LONG-TERM GROWTH HORMONE TREATMENT IN TWO GROWTH DISORDERS

PART "CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE"

ISBN 90-75561-03-2

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LANGDURIGE GROEIHORMOONBEHANDELING
BIJ TWEE GROEISTOORNISSEN

-DEEL
"KINDEREN MET EEN KLEINE LENGTE NA
KLEINE GEBOORTELENGTE VOOR ZWANGERSCHAPDUUR"

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFCUS
PROF. DR. P.W.C. AKKERMANS M.A.
EN VOLGEND BESLUIT VAN HET COLLEGE VOOR PROMOTIES.

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 15 DECEMBER 1999 OM 9.45 UUR

DOOR

THEO. C.J. SAS

GEBOREN TE
DEVENTER

PROMOTIECOMMISSIE

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

Opgedragen aan:

Jordy, Ilona, Renate, Jeffrey, Stefan, Nikki, Erika, Patty, Stacey, Denise, Lesley, Ricky, Randy, Wouter, Rick, Terence, Robin, Ben, Robert, Clinton, Simone, Timo, Fleur, Robin, Jordan, Lucien, Tim, Ker Cheung, Patricia, Judith, Muhammed, Patricia, Sigrid, Arno, Marloes, Stijn, Wesley, Richard, Eefje, Patrick, Eefje, Jan, Esmeralda, Renske, Sjors, Eva, Victor, Patricia, Sharon, Emiel, Fleur, Corné, Sonja, Mira, Nathalie, Mark, Ernst, Rik, Wouter, Toto, Martin, Daniëlle, Pedro, Martijn, Niels, Chead, Raymond, Jelmer, Bas, Michael, Amer, Joey, Ruud, Christian, Vincent, Sebastien, Abe, Robin, Chiel-Jan, Jessica, Naziha, Johan, Chrystal, Jouke, Daniël, Dai Tai, Betty, Marise, Bianca en Tynke.

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CHAPTER 1

INTRODUCTION

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INTRODUCTION

Short stature in children born small for gestational age

By definition, approximately three percent of infants are born small for gestational age (SGA). These children comprise a heterogenous group of newborns. SGA infants may be born either full-term or premature. SGA may be secondary to a chromosomal disorder, intrauterine infection, maternal disease, placental dysfunction, cigarette smoking, and multiple birth. However, in the majority of cases the etiology is not known[1].

It has been reported that most of the children born SGA without an underlying disorder do show catch-up growth within the first years of life[2,3]. The percentage of premature SGA infants with catch-up growth is not different from that of full-term SGA infants[3]. Approximately 15% of SGA children fail to show catch-up growth in the first two years of life[2,3]. A Swedish population-based study showed that during childhood this percentage decreases to approximately 8% at 18 years of age. These children represent about 22% of the total short adult population[4].

Little is known about the growth pattern of these children during childhood. In contrast to e.g. Turner syndrome[5,6] or idiopathic short stature[7], no reference growth diagrams are available for children with short stature born SGA. More data are available about a special subgroup of SGA children with the dysmorphic features of Silver-Russell syndrome (SRS), such as small triangular face, clinodactyly, and asymmetry of the body[8,9]. They are short at birth, show only minimal catch-up growth during infancy, and have a reduced pubertal growth spurt[10-12] . Davies *et al.* reported a mean adult height of -3.6 SDS in 18 children with SRS, corresponding to 142 cm for girls and 150.7 cm for boys[11]. Wollmann *et al.* found, however, a lower mean final height of 139.7 cm in 163 girls and 149.5 cm in 223 boys[12].

Previous reports have demonstrated that bone age was delayed in young children born SGA, irrespective of the presence of SRS[9,12,13]. Tanner *et al.* reported a gradual increase in bone maturation during the second half of the first decade of life resulting in only a small delay at the beginning of puberty[9]. Wollmann *et al.* showed that there was a catch-up of bone age in early puberty[12]. Despite the retardation of bone age in young childhood, the start of puberty has not been delayed compared to healthy children[9,11-13]. An even early pubertal development has been described in these children[8,14]. The acceleration of bone maturation and the absence of a delayed puberty may play an important role in the restriction of adult height of these children.

Pathophysiology of the postnatal growth failure

The mechanism of the stunted postnatal growth in short children born SGA is poorly understood. It has been previously shown that disturbances in the growth hormone (GH) / insulin-like growth factor (IGF) - axis may account ± 60 percent of the growth retardation. Short children with SGA spontaneously secrete less GH or showed other GH secretion patterns during 24-hour growth hormone profiles than healthy children with short stature born appropriate for gestational age and children with a normal height[15-17]. In addition, subnormal responses to arginine provocation and reduced mean plasma IGF-I, IGF-II, and IGF-binding protein-3 (IGFBP3) were reported in short children born SGA[15,16,18]. Furthermore, it has been reported that an increased proportion of non-22-kDa GH isoforms (in controls 70-75% 22-kDa) were found in children with short stature born SGA. These non-22-kDa GH isoforms may interact as weak agonists or antagonists of the GH-receptor[19]. However, the magnitude of the abnormality in the GH/IGF axis is not always directly related to the magnitude of the growth retardation and several children with short stature born SGA have no distinct GH/IGF axis abnormalities[16,19]. Therefore, other direct or

indirect mechanisms may play a role as well. We do not know whether the feeding problems being often present in short children born SGA and in children with SRS contribute to the postnatal growth failure. Even less is known about possible genetic abnormalities underlying the mechanisms leading to the absence of a postnatal catch-up growth[20,21].

Growth hormone treatment for short stature

Studies in the early 1970s with low frequency of GH administration and low dose in short children born SGA were quite disappointing, as has been described in other GH-treated children in those days[22-24]. Papers in the late 1980s showed that daily administration of GH treatment in varying dosages accelerates growth significantly in short children born SGA[15,25,26]. With the availability of recombinant human GH after 1985, greater numbers of SGA children were treated in standardized studies. In 1991 a GH dose-response trial in 79 children with short stature born SGA was started in four centers in the Netherlands. Preliminary data of a subgroup of the children showed that two years of GH treatment appeared to be efficacious in increasing height and height velocity. In addition, a significant improvement in the predicted adult height was achieved despite advanced bone maturation[27].

Possible side effects of GH treatment in children with short stature born SGA

No published data were available on whether an increase in height in children with short stature born SGA during treatment with supraphysiological GH dosages will be accompanied by proportional growth of other parts of the body.

SGA has been associated with increased prevalence of diabetes mellitus type II, hypertension, and hyperlipidemia at a relative young age in later life[28]. GH modulates tissue responses to insulin. Supra-physiological concentrations of GH in acromegalic patients[29] and in normal[30,31] and diabetic[31] adults showed a decrease in glucose sensitivity to insulin, both in liver and in extra-hepatic tissues. Concern has been expressed regarding possible adverse effects of long-term GH treatment on carbohydrate metabolism in children with short stature born SGA. Since in adults GH hypersecretion in acromegaly is associated with an increased incidence of hypertension[33], one may suggest that long-term GH treatment in short children born SGA may have negative effects on blood pressure. In addition, little is known about the effects of long-term GH treatment on lipid metabolism in these children.

