, after reaching final height in two multi-centre GH trials. Thirty-six girls participated in a randomised dose-response study (DRS) from mean (SEM) age 6.8 (0.4) years, and 14 girls participated in a frequency-response study (FRS) from age 13.2 (0.4) years. After discontinuation of long-term GH treatment, these 50 girls were evaluated for psychosocial functioning at a mean age of 18.8 (0.3) years. GH was given in a dosage of 4 IU/m^2/d (~ 0.045 mg/kg/d), 6 IU/m^2/d, or 8 IU/m^2/d. After a mean GH treatment duration of 7.1 (0.4) years mean final height (ref. normal girls) was –1.2 (0.2) SD-score.
Behavioral problem scores (Achenbach) of the TS women were comparable to normal Dutch peers. Although self-perception (Harter total scale: $P < 0.01$), and bodily attitude (Baardman: $P < 0.05$) was significantly less positive than for their normal Dutch peers, we found no evidence of depression. TS women rated their family functioning higher than their Dutch peers ($P < 0.0001$), and had a slightly different coping pattern.
These results show that even after reaching a height in most cases within the normal range and puberty induction at a pubertal age, some women with TS still experience psychosocial problems. It is likely, however, that GH and oestrogen treatment improved psychosocial functioning. Long term follow-up of these GH-treated patients will allow an evaluation of their life achievements.

Introduction

The most frequent clinical characteristics of Turner syndrome (TS) are short stature and the absence of spontaneous pubertal development. In most

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countries it is common practice to treat short stature in TS with GH treatment in a supra-physiological dosage. In addition, oestrogens are given to induce puberty at an age as close to normal puberty as possible. This approach has been shown to increase and even normalize height in childhood, adolescence, and adulthood in TS [41, 76, 101-103]. Little data is available, however, on the effect of this treatment strategy on psychosocial functioning. Untreated girls with TS have been described as being more immature, having a lower self-esteem, poor concentration, and being hyperactive [33, 34].
In the present study, we evaluated psychosocial functioning in a group of 50 women with TS, after reaching final height in two multi-centre GH trials.

Methods

GH group
All women who had participated in two GH trials (see below), had discontinued GH treatment for more than 6 months, and were able to fill in the questionnaires, were asked to participate in the psychosocial evaluation. Fifty women agreed to participate (response rate 50/69: 72%). Nineteen girls did not participate either because of practical reasons or because of losing interest in participating in a study.
Both GH trials evaluated the effect of GH on long-term growth and ultimately on final height. At time of the psychosocial evaluation, all participants were prescribed hormone replacement therapy in an adult dose.

Dose-response GH-trial
Sixty-eight previously untreated Dutch girls with TS, aged between 2 and 11 years, were enrolled in an open randomised multi-centre GH dose-response study. Biosynthetic GH (r-hGH NorditropinR, Novo Nordisk A/S, Denmark) was given subcutaneously once daily in a dosage of 4 IU, 6 IU , or 8 IU GH/m2 body surface area/day (~ 0.045-0.09 mg/kg /day). Puberty was induced at age 12 years (Study design as described previously [103]). Six girls dropped out of the study and were lost to follow-up. Six girls had not discontinued GH treatment for more than 6 months and 4 girls were unable to fill in the questionnaires due to mental retardation, leaving 52 girls able to participate in the psychosocial evaluation.

Frequency-response GH-trial
Nineteen previously untreated Dutch girls aged 11 years or older, with TS were enrolled in an open randomised multi-centre GH frequency-response study. Biosynthetic GH (r-hGH NorditropinR, Novo Nordisk A/S, Denmark) was given subcutaneously once or twice daily in a total dosage of 6 IU GH/m2/d. Puberty was induced at start of trial (Study design as described previously [41]). Two girls were unable to fill in the questionnaires due to mental retardation, leaving 17 girls able to participate in the psychosocial evaluation.
The GH trials and the psychological evaluation were approved by the Ethics Committees of the participating centres in the Netherlands. Written informed consent was obtained from the parents or custodians of each child.

Normal population sample
From a randomly selected population sample from three municipalities in the Netherlands (N=600, response rate 56%) only the females were selected for comparison (N=359) [104].

Psychosocial evaluation
In the GH group a psychosocial evaluation was performed after GH treatment had been discontinued for at least 6 months and (near) final height had been reached. Questionnaires for the GH trial groups and the Population sample were sent by post.

General information. Data on occupational and educational levels were provided by both parents and adolescents. Parental occupational level (SES) ranged from 1 (lower occupation) to 3 (higher occupation). When both parents were employed the highest of both SES levels was used. For unemployment the lowest SES was used [105].

Behavioral problems (YSR/YASR). Behavioral problems were measured by 3-point scale standardized questionnaires from Achenbach, translated and validated in the Dutch language [104, 106, 107]. For girls aged between 12 and 18 years, the 119-item Youth Self-Report (YSR; filled in by child) [108, 109] was used. For girls aged 18 years and older the 127-item Young Adult Self-Report (YASR; filled in by adolescent) was used [110]. As both questionnaires were constructed in a similar way, results from three scales could be combined for analysis (Internalizing, Externalizing, and Total Problem score). To allow combination of test scales (YSR/YASR), z-scores were constructed using the Population sample data as reference [104]. A higher test z-score indicated more problem behavior.

Self-perception (HSPP). The inventory, called in Dutch "Hoe ben ik" and in English "Harter Self Perception Profile", was designed by Harter to describe sense of self-worth and capability in several areas, using 4-point scales [111, 112]. In the evaluation the 45-item adolescent-version (HSPP-a) was used. Ten scales (Scholastic competence, Social acceptance, Athletic competence, Physical appearance, Behavioral conduct, Global self-worth, Romantic appeal, Close friendship, Job competence, and Total HSP-score) were used for analysis (median $\alpha = 0.76$). A higher test score indicated a more favorable self-perception.

Child Depression Inventory (CDI). The American Child Depression Inventory was designed to measure depressive thoughts and feelings in a child population and was designed by Kovacs [113]. Factor analysis of the Dutch version of the 27-item (3-point scales) "Gevoelens en Gedachten vragenlijst" produced one scale, the 'Total depression scale score' ($\alpha = 0.86$) [104]. A higher score indicated more depressive thoughts and feelings.

Body Attitude Scale (BAS). The Dutch questionnaire "Lichaamsbelevings vragenlijst" is a 45-item questionnaire and uses a 5-point Likert scale to assess bodily attitude. It has been constructed and validated by Baardman et al [114]. In an adolescent population three scales could be distinguished by factor analysis: 'Appraisal' (α = 0.94; e.g. 'Are you satisfied with the way your body looks?'), 'Attribution' (α = 0.88; e.g. 'Do you think people avoid you because of your appearance?'), and 'Physical contact' (α = 0.77; e.g. 'In general, how much do you like touching people?') [104]. High test scores indicated positive bodily attitudes.

Family Assessment Device (FAD). This shorter 12-item version of the General Functioning Subscale of the McMasters Family Assessment Device measured overall family functioning, using a 4-point scale. Reliability and validity were tested in the original language [115] and in Dutch [116]. A higher test score indicated more positive family functioning.

Coping (UCL). The "Utrecht Coping list" [117] was designed and validated in Dutch [118, 119]. The 47-item 4-point scale list was made to measure 7 ways of coping with stressful events: Active approach (e.g. 'In general, if I have a problem I tackle the problem immediately), Reassuring thoughts (e.g. 'In general, if I have a problem I encourage myself'), Expression of emotion (e.g. 'In general, if I have a problem I show I am annoyed'), Palliative reaction (e.g. 'In general, if I have a problem I seek distraction from it'), Passive reactional pattern (e.g. 'In general, if I have a problem I see dark clouds'), Seeking social support (e.g. 'In general, if I have a problem I share it with someone'), Avoiding/Anticipating (e.g. 'In general, if I have a problem I leave it the way it is')(median α = 0.69). A higher test score indicated the coping style was more prominent.

Statistical analysis

All data were expressed as mean (SEM) unless otherwise specified. Differences in SES between the Population sample and the GH group were analyzed by logistic regression analyses. To analyze differences in results between the GH group and the Population sample regression analyses were used, corrected for age, GH trial (1 dummy variable: FRS=1) and GH dosage (2 dummy variables). SES (2 dummy variables) was only corrected for when significant. The effect FH, corrected FH, and the increase in height from start of GH treatment were estimated by the addition of final height SD-score, corrected FH SD-score and height SD-score at start to the regression models, with correction for age, GH trial, and GH dosage. Results from the regression analyses were shown as unstandardized coefficients (B) with their two-tailed p-values. All calculations were done by SPSS 9.0. A p-value of 0.05 was considered significant for comparison between the GH group and the normal sample. A p-value < 0.01 was considered significant for within GH group comparison because of multiple testing.

Results

In Table 1 clinical data of the GH trials are shown for all women that participated in the psychosocial evaluation. We found no significant differences in clinical data between TS women participating in this psychosocial study and TS women who did not participate. Psychosocial data for the DRS and FRS were analyzed together (GH group). Correction for GH trial (FRS or DRS) and GH dosage did not have a significant influence on results.

Social economic status (SES)
In the GH group 35% had a low SES level, 20% an intermediate level, and 46% a high level. The differences in SES between the GH group and the Population sample (33/34/33%, respectively) were not significant. Correction for SES did not significantly change any of the following results.

Behavior (YSR/YASR)
Internalizing, Externalizing, and Total problem behavior SD-scores were comparable to the population sample mean (Table 2).

Self-perception (HSPP)
Total HSP-scores (Table 3) were significantly lower than the Population sample scores ($B=-0.30$, $P < 0.01$), while GH dosage had no significant effect. To explain this result, we examined the remaining scale-scores, which are the components of the Total HSP-scores. Social acceptance, Athletic competence, and Romantic appeal scores were also significantly lower than the Population sample scores ($B=-0.63$ $P < 0.001$, $B=-0.55$ $P < 0.01$, $B=-0.40$ $P < 0.05$, respectively). The remaining scale-scores were not significantly different to the Population sample scores.
Child Depression Inventory (CDI)
Total depression scale scores were not significantly different to the Population sample scores (Table 4).

Body Attitude Scale (BAS)
Attribution and Physical contact were scored slightly but significantly lower than the Population sample ($B=-0.26$ $P < 0.05$, $B=-0.41$ $P < 0.05$, respectively; Table 4). Appraisal scores, however, were not significantly different to the Population sample scores.

Family Assessment Device (FAD)
The Total FAD scale scores were significantly higher than the Population sample scores ($B=0.77$ $P <0.0001$; Table 4).

Coping (UCL)
Compared to the Population sample scores, only the scale Reassuring thoughts was significantly higher in the dose-response group ($B=0.32$ $P < 0.05$). Scores for the scales Active approach, Expression of emotion, Palliative reaction, Passive reactional pattern, Seeking social support, and Avoiding/Anticipating were not significantly different to the Population sample (Table 5).

TABLE 1. Mean (SEM) clinical data for the girls who participated in the psychosocial evaluation and for the Population sample.

GH	Dose-response group: 4-8 IU/m^2/day	Frequency-response group: 6 IU/m^2/day	Population sample
Number of girls	36	14	359
Chronological age at start GH trial (yr)	6.8 (0.4)##	13.2 (0.4)	- -
Height SD-score at start (ref. Normal Dutch girls)	-2.7 (0.2)	-3.2 (0.3)	- -
Final height SD-score (ref. Normal Dutch girls)	-1.0 (0.2) #	-1.9 (0.2)	- -
Target height SD-score	-0.2 (0.2)	-0.3 (0.2)	- -
Age start puberty (B2)(yr)	12.7 (0.1)	13.2 (0.4)	- -
GH duration (yr)	8.5 (0.3)##	3.6 (0.2)	- -
Karyotype: 45,X	30 (83%)	10 (71%)	- -
Karyotype: other	6 (17%)	4 (29%)	- -
Age at psychosocial evaluation (yrs)	18.2 (0.4)#	20.4 (0.4)**	17.1 (0.2)

GH = growth hormone. One-way ANOVA: * P < 0.05 ** P < 0.001; two-tailed (GH group versus Population sample) # P < 0.01 ## P < 0.001; two-tailed (Dose-response trial versus Frequency-response trial).

TABLE 2. Mean (SEM) of the SD-scores of the YSR/YASR questionnaires for self-reported behavioral problems.

	GH group	Population sample
Internalizing SD-score	0.3 (0.1)	0.0 (0.1)
Externalizing SD-score	-0.1 (0.1)	0.0 (0.1)
Total problem behavior SD-score	0.1 (0.1)	0.0 (0.1)

Regression analyses, corrected for age, GH trial, and GH dosage: No significant differences (GH group versus Population sample).

Correlations between tests
To assess possible relations between the results, which were significantly different from the Population sample, partial correlations were done, corrected for GH dosage. The Total HSP score was significantly correlated to the Total FAD score ($r=0.38$, $P = 0.01$), the BAS Attribution score ($r=0.61$, $P < 0.001$) and the BAS Physical contact score ($r=0.57$, $P < 0.001$). No correlation was found between the Total HSP score and the UCL Reassuring thoughts score. Total FAD score was significantly correlated with the BAS Physical contact score ($r=0.46$, $P = 0.001$), while no correlation was found between Total FAD score and the BAS Attribution score, or the UCL Reassuring thoughts score.

Effect of Final Height
Final height (FH) SD-score or corrected FH SD-score, with or without correction for height SD-score at start, showed no significant effect on Behavioral SD-scores, Self-perception (Total HSP) scores, Depression scores, Bodily Attitude scores or Family functioning scale (FAD) scores. Correction for height SD-score at start did not affect results.

Discussion

Our study presents psychosocial functioning results of women with Turner syndrome (TS) after reaching final height (FH) in two GH trials. We measured psychosocial functioning by standardized questionnaires on behavioral problems, self-perception, depression, bodily attitude, family functioning, and coping. We show that after long-term GH treatment behavior of the TS women was comparable to normal Dutch peers. In contrast, we show their self-perception, and their attitude towards their bodies was slightly less positive than for their normal Dutch peers. We found no evidence of increased symptoms of depression, TS women rated their family functioning higher, and had a slightly different coping pattern compared to their Dutch peers.
We show that self-rated problem behavior scores did not significantly differ from normal Dutch peers. Although we did not evaluate psychosocial functioning before start of GH treatment in our TS group, many studies have found that untreated adolescent girls with TS have more problem behavior than normal girls [53, 120]. Our results therefore suggest an improvement in problem behavior, after reaching a final height within the normal range for most and puberty induction at a pubertal age. According to Ross et al. improvement in (parent-reported) behavioral problems could be explained by the oestrogen substitution alone [34]. In other studies, similar improvement in behavior can be found after several years of GH treatment [52, 121].
Regarding self-perception, our results show that after reaching a final height, in most cases, in the normal range while puberty was induced at a relatively normal age (12 years in the majority), self-perception total score was significantly lower than in normal Dutch girls. We found that the TS women feel they are less socially accepted, are less athletic, and have a lower romantic appeal than normal girls.

TABLE 3. Mean (SEM) of the 10 scale scores of the Harter Self-perception profile.

	GH group	Population sample
Scholastic competence	2.7 (0.1)	2.9 (0.0)
Social acceptance	2.5 (0.1)***	3.0 (0.0)
Athletic competence	1.9 (0.1)**	2.5 (0.0)
Physical appearance	2.3 (0.1)	2.6 (0.0)
Job competence	3.1 (0.1)	3.2 (0.0)
Romantic appeal	2.2 (0.1)*	2.5 (0.0)
Behavioral conduct	3.2 (0.1)	3.1 (0.0)
Close friendship	3.1 (0.1)	3.3 (0.0)
Global self-worth	2.8 (0.1)	3.0 (0.0)
Total HSP	2.6 (0.1)**	2.9 (0.0)

Regression analyses, corrected for age, GH trial, and GH dosage:
* P < 0.05 ** P < 0.01 *** P < 0.001; two-tailed (GH group versus Population sample).

TABLE 4. Mean (SEM) of the scores for the Child Depression Inventory (CDI), the Bodily Attitude Scale (BAS), and the Family Assessment Device (FAD).

	GH group	Population sample
CDI Total depression scale	1.3 (0.0)	1.3 (0.0)
BAS Appraisal	3.6 (0.1)	3.7 (0.0)
BAS Attribution	4.4 (0.1)*	4.4 (0.0)
BAS Physical contact	3.4 (0.1)*	3.5 (0.0)
FAD Overall Family functioning	3.3 (0.1)***	2.5 (0.0)

Regression analyses, corrected for age, GH trial, and GH dosage:
* P < 0.05 *** P < 0.001; two-tailed (GH group versus Population sample).

TABLE 5. Mean (SEM) of the 7 scale scores of the Utrecht Coping List

	GH group	Population sample
Active approach	2.3 (0.1)	2.4 (0.0)
Reassuring thoughts	2.5 (0.1)*	2.4 (0.0)
Expression of emotion	2.0 (0.1)	2.2 (0.0)
Palliative reaction	2.4 (0.1)	2.2 (0.0)
Passive reactional pattern	1.7 (0.1)	1.7 (0.0)
Seeking social support	2.6 (0.1)	2.3 (0.0)
Avoiding/Anticipating	2.1 (0.1)	2.1 (0.0)

Regression analyses, corrected for age, GH trial, and GH dosage:
* P < 0.05; two-tailed (GH group versus Population sample).

Similar finding have been reported in untreated girls and women with TS [122, 123]. Several studies, however, have shown that treatment with oestrogens and/or GH treatment improved self-perception [34, 52, 53]. Whether, in our study, scores for self-perception were even lower before start of GH treatment, we cannot say. But, even if scores had improved during the GH trials, they did not normalize. A possible reason why self-perception remained lower than normal could be the insecurity brought about by having typical physical TS features. Another reason might be the incidental observation of both parents and clinicians that some girls seem to lack social graces. To substantiate this observation, a recent study showed TS women have an impairment in recognizing emotions on someone's face compared with normal women, possibly indicating anomalies in amygdala function [124].
Another reason why our TS group feels less athletic might be because they actually have a restriction in movement. Nijhuis- van der Sanden et al. found that, although TS girls move with the same accuracy as their normal peers, they move with a significantly lower speed and conclude that TS girls have a problem in execution of movement [125]. Due to the age of the participants (mean age of 18 years) the role of infertility was not investigated. Based on our professional experience, infertility has a larger role on self-perception later in life.
Several studies have found evidence of depression in TS women who were untreated or only treated with oestrogens [36, 126]. In our group of TS women, after long-term GH treatment and oestrogen substitution at a pubertal age, the results of the questionnaire show no significant signs of depression. A previous study on adolescent girls with TS treated with GH (100%) and oestrogen (61%) described severe depressive symptoms in 20% [127] of the girls. The factor related to these symptoms was teasing by peers about their physical appearance. Rickert et al., however, did not show any data on height gain or pubertal development during GH and oestrogen treatment. Therefore, the discrepancy in results might be explained by differences in height gain or pubertal development. Since we found no evidence of depression, this might indicate the girls suffered less from teasing as a result of adequate GH and oestrogen treatment.
Furthermore, our results show that after GH treatment and puberty induction TS women appraise their bodies similarly to their peers. Other studies have shown that TS girls and women when untreated score their physical appearance significantly lower than their peers [122, 128]. Interestingly, the TS women have a small but significant tendency to attribute problems to their appearance and avoid physical contact. Although our group was adequately treated with GH and oestrogen, we did not treat the typical physical TS features, other than short stature and absent pubertal development. It is therefore not surprising that they still feel insecure about their appearance.
Previous studies found girls with TS are often overprotected by their parents [52]. Overprotection, however, is also seen in other patient groups with short stature [129]. One of the reasons for parents to overprotect is lack of peer relations of their children. On the other hand, children with poor peer relations rely more on family functioning [130]. Several studies describe an increase in family functioning, parallel to an increase in self-concept and a decrease in behavioral problems during GH treatment [52, 53]. In our study, after reaching a final height within the normal range in most cases and

puberty induction at a relatively normal age, we found that TS women have a better family functioning than peers. This might indicate that these TS women still lack peer relations and take refuge in their family as a way of coping with problems [53]. Another explanation might be that, as a result of past medical and psychosocial problems these TS women and their family have faced, their family actually functions better than peer families. Rovet et al, in their controlled GH trial, found an increase in family functioning and explained this as a greater involvement of the parents due to the GH treatment [52].

Commonly, girls and women with TS are described as withdrawing from social interaction as a way of coping with problems [53, 131]. Interestingly, we show that women with TS, after long-term GH and oestrogen treatment, have only a slightly different coping strategy than their peers. They used more Reassuring thoughts to cope with problems, while they used strategies such as Avoiding problems but also Active approach and Expression of emotions as often as their peers. These results seem to indicate that, in general, TS women, after adequate GH and oestrogen treatment, have a 'normal' coping strategy.

When we looked at the relation between our results, we found that TS women, who have a good self-perception, also have a good family functioning, attribute problems less to their appearance and enjoy physical contact. These results strengthen the common suggestion that these TS women rely on family functioning more than other women [130], which for them leads to a more favorable self-perception and bodily attitude. Previous studies have described that the TS women rely on their families more than other women as a way to avoid problematic situations [53, 130]. In the GH group, we show that these women avoid problems similarly to their peers. As mentioned before, several studies show more behavioral problems in untreated TS girls and a decrease in problems during GH treatment [52, 53]. Similarly, for self-perception, an improvement has been found during GH and oestrogen treatment [52, 53]. Although it is therefore likely that in our study, GH and oestrogen treatment has had a positive effect on psychosocial development, we did not find significant relationships between test scores and (corrected) final height or a GH dosage effect. This might also indicate that GH treatment did not influence psychosocial functioning. Another explanation for this might be that the GH treatment regimen in the trials, regardless of the dosage, achieved its optimal effect on psychosocial functioning. Similarly, in short children born small for gestational age, while longitudinal results show a significant increase in psychosocial functioning during GH treatment, results did not differ between GH dosage groups [132]. In the present study, however, we are unable to show the extent of the effect of GH treatment, as we have no psychosocial data of start of the GH trials.

In conclusion, after long-term GH treatment behavior of the TS women was comparable to normal Dutch peers. Although their perception of themselves and their attitude towards their own bodies was slightly less positive than for their normal Dutch peers, we found no evidence of depression. In addition, TS women rated their family functioning higher, and had a coping pattern very similar to their Dutch peers. TS women with a more positive self-perception, also had a better attitude towards their bodies and had a better functioning family. These results show that even after reaching a height

within the normal range and puberty induction at a pubertal age, some women with TS still experience psychosocial problems. It is, however, likely that GH and oestrogen treatment improved psychosocial functioning. Long term follow-up of these GH-treated patients will allow an evaluation of their life achievements.

CHAPTER 5

Part 'Turner Syndrome': General Discussion and Conclusions

General Discussion and Conclusions

In this chapter we discuss the results of a randomised multi-centre dose-response growth hormone (GH) trial evaluating the efficacy, safety and psychosocial effect of long-term GH treatment in Turner syndrome (TS). The TS trial consisted of 68 untreated girls with TS who participated from 1989 in a GH trial using 4, 6, or 8 IU GH/m^2/day (~ 0.045, 0.067, or 0.090 mg/kg/day). Eleven paediatric departments in The Netherlands participated in this trial. In this chapter, we start by discussing the results of the TS trial. Finally, conclusions are drawn from the discussion and recommendations for future research are given.