Considerations, questions, and aims of the study

Efficacy of GH treatment

Although short-term data showed a catch-up growth during GH treatment in short children born SGA, we wondered if long-term GH treatment would result in an attainment of the target height percentile and subsequently if growth would persist along that percentile. In addition, we wanted to know whether GH treatment would result in gain in adult height prediction, and finally gain in attained adult height. Furthermore, we wanted to assess the effects of two GH doses and to find the optimal age to start treatment. Moreover, we wondered if there would be a relationship between gain in height during long-term GH treatment and the GH/IGF status before start of treatment. Therefore, we evaluated the effects of 5 years of GH treatment with 3 or 6 IU per m^2 body surface per day on **height, bone maturation, final height expectations, plasma IGF-I**, and **IGFBP3 levels** in short children born SGA with or without abnormalities of the GH/IGF axis.

Safety of GH treatment

Using supraphysiological GH dosages for a long period during childhood, we wanted to know if GH treatment would have side-effects. Therefore, we evaluated the development of the **body proportions** of short children born SGA before and during six years of GH treatment. In addition, we assessed the effect of GH on **carbohydrate metabolism** during six years of treatment. Furthermore, the **body composition, blood pressure, and lipids** were evaluated during long-term GH treatment.

Outline of the thesis, part "Children with short stature born SGA"

This part of the doctoral dissertation gives the results of the randomized, double-blind dose-response study on GH treatment in 79 children with short stature born SGA either with or without abnormalities of the GH/IGF axis. This study was started in 1991. Preliminary results concerning the first years of the study period were reported in the thesis of W de Waal entitled "Influencing the extremes of growth", Rotterdam 1996. **Chapter 2** describes the effects of 5 years of GH treatment on height, bone maturation, predicted final height, IGF-I, and IGFBP3 levels in short children born SGA. **Chapter 3** describes the body proportions of short children born SGA before and during six years of GH treatment. **Chapter 4** presents the effect of GH treatment on carbohydrate metabolism during six years of GH treatment. **Chapter 5** gives the results of body composition, blood pressure, and lipids during long-term GH treatment. **Chapter 6** discusses the significance of the presented data as such and in the relation to the current literature. Our final conclusions are listed and recommendations for future research are given. Finally, **Chapter 7** summarizes this dissertation in English as well as in Dutch.

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CHAPTER 2

GROWTH HORMONE TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE: FIVE-YEAR RESULTS OF A RANDOMIZED, DOUBLE-BLIND, DOSE-RESPONSE TRIAL

Adapted from the Journal of Clinical Endocrinology and Metabolism 84: 3064-3070, 1999.

GROWTH HORMONE TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE: FIVE-YEAR RESULTS OF A RANDOMIZED, DOUBLE-BLIND, DOSE-RESPONSE TRIAL

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Abstract

The growth promoting effect of continuous growth hormone (GH) treatment was evaluated over 5 years in 79 children with short stature (height SD-score < -1.88) born small for gestational age (SGA) (birthlength SD-score < -1.88). Patients were randomly and blindly assigned to either one of two GH dosage groups (3 vs 6 IU /m² body surface/day). GH deficiency was not an exclusion criterium.

After 5 years of GH treatment almost every child had reached a height well within the normal range for healthy Dutch children, being in the range of their target height SDS. Only in children who remained prepubertal during the study period, the 5-year increase in height SD-score ($HSDS_{CA}$) was significantly higher in the study group receiving 6 compared to 3 IU GH/m²/d. Remarkably, the 5-year increment in $HSDS_{CA}$ was not related to spontaneous GH secretion, maximum GH levels after provocation, nor to baseline insulin-like growth factor-I levels. GH treatment was associated with an acceleration of bone maturation, regardless of the GH dose given. Height SD-score for bone age and predicted adult height increased significantly. GH treatment was well tolerated.

In conclusion, our 5-year data show that long-term continuous GH treatment at a dose of 3 or 6 IU/m²/day in short children born SGA results in a normalization of height during childhood followed by growth along the target height percentile.

Introduction

Short stature in children born small for gestational age (SGA) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the SGA newborns, about 15% of these children fail to show catch-up growth (1, 2). They present with a height deficit during childhood that in almost all cases results in short adult stature (3-5). The mechanism of the stunted postnatal growth in short children born SGA is poorly understood. It has been previously shown that disturbances in the growth hormone (GH) / insulin-like growth factor I (IGF-I) - axis may account for some of the growth retardation: up to 60 percent of the short children born SGA have GH-secretory abnormalities and/or reduced levels of IGFs (6-12). GH treatment in these children has been explored from the early 1970s (13, 14). Initial data were disappointing probably due to the low dose and frequency of GH administration. Recent short-term studies have shown that daily administration of recombinant human GH therapy in varying dosages accelerates

growth significantly in short children born SGA (7, 15-22).

To assess whether GH treatment will also improve linear growth on the long-term as well as adult height, we started a randomized, double-blind, dose-response multicenter trial with continuous GH treatment until adult height in 79 prepubertal children with short stature born SGA. We now report a five-year analysis comparing the effects of two doses of GH (3 versus 6 IU/m^2 body surface/day).

Subjects and Methods

Study group

Seventy-nine prepubertal short children born SGA were included after meeting the following criteria: 1) birth length standard deviation score (SDS) below -1.88 (that is < 3rd percentile) for gestational age according to the standards of Usher and McLean (23), 2) chronological age (CA) between 3 and 11 years in boys and 3 and 9 years in girls at start of study, 3) height SDS for CA ($HSDS_{CA}$) below -1.88 according to Dutch references (24), 4) height velocity SDS for CA (HVSDS) ≤ zero (24, 25) to exclude children presenting spontaneous catch-up growth, 5) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys (26), 6) uncomplicated neonatal period, that is without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis neonatorum and without long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders or syndromes (emotional deprivation, severe chronic illness, chondrodysplasia), and previous or present use of drugs that could interfere with GH treatment. Patients with Silver-Russell syndrome (SRS), however, were included in this study. GH-deficiency was not an exclusion criterium.

Four centers in the Netherlands participated in the study. The study was approved by the Ethics Committee of each participating center. Written informed consent was obtained from the parents or custodians of each child.

Study design

Before GH treatment, 40 of the 79 children underwent a 24-hour plasma GH profile, as described previously (6). To stratify for the spontaneous GH secretion during the 24-hour GH profile, the total group of 79 children was divided into three groups: "normal profile", "GH insufficient profile" (area under the curve < 90 μg/L/24 h and mean GH < 2.0 μg/L), and "no profile performed". After stratification for spontaneous GH secretion during the 24-hour GH profile and CA all children were randomly and blindly assigned to either one of two GH dosage groups: group A, 3 IU/m^2 body surface/day, or group B, 6 IU/m^2 body surface/day (≈ 0.1 or 0.2 IU/kg/d, respectively). Biosynthetic GH (r-hGH Norditropin^R, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime with a pen injection system (Nordiject 24). Every three months the total GH dose was adjusted to the calculated body surface. The study was kept double-blind by using an equal volume of a reconstituted preparation. Criteria to discontinue the GH treatment were 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.