Evaluation of the effect of long-term GH treatment on final height in 60 girls with TS treated in a randomised dose-response trial comparing 3 dosage schedules

Although GH treatment for short stature in TS is now an accepted treatment in many countries, reports on final height are inconsistent and which dosage to use is an issue of debate [41, 43]. Previously, Th. Sas, in his thesis entitled "Long-term growth hormone treatment in two growth disorders", Rotterdam 1999, demonstrated that long-term GH treatment in TS leads to normalisation of height during childhood and adolescence [42]. In this thesis, in chapter 2 we showed that 83% of the 60 TS girls, who started GH treatment at a mean age of 6.6 years, reached a normal final height (FH SD-score above -2), while 63% reached a final height within their target height range (TH ± 1.3 SD). The mean final height using a conventional GH dosage of 4 IU/m^2/day was 157.6 cm, which is equal to an SD-score of -1.6 when using references for normal Dutch girls. Final height varied between 150.4 cm and 171.7 cm (5th and 95th percentile). The mean gain in final height varied between 11.9 cm and 16.9 cm, depending on the GH dosage used (estimation based on Lyon's predicted final height, adjusted for Dutch girls with TS). For all three dosage-groups together, gain in final height varied between 7.2 cm and 20.4 cm (5th and 95th percentile). These results are not confirmed by most other studies on final height in TS [43, 71]. They found a considerably lower gain in final height. Other studies, however, starting GH treatment at a younger mean age, also showed a greater gain in final height [60, 73, 74, 133]. In addition, we found that in a regression model a lower age at start predicted a greater gain in final height. Therefore, one of the reasons for these conflicting results probably lies in the age at which GH treatment is started. Other reasons might be the higher dose of oestrogens used in most studies and/or the difference in oestrogen-free GH treatment period (see next paragraph).
A second factor that predicted final height gain was the GH dosage. Our study showed that using a dosage of 6 or 8 IU/m^2/day instead of 4 IU/m^2/day significantly increased gain in final height. Using a dose of 8 IU/m^2/day, however, showed no advantage over 6 IU/m^2/day for gain in final height. Two non-randomised studies showed a similar dose-dependent increase in final height gain [75, 76]. A higher GH dosage of 6 IU/m^2/day, however, leads to a 50% increase in cost of treatment. But does the extra

gain in final height of approximately 3 ½ cm justify the increase in cost? Previously, we mentioned our finding that age at start predicted final height gain. Therefore, one justification for using a higher GH dosage could be to prevent a disappointing final height when GH treatment is initiated at an older age. In addition, a higher GH dosage also leads to higher IGF-I levels. We showed that at 7 years of GH treatment the highest dosage group had significantly higher IGF-I levels compared to the lowest dosage group. Recent studies have shown a potential danger of having high circulating IGF-1 levels. One study found that men (Physicians' Health Study) with higher IGF-1 levels in their blood had an increased risk of developing colorectal cancer after 14 years follow-up [78]. Another study found that after 7 years of follow-up, high circulating IGF-1 levels in nurses (Nurses' Health Study cohort) were associated with an increased risk of breast cancer [79]. These studies, however, did not show a causal relationship but only an association between high circulating IGF-1 levels and cancer. Therefore, whether GH-induced high IGF-I levels during childhood and adolescence might lead to an increased risk of cancer remains to be investigated. Pending these investigations, however, high dosages of GH should be given with caution.

Assessment of the effect of low dose oestrogen treatment begun at a relatively young age on final height in girls with TS during long-term GH treatment

Which age to start puberty induction is also still an issue of debate. Several paediatricians believe oestrogen treatment should be postponed as long as possible to achieve the highest possible height gain. Both research and professional experience of paediatricians, however, have shown the psychological importance of inducing puberty at an age as close as possible to their peers [34, 134]. In chapter 2 we show that starting a low dose of natural oestrogens at a normal pubertal age of 12 ½ years did not have a negative effect on height velocity and on bone maturation. Other studies, in contrast, found a decrease in height velocity and an increase in bone maturation leading to a decrease in height gain [73, 77]. An explanation for the different results might be the higher dose of oestrogens used. Another might be the difference in time-period between start of GH treatment and start of oestrogen treatment. In our study oestrogen treatment was started after at least four years of GH treatment while height had already increased by almost 2 SD-score. As most of the catch-up had already occurred before oestrogen treatment was initiated, a possible small negative effect might become less important. Confirming this explanation, Reiter et al. found that a longer duration of oestrogen-free GH treatment strongly predicted a higher gain in near final height [74]. Massa et al., however, in an analysis of the Belgian growth database of GH-treated TS girls, showed that, when using a low dose of ethinyl oestradiol, not the oestrogen free period, but the duration of treatment with GH and oestrogen predicted final height outcome in older TS girls (mean age of 11.6 years at initiation of GH treatment) [135]. In a Dutch study by Sas et al. in 19 girls with TS starting GH at an older age (mean (SD) 13.6 (1.7) years) mean (SD) attained final height was 155.5 (5.4) cm, while 13 of the 19 girls had a final height gain of more than 5.0 cm. In this study puberty was induced from start of GH treatment by a low dose of oral ethinyl

oestradiol. This study shows that simultaneous initiation of GH and low dose oestrogens, can lead to a substantial gain in final height without having to postpone puberty [41]. Therefore, the initiation of oestrogen treatment in older TS girls does not have to be postponed to a later age. Possible factors that might lead to reconsider postponing puberty in individual cases might be a very small stature and/or an older bone age [41]. In these cases, however, the individual psychological importance of inducing puberty at an age as close as possible to their peers, should also be taken into consideration.

Evaluation of several parameters to predict final height SD-score to aid individual treatment with GH in girls with TS during long-term GH treatment

When analysing the factors most likely to influence final height SD-score, we found that a regression model containing height SD-score at start, GH dosage, age at start, and first year height velocity explained 76 percent of the variation in final height SD-score. Our study showed that an increase in GH dosage might be worthwhile when a TS girl, for her age, has a low height SD-score at start, and/or a low height velocity in the first year of GH treatment. Without the influence of height SD-score at start and first year height velocity, age at start and final height SD-score showed a negative relationship. Therefore, another reason for increasing the GH dosage might be an older age at start of GH treatment. In other studies the same factors were found to predict final height or height gain after GH treatment [41, 133, 136]. In contrast, in an analysis of the growth database of GH-treated TS girls, one study found no relationship between age at start GH treatment and final height outcome [135]. Since this result has not been confirmed by other studies, it should not lead to postponing GH treatment. Both clinical observations and research have shown clear psychosocial implications of short stature during childhood in TS girls [52, 53]. Parameters that did not predict final height, among others, were karyotype (45, XO or other) and the increase in alkaline phosphatase in the first year of GH treatment. Several studies on natural growth in TS girls found a positive correlation between Xp disomy and final height [137, 138]. In our study only 9 girls had a karyotype other than 45, XO, all having different mosaic karyotypes. Therefore, whether GH treatment in girls with Xp disomy leads to a higher final height, we cannot say.
By filling in the different parameters, the model can also be used to aid clinicians to decide which dosage to use. For example, a girl with TS, with a height SD-score at start of GH treatment of −3, a first year height velocity of 10 cm/yr (1 year conventional dose of 4 IU/m^2/d), and an age at start of 6 years, would attain a final height SD-score of −1.5 when the GH dose is not increased, and a FH SD-score of −0.8 or −0.7 when the GH dose is increased by 50 or 100 percent. Depending on one's goal, for instance achieving a normal final height or reaching the target height range, the GH dosage could be adjusted accordingly. A higher GH dosage, however, leads to a 50-100% increase in cost, while the cost of long-term GH treatment is already considerable. In addition, a higher GH dosage also leads to a higher IGF-I SD-score while the long-term effects of GH-induced high IGF-I levels on the development of cancer remain to be investigated [78, 79]. To further optimize the model for clinical use it is crucial to validate it on other TS patient groups.

Although the model has a high prediction percentage, it does leave 24 percent to be explained by unknown factors. Furthermore, the predicted FH SD-score has a large prediction interval (residual SD of 0.54). A possible factor that may have contributed to the variation in final height and height gain is the origin of the X-chromosome (paternal or maternal). Chu at al. found a strong correlation between height SD-score of TS girls and their mothers, when the TS girls X-chromosome originated from their mother [139]. Other factors that may have contributed are unknown genetic aberrations, or non-compliance.

Investigation of carbohydrate metabolism in girls with TS after discontinuation of long-term GH treatment in a dose up to 8 IU/m^2/day (~ 0.090 mg/kg/day)

As GH treatment increases insulin levels, several authors have expressed their concern regarding long-term effect of GH treatment in children with a predisposition for diabetes mellitus such as girls with TS [25, 45, 46]. In chapter 3 we show that in TS girls, the increased fasting and stimulated insulin levels returned to normal after discontinuation of long-term GH treatment. Similarly, fasting glucose levels increased during GH treatment and decreased after discontinuation of GH treatment, while stimulated glucose levels showed no change during GH treatment. In addition, we found no evidence of a dose-dependent increase in glucose or insulin levels between the GH dosage groups.
Although insulin levels after discontinuation of GH treatment remained above pre-treatment levels, results were comparable to a similar aged group of normal girls [83]. The reason for the rise in stimulated insulin levels, both in normal and in TS girls after GH treatment, is that during puberty insulin sensitivity decreases [81, 86, 87]. A number of authors, however, have described an increased prevalence of insulin resistance in untreated women with Turner syndrome [22, 23]. Therefore, although the TS girls might have had a pubertal insulin resistance, the higher insulin levels might also be a result of having TS. In contrast, one study demonstrated that TS women (n=49), by comparing results of intravenous glucose tolerance tests (IVGTT) to those of age and body mass index matched normal women, have an increased insulin sensitivity and a reduced stimulated insulin response [30]. This might point to beta-cell dysfunction as a cause for impaired glucose tolerance and DM in TS.

Investigation of several factors that may predict development of cardiovascular disease, such as blood pressure, body mass index and blood lipid levels in TS women after discontinuation of GH treatment

Besides DM, girls with TS are also predisposed to develop cardiovascular disease (CVD). CVD is thought to be the main cause of death in TS [24, 25]. In addition, risk factors for CVD, such as hyperlipidaemia, hypertension, obesity, and impaired glucose tolerance occur more often in TS [25-28]. In chapter 3, after discontinuation of GH treatment, we show a decrease in age-corrected blood pressure, compared to pre-treatment. Blood pressure, however, remained slightly higher than in peers. Some think that having Turner syndrome predisposes for hypertension [25]. The aetiology of the

predisposition, however, remains unclear [31]. The decrease in blood pressure during treatment makes GH an unlikely cause. In addition, we found that in the first four years of GH treatment lipid levels became more favourable regarding the risk for CVD. After discontinuation of GH treatment, compared to the lipid levels in a group of normal children with a similar age, we found that lipid levels in the TS girls were still more favourable regarding the risk for CVD than in the normal peers. As most risk factors seem to have an independent influence on the development of CVD, clustering of several risk factors in TS girls would increase their chance of developing CVD. We found a positive correlation between BMI SD-score and insulin levels, but could not detect any other concurrence between the risk factors. It is not surprising we found a relation between BMI SD-score and insulin levels, as such a relationship is also found in normal children [89, 100]. Both risk factors, however, increase the chance of developing diabetes mellitus, while having TS possibly already predisposes for diabetes mellitus. We would therefore urge clinicians to do their utmost to prevent further weight gain in girls and women with TS.

Evaluation of psychosocial functioning in a group of 50 TS women with final height after long-term GH treatment

In the previous chapter we showed that GH treatment and induction of puberty at a normal pubertal age, in most cases, normalised final height in TS. The effect of such a treatment strategy on psychosocial functioning, however, has been relatively underreported. In chapter 4 we show that, after long-term GH treatment, behaviour in the TS women was comparable to normal Dutch peers. In contrast, we show that self-perception was significantly less positive than for their normal Dutch peers and they have a small but significant tendency to attribute problems to their appearance and avoid physical contact. In addition, we found no evidence of depression, TS women rated their family functioning higher, and had a slightly different coping pattern compared to their Dutch peers. TS women with a more positive self-perception, also had a better attitude towards their bodies and had a better functioning family. This seems to emphasise the importance of family functioning for these women. When looking at psychosocial functioning in untreated adolescent TS girls, more problem behaviour and a lower self-esteem than in normal girls have been described [53, 120 122]. Some even found signs of depression in untreated adolescent and adult TS women [36, 126 127]. Several studies show a positive effect of short-term GH and/or oestrogen treatment on problem behaviour and self-perception [52, 53]. In our study after long-term GH treatment, problem behaviour was comparable to normal peers, while self-perception and bodily attitude was less positive than for peers. Although most attained a normal height, we did not treat the other physical TS features. These other features of TS might be responsible for the less favourable results on self-perception and bodily attitude. Another reason might be the observed lack in social graces in some girls with TS. Lawrence et al. showed that TS women have trouble recognising facial emotions. He postulated that this was caused by anomalies in amygdala function [124].
Part of measuring self-esteem is perceived athletic competence. This was one of the categories the TS women scored less positive than normal. Nijhuis-van der Sanden et al. found that, although TS girls move with the same

accuracy as their normal peers, they move with a significantly lower speed [125]. Therefore, a possible reason for this result might be their problem in execution of movement.

Conclusions

GH treatment leads to a normalisation of final height in most girls. Using a dose of 8 IU/m^2/day showed no significant advantage over 6 IU/m^2/day for gain in FH. If GH is started at a young age final height will not be affected by early initiation of oestrogen. The optimal dosage to use depends on height and age at start of treatment, and first year height velocity. As twenty-four percent of the variance in final height remains unexplained, detailed phenotypic, genetic, and family data on GH-treated TS girls are required to find other predictive factors. High dosages of GH should be given with caution, not only because of the considerable increase in cost of treatment, but also because the very long-term safety of using a higher GH dosage remains to be investigated.
After discontinuation of long-term GH treatment in girls with TS the GH induced insulin resistance disappeared. The decrease in blood pressure both during and after discontinuation of GH treatment suggests a positive effect of GH and oestrogen treatment in TS. Lipid levels, after discontinuation of GH treatment, were more beneficial regarding the development of cardiovascular disease than in a normal control group, suggesting a positive effect of GH and oestrogen treatment in TS. The positive correlation between insulin resistance and BMI, together with the predisposition in TS for insulin resistance, should urge clinicians to do their utmost to prevent further weight gain in girls and women with TS.
Even after normalisation of height and puberty induction at a pubertal age, some TS women still experience psychosocial problems. Although it is likely that GH and oestrogen treatment improved psychosocial functioning, it did not normalise it for some women. Whether GH treatment also has an effect on their life achievements in the future, further follow-up will show.

Recommendations and future research

For most girls, long-term GH treatment using a dose of 4 IU/m^2/day (~ 0.045 mg/kg/day) in girls with TS will lead to a normal final height. Using a dose of 8 IU/m^2/day showed no significant advantage over 6 IU/m^2/day for gain in FH. Factors that might lead to consider an increase in GH dosage to 6 IU/m^2/day are very short height and older age at start of treatment, and low first year height velocity. In line with our recommendation, the 2005 guidelines of the Dutch Advisory group on Growth Hormone state that usage of a higher dose of 6 IU GH/m^2/day should be limited to girls older than 11 years with a height beneath the -2 SD-score for normal girls of the same age. In our study, early initiation of oestrogen does not have an apparent negative effect on final height. If GH is started at a young age, puberty induction can be started at 12 years of age. In case of late discovery of TS, low dose oestrogens can be started as soon as GH treatment is initiated, unless the girl is very short and/or has an older bone age. To optimise treatment results but also to prevent unduly expectations of GH treatment [134], GH treatment

should become more individualised. To find the best treatment strategy for each girl, more detailed data should be collected, based on larger groups of girls with TS. Potential genetic predictors might be, for instance, low frequency mosaicism or parental origin of the X chromosome. On the other hand, sub-clinically present autoimmune diseases, such as celiac disease, might have a negative effect on GH-induced height gain. The aim would be to create an advanced prediction model to aid the individualisation of treatment. High dosages of GH, however, should be given with caution, not only because of the considerable increase in cost of treatment, but also because the very long-term safety of using a higher GH dosage remains to be investigated. Recent studies have shown a potential danger of high circulating IGF-1 levels [78, 79]. Whether the relationship between high circulating IGF-1 levels and cancer is causal, remains to be investigated. Until causality can be ruled out, epidemiological studies on individuals treated with recombinant GH should monitor for an increased risk of colorectal or breast cancer in this group, keeping in mind the increased risk for colon cancer already present in TS, and the association between breast cancer and oestrogen treatment in post-menopausal women.

After discontinuation of long-term GH treatment in girls with TS the GH induced insulin resistance disappeared. A recent study suggested a beta-cell dysfunction as a cause of the increased prevalence of diabetes mellitus in TS women [30]. Studies on beta-cell function in other TS patient groups might confirm these results and could focus on the aetiology behind the dysfunction.

Results after discontinuation of GH treatment suggest a positive effect of GH and oestrogen treatment on blood pressure and lipid levels in TS. Whether the positive effect will remain on the long-term and will affect the prevalence of CVD in TS, remains to be investigated. Epidemiological studies on a large group of GH treated TS women will show whether long-term GH treatment will lead to a significant reduction in CVD.

After discontinuation of GH treatment, blood pressure remained slightly higher than in peers. The aetiology of the predisposition for hypertension in Turner syndrome, however, is unclear. In addition, no research has been done on the effect of current treatment strategies for hypertension in TS. Future studies should be aimed at finding the causes for hypertension in TS and optimising its treatment.

Regarding the clustering of risk factors for diabetes mellitus type 2 and CVD, we found a positive correlation between BMI SD-score and insulin levels. It is still under debate whether obesity, observed more frequently in TS women, is solely responsible for the predisposition for diabetes mellitus type 2 or whether missing a second sex-chromosome, i.e. having TS, attributes by causing insulin resistance and/or beta-cell dysfunction [22, 23, 30]. Investigating the cause for the predisposition will aid in preventing diabetes mellitus type 2 in TS. In addition, we should find out why women with TS are more obese than normal women. Epidemiological studies and clinical observational studies can help find risk factors for obesity in TS which should result in an obesity prevention programme for TS girls and women.

After normalisation of height and puberty induction at a pubertal age, most women have an improved psychosocial functioning compared to previous studies on untreated TS women. Some TS women, however, still experience

psychosocial problems. Having TS is associated with several factors that potentially influence psychosocial functioning, other than short stature. In the discussion we mention the typical facial features of TS, the deficit in recognition of facial emotion, and the problems in execution of movement [124, 125]. It would be important to determine which factors significantly influence the psychosocial status to optimise the treatment strategy for TS.

CHAPTER 6

Part 'Turner Syndrome': Summary

Summary

The most common clinical characteristic of Turner syndrome (TS) is short stature. Although girls with TS are not growth hormone (GH) deficient, studies show that long-term GH treatment in TS leads to normalisation of height during childhood. In this chapter the results and conclusions are summarised of the multi-centre randomised dose-response growth hormone (GH) trial evaluating the efficacy, safety and psychosocial effect of long-term GH treatment on girls with TS. The TS trial was an open trial consisting of 68 untreated girls, aged between 2 and 11 years, with TS. The girls were randomly assigned to a group using 4 IU GH/m^2/day, to a group using 4 IU GH/m^2/day in the first year, and 6 IU GH/m^2/day in the years thereafter, or to a group using 4 IU GH/m^2/day in the first, 6 IU GH/m^2/day in the second, and 8 IU GH/m^2/day in the years thereafter (~ 0.045, 0.067, or 0.090 mg/kg/day). After at least 4 years of GH treatment, at a minimum age of 12 years, a low dose of micronised oestradiol was given to induce puberty.
Chapter 1 introduces the characteristics and incidence of TS. In addition, it describes the development of height and bone maturation, the risk for diabetes mellitus type 2 and cardiovascular disease, and the psychosocial development in untreated TS girls and in TS girls treated with GH and oestrogens. Subsequently, it describes longer-term results of GH treatment and oestrogens on height in girls with TS.
Chapter 2 presents the results after long-term GH treatment (mean duration 8 years) and oestrogen treatment on final height, bone maturation and plasma IGF-I in 60 TS girls. We show that in TS a GH dosage of 4 IU/m^2/day combined with a puberty induction at a normal age, leads to a normal final height (FH) for most girls. Eighty-three percent of the girls with TS reach a normal final height and 63% reach a final height within their target height range. A higher dose of 6 IU/m^2/day (group B) leads to a final height that is slightly higher than for group A. Administration of an even higher dosage of GH (8 IU/m^2/day; group C) does not lead to a further increase in final height. Starting a low dose of natural oestrogen at a relatively young pubertal age has no apparent negative effect on height gain. Factors that seem to predict final height SD-score are height SD-score at start, GH dosage, age at start, and first year height velocity.
Chapter 3 shows the effect of discontinuation of long-term GH treatment on carbohydrate metabolism, body mass index, blood pressure, and serum lipid levels in girls with TS. After discontinuation, the effect of GH treatment on carbohydrate metabolism disappears. Girls with a higher body mass index, however, have a less favourable carbohydrate metabolism after discontinuation of GH treatment. Compared to pre-treatment blood pressure and lipid levels change favourably after discontinuation of GH treatment.
Chapter 4 describes psychosocial functioning of women with Turner syndrome (TS) after long-term GH treatment. After long-term GH treatment, behaviour of most TS women is comparable to peers. Self-perception and their attitude towards their bodies is slightly less positive than for their peers. We find no evidence of depression. TS women rate their family functioning higher, and have a slightly different coping pattern compared to peers. In comparison with previous literature data, GH and oestrogen treatment seem to improve

psychosocial functioning, for most women. Some women, however, still experience psychosocial problems.
Chapter 5 discusses the results presented in the previous chapters and relates them to relevant literature. In addition, conclusion are drawn and recommendations are given together with suggestions for future research. Finally, Chapter 13 synthesises the results from the TS trial and the SGA trial and discusses the similarities and differences.

Samenvatting

Het meest voorkomende klinische kenmerk van het Syndroom van Turner (TS) is een kleine gestalte. Hoewel meisjes met TS geen groeihormoon (GH) tekort hebben, blijkt uit onderzoek dat langdurige GH behandeling leidt tot een normalisatie van de lengte als kind. In dit hoofdstuk worden de resultaten en conclusies samengevat van een gerandomiseerd dosisrespons onderzoek dat heeft gelopen op verschillende kinderafdelingen verspreid door Nederland. Het onderzoek evalueerde het effect en de veiligheid van langdurige GH behandeling van meisjes met TS. De doseringen van het GH werden ad random toegekend aan drie groepen van onbehandelde meisjes tussen de 2 en 11 jaar: Groep A (*4 IU/m^2/dag*), Groep B (*4 IU/m^2/dag in het eerste jaar en vervolgens 6 IU/m^2/dag in de volgende jaren*), Groep C (*4 IU/m^2/dag, 6 IU/m^2/dag in het tweede jaar, en vervolgens 8 IU/m^2/dag in de volgende jaren*) . Zowel bij de 68 patiënten als hun behandelaars was de dosering bekend. Na minimaal 4 jaar GH behandeling en het bereiken van het twaalfde levensjaar, kregen de meisjes een lage dosering gemicroniseerd oestradiol om in de puberteit te komen.