Growth evaluation

Height (H) was measured at baseline and subsequently every three months, determined according to Cameron (27) using a Harpenden stadiometer. Four measurements per visit were

made by two trained observers (WdW and later on ThS) and the mean was used for the analysis. Height was expressed as SD-score for CA ($HSDS_{CA}$) (24). Target height (TH) was adapted from Dutch reference data with addition of 3 cm for secular trend: 1/2 x (H_{father} + H_{mother} + 12) + 3 for boys and 1/2 x (H_{father} + H_{mother} - 12) + 3 for girls (24). TH and body mass index (BMI) were expressed as SD-score using Dutch references (24). Bone age (BA) was determined by one investigator (ThS) according to Tanner & Whitehouse radius, ulna, short-bones score (BA_{RUS}) (28). Bone maturation was expressed as the ratio of the change in BA to the change in CA ($\Delta BA/\Delta CA$). Height SD-score for BA ($HSDS_{BA}$) and adult height prediction according to the Tanner & Whitehouse prediction method (TW2) (28) were used as indices of adult height prognosis. Pubertal stages were assessed by the same two investigators according to Tanner (26), using an orchidometer in boys.

Biochemical parameters

Before treatment a standard arginine tolerance test (ATT) was performed (6). A standard oral glucose tolerance test (OGTT) was done at baseline and after one year of GH treatment (29). Additional blood samples were taken at start of the study and subsequently every year for determination of the IGF-I levels and hemoglobin A_{1c} levels. IGFBP-3 was determined at start of the study, after the first and second year and after the fifth year of GH treatment. After centrifugation, all samples were frozen (-20°C) until assayed.

Hormone Assays

The RIA measurements of plasma GH, IGF-I, IGFBP-3, and insulin were performed as described previously (30-33). All measurements were performed in the same laboratories. Since 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 (34).

Statistical analyses

Results are expressed as mean ± SD, unless indicated otherwise. Differences between groups were tested using Student's t-tests. Differences between points in time were tested by paired Student's t-tests. To study the relation between the growth response variables (increment in $HSDS_{CA}$ after 5 years) and baseline parameters, multiple linear regression analyses, adjusted for GH dosage group, were done. For each possible predictive factor, separate analyses were performed. Possible predictive baseline factors were: CA, BA_{RUS}, BA delay (CA-BA), THSDS, IGF-I SD-score, pretreatment HV SD-score, maximum GH value during the GH provocation test, and the characteristics of the 24-hour GH profiles established at the start of the study (6). A subgroup analysis on prepubertal growth was performed in the group of children who remained prepubertal during the whole study period. Girls with Tanner breast stage I and boys with a testis volume ≤ 6 mL during the 5-year study period were included in this analysis on prepubertal growth. A p-value less than 0.05 was considered significant.

Since the study remains double-blind until final height, statistical analysis was performed by an independent statistician (PM) and therefore data are only expressed as mean ± SD.

Results

Clinical data, growth, and bone maturation

Table 1 lists the baseline clinical data of the 79 children. Both GH dosage groups had similar initial characteristics. Seven children had SRS. Five children dropped out of the study for

TABLE 1. Baseline clinical data (mean ± SD).

	group A 3 IU/m²/d (n=41)	group B 6 IU/m²/d (n=38)
Male/Female	31/10	21/17
Gestational age (wk)	37.3 ± 3.2	36.0 ± 4.1
Birthlength SDS	-3.6 ± 1.4	-3.7 ± 1.7
Birthweight SDS	-2.6 ± 1.2	-2.6 ± 1.0
Chronological age (yr)	7.3 ± 2.1	7.2 ± 2.4
Bone age (RUS) (yr)	6.6 ± 2.4	6.7 ± 2.9
Height SDS_{CA}	-3.0 ± 0.7	-3.1 ± 0.7
Height velocity SDS	-0.7 ± 1.1	-1.2 ± 1.3
Target height SDS	-1.0 ± 0.9	-0.5 ± 0.9

the following reasons: Three children were no longer motivated to inject GH daily after 15, 45, and 51 months of GH treatment, respectively, despite ongoing catch-up growth with GH treatment in two of the three children. One girl dropped out of the study because of treatment for early puberty after 27 months of GH treatment. In one boy GH treatment was discontinued after 27 months because of signs of GH insensitivity. During GH treatment his IGF-I levels and height velocity did not increase, despite good compliance with treatment. As these five children were lost to follow-up after discontinuation of GH, their data were not included in the analysis.

Figure 1 shows the height at baseline, as well as the height after 5 years of GH treatment. During the 5-year study period, onset of puberty was observed in 47 of the 74 children. The mean CA at the onset of puberty did not differ between the GH dosage groups (in girls 10.5 ± 0.9 yr vs 11.0 ± 1.1 yr; in boys 11.9 ± 0.9 yr vs 11.6 ± 0.6), being apparently within the normal range. One male adolescent discontinued GH treatment after 54 months because of satisfaction with the height achieved (165.3 cm). All other children were still growing and receiving GH treatment. Since the design of the study is still double-blind, in these figures no separate growth diagrams were made for the two GH dosage groups. After 5 years, almost every child had achieved a height well within the normal range for healthy Dutch children.

Figure 2 shows the $HSDS_{CA}$ at baseline and throughout the 5-year study period. After 5 years of GH treatment, the mean $HSDS_{CA}$ in both GH dosage groups have significantly increased compared to baseline ($p < 0.001$), being in conformity with the target height SDS. Although the 5-year increase in $HSDS_{CA}$ was higher in group B (2.6 ± 0.9) than in group A (2.2 ± 0.6), the difference was not statistically significant ($p = 0.057$). The increment in $HSDS_{CA}$ was not different between the seven children with SRS and those without (data not shown).

Figure 3 shows the ⌒BA/⌒CA ratio per year throughout the 5-year study period. The mean ⌒BA/⌒CA ratio per year was significantly higher than 1 for both GH dosage groups (1.4 ± 0.21 and 1.3 ± 0.2, respectively) ($p < 0.001$). No significant difference in bone maturation was found between the two GH dosage groups. At baseline, mean BA_{RUS} retardation was 0.6 ± 1.0 year, while after 5 years of GH treatment mean BA_{RUS} was advanced with 1.0 ± 1.1 years.

After five years of GH treatment, $HSDS_{BA}$ increased significantly compared to baseline ($p \leq 0.001$). The increase was significantly higher in the group B (from -2.4 ± 1.0 to 1.2 ± 0.8) compared to A (from -2.1 ± 1.1 to 1.5 ± 0.8) ($p = 0.004$).