Hoofdstuk 1 introduceert de kenmerken van TS en de frequentie van voorkomen. Het beschrijft verder de lengte- en botontwikkeling, het risico op diabetes mellitus type 2 en hart en vaatziekten, en de psychosociale kenmerken van onbehandelde en met GH behandelde meisjes met TS. Hoofdstuk 2 laat de resultaten zien van langdurige GH behandeling (gemiddelde 8 jaar), later aangevuld met oestrogeen behandeling, op eindlengte, botontwikkeling, en IGF-1 concentraties in 60 meisjes met TS. Uit de resultaten blijkt dat bij de meeste meisjes een GH dosis van 4 IU/m^2/dag met een puberteitsinductie op normale leeftijd leidt tot een normale eindlengte. Van de hele groep heeft 83 procent van de meisjes met TS een normale eindlengte behaald en 63 procent een eindlengte in hun target height interval (eindlengte gebaseerd op ouderlengte). Hogere dosering van GH (groep B) resulteert in een iets langere eindlengte dan in groep A, terwijl de hoogste dosering geen verdere toename laat zien in eindlengte. De start van een lage dosis oestrogenen in relatief jonge meisjes met TS laat ogenschijnlijk geen effect zien op lengtewinst. Factoren die eindlengte voorspellen zijn lengte bij start, gecorrigeerd voor leeftijd en geslacht (lengte SD-score), GH dosis, leeftijd bij start, en lengtewinst in eerste jaar. Hoofdstuk 3 beschrijft het effect van het staken van de GH behandeling op het koolhydraat metabolisme, op de body mass index (BMI), op de bloeddruk, en op het vetgehalte in het bloed. Na staken van de behandeling blijkt het effect van GH op het koolhydraat metabolisme te verdwijnen. Meisjes met een hoog BMI laten een negatief effect zien op het koolhydraat metabolisme. Vergeleken met voor de GH behandeling, hebben de meeste meisjes na de behandeling een betere bloeddruk en vetgehalte. Hoofdstuk 4 presenteert het psychosociaal functioneren van vrouwen met TS na langdurige GH behandeling. Hierin laten we zien dat na langdurige GH behandeling het gedrag van de meeste vrouwen met TS vergelijkbaar is met hun leeftijdsgenoten. Hun zelfbeeld en lichaamsbeeld blijkt iets negatiever te zijn dan voor hun leeftijdsgenoten. We hebben geen aanwijzingen gevonden

voor depressie. Vrouwen met het TS hebben een positiever beeld van het functioneren van hun gezin/familie en hebben een iets verschillend copinggedrag dan hun leeftijdsgenoten. Vergeleken met eerdere studies van onbehandelde vrouwen met TS, zijn er duidelijke aanwijzingen dat behandeling met GH en oestrogeen een positief effect heeft op het psychosociaal functioneren van de meeste vrouwen met TS. Een aantal vrouwen heeft echter nog steeds psychosociale problemen.
Hoofdstuk 5 bediscussieert de resultaten van de voorgaande hoofdstukken en betrekt daarin relevante literatuur. Daarnaast worden er conclusies getrokken en suggesties gedaan voor toekomstig onderzoek.
Als laatste wordt er in Hoofdstuk 13 een vergelijking gemaakt tussen de resultaten van het TS onderzoek en het SGA onderzoek.

Part ‘Small for Gestational Age’

CHAPTER 7

Part 'Small for Gestational Age': Introduction

TABLE 1. Factors associated with SGA.

Foetal factors	
	Chromosomal abnormalities
	Genetic diseases
	Congenital anomalies
Maternal factors	
	Hypertension
	Renal disease
	Diabetes mellitus
	Collagen vascular diseases
	Maternal hypoxemia
	Infection
	Nutritional status
	Cigarette smoking
	Substance abuse
	Therapeutic drugs
Uterine/placental factors	
	Gross structural placental defects
	Insufficient uteroplacental perfusion
	Suboptimal implantation site
Demographic factors	
	Maternal age (Very young or older)
	Maternal height
	Maternal weight
	Maternal and paternal race
	Parity (Nulliparity, Grand multiparity)
	Previous delivery of SGA infants
Environmental factors	
	Altitude
Other	
	Multiple gestation

Adapted from Bernstein and Divon [189].

Introduction

Children born small for gestational age

Until recently, several definitions have been used for small for gestational age (SGA), such as a birth weight and/or length below the 10th, or 3rd percentile for gestational age. In the Netherlands, SGA has been defined as a birth length of 2 or more standard deviations (SD) below the mean for gestational age from 1989 onwards [140]. The reference data from Usher and McLean are used for calculation of SD scores [141]. In 2002, an international consensus on the definition of SGA was reached by the International Small for Gestational Age Advisory Board, defining SGA as a birth weight and/or length of 2 or more standard deviations (SD) below the mean for gestational age [142]. Several associated factors have been mentioned as possible causes for the development of SGA. These factors can be divided into groups, such as maternal, foetal, and/or placental factors as shown in table 1. Maternal factors are, for instance, smoking, nutritional state, or several diseases. Foetal factors are chromosomal or congenital abnormalities, and placental factors are infarctions or placental development aberrations.

Postnatal growth

During the first two years of life, 10% of the children born SGA do not catch-up in height and have an increased risk to reach an adult height of 2 SD or more below the average height [140, 143]. According to the above definition of SGA, 1 in 43 (2.3%) of all live-born infants are born SGA per year. For the Netherlands this percentage could be translated into 4,648 children born SGA per year (2.3% of 202,083 life-born infants in 2002, Central Bureau of Statistics, www.cbs.nl). 10% of these children, approximately 460 per year, could be defined as persistently short children born SGA. Part of this group has an identified congenital or genetic cause for persistent short stature. This thesis focuses on persistently short children born SGA without a known congenital or genetic cause for their short stature, except for Silver-Russell syndrome. Of the total short population, it has been estimated that about 22% are born SGA [143].

Short children born SGA

The first 2 years of life are the most critical for determining whether a child born SGA will show adequate catch-up growth. After these 2 years short stature will most likely persist into adulthood [143]. The pathophysiology of inadequate postnatal catch-up growth is not yet understood. Given the heterogeneity of the SGA group, factors that attribute to the lack in postnatal catch-up will vary. Possible causes are low GH levels, low sensitivity to GH, low sensitivity to IGF-1, or a combination of these factors [144-146]. Associations between the extent of catch-up growth and parental height would suggest a genetic cause for short stature after being born SGA [143].

Bone maturation

Generally, bone development in early childhood after being born SGA progresses slowly. Longitudinal studies in children with Silver-Russell syndrome and pre-treatment data from several GH trials, confirm this [147-150].

By late childhood, the delay in bone maturation is followed by a spontaneous acceleration to an age appropriate bone age [147, 151]. The acceleration of bone maturation, however, is not associated with an adequate catch-up in height and adult height [152].

Physical appearance
Most short children are lean in prepuberty but many gain weight during puberty [150, 153]. Their hands and feet, shoulders and pelvis are on average in the upper range of normal for their stature [154]. A subgroup of short children born SGA has typical features of Silver-Russell syndrome, with frontal bossing, a triangular face, clinodactyly, and asymmetric extremities [148]. Natural growth in these children is comparable with that of short children born SGA without these features. For that reason, children with Silver-Russell syndrome are not excluded from trials studying growth in children born SGA.

Diabetes mellitus and cardiovascular disease
Epidemiological studies have found a propensity to diseases such as diabetes mellitus type 2 and cardiovascular disease in adults born with a low birth weight [155, 156]. Whether these associations depend on other factors such as developing obesity or adequate catch-up growth in childhood is under investigation [157-159]. Barker et al. suggested that the aetiology for the associations lies in the timing of the intrauterine growth retardation [160]. They hypothesise that foetal structure and function can be altered in response to adverse intrauterine influences during critical time windows. When the adverse influence, in this case malnutrition is timed during such a critical time, the foetus will develop a predisposition for adult disease. Gluckman and Hanson postulate reduction of foetal size is not the causal trigger but a non-obligatory side effect of the adverse influence [161]. Malnutrition is only one example of an adverse intrauterine influence that changes the receptiveness for adult disease [161-163]. The foetal insulin hypothesis by Hattersley and Tooke focuses on a genetic explanation for the association between low birth weight and adult disease [164]. They postulate genetic factors causing insulin resistance, affecting both prenatal growth and adult disease.

IQ and psychosocial development
Clinical studies in children born SGA have found a susceptibility for lower intelligence, poor academic performance, low social competence, and behavioural problems [165-171]. Possible physiological explanations are intrauterine malnutrition affecting brain development or relative deficiency of the GH-IGF-1 axis [172-174]. Intellectual and psychosocial capacity seem to be more reduced in case of an inadequate catch-up growth in height after being born SGA [171]. It can be debated, however, whether persistence of short stature in SGA individuals is the key factor to this relationship, or just a coinciding factor [175-177].

Growth hormone treatment
In the early seventies growth hormone (GH) treatment was initiated as a treatment for short stature in children born SGA. Mainly due to the low frequency of administration and low dosage used, at that time, GH

treatment had little success [178]. In the late eighties, however, it became apparent that daily GH treatment in a higher dosage caused a significant catch-up growth in height in short children born SGA at least on the short term [179-181]. In 1991, a Dutch multi-centre randomised double-blind dose-response trial (3 IU versus 6 GH/m^2/day) was started to investigate the efficacy of GH treatment until final height. A previous report on this trial showed that five years of continuous GH treatment in short children born SGA resulted in a normalisation of height during childhood with a slightly but significantly better catch-up in height in the children of the higher dosage group [150]. Interestingly, the SGA children diagnosed as partially growth hormone deficiency, who were also included in the trial, showed a similar catch-up in height as the non-GHD children born SGA [150]. Recent reports on final height, however, are inconsistent [182, 183].
During the first years of GH treatment, bone maturation accelerated during late childhood, similar to untreated SGA children of that age, resulting in either a normalisation of bone age or an advance [150, 184].
Since low birth weight has been associated with the development of diabetes mellitus type 2 and cardiovascular disease in later life, it is important to monitor the effect of GH treatment on risk factors for these diseases in children born SGA. In other patient groups, it was shown that GH treatment increased post-prandial insulin levels, probably due to a reduction in insulin sensitivity [185]. As expected, in SGA children a similar increase in insulin levels was found during GH treatment [186]. In a previous report from the Dutch SGA trial the expected increase in insulin levels during GH treatment was not accompanied by higher fasting glucose levels [91].
In the Dutch SGA trial mean systolic blood pressure was elevated before start of GH treatment and significantly decreased to normal during GH treatment, while mean diastolic blood pressure significantly decreased to the low normal range. This decrease resulted in a normal blood pressure during at least 6 years of GH treatment, while lipid levels also showed a favourable change [154]. In other patient groups, such as Turner syndrome, either a positive effect or no effect on blood pressure or lipid levels was found [47-50].
It is generally assumed that catch-up in height due to GH treatment will have a positive psychosocial effect, but only a few GH studies in other patient groups included a psychosocial evaluation during GH treatment. The majority of these studies showed a positive psychosocial result of GH treatment [53, 187]. The first data of the Dutch GH trial on intelligence and psychosocial functioning showed a significant increase during 2 years of GH treatment in total IQ-score, in 'social acceptance' scores, and in 'general self-worth' scores [169, 188].

Research questions and aims of the studies

Short children born small for gestational age
Previously, data analyses showed a positive effect of 5 years of GH treatment on height gain in prepubertal short children born SGA. We still, however, had to investigate whether GH treatment would lead to a normalisation of final height. In addition, to optimise individual treatment with GH in future, we wanted to know which parameters were important predictors for long-term growth response. Regarding side effects, we wanted to evaluate the effect of

discontinuation of GH treatment after long-term GH treatment on carbohydrate metabolism and several risk factors for the development of diabetes mellitus type 2 and cardiovascular disease (CVD) in later life. Regarding psychosocial development during long-term GH treatment, we wanted to know if and to what extent long-term GH treatment had an effect on intelligence, behaviour, and self-perception over the years and after reaching final height.
To answer these research questions, the following aims were identified:

1. Evaluation of the effect of long-term continuous GH treatment on final height in 54 short children born SGA without spontaneous catch-up growth, treated in a randomised, double-blind, dose-response GH trial with either 3 or 6 IU GH/m^2/day (1 or 2 mg/m^2/day; 0.033 or 0.067 mg/kg/day).
2. Determination of variables that predict final height SD-score.
3. Assessment of carbohydrate metabolism in short children born SGA after discontinuation of long-term GH treatment, compared to data before and during treatment.
4. Investigation of several risk factors for the development of DM type 2 and CVD by measuring blood pressure, body mass index and blood lipid levels in short children born SGA after discontinuation of GH treatment, compared to data before and during treatment.
5. Longitudinal evaluation of IQ, behaviour, and self-perception in a group of short children born SGA at start, during and after long-term GH treatment.

Outline of the thesis part SGA

In this thesis part SGA results are presented of a randomised multi-centre dose-response GH trials evaluating the efficacy, safety and psychosocial effect of long-term GH treatment in short children born SGA without spontaneous catch-up growth. The trial included the first participants in 1991.
In Chapter 7 the field of SGA is introduced. Chapter 8 describes the effect of long-term continuous GH treatment on final height in 54 short children born SGA without spontaneous catch-up growth. In addition, a model with predictive factors for final height SD-score is presented. Chapter 9 describes carbohydrate metabolism and several risk factors for DM type 2 and CVD in short children born SGA during and after discontinuation of long-term GH treatment. Chapter 10 describes IQ, behaviour, and self-perception in short children born SGA during and after long-term GH treatment. Chapter 11 discusses the results of the SGA trials in relation to other literature data and the implications of the results. In addition, it concludes and gives recommendations for future research. Chapter 12 summarises the SGA part of this thesis in English and Dutch.

Previous dissertations

W. de Waal published baseline and first-year results of the study in his thesis entitled "Influencing the extremes of growth", Rotterdam 1996. I. van der

Reyden-Lakeman presented results on IQ and psychosocial functioning during the first two years of GH treatment in her thesis entitled "Growing pains? Psychological evaluation of children with short stature after intrauterine growth retardation, before and after two years of growth hormone treatment", Rotterdam 1996. Th. C.J. Sas, in his thesis entitled "Long-term growth hormone treatment in two growth disorders", Rotterdam 1999, described 7-year data of the TS trial and 5-year data of the SGA trial.

CHAPTER 8

Part 'Small for Gestational Age': Final height after long-term, continuous GH treatment in short children born small for gestational age (SGA): Results of a randomised, double-blind, dose-response GH trial

Final height after long-term, continuous GH treatment in short children born small for gestational age (SGA): Results of a randomised, double-blind, dose-response GH trial

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Abstract

The GH-dose-response effect of long-term continuous growth hormone (GH) treatment on final height was evaluated in 54 short children born SGA, participating in a randomised double-blind dose-response trial. Patients were randomly and blindly assigned to treatment with either 3 IU (group A) or 6 IU GH/m2/d (group B)(~0.033 or 0.067 mg/kg/d, resp.). Mean (SD) birth length was –3.6 (1.4), age at start 8.1 (1.9) years and height SD-score at start –3.0 (0.7). Seventeen of the 54 children were partially GH deficient (stimulated GH peak 10-20 mU/l). Fifteen non-GH treated, non-GH deficient, short children born SGA, with similar inclusion criteria, served as controls (mean (SD) birth length –3.3 (1.2), age at start 7.8 (1.7) years and height SD-score at start –2.6 (0.5)).
GH treatment resulted in a FH above -2 SD-score in 85% of the children after a mean (SD) GH treatment period of 7.8 (1.7) years. Mean (SD) FH SD-score was -1.1 (0.7) for group A and -0.9 (0.8) for group B, resulting from a mean (SD) gain in height SD-score of 1.8 (0.7) for group A and 2.1 (0.8) for group B. No significant differences between group A and B were found for FH SD-score (mean difference 0.3 SD-score; 95% CI: -0.2, 0.6; p>0.2) and gain in height SD-score (mean difference 0.3 SD-score; 95% CI: -0.1, 0.7; p>0.1). When corrected for target height (TH), mean corrected FH SD-score was –0.2 (0.8) for group A and –0.4 (0.9) for group B. Mean (SD) FH SD-score of the control group (–2.3 (0.7)) was significantly lower than that of the GH-treated group (p<0.001). Multiple regression analysis indicated the following predictive variables for FH SD-score: TH SD-score, height SD-score and CA minus BA (yrs) at start. GH dose had no significant effect.
In conclusion, long-term continuous GH treatment in short children born SGA without signs of persistent catch-up growth leads to a normalisation of final height, even with a GH dose of 3 IU/m^2/d (~ 0.033 mg/kg/d).

Introduction

To be born small-for-gestational-age (SGA) may have considerable consequences. Not only a significantly increased risk for reaching a final

height below - 2 SD score has been reported [143]. There might also be an increased risk for diabetes mellitus type 2 and cardiovascular disease as has been described in adults who were born with a low birth weight [190]. It is yet unclear if this will also concern SGA patients with persistent short stature, as no distinction was made for those who had a complete catch-up growth in height after birth and those with a persistent short stature.
During the first two years of life about 10% of the children born SGA do not catch-up to a height above the - 2 SD-score. The majority of these children will reach a final height below - 2 SD-score [140, 143]. The reason for these children remaining short is not completely understood. Sixty percent of short children born SGA have low serum growth hormone (GH) levels during a 24-hour GH profile, but no relation was found between physiological GH levels and the growth response during GH treatment [145, 150, 191]. There are several theories to explain their persistent short stature. One suggests that it is the result of a reduced sensitivity for growth factors, another suggests that it might be influenced by intrauterine re-programming or genetic background [164, 192, 193]. Recent studies have demonstrated that 5 years of GH treatment in short children born SGA results in a normalisation of height during childhood.[150, 184] Final height results after long-term, continuous GH treatment, however, have not yet been published.
In this article we present final height results of 54 short children born SGA who have been treated in a randomised, double-blind, dose-response GH trial evaluating the efficacy and safety of long-term, continuous GH treatment with either 3 or 6 IU GH/m^2/day (0.033 or 0.067 mg/kg/day).

Patients and Methods

Study group
Seventy-nine prepubertal short children born SGA participated in a multi-centre double-blind randomised dose-response GH trial. Of these 79 children 6 children dropped out of the study for the following reasons: lack of motivation despite ongoing catch-up growth (n=4), treatment for precocious puberty (n=1), and biochemical signs of GH insensitivity (n=1). As these six children were lost to follow-up, their data were not included in the analysis. Of the remaining 73 children, 54 children reached final height and their data were evaluated for this study, whereas 19 children were not included because they had not yet reached final height. Those who had attained final height were approximately 3 years older at start of GH treatment compared to those who were still growing (mean (SD) CA 8.1 (1.9) years versus 5.4 (1.9) years, $p<0.001$), but the other clinical characteristics were similar at start of GH treatment (Table 1).
The dose-response GH trial evaluated the effect of two dosages of GH, 3 or 6 IU GH/m2 body surface/d (~ 0.033 or 0.067 mg/kg /d) on long-term growth and ultimately on FH. Inclusion criteria were: 1) birth length SD-score below -1.88 [141], 2) chronological age (CA) between 3 and 11 year in boys and 3 and 9 year in girls at start of study, 3) height SD-score for CA (height SD-score) below -1.88 [62], 4) height velocity SD-score for CA $\leq$ zero [62] to exclude children presenting spontaneous catch-up growth, 5) prepubertal stage, defined as Tanner breast stage 1 for girls and a testicular volume of less than 4 ml for boys [63], 6) uncomplicated neonatal period, that is without signs of

severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis neonatorum and without long-term complications of respiratory ventilation. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders (emotional deprivation, severe chronic illness, chondrodysplasia) or syndromes (except for Silver-Russell syndrome (SRS)) and previous or present use of drugs that could interfere with GH treatment. Twenty-seven of the 79 patients had partial GH-deficiency (GHD), which was defined as a maximal peak GH secretion between 10 and 20 mU/L during 2 GH provocation tests or during one provocation test and a 24-hour GH profile.[65, 145]
The GH trial started in 1991 and was approved by the Ethics Committees of the four participating centres in the Netherlands. Due to ethical considerations the Ethics Committees did not allow for a control group until FH. Written informed consent was obtained from the parents or custodians of each child.

Control group
In 1990, 107 children, born in three academic hospitals with a birth length below −1.88 SD-score in the same time period as the GH-treated group (1980 until 1989) were included in a cohort study to evaluate natural growth in SGA children with short stature at the age of two years.[140] Fifty-nine children were lost, due to either start of GH treatment (n=21), treatment for precocious puberty (n=1), spastic paraplegia (n=1), or lack of motivation or moving abroad (n=36). During the inclusion period of the GH trial twenty-nine children met the inclusion criteria of our GH trial, including persistent short stature without signs of catch-up growth (HV SD-score < 0), but they remained untreated because their paediatrician did not participate in the GH trial. None of these children were GH deficient. This group was followed as control group for the GH trial. In 2001, fifteen children had reached FH and served as controls for comparison of growth and FH of the 54 GH-treated children.

Design dose-response GH trial
After stratification for chronological age (CA) and spontaneous GH secretion during a 24-hour GH profile 79 children were randomly and blindly assigned to one of two GH dosage groups: group A, 3 IU/m^2/day, or group B, 6 IU/m^2/day (~ 0.033 or 0.067 mg/kg/day, respectively) [145, 150]. The inclusion period started in April 1991 and ended in January 1993. Biosynthetic GH (r-hGH Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime with a pen injection system (Nordiject 24). Every 3 months the total GH dose was adjusted to the calculated body surface. To ensure the double-blind design an equal volume of a reconstituted preparation was used.

Growth evaluation
During 11 years one physician (from '91-'95 W de Waal, '95-'98 Th Sas, '98-'02 YvPareren) examined all children every three months and measured height according to Cameron using a Harpenden stadiometer.[194] Four measurements per visit were taken and the mean was used for analysis. Height was expressed as SD-score for chronological age (CA) (height SD-

score).[62] Target height (TH) was adapted from Dutch reference data with addition of 3 cm for secular trend: 1/2 x (Heightfather + Heightmother + 12) + 3 for boys and 1/2 x (Heightfather + Heightmother - 12) + 3 for girls.[62] TH and body mass index (BMI) were expressed as SD-score using Dutch references.[62] Target height range was defined as the mean TH +/- 2SD. Bone age (BA) was determined by the same investigators ('91-'98 ThS, '98-'01 YvP) according to Tanner & Whitehouse radius, ulna, short-bones score (RUS TW-2).[66] Bone maturation was expressed as the ratio of the change in BA to the change in CA (dBA/dCA). The difference between CA and BA was calculated as CA minus BA (CA-BA) in years. The same investigators assessed pubertal stage according to Tanner, using an orchidometer in boys. Start of puberty was defined as a Tanner breast stage 2 in girls and a testicular volume of 4 ml in boys.[63]

Definition of final height
Final height (FH) in GH-treated children was defined as the condition when height velocity (HV) had dropped below 0.5 cm during the previous 6 months and bone age was ≥ 15 years for girls and ≥ 16.5 years for boys. FH was reached either during GH treatment or during the 2-year follow-up after discontinuation of GH treatment. Corrected FH was calculated by subtracting the target height SD-score from the FH SD-score. GH treatment was discontinued after reaching FH or on patient's decision at near final height (near FH), which was defined as a HV ranging between 0.5 and 2 cm during the previous 6 months. For the control group FH was defined as the condition when CA and/or BA had reached 18 years for boys and 16 years in girls.

Biochemical parameters
Before start and after discontinuation of GH treatment a standard arginine tolerance test (ATT) was performed.[145] A standard oral glucose tolerance test (OGTT) was done at start of GH treatment and after six years of GH treatment.[91] At start of GH treatment and during the dose-response GH trial additional blood samples were taken for determination of plasma levels of IGF-I and haemoglobin A1c levels. Plasma levels of IGFBP-3 were determined at start of GH treatment, after the first and second year and after the fifth year of GH treatment. After centrifugation, all samples were frozen (-20ºC) until assayed.

Hormone Assays
RIA measurements of plasma GH, IGF-I, IGFBP-3, and insulin were performed as described previously.[69, 195-197] All measurements were performed in the same laboratories. Since plasma levels of both IGF-I and IGFBP-3 are dependent on age and sex, values were transformed to SD scores using reference values for healthy children determined in the same laboratory.[70]

Statistical analyses
To maintain the double-blind design until all participants have reached FH, an independent statistician (PM) performed the statistical analyses and summarized the results per treatment group in such a way that it was impossible for the investigator to identify individual patients. Accordingly,

data are expressed as mean (SD) values unless otherwise specified. Differences in continuous variables were tested by paired Student's t-tests. Differences between zero and SD-score values at various time-points during the study were tested by one-sample Student's t-tests. Differences between groups were tested using a Student's two-sample t-test. To test for relationships between continuous variables correlations were estimated, after adjustment for GH dosage. Multiple linear regression analyses were used to construct the best model for predicting final height SD-score. For this purpose, the variables GH dose, TH SD-score, height SD-score at start of GH treatment, the difference CA-BA at start of GH treatment, birth length SD-score, gender, CA at start, bone maturation during the first year, age at onset of puberty and BMI SD-score at start were tested. A p-value < 0.05 was considered significant. All calculations were executed in SPSS version 9.0.

Results

GH trial
Fifty-four children reached final height (FH) after a mean (SD) GH treatment period of 7.9 (1.7) years for group A and 7.5 (1.7) for group B. Pretreatment clinical data are listed in Table 1 for the total group and for the 54 children who reached final height (FH). Both groups had similar clinical characteristics at start of GH treatment, except for the age at start.

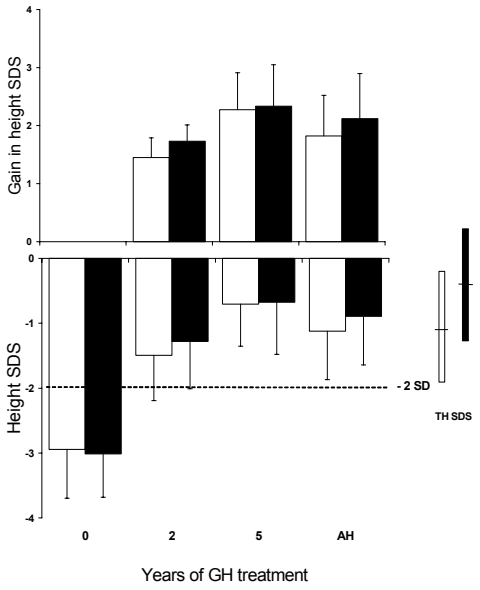
Final height
GH treatment resulted in a mean (SD) FH SD-score of -1.1 (0.7) for group A (3 IU/m^2/day) and -0.9 (0.8) for group B (6 IU/m^2/day) (Table 2). In both groups, FH SD-score was significantly higher compared to height SD-score at start of GH treatment (P<0.0001). The difference in FH SD-score between groups A and B did not reach significance (mean difference 0.3 SD-score; 95% CI: -0.2, 0.6; p=0.3). Figure 1 gives the height SD-score at start, after 2 and 5 years of GH treatment and at final height, and the gain in height SD-score from start until FH. Mean (SD) gain in height SD-score from start until FH was not significantly different for group A (from -2.9 [0.8] at start to -1.1 [0.7]) compared to group B (from –3.0 [0.7] to –0.9 [0.8]; mean difference 0.3 SD-score; 95% CI: -0.1, 0.7; p=0.1). Table 2 lists the height SD-score throughout the study, TH SD-score, FH SD-score and the corrected FH SD-score (FH SD-score minus TH SD-score). The mean (SD) TH SD-score was almost significantly lower for group A (–0.9 [1.0]) than for group B (–0.5 [0.9]; mean difference 0.5; 95% CI: 0.1, 1.0; p=0.08). As a result the mean (SD) corrected FH SD-score was not significantly different between group A (-0.2 [0.8]) and group B (-0.4 [0.9]; mean difference 0.2; 95% CI: -0.2, 0.7; p=0.2). Figure 2 shows the corrected height SD-score at the various time-points of the study. At start and after 2 years of GH treatment, the corrected height SD-score was significantly lower than zero for groups A and B (p<0.001, p<0.001). At final height the corrected height SD-score was comparable to zero for group A, but lower for group B (p<0.05). For boys, both TH SD-score and FH SD-score were significantly higher for group B than for group A (p<0.01, p<0.05, p<0.05, respectively)(Table 2).

TABLE 1. Baseline clinical data for the total group of 79 short children born SGA and for the 54 children who reached final height

	Total group N= 79		**Final height group** N=54	
	Group A (N=41)	Group B (N=38)	Group A (N=28)	Group B (N=26)
Male/Female	31/10	21/17	18/10	14/12
Gestational age (wk)	37.3 (3.2)	36.0 (4.1)	37.1 (3.4)	36.2 (4.3)
Birth length SD-score	-3.6 (1.4)	-3.7 (1.7)	-3.5 (1.3)	-3.6 (1.5)
Birth weight SD-score	-2.6 (1.2)	-2.6 (1.0)	-2.6 (1.1)	-2.6 (0.8)
Chronological Age (yr)	6.6 (2.4)	6.7 (2.9)	7.9 (1.9)*	8.2 (1.9)*
Height SD-score	-3.0 (0.7)	-3.1 (0.7)	-2.9 (0.8)	-3.0 (0.7)
Height velocity SD-score	-0.7 (1.1)	-1.2 (1.3)	-0.6 (1.1)	-1.4 (1.4)

Data expressed as mean (SD). *p < 0.001, mean (SD) CA of 54 children with FH (8.1 (1.9)) versus 19 who were still growing (5.4 (1.9))

FIGURE 1. (Bottom panel) Height SD-score (+SD) during GH treatment and at final height, in relation to target height (TH); (Top panel) Gain in height SD-score (+SD) from start until 2 years of GH treatment, until 5 years of GH treatment, and until final height (FH). Group A (white bar) and group B (black bar).



For girls, however, no difference was found for TH SD-score and FH SD-score between groups A and B (p>0.1).
Final height of non-GH deficient versus partially GH deficient SGA children
Final height SD-score of the non-GHD children was –1.1 (0.8) for group A and –0.9 (0.8) for group B, compared to –1.2 (0.7) for group A and –0.9 (0.7) for group B in the partially GHD children (Table 2). Mean height gain SD-score of the non-GHD children was 1.9 (0.8) for group A and 2.1 (0.8) for group B, compared to 1.8 (0.5) for group A and 2.2 (0.6) for group B in the partially GHD children. Final height SD-score as well as height gain SD-score were not significantly different between the non-GHD and the partially GHD SGA children, even when corrected for GH dose and TH SD-score.

Puberty
Mean (SD) age at onset of puberty for boys was 11.7 (0.9) years in group A and 11.8 (0.7) years in group B, and for girls 10.9 (1.1) years in group A and 10.8 (1.1) in group B, without significant differences between the GH dosage groups (p>0.1 for both sexes). For boys, the mean age (SD) at final height was 16.8 (0.9) years in group A versus 16.9 (1.1) years in group B, and for girls 14.8 (0.8) years versus 15.1 (1.2) years in groups A and B, respectively. The age at final height was not different between the GH dosage groups (p>0.1 for both sexes). Mean duration of puberty (from start of puberty until FH) for boys was 5.1 (1.2) years in group A and 5.2 (1.0) years in group B, and for girls 3.9 (1.0) years in group A and 4.3 (1.1) years in group B.

Bone maturation
The average bone maturation expressed as delta BA / delta CA per year, was throughout the study significantly higher than 1, regardless of GH dosage. During the first year of GH treatment median (range) bone maturation was 1.5 (0.6-2.7) year for group A and 1.1 (0.2-3.2) years for group B. From 4 to 5 years of GH treatment bone maturation was 1.1 (0.3-2.5) years for group A and 1.0 (0.3-2.2) year for group B, and from 5 years until discontinuation of GH treatment bone maturation was 1.0 (0.3-1.6) for group A and 1.1 (0.4-1.7) for group B. The average difference between CA and BA (CA minus BA) at start of GH treatment, at 5 years of GH treatment, and at discontinuation of GH treatment was 0.5 (1.1), -1.0 (1.1), and -1.1 (1.1) respectively, without significant differences between groups A and B.

GH, IGF-I and IGFBP-3
The non-GHD SGA children who attained FH had at start of GH treatment a mean (SD) maximal GH peak during GH provocation test of 28.7 (11.5) mU/L, a mean (SD) GH peak during the 24-h GH profile of 37.2 (16.1) mU/L and mean IGF-1 SD-score of –0.5 (0.8). Seventeen of the 54 SGA children who attained FH had a maximal GH peak between 10 and 20 mU/L. In these partially GHD SGA children mean (SD) maximal GH peak during GH provocation test, GH peak during the 24-h GH profile, and mean IGF-1 SD-score at start were 13.0 (5.8) mU/L, 20.9 (8.5) mU/L, and –1.8 (1.2), respectively, all being significantly lower than in the non-GHD SGA children (p<0.001, p<0.01, p<0.001, respectively).

TABLE 2. Final height SD-score and other auxological data in 54 SGA children at various time-points during the study.

		All children SGA				Non-GH deficient SGA				Partially GH deficient SGA			
		(n=28) Group A		(n=26) Group B		(n=17) Group A		(n=20) Group B		(n=11) Group A		(n=6) Group B	
Duration of GH treatment		7.9	(1.7)	7.5	(1.7)	8.1	(1.9)	7.6	(1.8)	7.7	(1.5)	7.2	(1.2)
Height SD-score													
	At start	-2.9	(0.8)	-3.0	(0.7)	-2.9	(0.7)	-3.0	(0.8)	-3.0	(0.8)	-3.1	(0.3)
	2 years	-1.5	(0.7)	-1.3	(0.7)	-1.5	(0.7)	-1.3	(0.8)	-1.5	(0.7)	-1.3	(0.3)
	5 years	-0.7	(0.7)	-0.7	(0.8)	-0.7	(0.7)	-0.6	(0.8)	-0.8	(0.6)	-0.9	(0.7)
	FH	-1.1	(0.8)	-0.9	(0.8)	-1.1	(0.8)	-0.9	(0.8)	-1.2	(0.7)	-0.9	(0.7)
Corrected height SD-score (Height SD-score – TH SD-score)	At start	-2.0	(0.9)	-2.6	(0.8)*	-2.2	(0.9)	-2.7	(0.9)	-1.9	(0.9)	-2.2	(0.4)
	2 years	-0.6	(0.9)	-0.8	(0.8)	-0.7	(1.0)	-1.0	(0.9)	-0.4	(0.9)	-0.3	(0.4)
	5 years	0.2	(0.8)	-0.2	(1.0)	0.1	(0.9)	-0.3	(1.1)	0.4	(0.8)	0.0	(0.4)
	FH	-0.2	(0.8)	-0.4	(0.9)	-0.3	(0.8)	-0.6	(1.0)	-0.1	(0.7)	0.0	(0.2)#
Target height SD-score		-0.9	(1.0)	-0.5	(0.9)	-0.8	(1.0)	-0.3	(0.9)	-1.1	(0.9)	-0.9	(0.6)
Final height (cm)													
	Boys	169.3	(6.7)	173.7	(5.8)	-	-	-	-	-	-	-	-
	Girls	160.1	(3.1)	159.2	(4.0)	-	-	-	-	-	-	-	-
Pubertal height gain (cm)													
	Boys	27.3	(7.6)	30.0	(5.3)	-	-	-	-	-	-	-	-
	Girls	18.8	(6.9)	19.4	(5.8)	-	-	-	-	-	-	-	-

Data expressed as mean (SD). FH: final height. TH: target height. Data on Final height (cm) and Pubertal height gain (cm) for non-GHD versus GHD are not shown due to the small number of children in each subgroup.
*$p < 0.05$, Group A versus Group B; # $p < 0.05$, non-GH deficient versus partially GH deficient children

The changes in IGF-I SD-score and IGFBP-3 SD-score of the total group during 5 years of GH treatment were described previously [150]. At discontinuation of GH treatment, mean IGF-I SD-score was 1.0 (1.1) for group A and 1.3 (1.2) for group B, being significantly different to zero for both groups ($p<0.001$ for both). Mean IGFBP-3 SD-score at discontinuation of GH treatment was –0.8 (0.9) for group A and –0.06 (0.7) for group B, being significantly lower than zero for group A only ($p<0.001$). Only for IGFBP-3 SD-score the difference between group A and B at discontinuation of GH treatment was significant ($p<0.01$).

Predictors for final height
All correlations were done for groups A and B together, after adjustment for GH dose, although no significant differences in FH SD-score and gain in height SD-score were found between groups A and B. FH SD-score correlated positively with height SD-score at start of GH treatment ($r=0.49$, $p<0.001$), with TH SD-score ($r=0.49$, $p<0.001$), and pre-treatment HV SD-score ($r=0.32$, $p<0.05$). FH SD-score was not significantly related to the following variables: CA and BA at start of GH treatment, birth length SD-score, maximal GH peak during ATT, characteristics of the 24-hour GH profiles, IGF-I SD-score values at start of GH treatment, and the increment in IGF-I SD-score during the first year of GH treatment.
The gain in height SD-score from start of GH treatment until FH had a negative correlation with CA and BA at start of GH treatment ($r=-0.36$, $p<0.01$; $r=-0.46$, $p<0.01$, respectively). Birth length SD-score, TH SD-score, pre-treatment HV SD-score, IGF-I SD-score at start of GH treatment, the increase in IGF-I SD-score, mean maximal plasma GH response during ATT, and the characteristics of the 24-hour GH profiles did not correlate significantly with the gain in height SD-score from start of GH treatment until FH.
Multiple regression analysis showed that a model, using the variables TH SD-score, height SD-score at start of GH treatment, the difference CA-BA at start of GH treatment and the GH dose accounted for 42% (residual SD 0.60) of the variation in FH SD-score. Table 3 shows the results of the multiple regression analysis. Variables, which showed a non-significant effect on FH SD-score were gender, CA at start, bone maturation during the first year, age at onset of puberty, BMI SD-score at start, birth length SD-score, and GH dose. The model provided the following equation: Final height SD-score = 0.02 + 0.29 x TH SD-score + 0.42 x height SD-score at start + 0.20 x (CA-BA in years) at start + 0.07 x GH dose.

Safety
Treatment was well tolerated and no adverse events were detected that were considered to be GH-related. Our group has recently published 6-year results on fasting and stimulated glucose and insulin levels during an oral glucose tolerance test [91]. In short, continuous GH treatment over a 6-year period showed no adverse effects on glucose levels. GH treatment induced higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance. No significant differences between the two GH-dosage groups were found. None of the children developed diabetes mellitus.

FIGURE 2. Corrected height SD-score (+SD) during GH treatment and at final height (FH) for group A (white bar) and group B (black bar).

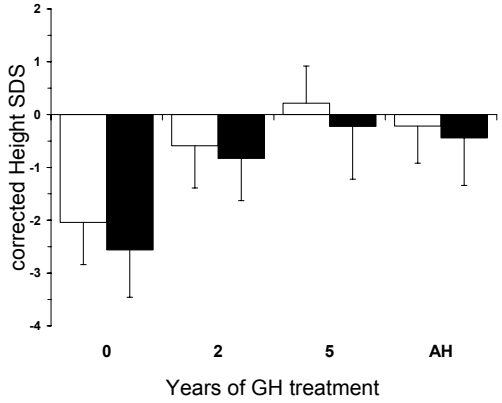


TABLE 3. Multiple regression analysis on final height SD-score.

Independent Variable	Regression Coefficient	SE	P-value
TH SD-score	0.29	0.10	< 0.01
Height SD-score at start	0.43	0.14	< 0.01
Difference CA-BA at start (yr)	0.20	0.08	< 0.05
GH Dose (3 vs 6 IU/m^2/day)	0.07	0.06	0.2

TH: target height; SE: standard error; CA: chronological age; BA: bone age. Regression equation: FH SD-score = 0.02 + 0.29 x TH SD-score + 0.43 x height SD-score at start + 0.20 x (CA-BA) at start + 0.07 x GH dose.

TABLE 4. Examples of predicted final height SD-score resulting from the regression model.

	TH SD-score = 0		TH SD-score = -1		TH SD-score = -2	
GH dose (IU/m^2/day)	3	6	3	6	3	6
Height SD-score at start						
-2	-0.6	-0.4	-0.9	-0.7	-1.2	-1
-3	-1	-0.8	-1.3	-1.1	-1.6	-1.4
-4	-1.5	-1.3	-1.8	-1.5	-2	-1.8

TH: target height.

Control group
The control group, 15 children (5 boys, 10 girls), had a mean (SD) birth length SD-score of −3.3 (1.2), birth weight SD-score of −2.7 (0.7) and a mean (SD) gestational age of 34.3 (2.3) weeks. At inclusion in the control group, mean (SD) age was 7.8 (1.7) years, height SD-score −2.6 (0.5), TH SD-score −1.1 (1.0) and corrected height SD-score −1.5 (0.9). No significant difference was found between the GH-treated group and the control group regarding pretreatment clinical characteristics except for gestational age ($p<0.05$).

Final height of GH-treated versus control group
The control group attained a mean (SD) FH SD-score of −2.3 (0.7), after a mean (SD) follow-up of 7.5 (1.2) years from inclusion in the control group until FH. The mean (SD) gain in height SD-score until FH was 0.3 (0.7) SD-score and the corrected FH was −1.2 (0.6) SD-score. Mean FH SD-score, gain in height SD-score until FH and corrected FH SD-score were all significantly lower than in the GH-treated group (mean differences [95% CI]: 1.3 [0.9, 1.8], 1.7 [1.3, 2.3], 0.9 [0.4, 1.4], respectively; $p<0.001$ for all).

Discussion

Our study shows that in short children born SGA long-term continuous treatment with GH results in a normalisation of height during childhood and a normalisation of final height (FH) in most children. After a mean duration of 7.8 years children treated with 3 IU GH/m^2/day (group A) attained a mean (SD) FH SD-score of -1.1 (0.7), whereas those treated with 6 IU GH/m^2/day (group B) attained a mean FH SD-score of −0.9 (0.8). GH treatment resulted in a FH within the normal range (above −2.0 SD-score) in 85% of the children and a FH within the target height range in 98%. Interestingly, FH SD-score was not significantly different between the two GH-dosage groups. Also, the mean gain in height SD-score from start of treatment until FH, being 1.8 (0.7) SD-score (~ an improvement of 12 cm for boys and 11 cm for girls) for group A and 2.1(0.8) SD-score (~ an improvement of 14 cm for boys and 13 cm for girls) for group B, were not significantly different between the two GH-dosage groups.
When we corrected FH SD-score for TH SD-score, because group A had a significantly lower TH SD-score than group B, the mean corrected final height SD-score (FH SD-score minus TH SD-score) proved to be comparable for group A (-0.2[0.8]) and group B (-0.4 [0.9]). This means that when FH SD-score was corrected for the genetic potential (TH), children who had been treated with the lower GH dose of 3 IU/m^2/day had the same results. Our study shows that 98% of short children born SGA treated with long-term GH reached a final height within their target height range.
Two non-randomised studies reported on final height in a group of short children born SGA with growth hormone deficiency (GHD).[182, 183] They found a mean height SD-score gain from start until final height of 0.5 and 0.9 SD-score, which is much lower than we now report. There are, however, several factors that can explain the discrepancy between their results and ours. Their children were older (10.9 and 10.7 years) at start and were treated for a much shorter period than our children (4.6 years for both studies). In

addition, in the study by Coutant et al. children were treated with a wide range of GH dosages and with a low mean GH dose of 1.8 IU/m^2/day (=almost half of our lowest GH dose).
As including a randomised control group as part of our GH trial until FH was not allowed by the Medical Ethics Committees, we compared our GH-treated patients with a longitudinally followed control group with the same inclusion criteria and age as the GH-treated patients at start of treatment who did not receive GH treatment because the paediatrician did not participate in the GH trial. The control group attained a mean (SD) final height SD-score of −2.3 (0.7), had a mean gain in height SD-score of 0.3 (0.7) until FH during the follow-up period of 7 ½ years and a corrected FH of −1.2 (0.6) SD-score. These data show that compared to untreated short children born SGA, those treated with GH significantly gained in final height SD-score. The proportion of girls in the control group, however, was larger than in the GH groups. It is unlikely this will affect the results as we show FH, expressed as SD-score, did not differ between genders.
Our study demonstrates that most children reached a normal height after the first years of GH treatment and remained in the normal range until final height. This means that most of them had a normal height during childhood and puberty. At the end of GH treatment a slight decrease in height SD-score was found in comparison with height SD-score after 5 years of GH treatment. Possible explanations for this decline might be an early onset of puberty, short duration of puberty, and/or an acceleration of bone maturation. This would lead to attainment of final height at a relatively young age in comparison with peers, and/or a reduced pubertal height gain. We found, however, that for both GH dosage groups, mean age at onset of puberty was comparable to normal Dutch children (median age for healthy boys is 11.5 years and for girls 10.7 years), whereas from the onset of puberty until final height the mean pubertal height gain was 29.8 cm for boys and 18.9 cm for girls [9]. In accordance, during puberty, no acceleration of bone maturation was found until discontinuation. Previous publications have shown, however, that untreated children born SGA start their pubertal growth spurt earlier than normal children.[148, 198] We found that the difference between CA and BA (CA-BA) after 5 years of GH treatment was -1.0, indicating a 1-year bone age advance. This might explain the slight decrease in height SD-score. As we found no significant difference between the two GH dosage groups, it is unlikely that the 1-year bone age advance was related to the GH treatment. Another factor that might explain the decrease in height SD-score is that some of the children did not reach their full height potential because they stopped GH treatment at near-final height.
Final height SD-score, when corrected for GH dosage was higher in children with the highest TH SD-score and height SD-score at start of GH treatment. Although age at start was weekly correlated with height gain from start until FH, it was not associated with FH SD-score. Why other studies did find an association might be explained by the fact that they started treatment at a later mean CA, which implied a shorter duration of GH treatment (4.6 years) until FH than in our group.[182] On the long-term, other factors that influence FH (i.e. genetic background) might become more important. In our opinion, however, this should not lead to postponing GH treatment until puberty. Not only would the treatment period then be too short, but also the important

advantages of a normal height during childhood and adolescence would be lost.