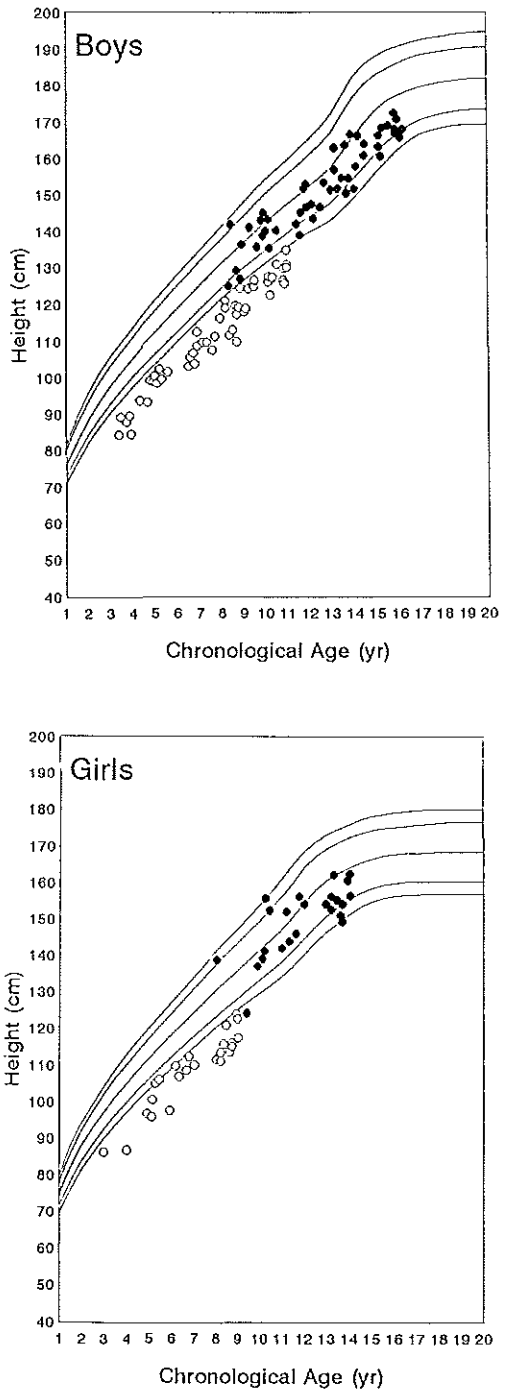

Fig. 1. Individual heights at start of the study (open circles) and after 5 years of GH treatment (filled circles). Reference curves for healthy Dutch children (P3, P10, P50, P90 and P97) are given.

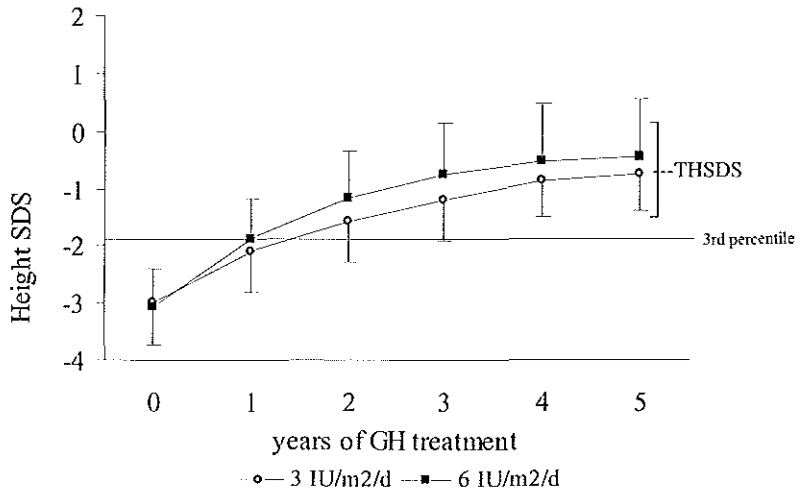

Fig. 2. Mean (±SD) height SD-score for chronological age for both GH dosage groups during 5 years of GH treatment. The target height SDS (±1SD) is indicated in the figure.

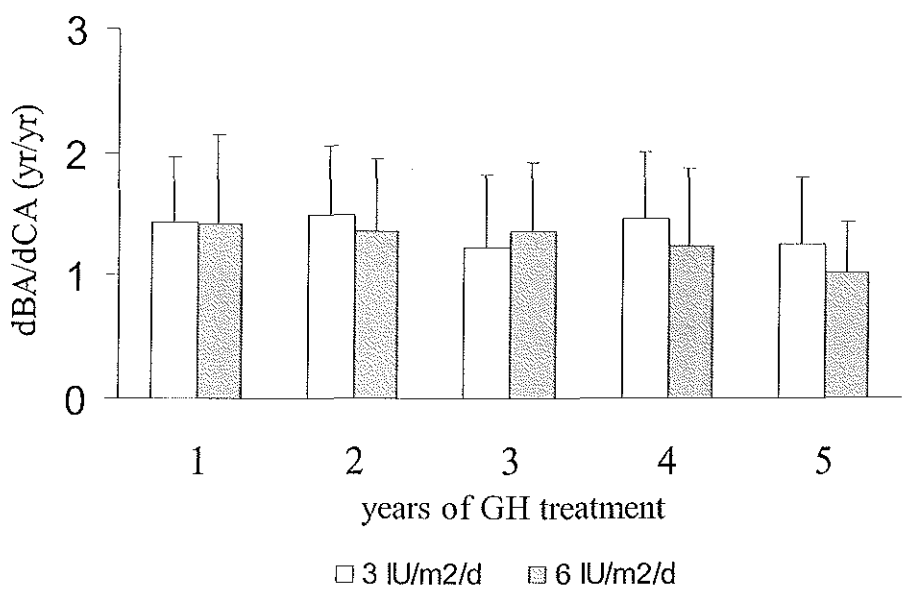

Fig. 3. Mean (+SD) ratio (△BA (yr) / △CA (yr)) per year for both GH dosage groups during 5 years of GH treatment.

In the subanalysis on prepubertal growth (n=23 in group A, n=16 in group B), the $HSDS_{CA}$ was significantly increased in both GH dosage groups ($p < 0.001$) (Figure 4). The increment in $HSDS_{CA}$ was significantly higher in group B (3.30 ± 0.73) than in group A (2.35 ± 0.51) ($p < 0.001$). The mean △BA/△CA ratio per year was significantly higher than 1 for both GH dosage groups (1.39 ± 1.17 and 1.37 ± 0.22, respectively) ($p < 0.001$), without significant differences

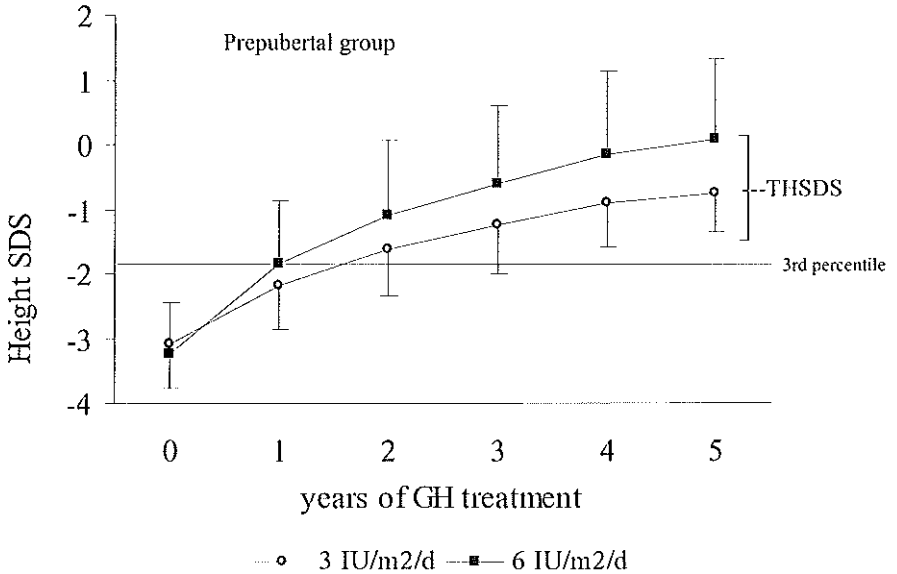

Fig. 4. Mean (±SD) height SD-score for chronological age for both GH dosage groups during 5 years of GH treatment in the children who remained prepubertal during the study period. The target height SDS (±1SD) is indicated in the figure.

between the two GH dosage groups. $HSDS_{BA}$ increased significantly compared to baseline ($p < 0.05$). The increase in $HSDS_{BA}$ was significantly higher in group B (from -2.06 ± 1.17 to -0.88 ± 0.93) compared to A (from -1.86 ± 1.11 to -1.49 ± 0.89) ($p = 0.02$).
The increase in predicted adult height after 5 years of GH treatment was 9.1 ± 2.8 cm in group A and 14.0 ± 5.5 cm in group B, being significantly increased compared to baseline in both GH dosage groups ($p < 0.005$), and being significantly higher in the group B compared to group A ($p = 0.02$).

Body mass index

At baseline, the BMI SD-score (BMI-SDS) was -1.2 ± 1.3 in group A and -1.2 ± 1.1 in group B, being significantly lower than zero ($p < 0.001$). After 5 years of GH treatment BMI-SDS was significantly increased to -0.3 ± 1.2 in group A and to -0.2 ± 0.8 in group B ($p < 0.001$). The increase in BMI-SDS was not significantly different between the two GH dosage groups.

GH, IGF-I and IGFBP-3

The results of the 24-hour plasma GH profiles at baseline have been described previously (6). The mean ± SD maximum serum GH concentration during the arginine tolerance test at baseline was not significantly different between the two GH dosage groups (11.2 ± 6.3 vs 14.0 ± 6.3 μg/L). In 27 children, the maximal GH peak was below 10 μg/L.

Table 2 shows the IGF-I and IGFBP-3 SDS at baseline and during 5 years of GH treatment. Baseline IGF-I SDS was significantly lower than zero. During GH treatment, IGFI-SDS was significantly higher compared to baseline at each point in time for both GH dosage groups. The IGF-I SDS was significantly higher in group B compared to group A during the first three years. Thereafter, this difference was no longer statistically significant. The mean baseline IGFBP-3 SDS was significantly lower than zero. During the first year of GH treatment the

IGFBP-3 concentrations normalized and after 5 years the IGFBP-3 SDS was significantly higher than zero for both GH dosage groups. The IGFBP-3 SDS after five years of GH treatment was not significantly different between group A and B.

TABLE 2. Mean (± SD) of IGF-I and IGFBP-3 SD-scores.

	group A 3 IU/m²/d	group B 6 IU/m²/d
IGF-I SDS		
baseline	-1.2 ± 1.2[1]	-0.9 ± 1.0[1]
1 year	1.2 ± 1.1[1]	1.9 ± 1.1[1**]
2 year	1.2 ± 1.0[1]	1.9 ± 1.2[1*]
3 year	1.4 ± 1.0[1]	2.0 ± 1.2[1*]
4 year	1.8 ± 0.8[1]	2.0 ± 1.2[1]
5 year	1.7 ± 0.7[1]	2.0 ± 0.9[1]
IGFBP-3 SDS		
baseline	-1.7 ± 1.3[1]	-1.3 ± 1.1[1]
1 year	0.2 ± 1.2	0.5 ± 1.1[2]
2 year	0.0 ± 1.2	0.2 ± 1.0
5 year	1.0 ± 0.8[1]	1.2 ± 1.0[1]

Significantly different from zero, (1) $p < 0.001$, (2) $p = 0.021$. Significantly higher in the group receiving 6 than the group receiving 3 IU/m2/d, (*) $p < 0.05$, (**) $p = 0.01$

Predictors for growth response

After adjustment for GH dosage group, the 5-year increase in $HSDS_{CA}$ correlated negatively with baseline CA (ß = -0.216 $p < 0.001$) and with baseline BA_{RUS} (ß = -0.173 $p < 0.001$). The 5-year change in $HSDS_{CA}$ was not significantly related to THSDS, baseline BA delay, pretreatment HVSDS, nor to baseline IGF-I SD-score, the mean maximal plasma GH response during ATT, or the characteristics of the 24-hour GH profiles established at the start of the study (6). In addition, no difference in the 5-year increment in $HSDS_{CA}$ was found between the children with GH-deficiency and those with normal GH levels.

Safety

Treatment was well tolerated and no adverse events were detected that were considered to be drug related. In both GH dosage groups the mean fasting glucose level and area under the curve (AUC) for glucose during OGTT did not significantly change during 1 year of GH treatment compared to baseline. In contrast, the mean fasting insulin levels increased significantly in both GH dosage groups after one year of GH treatment, from 6.4 to 7.6 mU/L and from 4.9 to 8.4 mU/L, for group A and B, respectively ($p < 0.01$). In addition, the AUC for insulin during OGTT was significantly higher after one year of GH treatment compared to baseline ($p < 0.001$), from 1433 at baseline to 2101 mU/L*1h after 1 year for group A and from 1161 to 2634 mU/L*1h for group B, however, without a significant difference between the two GH dosage groups. Hemoglobin A_{1c} levels remained within the normal range and none of the children developed diabetes mellitus.

Discussion

Our study shows that long-term continuous GH treatment (3 vs 6 IU/m²/day) in children with short stature born SGA results in a normalization of height during childhood followed by growth along the target height percentile. The difference in gain in $HSDS_{CA}$ between the GH dosage groups was just not statistically significant. Only in children who remained prepubertal during the study, the mean gain in $HSDS_{CA}$ after 5 years of GH treatment was significantly greater in those treated with 6 compared to 3 IU/m²/d. Both GH dosage groups reached their target height SDS well within 5 years of GH treatment, indicating that long-term GH treatment with a lower GH dose of 3 IU/m²/d is also able to normalize height of short children born SGA.

Most controlled trials have shown a beneficial effect of GH treatment over a period of 2-3 years in comparison with a randomized untreated group of children born SGA for 1 or 2 years. The untreated children did not show any spontaneous catch-up growth indicating that they are destined to remain short (18, 20, 21). For our long-term study, a randomized controlled trial with an untreated group until adult height was considered unethical by several Ethics Committees. Therefore, a dose-response design comparing two GH dosages (3 and 6 IU/m²/day) was chosen to evaluate the long-term use of continuous GH treatment.

Our 5-year results consolidate the previously described effects of short-term GH treatment in short children born SGA (18-22). In the 3-year study reported by Boguszewski et al., similar daily GH dosages (3 and 6 IU/m²) have been used as in our study. Interestingly, the GH dose-dependent 3-year increase in $HSDS_{CA}$ in prepubertal children was found to be almost identical in both studies (21). A metanalysis of four European trials showed that the 4-year growth response was similar between continuous GH use, 3 IU/m²/d for 4 years, and discontinuous GH use, 6 IU/m²/d for 2 years followed by 2 years without GH (22), suggesting that the cumulative GH dose received and not the daily GH dose determines the growth response. All studies demonstrate that GH treatment induces an acceleration in linear growth in short children born SGA. In addition, our 5-year study shows that continuous GH treatment can normalize height and is followed by a persistent growth within the normal height range.