The GHD children included in our study were not severely GHD but only partially GHD. Their growth and FH results were similar to those without GHD. Also, in the total study group no correlation was found between spontaneous or stimulated GH secretion before start of GH treatment and FH SD-score or gain in height SD-score. Our findings agree with other reports showing no association between response to GH treatment and GH status before start [199, 200]. This indicates that GH treatment is effective in short children born SGA, leading to a normal FH for most of them, regardless of the GH status at start.

The recommended GH dose will depend on the ultimate goal one aims for in short children born SGA. First of all, one might aim for a normal FH, meaning a FH above −2 SD-score. Secondly, one could set out for a FH within the target height range SD-score. To visualize the effects of each of these goals on the decision of which GH dose to use, we constructed a prediction model (Table 3). The prediction model for FH SD-score shows that several variables influence FH SD-score: TH SD-score, height SD-score at start, and the difference CA-BA at start. For all these variables a higher value predicts an increment in FH SD-score. These variables are not surprising as they have also been found to predict height gain and final height in other patient groups, but it indicates that similar factors play a role in response to GH treatment in short SGA children [102, 201]. Suppose a child born SGA starts GH treatment when he or she has a persistent short height of −2 SD-score and no bone age delay (CA-BA = 0) (Table 4). Our model then predicts that when this child has an average TH SD-score (TH SD-score = 0) it would achieve a FH of −0.6 SD-score after a dose of 3 IU GH/m^2/day (95% prediction interval [PI] between −1.8 and 0.6 SD-score). For a child with a TH SD-score of −2, treated with the same GH dose, the predicted FH would be −1.2 SD-score (95% PI between −2.4 and 0). When one chooses the first goal, a final height above −2 SD-score, both children can be given 3 IU GH/m^2/day. When one decides on the second goal, a final height near the target height SD-score, a higher GH dose of 6 IU GH/m^2/day might be considered for a child with a higher target height SD-score. Our model, however, shows that by giving the child with a TH of 0 SD-score the higher dose, the predicted FH SD-score would only increase by 0.2 SD-score (−0.4 SD-score with 95% PI between −1.6 and 0.8), while doubling the costs of treatment. Another possible reason for considering a higher GH dose could be when the child is very short at start of GH treatment. The model shows that, using the same values as before (no BA delay and TH SD-score=0), a child with a height SD-score at start of GH treatment of −3 would reach a FH SD-score of −1.0 (95% PI between −2.2 and 0.2) when using the low dose, and −0.8 SD-score (95% PI between −2.0 and 0.4) when using the high dose of GH. As before, the difference in final height would be quite small, while doubling the costs of treatment. Obviously, one should keep in mind that the model only predicts 42% of the difference in FH, leaving 58% of the variation to be explained by other factors, such as genetic background.[164, 193] For that reason, larger numbers of GH-treated short SGA children with detailed phenotypic and genetic data are required to allow for a prediction model with a higher predictive value.

In conclusion, long-term, continuous GH treatment in short children born SGA leads to a normalisation of height in childhood and adolescence. Eighty-five percent will reach a normal final height, whereas 98% will reach a final height within their target height range. Based on our study we recommend considering GH treatment for short SGA children without signs of persistent catch-up growth and who are therefore at risk of significant height disability as adults. Interestingly, a dose of 3 IU/m^2/day proved to be as effective as the higher GH dose of 6 IU/m^2/day, for most children. Only children with extreme short stature or/and a target height below the normal range may need a higher GH dose to normalize height during childhood and in adulthood. Further studies should aim at optimizing GH treatment by developing advanced prediction models indicating the best treatment options for each child.

CHAPTER 9

Part 'Small for Gestational Age': Effect of discontinuation of GH treatment on risk factors for cardiovascular disease in adolescents born small for gestational age

Effect of discontinuation of GH treatment on risk factors for cardiovascular disease in adolescents born small for gestational age

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Abstract

Hyperlipidaemia, diabetes mellitus type 2, and coronary heart disease have been associated with being born small for gestational age (SGA). It has been reported that GH treatment induced higher insulin levels, which has lead to concern regarding long-term effect of GH treatment in predisposed individuals such as children born SGA. In this study, we assessed the effect of discontinuation of long-term GH treatment in 47 adolescents born SGA on oral glucose tolerance tests, blood pressure (BP), and serum lipid levels, for two GH dosage groups (3 versus 6 IU/m^2/day).
At 6 months after discontinuation of GH treatment mean (SD) age was 16.0 (2.1) years. Mean duration of GH treatment had been 6.9 (1.5) years. Fasting glucose levels and 120-minute area under the curve (AUC) for glucose 6 months after discontinuation of GH treatment showed no difference with pre-treatment levels for both GH dosage groups. After discontinuation of GH treatment fasting insulin levels returned to pre-treatment levels (8.4 mU/l), whereas the 120-min AUC for insulin decreased compared to 6 year-levels ($P < 0.01$), regardless of GH dosage group. No significant difference was found when levels were compared with a control group. In addition, for both GH dosage groups, no significant changes in systolic and diastolic BP SD-score, total cholesterol (TC) and atherogenic index (TC/HDL-c) were seen from 6-years of GH until 6 months after discontinuation of GH treatment.
In conclusion, in children born SGA the GH induced insulin insensitivity disappeared after discontinuation of GH, even after long-term GH treatment. Furthermore, the beneficial effect of GH on blood pressure was not changed after discontinuation of GH and most children had normal lipid levels.

Introduction

Since the first reports of correlation between low birth weight (LBW) and high blood pressure in the 80's [202], a large number of studies have elaborated on the consequences of LBW in relation to adult disease. Several other diseases such as hyperlipidaemia, diabetes mellitus (DM) type 2, and coronary heart disease (CHD) have also been associated with LBW [155, 190]. Based on their findings, Barker and co-workers [203] suggested that the associated adult diseases, so-called syndrome X, are programmed by undernutrition in utero. Impaired foetal growth, especially when timed during the mid- to late

gestation, would lead to permanent changes in organ structure and physiology.
Failure to show sufficient catch-up growth in childhood is a known phenomenon in about 10% of children born small for gestational age (SGA) [140, 198]. Several studies [150, 184] have shown accelerated growth during GH treatment. However, it has been established that GH treatment increases post-prandial insulin levels, probably due to a reduction in insulin sensitivity [185]. This finding has instigated several authors to express their concern regarding long-term effects of GH treatment in predisposed individuals such as SGA children [46].
We previously reported on the effect of 6 years of GH treatment on carbohydrate metabolism, blood pressure and serum lipid levels in children born SGA [91, 204]. The present paper focuses on the effect of discontinuation of long-term GH treatment on carbohydrate metabolism, blood pressure and serum lipid levels in children born SGA.

Subjects and Methods

Study group
The study group comprised 47 children born SGA who were examined 6 months after discontinuation of GH treatment. Thirty children had an OGTT at 6 months after discontinuation of GH. They had participated in a multi-centre double-blind randomised dose-response GH trial in prepubertal short children born SGA. Four children who had discontinued GH treatment did not agree to an examination 6 months after discontinuation of GH treatment and 28 were still receiving GH treatment.
The ongoing dose-response trial evaluates the effect of two dosages of GH (3 or 6 IU GH/m2 BSA/day; approximately 0.03 or 0.07 mg/kg BW/day) on long-term growth and ultimately on final height.
Inclusion criteria for the dose-response trial were described previously [150]. In short, the children were included when prepubertal with a birth length SD-score and height SD-score below -1.88, without spontaneous catch-up growth, and without growth failure caused by other disorders. Patients with Silver-Russell syndrome (SRS) and GH-deficiency, however, were included in this dose-response trial. The Ethics Committees of the four participating centres in the Netherlands approved the dose-response trial. Written informed consent was obtained from the parents or custodians of each child.

Dose-response trial design
After stratification for chronological age (CA) and for spontaneous GH secretion during a 24-hour GH profile, all children were randomly and blindly assigned to either 1 of 2 GH dosage groups: group A, 3 IU/m^2 body surface/day, or group B, 6 IU/m^2 body surface/day (approx. 0.03 or 0.07 mg/kg/d, respectively) [145, 150]. Biosynthetic GH (recombinant human GH Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily. To ensure the double-blind design an equal volume of a reconstituted preparation was used. Growth hormone treatment was discontinued after either reaching final height, defined as a height velocity (HV) below 0.5 cm over the last 6 months and/or bone age ≥ 15 years for girls and ≥ 16.5 years

for boys, or on the decision of the patient due to satisfaction with near final height.

Physical examination
Every three months one physician ('91-'95 WdW, '95-'98 ThS, '98-'01 YvP) visited all children and measured height (H) [194] and weight (W). Height was expressed as SD-score for chronological age (CA) (Height SD-score) [62]. Body mass index (BMI) was expressed as SD-score for sex and CA using Dutch references [62]. Every 6 months blood pressure (BP) was measured. The same Dynamap Critikon 1846SX determined Systolic and diastolic BP with the children in sitting position using a cuff size corresponding to the size of their arm. BP was expressed as SD-score, using age and sex specific reference values [82]. Pubertal stage was assessed by the same investigators according to Tanner [63], using an orchidometer in boys.

Biochemical parameters
At start, after one, and six years of GH treatment all children underwent an OGTT (oral glucose tolerance test) as previously described by Sas et al [91]. For the present study we performed an OGTT 6 months after GH treatment in three of the four participating centres. To evaluate the overall responses to the oral glucose load, the following variables were described: 1) Impaired glucose tolerance (IGT) was defined according to The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus [80]: the 120-minute (120-min) level > 7.8 mmol/L (140 mg/dl) and < 11.1 mmol/L (200 mg/dl). 2) The 120-minute area under the curve for time-concentration (AUC) during the OGTT was calculated using the trapezoidal rule. 3) The ratio insulin to glucose at 30 minutes (30' ratio) and the ratio at 120 minutes (120' ratio) was calculated as an index for relative insulin resistance. Results were compared with 24 normal adolescent girls aged 14.7 (0.98) years [81].
At start, during the dose-response trial, and at 6 months after discontinuation of GH treatment additional non-fasting blood samples were taken for determination of haemoglobin A1c (HbA1c) levels, total serum cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c). The atherogenic index was calculated as the ratio of TC to HDL-c. After centrifugation, all samples were frozen (-20ºC) until assayed. Serum TC, LDL-c, HDL-c levels, and atherogenic index were compared to a Dutch control group of the same age [83].

Assays
The plasma glucose level was measured at the local hospital laboratories with automatic analysers using a hexokinase catalysed-glucose oxidase method. Plasma insulin was determined in one laboratory by radioimmunoassay (RIA) (Medgenix, Fleurus, Belgium). The intra-assay coefficient of variation (CV) was 6% to 10% and the inter-assay CV was 6% to 11%. Fasting normal range was < 20 mU/L. Control samples were measured by a RIA, comparable to the RIA we used [81].
HbA1c levels were measured in one laboratory using an automatic HPLC analyser (DIAMAT, BioRad, Edgemont, CA, USA). The upper-normal assay limit is 6.6%.

TABLE 1. Clinical data.

N=47	Group A (3 IU/m^2/day)	Group B (6 IU/m^2/day)
Male/Female	16/7	11/13
Gestational age (wk)	37.7 (3.1)	36.3 (4.1)
Birth length SD-score	-3.4 (1.4)	-3.8 (1.7)
Birth weight SD-score	-2.3 (1.2)	-2.8 (0.9)
Chronological Age (yr.) at start	8.6 (1.5)	8.3 (1.9)
Height SD-score at start	-2.9 (0.8)	-2.8 (0.6)
Body mass index SD-score at start	-0.9 (1.5)	-1.0 (1.0)
GH duration	7.2 (1.2)	6.6 (1.7)

Data expressed as mean (SD)

FIGURE 1. Mean (+SD) fasting glucose levels and fasting insulin levels, before treatment, at 6 years of GH treatment, and at 6 months after discontinuation of treatment for group A (white bars) and for group B (black bars), respectively.

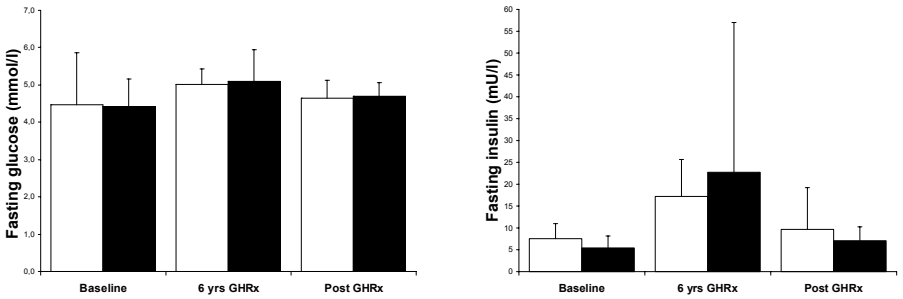
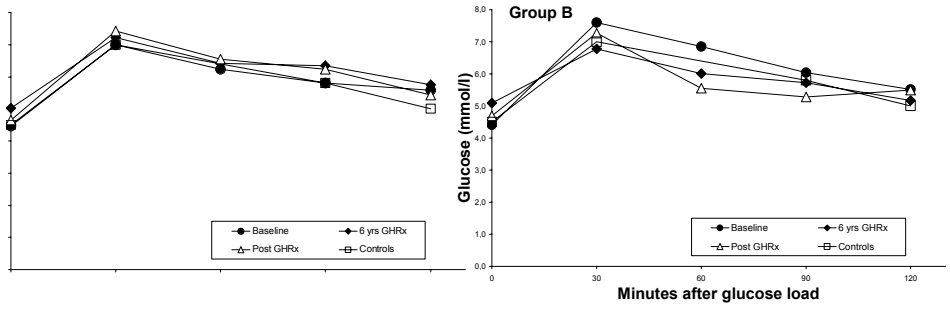


FIGURE 2. Mean glucose levels during OGTT for group A and group B, before treatment (black circles), at 6 years of GH treatment (black diamonds), at 6 months after discontinuation of treatment (white triangles), and for the control group (white squares).



The TC level was measured using an automated enzymatic method [205] with the CHOD-PAP High Performance reagent kit (Boehringer, Mannheim, Germany). TC analysis was subject to the quality-assessment program of the World Health Organisation Regional Lipid Reference Centre (Prague, Czech Republic). HDL and LDL-c were measured by the same method after precipitation. For HDL-c, the phosphotungstate method of Burstein was modified [206]. LDL-c precipitation was performed with polyvinylsulfate (Boehringer). The overall coefficient of variance for TC, HDL-c, and LDL-c was 2.9%, 3.7%, and 5.8%, respectively. Lipid levels for the control group were measured by the same assays in the same laboratory [83]. Except for plasma glucose, all determinations were performed in the same laboratories.

Statistical analyses
To maintain the double-blind design until all participants have reached FH, an independent statistician (PM) performed the statistical analyses and summarised the results per treatment group in such a way that it was impossible for the investigator to identify individual patients. Accordingly, data are expressed as mean (SD) values unless otherwise specified. For the 30' ratio, the 120' ratio, and the atherogenic index the geometric mean (95% range: the back transformed +/- 2 SD range of the log transformed variable) was used because of the positively skewed distributions involved. Differences in continuous variables were tested by paired Student's t-tests. Differences between zero and SD-score values at various time-points during the study were tested by one-sample Student's t-tests. Differences between groups were tested using a Student's two-sample t-test unless otherwise specified. Correlations were estimated after adjustment for GH dosage. A P-value < 0.05 was considered significant. All calculations were performed by SPSS version 9.0.

Results

Table 1 shows the clinical data of the 47 children. Both GH dosage groups had similar initial characteristics. Three children had SRS. Seventeen children had GHD at start. Mean (SD) age at 6 months after discontinuation of GH treatment was 16.0 (2.1) years. No significant differences were found in clinical data and serum glucose or insulin levels between the 30 children who underwent an OGTT at 6 months after discontinuation of GH treatment, and the 17 children who did not.
Fasting glucose levels at 6 years of GH treatment had increased significantly compared to pre-treatment ($P < 0.05$) but returned to pre-treatment level for both GH dosage groups at 6 months after discontinuation of GH treatment (Figure 1). The AUC for glucose showed neither a change at six years of GH treatment or at 6 months after discontinuation of GH treatment. Fasting glucose levels and the AUC for glucose were not significantly different between the GH dosage groups. Similarly, no significant differences were found between the GH-treated group and the control group (Figure 2).

FIGURE 3. Mean insulin levels during OGTT for group A and for group B, before treatment (black circles), at 6 years of GH treatment (black diamonds), at 6 months after discontinuation of treatment (white triangles), and for the control group (white squares).

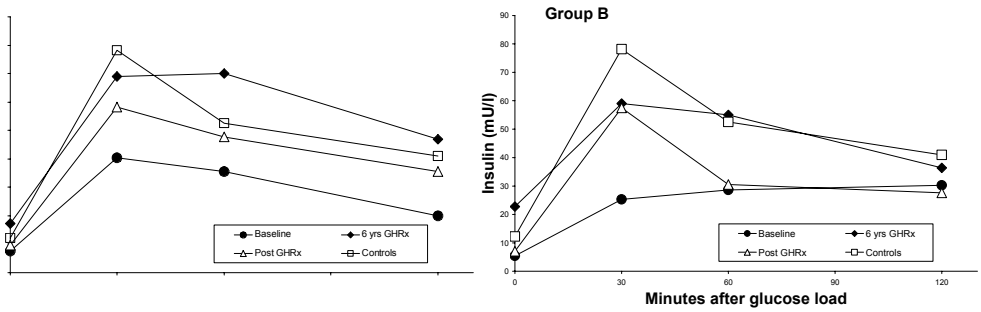


FIGURE 4. Mean (SD) systolic blood pressure (BP) SD-score and diastolic BP SD-score using age-matched reference values, before GH treatment, at 6 years of GH treatment, and at 6 months after discontinuation of treatment for group A (white bars) and for group B (black bars).

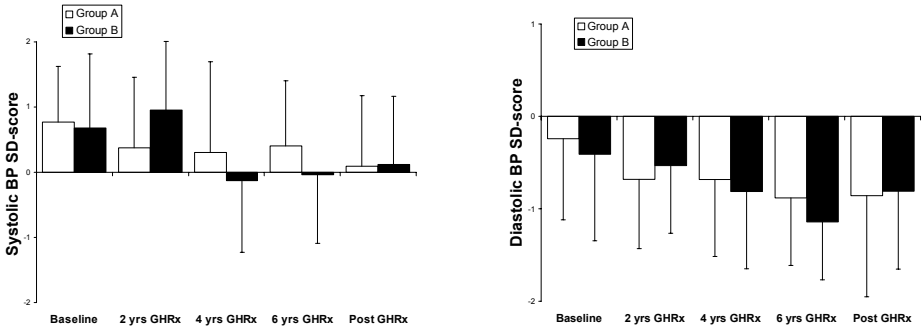


TABLE 2. Serum lipid levels.

		Group A	Group B	Control group
TC mmol/l	Pre GH	4.9 (0.7)	4.6 (0.8)	
	6 yr. GH	4.1 (0.5)	3.8 (0.6)	
	Post GH	4.1 (0.5)	4.0 (0.7)	4.6 (0.8)
LDL-c mmol/l	Pre GH	2.9 (0.5)	2.7 (0.9)	
	6 yr. GH	2.4 (0.5)	2.0 (0.7)	
	Post GH	2.5 (0.5)	2.3 (0.7)	2.8 (0.7)
HDL-c mmol/l	Pre GH	1.5 (0.3)	1.4 (0.2)	
	6 yr. GH	1.1 (0.2)	1.0 (0.2)	
	Post GH	1.1 (0.2)	1.1 (0.4)	1.3 (0.3)
Atherogenic index* (TC/HDL-c)	Post GH	3.0 (2.0-4.5)	3.0 (1.3-6.7)	3.7 (2.3-6.1)

Data expressed as mean (SD). TC = Total Cholesterol; LDL-c = Low Density Lipoprotein-cholesterol; HDL-c = High Density Lipoprotein-cholesterol; * Geometric mean (95% range).

Fasting insulin levels had increased at 6 years of GH treatment compared to at pre-treatment ($P < 0.001$) but returned to pre-treatment levels at 6 months after discontinuation of GH treatment (Figure 1). Also, the AUC for insulin showed a significant increase from 3349 (1643) min*mU/l pre-treatment to 6157 (2368) min*mU/l at 6 years of GH treatment ($P < 0.001$). At 6 months after discontinuation of GH treatment the AUC for insulin, 4645 (3641) min*mU/l, had significantly decreased compared to the 6 year-level ($P < 0.01$) but remained higher than pre-treatment ($P < 0.05$). Compared to the control group, however, the AUC for insulin at 6 months after discontinuation of GH treatment showed no significant difference. No significant differences between GH dosage groups were found for change in fasting insulin levels and AUC for insulin (Figure 3).
The ratio for insulin to glucose at 30' (30' ratio) and 120' (120' ratio) had increased significantly at 6 years of GH treatment compared to pre-treatment levels ($P < 0.001$, $P < 0.05$, respectively). At 6 months after discontinuation of GH treatment the 30' ratio, returned to pre-treatment values for group A. For group B, however, the 30' ratio remained increased compared to pre-treatment values ($P < 0.001$). The 120' ratio, at 6 months after discontinuation of GH treatment, was comparable to pre-treatment values, showing no differences between GH dosage groups.
After six years of GH treatment, we found impaired glucose tolerance (IGT) in 1 of 27 children (4%), and at 6 months after discontinuation of GH treatment IGT was present in 3 of 29 children (10%). One of the children with IGT at six years of GH treatment also had IGT at 6 months after discontinuation of GH treatment. The other children had IGT only once during GH treatment or after discontinuation of GH treatment. None of the children developed diabetes mellitus type 1 or 2.
The HbA1c values after six years of GH treatment had significantly decreased from 5.1 (0.3) at pre-treatment to 4.8 (0.4) at 6 years of GH treatment ($P < 0.001$), whereas no change was found at 6 months after discontinuation of GH treatment (4.7 (0.3)). Throughout the years, all individual HbA1c levels remained within normal range.
BMI SD-score after 6 years of GH treatment had increased significantly compared to the pre-treatment BMI SD-score of -1.0 (1.2) ($P < 0.001$). As a result the mean BMI SD-score of -0.2 (1.1) at 6 years was not significantly different from zero. At 6 months after discontinuation of GH treatment mean BMI SD-score was –0.1 (1.3), showing no significant difference compared to 6-year values. The change in BMI SD-score was not significantly different between GH dosage groups.
Systolic BP and diastolic BP decreased significantly during 6 years of GH treatment ($P < 0.05$, $P < 0.001$, respectively) to values not significantly different from zero for systolic BP and significantly lower than zero for diastolic BP ($P < 0.001$)(Figure 4). After discontinuation of GH treatment no change in systolic and diastolic BP was seen. The changes in BP were not significantly different between GH dosage groups.
Serum TC, LDL-c, and HDL-c levels significantly decreased after 6 years of GH treatment compared to start ($P < 0.001$ for all)(Table 2). After discontinuation of GH treatment, TC levels showed no change compared to 6-year levels for both sexes, whereas LDL-c and HDL-c levels increased significantly for girls only ($P < 0.01$, $p=0.01$, respectively). Discontinuation of

GH treatment did not result in a change in atherogenic index compared to 6-year values. Compared to the control group, serum TC, LDL-c, but also HDL-c levels were significantly lower in the GH-treated group at 6 months after discontinuation of GH treatment, after correction for age and gender ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively). The atherogenic index, however, was not significantly different at 6 months after GH treatment, compared to the control group (Table 2). The changes in serum lipid levels were not significantly different between the GH dosage groups.
A significant correlation was found between AUC for glucose and AUC for insulin at 6 months after discontinuation of GH treatment ($r=0.67$, $P < 0.0001$). BMI SD-score correlated significantly with fasting insulin levels, systolic BP SD-score, and the atherogenic index at 6 months after discontinuation of GH treatment ($r=0.58$ ($P < 0.01$), $r=0.32$ ($P < 0.05$), and $r=0.42$, $P < 0.05$, respectively), but no correlation was found between BMI SD-score and diastolic BP SD-score, or 120-minute glucose level at 6 months after discontinuation of GH treatment. The atherogenic index at 6 months after discontinuation of GH treatment was also significantly correlated to diastolic BP SD-score ($r=0.43$, $P < 0.05$), but did not significantly correlate with systolic BP SD-score, fasting insulin, or 120-minute glucose levels. Regarding the change in fasting glucose, fasting insulin, 30' ratio, 120' ratio or AUC for insulin from start until 6 months after discontinuation of GH treatment, no correlation was found with the change in BMI-SD-score.