Comparing our growth results with other GH-treated patient groups receiving long-term GH treatment, the gain in height in short children born SGA is comparable with that in GHD patients with a GH dose that is slightly higher than the conventional GH dose (2 IU/m²/d) (35).

In some previous short-term studies and in the present study bone maturation was accelerated compared to untreated short children born SGA and compared to healthy children, respectively (18, 20, 22). However, it was remarkable that during the 5-year treatment period the bone maturation in the total group, as well as in the children who remained prepubertal during the study period, appeared to be independent of the given GH dose, whereas no progressive acceleration of the bone maturation was found. Previous reports have demonstrated a spontaneous acceleration of bone maturation from the age of 6-10 years in untreated children with short stature born SGA (3, 36, 37). Therefore, the acceleration of bone maturation seen in GH-treated short children born SGA may be partly explained by the effects of GH treatment, but may also be explained by the spontaneous acceleration of bone maturation observed in untreated SGA children.

Data on final height are very limited. Therefore, an indication of final height is often given by the change in $HSDS_{BA}$ and the predicted adult height according to the Tanner & Whitehouse prediction method, during GH treatment. In our study, $HSDS_{BA}$ and predicted adult height increased significantly after 5 years of GH treatment, in conformity with results described in short-term studies (20, 22). Thus, the gain in height outweighs the faster bone maturation.

However, we realize that both prediction methods have limitations and therefore, data on adult height have to substantiate our results. Only two studies reported data on final height in relatively small numbers of patients (38, 39). One study reported data from a pharmaceutical registration database showing that 16 SGA patients, treated with GH at a median daily GH dose of approximately 3 IU/m² until near adult height, achieved an adult stature that was 1.0 SDS greater than the pretreatment $HSDS_{CA}$ (38). However, in these patients the median age at start of treatment was 12.7 years. Preliminary data of the study of Albanese et al., demonstrated that in 12 GH-treated children with short stature born SGA, GH treatment with approximately 4 IU/m²/day, starting at a mean age of 7.6 years, significantly improved final height. Although $HSDS_{BA}$ did not improve throughout the study, the $HSDS_{CA}$ had increased from -2.9 at baseline to -1.5 at final height (39).

Some studies reported earlier onset of puberty in untreated short children born SGA (36, 37). The question arose as to whether GH could further advance the timing of puberty and consequently reduce the growth period. In our study the mean pubertal onset seemed not to be advanced by GH treatment (girls 10.8 year, boys 11.8 year). However, longer follow-up is required to establish whether all of our children will start puberty at an appropriate age and whether the overall duration of puberty is not altered.

As described in other studies, the variability in growth response to GH treatment was considerable. For that reason, we looked for clinical predictors of the growth response to GH treatment. The increase in $HSDS_{CA}$ over 5 years of treatment was negatively related to baseline chronological age and bone age: the younger the child at baseline, the better the 5-year increase in $HSDS_{CA}$. In contrast, neither the height of the parents, the pretreatment height velocity, nor the baseline bone age retardation were related to the 5-year increment in height.

The etiology underlying the insufficient spontaneous catch-up growth in short children born SGA is poorly understood. Findings in previous studies (6-12) and in our study suggest that disturbances in the GH/IGF-I axis play a role in the absence of spontaneous catch-up growth. However, prior to treatment, we found no clear relation between the GH secretory status and spontaneous growth (6). To study the relation between the baseline parameters of the GH secretion status and the growth response to GH treatment, we included, in contrast to other prospective studies, patients regardless of their GH secretion. We found that the maximum GH levels during the provocation tests before the start of the GH treatment were not significantly related to the growth response. Two previous studies also found no differences in growth response on GH treatment between SGA children who were GH deficient and those who were not (35, 40). In addition, our study showed that neither the IGF-I levels at baseline, nor the GH levels during the 24-hour plasma GH profile test were significantly related to the growth response. Thus, although the stunted growth in short stature born SGA may be partly explained by disturbances in the GH/IGF-I axis, the growth promoting effect of GH treatment at a dose of 3-6 IU/m²/d seems to be independent of the baseline GH/IGF-I status.

It is well known that many short children born SGA are lean and have lack of appetite. In the present study, we did not systematically investigate a possible change in appetite of the children. Several parents, however, mentioned that the children had better appetite and food intake after the start of the GH treatment. Although we do not know the natural development of the body mass in short children born SGA during childhood, it is remarkable that the normalization of height during GH treatment was accompanied by a normalization of the BMI. We may speculate that the anabolic effect of GH treatment has a positive influence on the food intake of the child and that this plays an additional role in the catch-up growth in short children born SGA.

Our study showed that the height gain after 5 years of GH treatment with 6 IU/m²/d was only statistically significant higher than with 3 IU/m²/d in the children who remained prepubertal during the study period. In addition, this difference was relatively modest, suggesting that near-maximal GH effects in SGA children are reached in this dose-range. Follow-up until adult height is needed to assess whether the higher GH dose results in a significantly better improvement of adult height. Therefore, we will continue our double-blind, dose-response study until all children have attained adult height.

In conformity with other studies (18-22), tolerance to GH treatment was good. No adverse events were detected that were GH related. In our study, 1-year GH treatment resulted in a significant rise in insulin levels with normal glucose and glycosylated hemoglobin levels. SGA has been reported to be associated with an impairment in insulin sensitivity and noninsulin-dependent diabetes mellitus in later life (41, 42). Further studies on the effects of long-term GH treatment on carbohydrate metabolism are underway.

The most important aim of GH treatment in short children born SGA is a normalization of height and consequently an improvement of the psychosocial burden of being small in childhood, as well as in adulthood. A two-year psychosocial evaluation in the children of our study showed a beneficial effect on behavioral and emotional problems and on the self-concept of the children (43, 44). Further psychosocial research will be performed to confirm this improvement.

In conclusion, our 5-year data show that long-term continuous GH treatment (3 or 6 IU/m²/day) in short children born SGA results in a normalization of height and a subsequent growth along the target height percentile. The increase in height appears to be independent of the baseline GH/IGF-I status. Adult height prognosis and height SDS for bone age increased significantly, despite acceleration of bone maturation. The difference in growth response between the children receiving daily 6 IU/m² and those receiving 3 IU/m² was small and only significant in the children who remained prepubertal during the study. Further studies should be directed at optimizing GH modalities and establishing adult height results and long-term safety data, and at the predictors indicating the small children born SGA who will benefit most from GH treatment.

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CHAPTER 3

BODY PROPORTIONS DURING SIX YEARS OF GROWTH HORMONE TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE PARTICIPATING IN A RANDOMIZED, DOUBLE-BLIND, DOSE-RESPONSE TRIAL

Submitted for publication 1999

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Abstract

To assess body proportions in children with short stature born small for gestational age (SGA) before and during six years of growth hormone (GH) treatment, height, sitting height (SH), hand (Hand) and foot length (Foot), and biacromial (Biac) and biiliacal diameter (Biil) were measured in 79 children (height SD-score < -1.88 and birth length SD-score < -1.88) participating in a double-blind randomized dose-response study, receiving 3 or 6 IU/m²/day. All results were adjusted for age and sex, and expressed as SD-scores (SDS) using reference values for healthy Dutch children. To describe the proportions of SH, Hand, Foot, Biac, and Biil in relation to height, these values were adjusted for the SDS of height expressing it as shape values, using the formula, e.g. for SH: shape SH = (SH SDS - height SDS) / $\sqrt{}$(2-2 x correlation coefficient between SH and height in the reference population). Values being less than -2 or more than +2 were considered outside the normal range.

At baseline, the mean SD-scores of height, SH, Hand, Foot, Biac, and Biil were lower than zero, indicating that the stunted growth is concerning several parts of the body. However, height was more affected than Hand, Foot, Biac, and Biil. Consequently, the means of these shape values were above zero, however, within the normal range. During six years of GH treatment the SD-scores of all measurements increased significantly towards values more close to zero. The shape values of Hand, Foot, and Biil showed a decrease, while the mean shape value of SH had increased to values higher than zero, however, still being well within the normal range. The shape value of Biac had not changed after six years of GH treatment and was still higher than zero. While the six-year increase in height was significantly higher in the group receiving 6 IU/m²/d compared to 3 IU/m²/d, no differences in the six-year changes in shape values between the two GH dosage groups were found.

In conclusion, untreated short children born SGA have, on average, relatively large hands and feet, and broad shoulders and pelvis, but a normal sitting height compared to height. The dose-dependent significant increase in height during six years of GH treatment is accompanied by a dose-independent improvement of the proportions of the size of hands, feet, and biiliacal diameter, respectively, in relation to height. The increase in height appears to be the result of a relatively greater increase in sitting height than that of leg length. The dose-independency of the changes in body proportions suggests that the changes are more the result of the natural development of proportions in short children born SGA during childhood or part of the catch-up growth rather than due to a direct effect of GH treatment. Thus, six-year continuous GH treatment

with 3 or 6 IU/m²/day in children with short stature born SGA does not negatively influence body proportions.

Introduction

Short stature in children born small for gestational age (SGA) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the SGA newborns, about 15% of these children fail to show catch-up growth resulting in short adult stature in most of the cases (1-5). Although the mechanism of the stunted postnatal growth in short children born SGA is poorly understood, studies have shown that continuous or discontinuous treatment with recombinant human growth hormone (GH) in varying dosages accelerates growth significantly in short children born SGA resulting in catch-up growth to values within the normal range followed by growth along their target height percentile (6-14). We do not know if height is affected to the same extent as other parts of the body in children with short stature born SGA. In addition, to our knowledge, no published data are available on whether the normalization of height in these children during GH treatment with supraphysiological GH dosages is accompanied by proportional growth of the other parts of the body.

To assess the body proportions of children with short stature born SGA, before and during long-term GH treatment, we measured height, sitting height, hand length, foot length, biacromial diameter and biiliacal diameter in 79 children participating in a randomized, double-blind, dose-response multicenter trial. The effect of 5 years of continuous GH treatment on height was described earlier (14). We now report six-year data on body proportions comparing two GH dosage groups (3 versus 6 IU/m² body surface/day).

Methods

Study group

Seventy-nine prepubertal short children born SGA were included after meeting the following criteria: 1) birth length standard deviation score (SDS) below -1.88 (that is < 3rd percentile) for gestational age according to the standards of Usher and McLean (15), 2) chronological age (CA) between 3 and 11 years in boys and 3 and 9 years in girls at start of study, 3) height SDS for CA ($HSDS_{CA}$) below -1.88 according to Dutch references (16), 4) height velocity SDS for CA (HVSDS) ≤ zero (16,17) to exclude children presenting spontaneous catch-up growth, 5) prepubertal stage, defined as Tanner breast stage I for girls and testicular volume less than 4 mL for boys (18), 6) uncomplicated neonatal period, that is without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without sepsis neonatorum and without long-term complications of respiratory ventilation such as bronchopulmonary dysplasia. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders or syndromes (emotional deprivation, severe chronic illness, chondrodysplasia), and previous or present use of drugs that could interfere with GH treatment. Patients with Silver-Russell syndrome (SRS), however, were included in this study. GH-deficiency was not an exclusion criterium.

Four centers in the Netherlands participated in the study. The study was approved by the Ethics Committee of each participating center. Written informed consent was obtained from the parents or custodians of each child.

Study design

After stratification for spontaneous GH secretion during a 24-hour GH profile (14,19) and

CA, all children were randomly and blindly assigned to either one of two GH dosage groups: group A, 3 IU/m² body surface/day, or group B, 6 IU/m² body surface/day (≈ 0.1 or 0.2 IU/kg/d, respectively). 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 three months the total GH dose was adjusted to the calculated body surface. The study was kept double-blind by using an equal volume of a reconstituted preparation. Criteria to discontinue the GH treatment were 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. Bone age was determined by one investigator (TS) according to Tanner & Whitehouse radius, ulna, short-bones (RUS) score (20).

Height, sitting height (SH), left hand length (Hand), left foot length (Foot), biacromial diameter (Biac) and biiliacal diameter (Biil) were measured at baseline and subsequently every six months by two trained observers (WW,TS). Measurements were taken according to Cameron (21). Height and sitting height were obtained using a Harpenden stadiometer and sitting height table. The other measurements were taken with the Harpenden anthropometer. Pubertal stages were assessed by the same two investigators according to Tanner (18), using an orchidometer in boys. The results of each measurement were adjusted for age and sex expressing it as a SD-scores (SDS). When GH treatment was stopped because of final height achievement the last available measurements at discontinuation of GH treatment was used for analysis. The data of the Oosterwolde Study were used as references for healthy Dutch children (22). This reference population consisted of 1240 boys and 1093 girls, ranging from birth to 18 years of age. To calculate SD-scores, data of the reference population were transformed using the LMS method (23,24). This method transforms the reference data at each age to a normal distribution. In addition, the SD-scores of the measurements of SH, Hand, Foot, Biac and Biil were adjusted for the height SD-score and expressed as the shape value. The shape value, in this example defined as the SH SD-score adjusted for the height SD-score, was calculated using the formula (25,26),

$$\text{shape SH} = (\text{SH SDS} - \text{height SDS}) / \sqrt{(2-2r)}$$

in which r is the correlation coefficient between height and SH in the reference population. Values less than -2 or more than +2 were considered outside the normal range. Fourteen children were of non-Caucasian origin. Although references of body proportions of non-Caucasian children might be somewhat different compared to our Caucasian references, all children were included in the statistical analysis.

Since the study remains double-blind until final height, statistical analysis was performed by an independent statistician (PM) and therefore data are only summarized as mean and standard deviation (SD). SD-scores and shape values at baseline and after six years were compared with zero using Student's one-sample t-tests. Differences between SD-scores or shape values after 6 years of GH treatment and baseline values were tested by paired Student's t-tests. Differences in the six-year change in height SDS and the shape values between the GH dosage groups were tested using Student's two-sample t-tests. A p-value < 0.05 was considered significant.