Discussion

We present the results describing the effect of discontinuation of long-term GH treatment on carbohydrate metabolism, body mass index, blood pressure, and serum lipid levels in children born small for gestational age. Our results show that discontinuation of long-term GH treatment in adolescents born SGA normalised both fasting insulin and stimulated insulin levels, after a significant increase during GH treatment. Furthermore, we found that discontinuation of GH did not alter the positive influence of GH on BMI and blood pressure and had no effect on the atherogenic index (TC/HDL-c). Previously, we have shown that stimulated glucose levels remained unchanged and fasting glucose levels rose slightly during 6 years of GH treatment. In addition, both fasting and stimulated insulin levels increased significantly [91]. It has been reported that GH treatment increases serum insulin levels in conditions such as GHD, Turner syndrome, and renal diseases [50, 51]. This has been attributed to a GH induced reduction of insulin sensitivity. Also, it has been reported that post-prandial glucose levels increase in individuals with a reduced insulin sensitivity [207].

Our present results show that after discontinuation of GH treatment, fasting glucose and fasting insulin levels returned to pre-treatment levels. This indicates that the rise in glucose and insulin levels were indeed a result of GH treatment. In other patient groups, such as girls with Turner Syndrome, GHD adolescents, and non-GHD adolescents similar results were found after discontinuation of GH treatment [51, 84, 186]. While fasting insulin levels at 6 months after discontinuation of GH treatment decreased to pre-treatment levels, stimulated insulin levels did not. The reason why post-treatment stimulated insulin levels did not completely return to pre-treatment levels might have various reasons. Firstly, patients were prepubertal at start and postpubertal after discontinuation of GH treatment. Euglycaemic clamp tests or frequently sampling intravenous glucose tolerance tests have shown that insulin sensitivity in healthy children decreases during puberty. Also, in these studies, post-pubertal insulin sensitivity did not return to prepubertal values [81, 86, 87]. The higher insulin levels were attributed to a reduced insulin sensitivity in normal puberty and post-puberty [86]. Thus, the reduced insulin sensitivity compared to baseline in our patients might be explained by their post-pubertal stage. This is supported by the fact that post-treatment stimulated insulin levels were comparable to those of healthy adolescent peers. Secondly, the BMI SD-score increased during GH treatment. Since a higher BMI is associated with higher insulin levels, this might also explain the higher post-treatment stimulated insulin levels compared to pre-treatment levels [88, 89]. In accordance, we found that adolescents with high fasting and stimulated insulin levels after discontinuation of GH treatment had a significantly higher BMI SD-score. Thirdly, post-treatment stimulated insulin levels might be influenced by the fact that these adolescents were born SGA. Significantly higher insulin levels have been found after an oral glucose load in young adults born SGA compared to normal controls [208, 209].

During the 6 years of GH treatment we found no differences between the two GH dosage groups regarding fasting or post-prandial glucose and insulin levels. After discontinuation of GH treatment, however, we observed that in the higher GH-dosage group (group B) the decrease in the 30-minute ratio for insulin/glucose was significantly less profound than in the lower GH-dosage group (group A). As the 30-minute ratio for insulin/glucose is an indicator for insulin resistance, this finding might suggest that long-term treatment with a higher GH dose of 6 IU/m^2/day (~ 0.07 mg/kg/day) increases the degree of insulin resistance in children born SGA even after discontinuation of GH treatment. Compared to the control group, however, the mean 30-minute ratio of group B still falls into the normal range.

As insulin resistance, either on its own or in combination with beta-cell dysfunction, causes IGT, we evaluated the number of children in our group with IGT. We found that after six years of GH treatment 1 of 27 children had IGT (4%), and at 6 months after discontinuation of GH treatment IGT was present in 3 of 29 children (10%). This result might be explained by the fact that puberty induces higher 120-min glucose levels [86, 87]. The predisposition for insulin resistance and IGT in this group of adolescents born SGA, however, might also be responsible. Several studies found evidence for insulin resistance in untreated short children born SGA [210, 211]. Also, being born with a low birth weight is associated with IGT and diabetes mellitus type 2 [190, 212]. On the other hand, mean glucose levels at 6 months after

discontinuation of GH decreased to pre-treatment levels, individual HbA1c levels never exceeded the normal range, and none of the children developed diabetes mellitus type 1 or 2.
Systolic and diastolic blood pressure did not change after discontinuation of GH treatment. Another study evaluating the effect of discontinuation of GH in adolescents with GHD, showed similar results [92]. It has been reported that children and young adults born with a low birth weight had a significantly higher systolic blood pressure [213-216]. In some studies this relationship was also found when low birth weight was corrected for gestational age [216], but in others it was not [208, 217]. We reported, previously, that before start of GH treatment children born SGA had a significantly higher systolic blood pressure than reference values, which decreased to normal during GH treatment [91]. Our present study shows that after discontinuation of GH treatment, systolic blood pressure was not different to age-and sex-matched reference values, whereas diastolic blood pressure was even significantly lower than reference values. These results contrast with data of untreated subjects born SGA and might reflect a positive influence of GH on blood pressure in this patient group.
During GH treatment serum TC, LDL-c, and HDL-c levels decreased. Discontinuation of GH treatment resulted in a slight increase in both LDL-c and HDL-c levels and no change in total cholesterol levels. Previous reports have shown that discontinuation of GH treatment in GHD adolescents had either no effect on serum lipids [218] or resulted in an increase in total cholesterol and LDL-c levels, without change in HDL-c levels [92, 93]. The increase in both LDL-c and HDL-c in our children might also be an age-effect [95, 96]. We found that the atherogenic index after discontinuation of GH treatment was comparable with a control group of similar age. The lack of difference in serum lipid levels between the two GH dosage groups suggests that the changes in lipid levels were not related to GH treatment.
After discontinuation of GH treatment, we found a positive correlation between the atherogenic index, BMI SD-score, systolic and diastolic blood pressure SD-score, and fasting insulin. This might suggest that some adolescents born SGA, a clustering of risk factors for cardiovascular disease, as described by Barker and co-workers, is already present [203]. Since fasting insulin, systolic BP, and atherogenic index were positively correlated with BMI SD-score, a possible strategy to decrease risk for cardiovascular disease in subjects born SGA might be to prevent weight gain.
In conclusion, in children born SGA the GH induced insulin insensitivity disappeared after discontinuation of GH, even after long-term GH treatment. Furthermore, the beneficial effect of GH on blood pressure was not changed by discontinuation of GH. Although most children had normal serum lipid levels, we did find a clustering of risk factors for cardiovascular disease, which may point to their predisposition. Whether long-term GH treatment will contribute to longevity, however, remains to be investigated

CHAPTER 10

Part 'Small for Gestational Age': Intelligence and psychosocial functioning during long-term GH therapy in children born small for gestational age

Intelligence and psychosocial functioning during long-term GH therapy in children born small for gestational age

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Abstract

Short stature is not the only problem faced by small for gestational age (SGA) children. Being born SGA has also been associated with lowered intelligence, poor academic performance, low social competence, and behavioural problems. Although GH treatment in short children born SGA can result in a normalisation of height during childhood, the effect of GH treatment on intelligence and psychosocial functioning remains to be investigated. We show the longitudinal results of a randomised, double blind, GH-dose response study initiated in 1991 to follow growth, IQ and psychosocial functioning in SGA children during long-term GH treatment. Patients were assigned to one of two treatment groups (3 or 6 IU GH/m^2 body surface/day ~ 0.035 or 0.07 mg/kg/day). Intelligence and psychosocial functioning were evaluated at start of GH treatment (N=74), after 2 years of GH treatment (N=76), and in 2001 (N=53). IQ was assessed by a short form WISC-R or WAIS (Block-design and Vocabulary subtest). Behavioural problems were measured by the Achenbach CBCL or YABCL and self-perception by the Harter Self-Perception Profile.
Mean (SEM) birth length SD-score was −3.6 (0.2), mean age and height at start was 7.4 (0.2) years and −3.0 (0.1) SD-score, mean duration of GH treatment was 8.0 (0.2) years, mean age in 2001 16.5 (0.3) years. After 2 years of GH treatment 96% of both GH groups showed a height gain SD-score from start of 1 SD or more, resulting in a normal height (i.e. height ≥ −2.0 SD for age and sex) in 70% of the children. In 2001 48 of the 53 children, participating in this study, had reached a normal height (91%). Block-design s-score and the estimated Total IQ significantly increased ($P < 0.001$, $P < 0.001$, respectively) from scores significantly lower than Dutch peers at start ($P < 0.001$, $P < 0.001$, respectively) to comparable scores in 2001. The increase over time for the Vocabulary s-score was not significant. Internalizing behavior SD-scores remained comparable to Dutch peers, while Externalizing behavior SD-scores and Total problem behavior SD-scores improved significantly during GH therapy ($P < 0.01$, $P < 0.05$, respectively) to scores comparable to Dutch peers. Self perception SD-scores, improved

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from start of GH treatment until 2001 ($P < 0.001$) to scores significantly higher than Dutch peers ($P < 0.05$). No significant differences between the two GH dosage groups were found. Improvement in Externalizing and Total problem behavior SD-scores over time was significantly related to change in height SD-score ($P < 0.05$, $P < 0.01$, respectively), while scores over time for Vocabulary, Block-design, Internalizing, or Total HSP were not related to change in height SD-scores.
In conclusion, parallel to a GH-induced catch-up growth in adolescents born SGA, IQ, behaviour, and self-perception showed a significant improvement over time from scores below average to scores comparable to Dutch peers. In addition, children whose height over time became closer to that of their peers showed less problem behaviour.

Introduction

Ten percent of children born SGA do not catch-up in height and remain short [140, 198]. The reason for these children remaining short is not completely understood. Sixty percent of short children born SGA have low serum growth hormone (GH) levels during a 24-hour GH profile and most have low IGF-1 levels, but no relation was found between physiological GH levels and the growth response during GH treatment [145, 150, 191, 219]. Short stature, however, is not the only problem which SGA children face. Being born SGA, with or without catch-up growth, has also been associated with lower intelligence, poor academic performance, low social competence, and behavioural problems [165-171]. Suggested explanations for this association are intrauterine malnutrition affecting brain development or relative deficiency of the GH-IGF-1 axis [172-174]. Although recent studies have demonstrated that GH treatment in short children born SGA can result in a normalisation of height during childhood [150, 184], the effect of GH treatment on intelligence and psychosocial functioning remains to be investigated. Therefore, in 1991, a randomised, double blind, GH-dose response study was initiated to follow growth, IQ and psychosocial functioning during long-term GH treatment. Previously, our group described the effect of two years of GH treatment in short children born SGA on IQ and psychosocial functioning. We then found a significant increase in total IQ-score, in 'social acceptance' scores, and in 'general self-worth' scores [169, 188]. In the present study, during 8 years of GH treatment, we evaluated IQ and psychosocial functioning in the same group of short children born SGA without spontaneous catch-up growth. Recent analysis of the growth data showed a further increase in height SD-scores and a normalisation of final height for most participants [220]. In accordance with our clinical observation and the GH-induced catch-up growth, in the present study we expected to find a similar IQ to 2 years data and a further improvement in psychosocial functioning.

Methods

Patient group (GH group)
Seventy-nine short children born SGA participated in a multi-centre randomised double-blind dose-response GH trial (Table 1). Of these 79 children 6 children dropped out and were lost to follow-up. Fifty-three

children agreed to participate in the evaluation of IQ and psychosocial functioning in 2001, during a mean of 8 years of GH treatment, whereas 20 children were not motivated to participate (T3: response rate 73%). In the previous evaluations at start (T1) and after 2 years of GH treatment (T2), a small part of the GH groups had not been able to participate due to the age minimum of the questionnaires. The response rates for the previous evaluations at start and after 2 years of GH treatment were 100% (74/74) and 97% (76/78), respectively. Sixty-three percent (50/79) was evaluated three times and 94% (74/79) participated at least twice in the evaluation of IQ and psychosocial functioning.
The GH trial started at 1991 (T1) and evaluated the effect of GH on long-term growth and ultimately on final height. Inclusion criteria for the GH dose-response trial were: birth length SD-score below -1.88 (< P3), chronological age (CA) between 3 and 11 year in boys and 3 and 9 year in girls, height SD-score for CA below -1.88, no spontaneous catch-up growth, prepubertal stage, uncomplicated neonatal period without severe asphyxia. Biosynthetic GH (r-hGH NorditropinR, Novo Nordisk A/S, Denmark) was given subcutaneously once daily. GH was given in a dosage of 3 or 6 IU (1 or 2 mg) GH/m^2 body surface/day (~ 0.035 or 0.07 mg/kg /day). GH treatment was discontinued after reaching final height (height velocity < 0.5 cm in 6 months) or on patient's decision after reaching a satisfactory height (near final height). Of the participants in the evaluation of IQ and psychosocial functioning in 2001, 37 of the 53 adolescents (70%) had discontinued GH treatment.
The evaluation of IQ and psychosocial functioning, being part of the GH trial, started in 1991 and was approved by the Ethics Committees of the participating centres in the Netherlands. Due to ethical considerations, the Ethics Committees did not allow for a control group until final height as part of the GH trial. Written informed consent was obtained from the parents or custodians of each child.

Clinical evaluation
Height was measured using a Harpenden stadiometer [194]. Four measurements per visit were taken and the mean was used for analysis. Target height (TH) was adapted from Dutch reference data with addition of 3 cm for secular trend: 1/2 x (Heightfather + $Height^{mother}$ + 12) + 3 for boys and 1/2 x ($Height^{father}$ + $Height^{mother}$ - 12) + 3 for girls.[62] Height, TH, and head circumference were expressed as SD-score for chronological age (CA) and gender [9, 62].

Evaluation of IQ and psychosocial functioning
The evaluation of IQ and psychosocial functioning was performed at start (T1), after 2 years of GH treatment (T2), and in the year 2001 (T3) by an experienced psychologist. At start, all parents returned the questionnaires. In the evaluation at 2 years of GH treatment 2 parents did not return the questionnaires and in 2001 8 parents. Data on occupational levels were provided by both parents and adolescents. 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 [105].

Intelligence. To assess intelligence a short form of two subtests (Block-design, a Performance IQ subtest and Vocabulary, a Verbal IQ subtest) of the Wechsler Intelligence Scale for Children – Revised, Dutch version (WISC-R) [221], was used for children aged between 6 and 16 years and a short form (also Block-design and Vocabulary subtest) of the Wechsler Adult Intelligence Scale, Dutch version (WAIS) [222], for adolescents aged 17 years and older (only used in 2001). Good correlations have been found between the short form IQ and the full scale IQ both for the WISC-R and the WAIS [223]. The subtest scores for Block-design (Bd) and Vocabulary (Vo) were expressed as normalised standard scores (s-scores) with a mean of 10, ranging from 1 (- 3 SD) to 19 (+ 3 SD), based on Dutch population sample data for the same age [221, 222]. S-scores for the WAIS and WISC-R were combined to enable time trend analysis of the subtests. In the time trend analysis a dummy variable (WAIS yes/no) was added, when significant, to correct for type of IQ test used in 2001. A higher s-score indicated a better result in the subtest. Total IQ score was calculated according to an equation, based on the outpatient population reference data of the Child Psychiatry Department of the Sophia Children's Hospital (TIQ=45.3 + 2.91*Vocabulary s-score + 2.50*Block-design s-score).

Behaviour. Behavioural problems were measured by 3-point scale standardised questionnaires, designed by Achenbach, translated and validated in the Dutch language [104, 106, 107, 224, 225]. For children aged between 4 and 18 years, the 120-item Child Behavior Checklist (CBCL; filled in by parents) was used and for adolescents aged 19 years and older the 115-item Young Adult Behavior Checklist (YABCL; filled in by parents). Since all questionnaires were constructed in a similar way, SD-scores of three scales (Internalizing, Externalizing, and Total Problem score) were combined (CBCL/YABCL). A higher SD-score indicated more problem behaviour. Self-perception. The inventory, called in Dutch "Hoe ben ik" and in English "Harter Self Perception Profile" (HSPP), was designed by Harter to describe sense of self-worth and capability in several areas, using 4-point scales [111, 112]. At the T1 and T2 evaluation the 36-item child-version for children aged 8 to 12 years (HSPP-c) was used, at the T3 evaluation the 45-item adolescent-version (HSPP-a). Since both versions were constructed in a similar way, SD-scores of one scale (Total HSP-score) could be combined to enable analysis of time-trend.

SD-scores
To allow combining outcome variables over time (CBCL/YABCL, HSPP-c/HSPP-a) the psychosocial test scores were transformed into SD-scores. For the CBCL, YABCL, and HSPP-a a Dutch general population sample aged between 12 and 22 years was used to calculate SD-scores (N=600, SES 1/2/3: 30/36/34%, male 40%, mean age 16.4 yr. +/- 0.13 SEM) [104]. Comparison between the GH groups at T3 and the population sample by logistic regression (group as dependent variable and SES, gender, and age as independent variables) showed no significant differences in SES and age between the normative group and the GH groups, but a difference for gender (more males in GH group: $P < 0.01$). For the child-form HSPP (HSPP-c), used at T1 and T2, Dutch normative data of the same age-range (8-12 years) as

the GH groups at start were used (n=300, male 48%, mean age 9.7 +/- 0.06 SEM) [226]. Comparison between the GH groups who filled in the HSPP-c at T1 and the population sample showed a significant difference for gender (more males in GH groups, $P < 0.05$), when logistic regression was applied (group as dependent variable, gender and age as independent variables).
To correct for the skewed distribution in gender of the population sample, the mean and SD of the female and male outcome was averaged and used to calculate SD-scores.

Statistical analysis
To maintain the double-blind design until all participants have reached FH, an independent statistician (HJD) performed the statistical analyses. All data were expressed as mean (SEM) unless otherwise specified. To compare mean outcome scores with the normative means, one-sample t-tests were performed, using test value 10 for the s-scores and test value 0 for the SD-scores. To estimate the time trend of the IQ and psychosocial test scores, the effect of height SD-score at time of evaluation, and the effect of GH dosage on the IQ and psychosocial test scores, Random Regression Models were used for continuous data (RRMs). The effect on IQ and psychosocial test scores was expressed as an unstandardised estimate (b). To find the optimal model fit, the following strategy was used: 1) fixed linear time trend with and without random linear time effect, 2) fixed linear and quadratic time trend, 3) model 1 or 2 with covariables age, gender (1/2: male/female), and GH dosage (1 or 2 mg/m^2/day). To limit the effect of collinearity, variables height SD-score and age were centred (individual value minus mean value), and the variable time was divided by 2 (0, 1, 4.65 yr.). To correct for skewed distributions, the square roots of the CBCL/YABCL scores plus 1.5 were used for analysis. The effect of GH dosage was estimated by the addition of the interaction term GH dosage*time to the optimal model. The effect of change in height was estimated by the addition of height SD-score at time of evaluation to the optimal model. The advantage of using RRM, instead of a MANOVA for repeated measurements, is that RRM copes better with missing data because it estimates missing data based on non-missing data for that individual, assuming that missing data are missing at random [227, 228]. In addition, in RRM using an "unstructured" error structure, variance and covariance are not assumed to be the same between time-points. All calculations were done by SPSS 9.0, except for RRMs (SAS 6.12). A two-tailed P-value < 0.05 was considered significant.

TABLE 1. Characteristics at start of GH treatment for the total group of 79 short children born SGA at start of GH treatment and for the children who participated in the evaluation at 2001.

Evaluation	**At start** (N=79)		**In 2001** (N=53)	
	3 IU/m^2/day	6 IU/m^2/day	3 IU/m^2/day	6 IU/m^2/day
Male/Female	31/10	21/17	22/6	14/11
Gestational age (wk)	37.3 (0.5)	36.0 (0.7)	37.8 (0.6)	36.3 (0.8)
Birth length (SD-score)	-3.6 (0.2)	-3.7 (0.3)	-3.2 (0.2)	-3.5 (0.3)
Birth weight (SD-score)	-2.6 (0.2)	-2.6 (0.2)	-2.5 (0.2)	-2.5 (0.2)
Chronological age (yr.) at start	7.3 (0.3)	7.6 (0.4)	7.3 (0.4)	7.0 (0.5)
Height (SD-score) at start	-3.0 (0.1)	-3.1 (0.1)	-2.9 (0.1)	-3.1 (0.1)

Data expressed as mean (SEM).

TABLE 2. Clinical characteristics of the participants of the IQ and psychosocial evaluations at start, after 2 years of GH treatment, and in 2001.

Evaluation	**At start** (N=74)#		**2 yr. GH** (N=76)		**2001** (N=53)	
	3 IU/m^2/d	6 IU/m^2/d	3 IU/m^2/d	6 IU/m^2/d	3 IU/m^2/d	6 IU/m^2/d
Chronological age (yr.) at evaluation	7.4 (0.3)	7.4 (0.4)	9.4 (0.3)	9.6 (0.4)	16.7 (0.4)	16.4 (0.6)
Height (SD-score) at evaluation	-3.0 (0.1)	-3.0 (0.1)	-1.6 (0.1)*	-1.2 (0.1)*	-1.0 (0.2) #	-0.5 (0.3) #
Target height (SD-score)	-0.9 (0.1)	-0.4 (0.2)	-0.9 (0.1)	-0.4 (0.2)	-0.9 (0.2)	-0.5 (0.2)
Head circumference (SD-score) at evaluation	-0.9 (0.1)	-0.8 (0.2)	-0.5 (0.2)*	-0.2 (0.2)*	-0.8 (0.2) #	-0.6 (0.2)#

Data expressed as mean (SEM). # At start of GH treatment 5 children were too young to participate in the evaluation. Paired t-test on both GH groups: * $P < 0.001$ (start GH versus 2 yr. GH); # $P < 0.001$ (2 yr. GH versus 2001).

Results

Table 1 shows characteristics at start of GH treatment for all short children born SGA who were included in the GH trial, and for those who participated in the IQ and psychosocial evaluation in 2001. No significant differences in baseline characteristics were found between the 53 GH-treated children who participated in the IQ and psychosocial evaluation in 2001 and the 26 who did not participate. Table 2 shows the clinical characteristics at time of evaluation for the children who participated in the IQ and psychosocial evaluation at start (T1), after 2 years of GH treatment (T2), and in 2001 (T3). After 2 years of GH treatment, 96% of both GH groups (73/76) showed a gain in height SD-score of 1 SD or more, measured from start of treatment. This already resulted in a normal height (i.e. height ≥ –2.0 SD for age and sex) after 2 years of GH treatment in 70% of the children (53/76). In 2001, 48 of the 53 children who participated in the IQ and psychosocial evaluation had reached a normal height (91%). Mean (SEM) duration of GH treatment was 8.0 (0.2) years. In 2001, 24% of the parents had a lower occupation, 35% an intermediate occupation, 41% a higher occupation.

Psychosocial functioning and IQ over time

Intelligence
Block-design s-score, corrected for gender, age at start, and GH dosage, showed a significant linear increase of 2.2 s-score from start to 2001 ($b=0.48$, $P < 0.001$) (Figure 1). Compared to the Dutch population sample, mean Block-design s-scores for the GH groups at start and after 2 years of GH treatment were significantly lower ($P < 0.001$ at both times). In 2001, mean Block-design s-score was comparable to the Dutch population sample mean. The increase over time for Vocabulary s-score was not significant. Compared to the Dutch population sample, mean Vocabulary s-scores for the GH groups at start, after 2 years of GH treatment, and in 2001 were significantly lower ($P < 0.01$, $P < 0.001$, and $P < 0.05$, respectively) (Figure 2). To analyse whether the type of IQ test used in 2001 could explain the change in IQ s-scores, a dummy variable was added (1=WAIS at 2001, 0=WISC-r at 2001). The dummy variable, however, had no significant explanatory effect on both the changes in Block-design and Vocabulary s-scores. The estimate of the Total IQ, corrected for gender, age at start, GH dosage, and type of IQ test used in 2001, increased significantly by 7.0 s-score over time ($b=1.51$, $P < 0.001$) (Figure 3). Compared to the mean of the Dutch population sample (Total IQ=100), mean Total IQ score for the GH groups at start and after 2 years of GH treatment were significantly lower ($P < 0.001$ and $P < 0.001$). In 2001, mean Total IQ score was comparable to the Dutch population sample mean. No significant difference between the two GH dosage groups was found in change over time for both subtests and Total IQ.

FIGURE 1. Block-design s-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU) Significant increase from start: * P < 0.001.

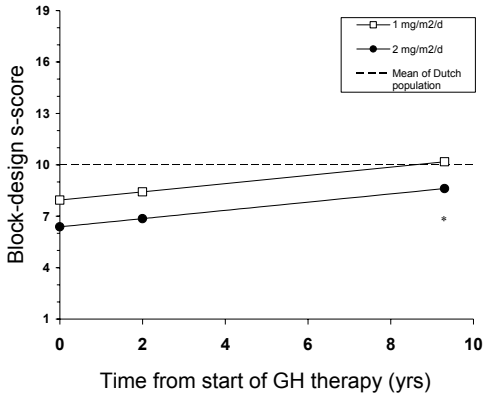


FIGURE 2. Vocabulary s-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU)

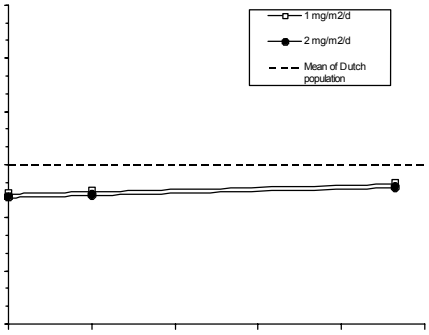
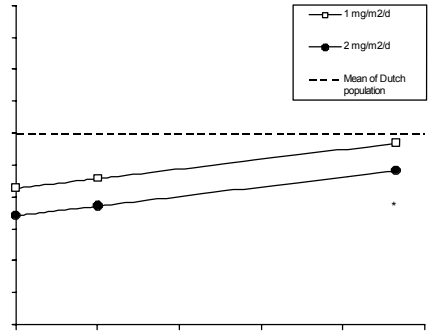


FIGURE 3. Estimated Total IQ s-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU) Significant increase from start: * P < 0.001.



Behaviour
The 3 scales of the behaviour checklist (CBCL/YABCL), Internalizing SD-scores, Externalizing SD-scores, and Total problem behavior SD-scores, were analysed over time, corrected for gender and age at start. Over time Internalizing SD-scores did not change significantly. At start, after 2 years of GH treatment, and in 2001, mean Internalizing SD-score were comparable to the population sample mean (Figure 4). Externalizing SD-scores and Total problem behavior SD-scores, however, decreased linearly and significantly by 0.7 and 0.5 SD-score, respectively, during GH therapy ($b=-0.05$, $P < 0.01$; $b=-0.04$, $P < 0.05$, respectively) (Figures 5 and 6). When comparing Externalizing SD-scores and Total problem behavior SD-scores for the GH groups with the Dutch population sample mean, at start and after 2 years of GH treatment, the GH groups had higher scores (Ext.: $P < 0.001$, $P < 0.01$, respectively; Tot.: $P < 0.001$, $P < 0.01$, respectively), indicating more problem behaviour. In 2001, however, the GH groups had Externalizing and Total problem behavior SD-scores comparable to the population sample mean. No significant difference between the two GH groups was found in change over time for Internalizing, Externalizing, and Total problem behavior SD-scores.

Self-perception
Mean self-perception scale, Total HSP SD-score, increased quadraticly by 1.6 SD-score from start of GH treatment until 2001 ($P < 0.001$) (Figure 7). Compared to the Dutch population sample mean, the GH groups had significantly lower scores at start ($P < 0.001$). After 2 years of GH treatment mean Total HSP SD-score had increased to a mean SD-score comparable to the normative mean, while in 2001, mean Total HSP SD-score was significantly higher than the population sample mean ($P < 0.05$). No significant differences were found in change over time between the two GH groups.

Effect of height
Externalizing and Total problem behavior SD-scores over time were inversely related to change in height SD-score ($b=-0.05$, $P < 0.05$;$b=-0.06$, $P < 0.01$, respectively). To demonstrate the size of the effect of height SD-score on behavioural problems, suppose a short SGA child commenced GH treatment with a height of –3 SD-score, gained 1 SD-score after 2 years of GH treatment, and attained a final height of –1 SD-score in 2001. The child's Externalizing problem behavior would have decreased 0.8 SD-score from start of GH treatment until 2001, while a short SGA child with no increase in height SD-score, would have a Externalizing problem behavior decrease of 0.5 SD-score. When using the same height increase as in the previous example, the child's Total problem behavior would have decreased 0.5 SD-score from start of GH treatment until 2001, while a short SGA child with no increase in height SD-score, would have a Total problem behavior decrease of 0.1 SD-score. The scores over time for Vocabulary, Block-design, Internalizing, or Total HSP were not related to change in height SD-score. In 2001, scores for Vocabulary, Block-design, Internalizing, Externalizing, Total problem behavior, or Total HSP were not related to height SD-score in 2001, neither with nor without correction for age, gender, or GH dosage.

FIGURE 4. Internalizing problem behavior SD-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU)

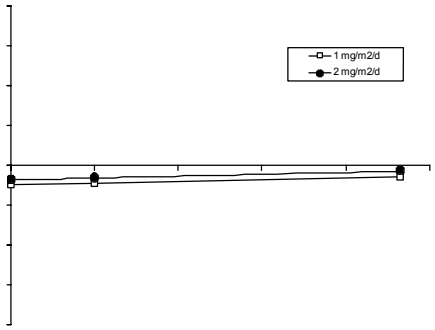


FIGURE 5. Externalizing problem behavior SD-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU) Significant decrease from start: * P < 0.01.

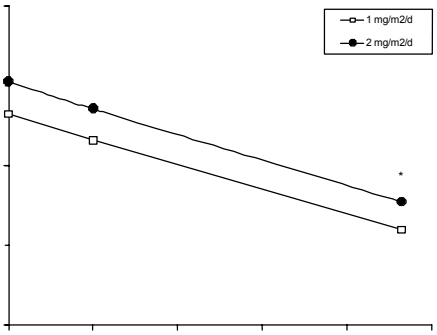
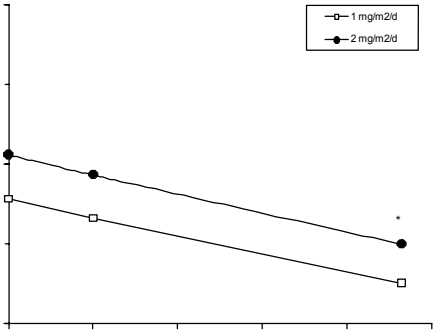


FIGURE 6. Total Problem Behavior SD-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU) Significant decrease from start: * P < 0.05.

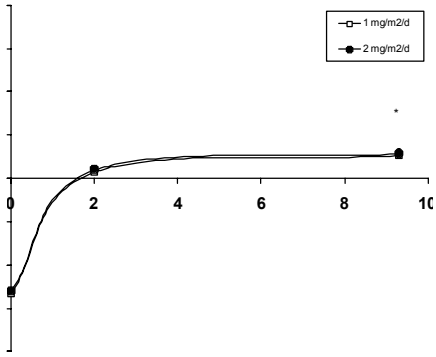


Effect of head circumference
Head circumference SD-score at start was positively and significantly related to Block-design and Vocabulary s-scores over time (b=0.65, P < 0.05; b=1.06, P < 0.01, respectively). Head circumference SD-score at start, however, was not related to the change over time for both Block-design and Vocabulary s-scores (interaction term: head circumference SD-score at start*time).
In addition, change in head circumference SD-score was positively and significantly related to Block-design and Vocabulary s-scores over time (b=0.72, P < 0.01; b=0.75, P < 0.01, respectively). Correction of head circumference SD-score for height SD-score did not contribute to the models.

Discussion

Our study presents the first longitudinal results on IQ and psychosocial functioning after GH-induced catch-up growth in adolescents born small for gestational age (SGA) during long-term growth hormone treatment. We show that during 8 years of GH treatment IQ scores, behaviour, and self-perception had improved significantly over time to values comparable to their Dutch peers. In addition, we show that over time children whose height became closer to that of their peer group had less problem behaviour.
Before start of GH treatment, all children had short stature, as this was an inclusion criterion for the trial. After 2 years of GH treatment 96% of both GH groups showed a gain in height SD-score of 1 SD or more, measured from start of treatment. GH treatment, therefore, already resulted in a normal height (i.e. height ≥ −2.0 SD for age and sex) after 2 years of GH treatment in 70% of the children (53/76), while this percentage had increased to 91% in 2001. In the present study, as part of a randomised, double-blind, GH-dose response study, we evaluated the effect of long-term GH therapy on IQ and psychosocial functioning, measured by standardised IQ subtests and standardised questionnaires on behavioural problems, and self-perception. From start of GH treatment until 2001 we found a significant linear improvement in mean Block-design s-score, a Performance IQ subtest, and estimated total IQ. Both s-scores increased from values significantly lower than the mean of Dutch peers to values comparable to the mean of Dutch peers. Vocabulary s-score, which is a Verbal IQ subtest, however, remained lower than the mean of their Dutch peers. Regarding behavioural problems reported by the parents, we found a significant linear decrease over time of Externalizing behavior SD-score, indicating a decrease in behavioural problems. Both Externalizing and Total problem behavior scores decreased from values above the mean of the reference population sample to values comparable to the reference mean. Also, Self-perception (total HSP SD-score) improved quadraticly over time during GH treatment, showing that the largest improvement occurred in the first 2 years of GH treatment. Self-perception increased from a score below the mean of their Dutch peers to a score above the mean.

FIGURE 7. Self Perception total HSP SD-score for both GH groups during GH treatment, corrected for gender and age at start. (1 mg=3 IU) Significant increase from start: * P < 0.001.



While one study found little difference in quality of life between growth hormone deficient adults and same sex siblings [229], several other studies in growth hormone deficient children and adults, some placebo-controlled, have shown that GH treatment had a beneficial effect on cognition, energy, mood, and behaviour [187, 230-234]. The increase in test scores might therefore be a direct effect of GH itself on cerebral functioning. Only Block-Design s-scores, a subtest for Performance IQ, improved over time, without change in the Verbal IQ subtest, Vocabulary s-scores, possibly indicating a GH effect on the right hemisphere of the brain [235]. Hopefully, further studies, for instance into processing speed, will show the mechanisms behind this improvement.
Although we did not find any significant differences in IQ test results and psychosocial functioning between the two GH dosage groups, this might have been because 3 IU/m^2/day was enough to achieve the optimal effect on IQ and psychosocial functioning. This is similar to our findings regarding the effect of GH dosage on final height [220]. Another possible explanation for the improvement in IQ and psychosocial functioning might be the extra medical attention. Unfortunately, due to ethical considerations, it was not possible for us to include a non-treated randomised control group as part of the GH trial to explore the effect of the extra medical attention. We did not, however, provide psychological counselling to treat the behavioural problems found at start of GH treatment.
Interestingly, no differences in Internalizing problem behavior scores were found compared to Dutch peers, only in Externalising problem behavior scores. A possible reason might be the way short children are treated, unintentionally, by adults. They tend to treat short children as younger and evade age-appropriate demands on their intellect and behaviour. This may lead to feelings of frustration, which, in this study group, was acted out by 'childish' (age-inappropriate) and/or aggressive externalising behaviour.
Most children showed a considerable catch-up in height during 8 years of GH treatment, paralleled by an improvement in scores for IQ, behaviour, and self-perception. Previous studies in other patient groups, such as idiopathic short stature, childhood GH deficiency, and Turner syndrome, an effect of height gain during GH treatment on psychosocial functioning was found [53, 236, 237]. To test whether the considerable increase in height SD-score in our study group could explain the improvement of the scores, we added height SD-score over time to the models. Interestingly, the addition of height SD-score to the regression model for Externalizing behavior SD-score, corrected for age and gender, showed a significant negative correlation, indicating that over time children whose height became closer to that of their peer group had less problem behaviour. This finding might suggest that the decrease in externalising behavioural problems was caused by the increase in height. For IQ and Self-perception, however, the addition of height SD-score had no effect on the model, indicating that height SD-score at time of evaluation was not related to IQ or Self-perception test scores. Several reasons might explain why we did not find such a relation. For IQ, the influence of other factors, such as genetic predisposition, might have masked the effect of increase in height. As all children showed GH-induced catch-up growth after start of GH treatment, the GH groups might have been too homogenous regarding height and too heterogeneous regarding factors such as genetic predisposition, to show a relation between increase in height and IQ. Another

possibility is that the effect of GH treatment was a result of its direct action on cerebral functioning, as mentioned above, and was therefore not related to its effect on increase in height. For self-perception, factors such as physical appearance and coping strategy but also the non-linear increase in self-perception might have masked the effect of increase in height.
One factor, previously reported to be positively related to intelligence, is head circumference [173]. We show that this relation between head circumference and IQ also exists in short children born SGA, both at start of the GH trial as well as during GH treatment. In addition, we show that the head circumference SD-score was low normal at start of treatment and remained so during GH treatment. Thus, head circumference SD-score did not improve with IQ scores during GH treatment. Height SD-score, however, also increased during GH treatment, leading to a head circumference SD-score more in proportion to height SD-score. One of the suggested reasons for a decreased IQ in SGA children was that brain development was affected by intrauterine malnutrition [172]. As a deficit in brain development is often accompanied by a smaller head circumference, it would be interesting to see whether head circumference before GH treatment would predict IQ development during treatment. We show that head circumference SD-score at start of treatment did not predict the IQ increase during GH treatment. This indicates that IQ scores improved in short children born SGA during GH treatment, regardless of whether head circumference had been spared from previous growth retardation.
In conclusion, parallel to a considerable GH-induced catch-up growth, children born small for gestational age showed a significant improvement in IQ scores, behaviour, and self-perception over time. During eight years of GH treatment, IQ and psychosocial functioning had improved from scores significantly below average to scores comparable to Dutch peers. In addition, the taller the child became over time, the less problem behaviour it showed. Although, in general, a child with a smaller pre-treatment head circumference had a lower IQ at start of GH treatment, the increase in its IQ score during GH treatment occurred regardless of whether its head circumference had been spared from growth retardation. Considering the positive effects on IQ and psychosocial functioning, in addition to the catch-up growth during GH treatment, we recommend GH treatment for short SGA children without signs of persistent catch-up growth. Whether GH treatment also has an effect on their life achievements in the future, further follow-up will show.

CHAPTER 11

Part 'Small for Gestational Age': General Discussion and Conclusions

General Discussion and conclusions

In this chapter we discuss the long-term results of a randomised multi-centre dose-response growth hormone (GH) trial evaluating the efficacy, safety and psychosocial effect of long-term GH treatment. A group of short children born small for gestational age (SGA) participated in a double-blind dose-response trial using 3 or 6 IU GH/m^2/day (~1 or 2 mg/m^2/day; 0.033 or 0.067 mg/kg/day). The trial started in 1991 and four paediatric departments in The Netherlands participated. We start by discussing the results of the SGA trial. Finally, conclusions are drawn from the discussion and recommendations for future research are given.

Effect of long-term continuous GH treatment on final height in 54 short children born small for gestational age (SGA)

In chapter 8 of this thesis we described the first results on final height after long-term, continuous GH treatment with either a GH dosage of 3 or 6 IU/m^2/day. We showed that GH treatment resulted in a final height within the normal range (above –2.0 SD score) in 85% of the children and a final height within the target height range in 98%. The mean final height using a GH dosage of 3 IU/m^2/day was –1.1 (0.7) SD score and the mean final height using 6 IU GH/m^2/day was –0.9 (0.8) SD score when using references for normal Dutch children of the same age. As target height SD score was a strong predictor for final height, and the 3 IU group (1 mg) had a significantly lower target height SD score than the 6 IU group (2 mg), we corrected the final height for target height. The mean corrected final height SD score (final height SD score minus target height SD score) proved to be comparable for the 3 IU group (–0.2[0.8]) and the 6 IU group (–0.4 [0.9]). The mean gain in height SD score from start of treatment until final height was 1.8 (0.7) SD score (~ an improvement of 12 cm for boys and 11 cm for girls) for the 3 IU dosage group (1 mg). For the 6 IU dosage group (2 mg) the gain was 2.1(0.8) SD score (~ an improvement of 14 cm for boys and 13 cm for girls). The difference in height gain between the two dosage groups was not significant.
To estimate the effect of GH treatment, we compared our final height results with non-GH treated short children born SGA from a longitudinally followed control SGA group with the same inclusion criteria and age as the GH-treated patients at start of treatment. Including a randomised control group as part of our GH trial until attainment of final height was not allowed by the Medical Ethics Committees. The group attained a mean (SD) final height SD score of –2.3 (0.7) and had a mean gain in height SD score of 0.3 (0.7) until final height during the follow-up period of 7½ years.
These results showed that GH treatment in short children born SGA leads to a significant gain in final height, while a dose of 3 IU/m^2/day (1 mg/m^2/day) proved to be as effective as the higher GH dose of 6 IU/m^2/day (2 mg/m^2/day), for most children. Based on these results, in 2003, the European Agency for the Evaluation of Medicinal Products (EMEA) and the Dutch government acknowledged GH as a treatment for short stature in

children born SGA. GH treatment for short stature in children born SGA has now become an accepted treatment in many countries.
Other studies reporting on final height in short children born SGA found a considerably lower height gain than in our group [182, 183, 238]. Several factors can explain the discrepancy between their results and ours. Their children were older at start, were treated for a much shorter period, or they were treated with a lower GH dose.
Recent studies have shown a potential danger of having high circulating IGF-1 levels. As mentioned in the Turner syndrome Discussion, it was reported that men (Physicians' Health Study) with serum IGF-I levels in the upper quartile showed an increased risk of developing colorectal cancer after 14 years of follow-up [78]. Another study found that after 7 years of follow-up, serum IGF-I levels in premenopausal nurses (Nurses' Health Study cohort) in the upper tertile were associated with an increased risk of breast cancer [79]. These studies, however, did not show a causal relationship between high circulating IGF-I levels and cancer. Therefore, whether recombinant GH treatment during childhood and adolescence might lead to an increased risk of cancer remains to be investigated. Pending these investigations, however, high dosages of GH should be given with caution. Although in this trial we did not find a significant difference between the two GH dosage groups in IGF-1 SD-scores at discontinuation, Sas et al. described a significantly higher IGF-1 SD-score in the 6 IU group (2 mg) during the first years of GH treatment [44, 150]. In addition, a higher dose increases the already high cost of treatment. Our study showed that the majority of short SGA children can be effectively treated and reach a normal final height with a dose of 3 IU GH/m^2/day (1 mg GH/m^2/day). We therefore suggest to limit high dose treatment to children with extremely short stature and/or advanced chronological age and in that case only for induction of a fast catch-up growth during a few years.
Previous publications have shown that untreated children born SGA start their pubertal growth spurt relatively early in the normal age range [148, 198]. As a shorter growth spurt might result in a reduction in final height, we investigated the growth spurt and its effect on height SD score in our GH treated group. For both GH dosage groups, mean age at onset of puberty was comparable to normal Dutch children (median age for healthy boys is 11.5 years and for girls 10.7 years) [9]. In addition, mean pubertal height gain (from the onset of puberty until final height) was 29.8 cm for boys and 18.9 cm for girls. During puberty no acceleration of bone maturation occurred until discontinuation. The slight decrease in height SD-score at the end of GH treatment might indicate a slightly shorter growth spurt than normal Dutch children. Another explanation might be that some of the children did not reach their full height potential because they discontinued GH treatment at near-final height.

Several parameters to predict final height SD score

Multiple regression analysis with final height as dependant variable which is presented in chapter 8 showed the following variables significantly influencing final height SD score: target height SD score, height SD score at start, and chronological age minus-bone age at start. For all variables a higher value predicted an increase in final height SD score. To explain the impact of a

higher GH dose, we considered the following case. Suppose a child born SGA started GH treatment when he or she had a persistent short stature of –3 SD-score, no bone age delay, and a mean target height of 0 SD score. According to the model, this child would reach a final height SD score of –1.0 (95% Probability interval between –2.2 and 0.2) when using 3 IU GH (1 mg), and –0.8 SD-score (95% Probability interval between –2.0 and 0.4) when using 6 IU (2 mg) of GH. The difference in final height would be quite small, while doubling the costs of treatment. The model, however, could only explain 42 percent of the variation in final height SD score, leaving 58 percent of the variation to be explained by other factors, such as genetic background [164, 193]. Future research should be aimed at creating a prediction model based on larger groups of short children born SGA, using more detailed phenotypic and genetic data. In future, this would support individualisation of GH treatment.

Carbohydrate metabolism in short children born SGA after discontinuation of long-term GH treatment

It has been established that GH treatment increases post-prandial insulin levels, probably due to a reduction in insulin sensitivity [185]. Especially in individuals born SGA, who might have a predisposition for diabetes mellitus type 2, monitoring of the carbohydrate metabolism is an important safety parameter during long-term GH treatment [190]. Previously, Sas et al. showed that stimulated glucose levels remained unchanged and fasting glucose levels rose slightly during 6 years of GH treatment, while both fasting and stimulated insulin levels increased significantly [44, 91]. In chapter 9 we showed that 6 months after discontinuation of long-term GH treatment in adolescents born SGA both fasting glucose and insulin levels returned to pre-treatment levels. Stimulated insulin levels remained slightly higher than pre-treatment levels, but we could not expect stimulated insulin levels to return to prepubertal levels since all participants were postpubertal after discontinuation of GH treatment [81, 86, 87]. The stimulated insulin levels were, however, comparable to those of healthy adolescent peers indicating that discontinuation of GH treatment resulted in a normalisation of stimulated insulin levels [81]. In different patient groups, such as Turner syndrome, similar results were found after discontinuation of GH treatment [51, 84, 186]. All insulin levels showed no significant differences between the GH dosage groups. After discontinuation of GH treatment we found an increase in the prevalence of Impaired Glucose Tolerance, according to The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (120-minute stimulated glucose level above 7.8 mmol/L), from three percent during treatment to ten percent after discontinuation of treatment [80]. These results emphasise the importance of regular monitoring of fasting glucose and insulin levels, also after GH treatment has been discontinued [210, 211].

Several factors that may predict development of cardiovascular disease, such as blood pressure, body mass index and blood lipid levels in short children born SGA after discontinuation of GH treatment

In chapter 9 we described that discontinuation of GH treatment did not alter the previously described normalisation of blood pressure and body mass index (BMI) during GH treatment [44, 91]. Another study evaluating the effect of discontinuation of GH on blood pressure in adolescents with GHD, showed similar results [92]. After discontinuation of GH treatment, in our study, the decrease in serum total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-c), and high-density lipoprotein-cholesterol (HDL-c) during GH treatment, remained present for TC levels, whereas only for the female participants LDL-c and HDL-c levels increased significantly. Compared to a control group, serum lipid levels after discontinuation of GH treatment, including HDL-c, were significantly lower in the GH-treated group. The atherogenic index (TC/HDL-c), a more potent risk factor for the development of cardiovascular disease (CVD), did not change after discontinuation of GH treatment and was comparable to the control group. BMI SD-score correlated significantly with fasting insulin levels, systolic BP SD-score, and the atherogenic index at 6 months after discontinuation of GH treatment which might indicate that the clustering of risk factors for cardiovascular disease in adults with low birth weight was already present in the more obese subjects in young adulthood. These results also suggest prevention of obesity as a possible strategy to decrease risk for CVD in subjects born SGA.

IQ and psychosocial functioning of short children born SGA during and after long-term GH treatment

In chapter 8 we showed that long-term GH treatment for most SGA children resulted into a normalisation of height. Previously, children born SGA have been associated with lower intelligence, poor academic performance, low social competence, and behavioural problems [165-171]. The effect of GH treatment and the resulting normalisation of intelligence and psychosocial functioning were discussed in chapter 10. We found that during GH treatment both Block-design s-score, a Performance IQ subtest, and estimated total IQ increased significantly from low values to values comparable to scores for Dutch peers, whereas Vocabulary s-score, which is a Verbal IQ subtest, remained normal during treatment. Externalising problem behaviour of the short SGA children, as reported by the parents, decreased significantly over time. Both scores for Externalizing behavior and Total problem behavior decreased to values comparable to the same age reference sample. Similarly, self-perception (total HSP SD-score) improved over time during GH treatment, showing the largest improvement during the first 2 years of GH treatment. Self-perception scores rose from below the mean of Dutch peers to a score above the mean.
A possible explanation for the increase in performance IQ was a direct effect of GH itself or IGF-I on cerebral functioning. Support for this explanation could be found in the beneficial effect of GH treatment on cognition, energy, mood, and behaviour in growth hormone deficient children and adults [187, 230-234]. The finding that only Block-Design s-scores, a subtest for Performance IQ, improved over time, without change in the Verbal IQ subtest, (Vocabulary s-scores), might indicate a GH effect targeting the right hemisphere of the brain [235]. The improvement in psychosocial functioning, and possibly IQ, could also be explained by the extra medical attention. Although we did not

provide psychological counselling for behavioural problems, the effect of extra medical attention could not be excluded without the presence of a non-treated randomised control group as part of the GH trial. Another possible aetiology for our findings might be the unintentional lack of age-appropriate demands on intellect and behaviour before start of GH treatment on our group of SGA children due to their short stature. The subsequent frustration might have led to 'childish' (age-inappropriate) and/or aggressive behaviour. This mechanism might explain lower psychosocial functioning scores and possibly lower IQ scores at start of GH treatment and normalisation of scores coinciding with normalisation of height during and after GH treatment. The positive association between increase in height and decrease in problem behaviour supported this theory.

Conclusions

Long-term, continuous GH treatment in short children born SGA leads to a normalisation of height in childhood and a normal final height for most children. A dose of 3 IU/m^2/day (1 mg/ m2/day) proved to be as effective as the higher GH dose of 6 IU/m^2/day (2 mg/m^2/day), for most children. Only children with extreme short stature may need a higher GH dose to normalise final height. Further studies should aim at optimising GH treatment by developing advanced prediction models indicating the best treatment options for each child.
GH induced higher insulin levels reduced after discontinuation of GH, while the beneficial effect of GH on blood pressure remained after discontinuation of GH. Although most children had normal serum lipid levels, we found an indication for clustering of risk factors for diabetes mellitus type 2 and cardiovascular disease, in adolescents with relatively higher BMI. Whether long-term GH treatment will contribute to a reduction of adult diseases and longevity, however, remains to be investigated.
During eight years of GH treatment, for most children test scores for IQ and psychosocial functioning improved from scores significantly below average to scores comparable to Dutch peers. In addition, the taller the child became over time, the less problem behaviour it showed. Whether GH treatment also has an effect on their life achievements in future, further follow-up will show.
Based on our studies we recommend considering GH treatment for short SGA children without signs of persistent catch-up growth and who are therefore at risk of short stature as adults. GH treatment, however, not only improves final height, it also improves blood pressure and serum lipids, without a rebound effect after discontinuation of GH treatment. Furthermore, it improves IQ and psychosocial functioning. In other words, GH treatment in short children born SGA might have several positive effects on the long-term.

Recommendations and future research

For most children, long-term, continuous GH treatment in a dose of 3 IU/m^2/day (1 mg/ m2/day) in short children born SGA leads to a normal final height. A higher GH dose of 6 IU/m^2/day (2 mg/m^2/day) is not more effective for most children. We therefore suggest to limit high dose treatment to

children with extremely short stature and/or advanced chronological age and in that case only for induction of a fast catch-up growth during a few years. To find the best treatment strategy for each child GH treatment should become more individualised. Based on larger groups of short children born SGA more detailed phenotypic and genetic data could be collected. The aim would be to create an advanced prediction model to aid individualisation of treatment.

As mentioned previously in the TS part of the thesis, recent studies have shown a potential danger of high circulating IGF-1 levels [78, 79]. The causality in the relationship between high circulating IGF-1 levels and cancer remains to be investigated. Until causality can be ruled out, epidemiological studies on individuals treated with recombinant GH should monitor for an increased risk of colorectal or breast cancer in this group.

GH treatment in short SGA children improves blood pressure and serum lipids, without a rebound effect after discontinuation of GH treatment. Follow-up of larger groups of GH treated short SGA children will show whether long-term GH treatment will lead to a significant reduction in adult disease.

In addition, we found an indication for clustering of risk factors for diabetes mellitus type 2 and cardiovascular disease, in adolescents with relatively higher BMI. Whether being born SGA causes a clustering of risk factors for CVD or whether factors associated with SGA, such as obesity or (lack of) catch-up growth, are responsible, is currently under debate. Investigating the cause for the predisposition will aid in preventing CVD in adult life.

IQ and psychosocial functioning improved during GH treatment for most children. Whether GH treatment also has an effect on life achievements in future, epidemiological studies of larger groups of GH-treated SGA adults in comparison to untreated SGA adults will show.

CHAPTER 12

Part 'Small for Gestational Age': Summary

Summary

During the first two years of life about 10% of the children born SGA do not catch-up to a normal height. The majority of these children will become short adults. The reason for these children remaining short is not completely understood. There are several theories, such as reduced sensitivity for growth factors, intrauterine re-programming or genetic background. Recent studies have demonstrated that 5 years of GH treatment in short children born SGA results in a normalisation of height during childhood.
In this chapter the results and conclusions are summarised of a multi-centre randomised double-blind dose-response growth hormone (GH) trial evaluating the efficacy, safety and psychosocial effect of long-term GH treatment on short children born small for gestational age (SGA). The SGA trial consisted of 79 untreated short children, aged between 3 and 11 years, born SGA. The children were randomly assigned to a group using 3 or 6 IU GH/m^2/day.

Chapter 7 introduces the characteristics and incidence of short children born SGA. In addition, it describes the aetiology, post-natal growth and risk factors for cardiovascular disease (CVD) associated with SGA. Subsequently, it describes short-term results of GH treatment on final height and CVD risk factors in short children born SGA.
Chapter 8 shows the effect of long-term continuous GH treatment on final height (FH) in a group of 54 children born SGA. Long-term treatment with GH in short children born SGA results in a normalisation of FH in most children. After a mean duration of almost 8 years of GH treatment 85% of the children reach a FH within the normal range, and 98% within the target height range. A higher GH dose of 6 IU GH/m^2/day does not result in a significantly higher FH. Compared to a group of short children born SGA of the same age who did not receive GH treatment, those treated with GH significantly gain in final height. Factors that seem to predict gain in final height are target height, height at start, and bone age delay at start.
Chapter 9 presents the effect of discontinuation of long-term GH treatment on carbohydrate metabolism, body mass index, blood pressure, and serum lipid levels in children born SGA. Discontinuation of long-term GH treatment in adolescents born SGA normalises carbohydrate metabolism, after a significant change during GH treatment. Furthermore, discontinuation of GH does not alter the positive influence of GH on body mass index (BMI) and blood pressure, while serum lipid levels remain normal.
Chapter 10 describes longitudinal results on IQ and psychosocial functioning after GH-induced catch-up growth in adolescents born small for gestational age (SGA) during long-term growth hormone treatment. During 8 years of GH treatment IQ scores, behaviour, and self-perception had improved significantly over time to values comparable to their Dutch peers. In addition, we show that over time children whose height became closer to that of their peer group had less problem behaviour.
Chapter 11 discusses the results presented in the previous chapters and relates them to relevant literature. In addition, conclusion are drawn and recommendations are given together with suggestions for future research.
Chapter 13 synthesises the results for the SGA trial.

Finally, Chapter 13 synthesises the results from the TS trial and the SGA trial and discusses the similarities and differences.

Samenvatting

Ongeveer tien procent van de kinderen die te klein worden geboren slaagt er niet in om de groeiachterstand in de eerste twee jaar in te halen. De meeste van deze kinderen zullen eindigen als volwassenen met een te kleine lengte. De oorzaak van de te kleine lengte is vaak onbekend. Er zijn verschillende theorieën, zoals verminderde gevoeligheid voor groeifactoren , re-programmering in de baarmoeder, en genetische achtergrond. Recent onderzoek heeft uitgewezen dat vijf jaar GH behandeling in te kleine kinderen die te klein zijn geboren (SGA) resulteert in normalisatie van lengte als kind. In dit hoofdstuk worden de resultaten en conclusies samengevat van een gerandomiseerd dubbelblind dosisrespons onderzoek dat heeft gelopen op verschillende kinderafdelingen verspreid door Nederland. Het onderzoek evalueerde het effect en de veiligheid van langdurige GH behandeling van te kleine SGA kinderen. De doseringen van het GH werden ad random toegekend aan twee groepen van onbehandelde te kleine SGA kinderen tussen de 3 en 11 jaar: Groep A (*4 IU/m^2/dag*), Groep B (*6 IU/m^2/dag*). De doseringen werden niet bekend gemaakt aan de 79 patiënten, hun behandelaars, en degenen die de studie uitvoerden.

Hoofdstuk 7 introduceert de kenmerken van SGA en de frequentie van voorkomen. Het beschrijft verder de etiologie, de postnatale groei en het risico op hart- en vaatziekten. Vervolgens beschrijft het hoofdstuk het korte termijn effect van GH behandeling op eindlengte en op risicofactoren voor hart- en vaatziekten.
Hoofdstuk 8 laat het effect zien van langdurige continue GH behandeling (bijna 8 jaar) op eindlengte in 54 SGA kinderen. Langdurige GH behandeling resulteert in een normale eindlengte bij de meeste SGA kinderen. Van de hele groep heeft 85 procent van de SGA kinderen een normale eindlengte behaald en 98 procent een eindlengte in hun target height interval (eindlengte gebaseerd op ouderlengte). Hogere dosering van GH (groep B) resulteert niet in een langere eindlengte dan in groep A. In vergelijking met een groep onbehandelde SGA kinderen, bereikt de behandelde groep SGA kinderen een significant hogere eindlengte. Factoren die toename in eindlengte voorspellen zijn target height, lengte bij start, gecorrigeerd voor leeftijd en geslacht (lengte SD-score), en achterstand in botleeftijd bij start.
Hoofdstuk 9 beschrijft het effect van het staken van de GH behandeling op het koolhydraat metabolisme, op de body mass index (BMI), op de bloeddruk, en op het vetgehalte in het bloed. Na staken van de behandeling blijkt het effect van GH op het koolhydraat metabolisme te verdwijnen. De positieve effecten van GH op bloeddruk en vetgehalte blijven bestaan na het staken van de GH behandeling, terwijl het vetgehalte gedurende en na de GH normaal blijft.
Hoofdstuk 10 presenteert het psychosociaal functioneren tijdens en na langdurige GH behandeling. Gedurende de GH behandeling zien we een verbetering van IQ, gedrag, en zelfbeeld in SGA kinderen. De resultaten verschuiven van waarden onder het gemiddelde, voor de behandeling, naar waarden vergelijkbaar met het gemiddelde van leeftijdgenoten tijdens en na

de behandeling. Daarnaast blijkt dat hoe normaler de lengte is tijdens de GH behandeling, des te minder probleemgedrag de kinderen vertonen.
Hoofdstuk 11 bediscussieert de resultaten van de voorgaande hoofdstukken en betrekt daarin relevante literatuur. Daarnaast worden er conclusies getrokken en suggesties gedaan voor toekomstig onderzoek.
Als laatste wordt er in Hoofdstuk 13 een vergelijking gemaakt tussen de resultaten van het SGA onderzoek en het TS onderzoek.

General Part

CHAPTER 13

General Part: Synthesis

Synthesis

In the previous chapters we have discussed the effect of long-term GH treatment on final height, carbohydrate metabolism, risk factors for cardiovascular disease, and psychosocial functioning. The first chapters described and discussed the results of the TS trial, and the following chapters described and discussed the results of the SGA trial. In this part of the thesis we compare results of both trials.

Effect of long-term GH treatment on final height

In the TS trial we showed that 83% of the TS girls reached a normal final height (SD-score above -2). In the SGA trial we showed GH treatment resulted in a final height (FH) within the normal range in 85% of the children. These percentages are similar for the two patient groups. Although the percentages for a final height within the target height range (TH ± 1.3 SD) were different, namely 63% in the TS trial and 98% in the SGA trial, this result does not indicate a different growth response. It only reflects the difference in TH between the two patient groups. In the TS trial the mean TH was normal, while in the SGA trial TH was in the low normal range. A Swedish study, investigating an untreated SGA cohort, also found a low mid-parental height in the SGA children with reduced post-natal catch-up growth [143]. This might indicate that at least part of the post-natal persistent short stature in SGA can be explained by an inherited genetic background. To reach the same percentage in the normal range for FH, however, a different dosage is necessary for TS and SGA. In the TS trial a dosage of 6 IU/m^2/day (2 mg) led to a significantly higher FH than a lower dosage of 4 IU/m^2/day (1.3 mg), while in the SGA trial the 3 IU/m^2/day (1 mg) dosage was as effective as 6 IU/m^2/day. The mean gain in height SD-score (increase in height SD-score from start until FH) was similar in both patient group using the most effective dosage (TS trial: 1.9 (0.5) SD-score in the 6 IU group; SGA trial: 1.8 (0.7) SD-score in the 3 IU group). Most likely, the difference in GH dosage reflects the different aetiology of the growth retardation. The higher GH dose required for optimal height gain in TS girls might confirm the previously suggested diminished sensitivity for growth factors [12, 14]. Given the heterogeneity in aetiology of SGA (Chapter 7, Table 1), it is likely that factors that contribute to the lack in postnatal catch-up will also vary. Suggested causes for post-natal persistent short stature in SGA are low GH levels, or a diminished sensitivity for growth factors [144-146]. The fact that short SGA children require a lower GH dose than the TS girls for reaching normal stature might suggest that in short SGA children either the main cause for the growth failure is low GH levels, or the lower sensitivity for growth factors is of a less severe nature than in TS.

Parameters to predict final height SD-score

When analysing the factors most likely to influence final height SD-score, different factors were found to be significant. In the TS trial we found height SD-score at start, GH dosage, age at start, and first year height velocity

influencing FH SD-score outcome. In contrast, in the SGA trial, the factors target height SD-score, height SD-score at start, and bone age delay at start predicted FH SD-score outcome. Another difference in the two trials was that the explained part of the variation in final height SD-score in the TS trial was higher than in the SGA trial, respectively 76% and 42%. A likely explanation is that the SGA patient group is a more diverse group, as mentioned previously. Diversity in aetiology of post-natal catch-up growth might lead to a difference in factors that influence growth during GH treatment. It has been suggested that genetic factors, such as deletions or polymorphism in the genes encoding for growth factors or growth factor receptors, might play a role in the aetiology of the post-natal growth retardation. Possibly, identifying such factors will lead to a more reliable prediction of response to GH treatment in SGA [164, 193].

Carbohydrate metabolism after discontinuation of long-term GH treatment

After discontinuation of GH treatment, in both patient groups the glucose-stimulated insulin levels remained above pre-treatment levels, while levels were similar to normal peers of the same age range [83]. The reason for the increased stimulated insulin levels, in normal peers and in both patient groups after GH treatment, is that during puberty insulin sensitivity decreases [81, 86, 87].
Fasting insulin levels in the SGA trial decreased to pre-treatment levels after discontinuation of GH treatment. In the TS trial, however, fasting insulin levels remained higher than pre-treatment after discontinuation. Although the levels were still comparable to normal peers, a possible explanation for this result might be the difference in BMI SD-score. In the TS group the BMI SD-score after GH discontinuation was significantly higher than for normal girls, while in the SGA group the mean BMI SD-score after GH discontinuation was normal. Another explanation might be that slightly higher insulin levels are an early sign of the TS predisposition for type 2 diabetes mellitus. If the TS predisposition for diabetes is based primarily on beta-cell dysfunction in stead of on insulin resistance, this explanation does not hold [30]. In both trials no significant differences in glucose or insulin levels were found between dosage groups.

Several factors that may predict development of cardiovascular disease after discontinuation of GH treatment

Adult cardiovascular disease (CVD) and risk factors for CVD seem to occur more often in both TS and SGA populations [24-29, 155, 156]. In this thesis, we describe that blood pressure, in both patient groups, declined significantly compared to pre-treatment data. In the TS group, however, blood pressure remained slightly higher than in peers. It is generally taken that having Turner syndrome predisposes for hypertension [25]. But the aetiology of the predisposition remains unclear [31].
The atherogenic index (TC/HDL-c) in the TS group decreased to pre-treatment levels during and after discontinuation of GH treatment, to levels even lower than for normal peers. For the SGA group the atherogenic index

remained at pre-treatment level during and after discontinuation of GH treatment, and was similar to normal peers.
Clustering of risk factors for CVD at 6 months after discontinuation of GH treatment was found only for insulin levels and BMI SD-score in TS group, while in the SGA group BMI SD-score correlated significantly with fasting insulin levels, systolic BP SD-score, and the atherogenic index. In normal children a relationship between BMI SD-score and insulin levels is also found [89, 100]. The clustering of risk factors for CVD in the SGA group might indicate that obese SGA subjects have an increased risk for adult CVD which is already present in young adulthood. Prevention of obesity, however, appears to be important for both patient groups.

Psychosocial functioning after long-term GH treatment

In the TS group, in Chapter 4, we describe psychosocial functioning after discontinuation of GH treatment. In the SGA group, in Chapter 10, we describe IQ and psychosocial functioning before, during, and after discontinuation of GH treatment. In both patient groups, we investigated problem behaviour scores and self-perception after discontinuation of GH treatment. Problem behaviour after discontinuation of GH treatment were comparable to normal peers in both trials. The longitudinal data in the SGA group showed that scores for in particularly externalising problem behaviour during GH treatment had decreased to values comparable to the same age reference sample. In addition, in the SGA trial, children whose height became closer to that of their peers during GH treatment, had less problem behaviour.
Self-perception in the TS group after discontinuation of GH treatment was less positive than for peers, while in the SGA group self-perception was slightly better than for peers. Longitudinal self-perception scores in the SGA group demonstrated an increase in self-perception during the first years of GH treatment, in parallel with the improvement in height. The difference in self-perception between the two patient groups after discontinuation of GH treatment seems to indicate that in TS not only short stature has an influence on self-perception, but also that other factors, most likely related to other TS features, are partly accountable for a lower self-perception score. In the discussion of the TS results in Chapter 5, it is postulated that the typical physical TS features or a suggested lack in social graces play a part in their lower self-perception.
In addition to psychosocial functioning, in the SGA trial, intelligence was investigated over time. As described in Chapter 10, during GH treatment, a significant increase in IQ was found.

In conclusion

After synthesis of results of the two trials, it can be concluded that for both patient groups GH treatment results in normalisation of final height in most children. For the TS group, a higher dosage is necessary to achieve the highest percentage of normalisation. This finding seems to reflect the difference in aetiology of the growth retardation in the two patient groups.

The GH-induced increase in insulin levels during GH treatment reduces after discontinuation of GH treatment to levels comparable to normal peers, in both patient groups. In the TS group, however, insulin levels remain slightly higher than pre-treatment, after discontinuation of GH treatment. Most likely this can be explained by the higher BMI SD-score in the TS group after GH discontinuation.

Clustering of risk factors for CVD in TS girls seems to be limited to a clustering of insulin levels and BMI SD-score, which is also found in normal children. In the SGA children, there is a clustering of several risk factors. It is not yet known whether the clustering is an early sign of the SGA predisposition for adult CVD. The lack of clustering in the TS girls of the slightly higher post-treatment blood pressure with other risk factors possibly indicates a different aetiology or timing for the TS predisposition for CVD.

Long-term GH treatment, and additional oestrogen therapy in TS, in most children, improved psychosocial functioning in both GH trials. In case of TS, however, other TS features which were not treated, most likely prevented the self-perception scores to normalise. This results emphasise the importance of further studies determining factors which significantly influence the psychosocial status in TS, and by doing so optimise the treatment strategy for TS. In the SGA group the normalisation of behaviour and self-perception during GH treatment most likely demonstrates the psychosocial importance of GH treatment for short SGA children.

Chapter 14
General Part: References

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