Results

Clinical data

Table I lists the baseline clinical data of the 79 children. Both GH dosage groups had similar initial characteristics. Seven children had SRS. Five children dropped out of the study for the following reasons long before reaching adult height: treatment of early puberty (n=1), signs of GH insensitivity and no catch-up growth (n=1), and lack of motivation to inject GH (n=3). More details are described in a previous paper (14). As these five children were lost to follow-up after

TABLE 1. Mean (SD) baseline data for each treatment group.

	group A 3 IU/m²/day (n=41)	group B 6 IU/m²/day (n=38)
Male/Female	31/10	21/17
Gestational age (wk)	37.3 (3.2)	36.0 (4.1)
Birth length SDS	-3.6 (1.4)	-3.7 (1.7)
Birth weight SDS	-2.6 (1.2)	-2.6 (1.0)
Chronological Age (yr)	7.3 (2.1)	7.2 (2.4)

discontinuation of GH, their data were not included in the analysis. After six years of GH treatment, five other children had discontinued GH treatment because of reaching (near) final height. All other children were still growing and receiving GH treatment.

Body proportions

Figure 1 shows the SD-scores of the measurements at baseline and during 6 years of GH treatment in both GH dosage groups. At start of GH treatment, all mean SD-scores were significantly lower than zero, indicating a stunted growth in several parts of the body. However, the baseline SDS of height and SH appeared to be lower than the SD-scores of the other measurements. After six years of GH treatment all SD-scores had increased significantly compared to baseline to values more close to zero. After six years of GH treatment, the SDS of Biac in group B was even higher than zero. The largest changes in the six measurements appeared to be mainly during the first three years of treatment, followed by a sustained growth along the attained percentile. The six-year increase in the SDS of height was significantly higher in group B (2.5 (1.0)) compared to A (2.0 (1.02)) ($p = 0.04$).

Figure 2 shows the shape values at baseline and during 6 years of treatment in both GH dosage groups. At start of the treatment all shape values, except for SH, were significantly higher than zero, indicating relatively large hands and feet, relatively broad shoulders and pelvis, but a normal sitting height compared to height. However, all baseline mean shape values were within the normal range. After six years of GH treatment, the shape values of Hand, Foot, and Biil had decreased significantly to values more close to zero. In contrast, the shape value of SH had increased significantly to mean values higher than zero, however still well within the normal range. The shape value of Biac showed an increase in the first two years of treatment followed by a decrease resulting in no overall change after six years of treatment. No significant differences in the six-year changes in body proportions between the two GH dosage groups were found.

Children who remained prepubertal throughout the whole study period (n=17) showed similar patterns in shape values as the whole study group. In addition, during GH treatment similar patterns of the results were found in boys as in girls. Morover, the shape values of the seven children with SRS were also comparable with the total group (data not shown).

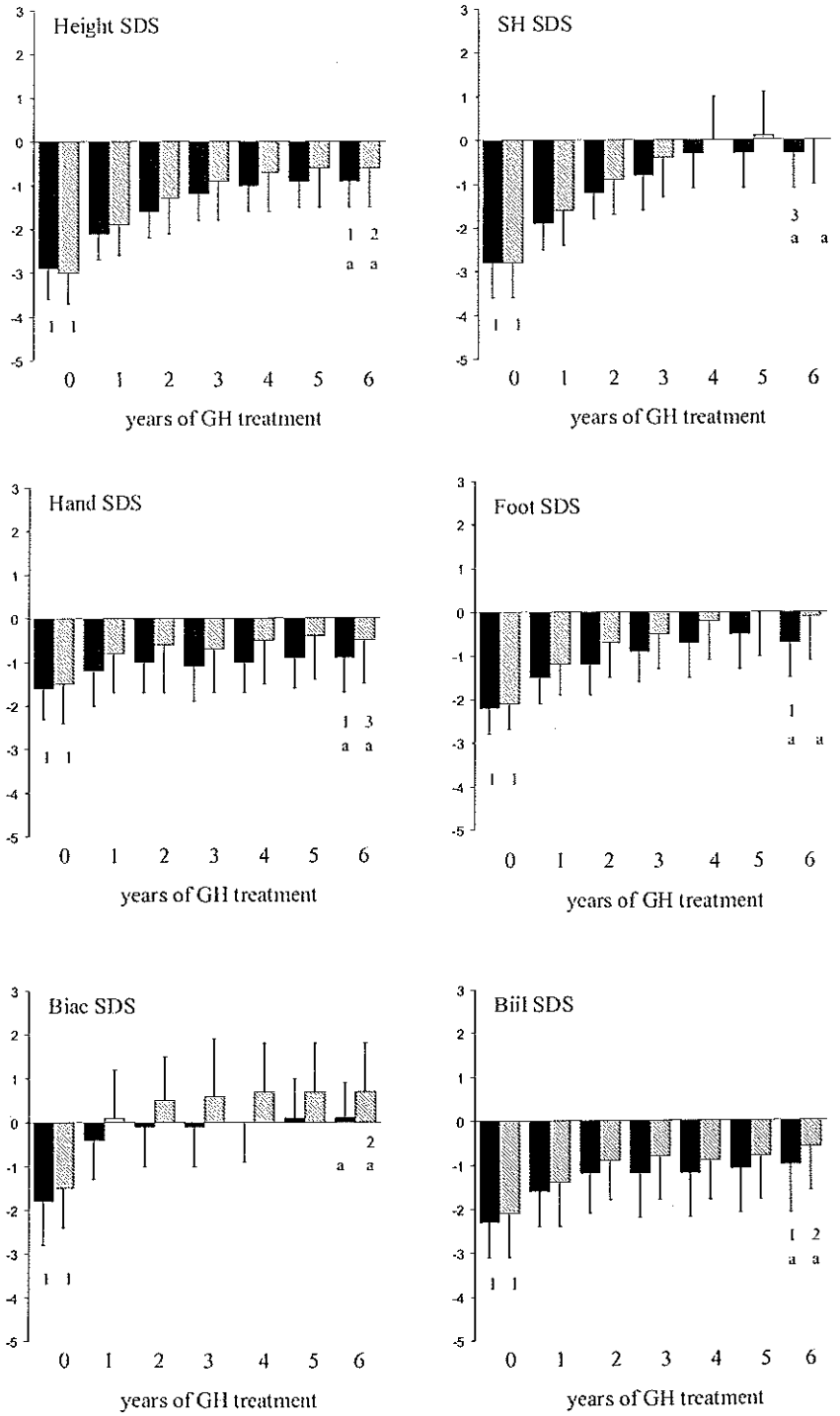

Fig. 1. Mean (SD) SD-scores using reference values of healthy Dutch children at baseline and during 6 years of GH treatment in group A, (3 IU/m²/d, black bars) and group B, (6 IU/m²/d, grey bars), respectively. Significantly differences from zero (1) $p < 0.001$, (2) $p \leq 0.005$, and (3) $p < 0.05$ and significant changes from baseline to 6 years of GH treatment (a) $p < 0.001$ are indicated.

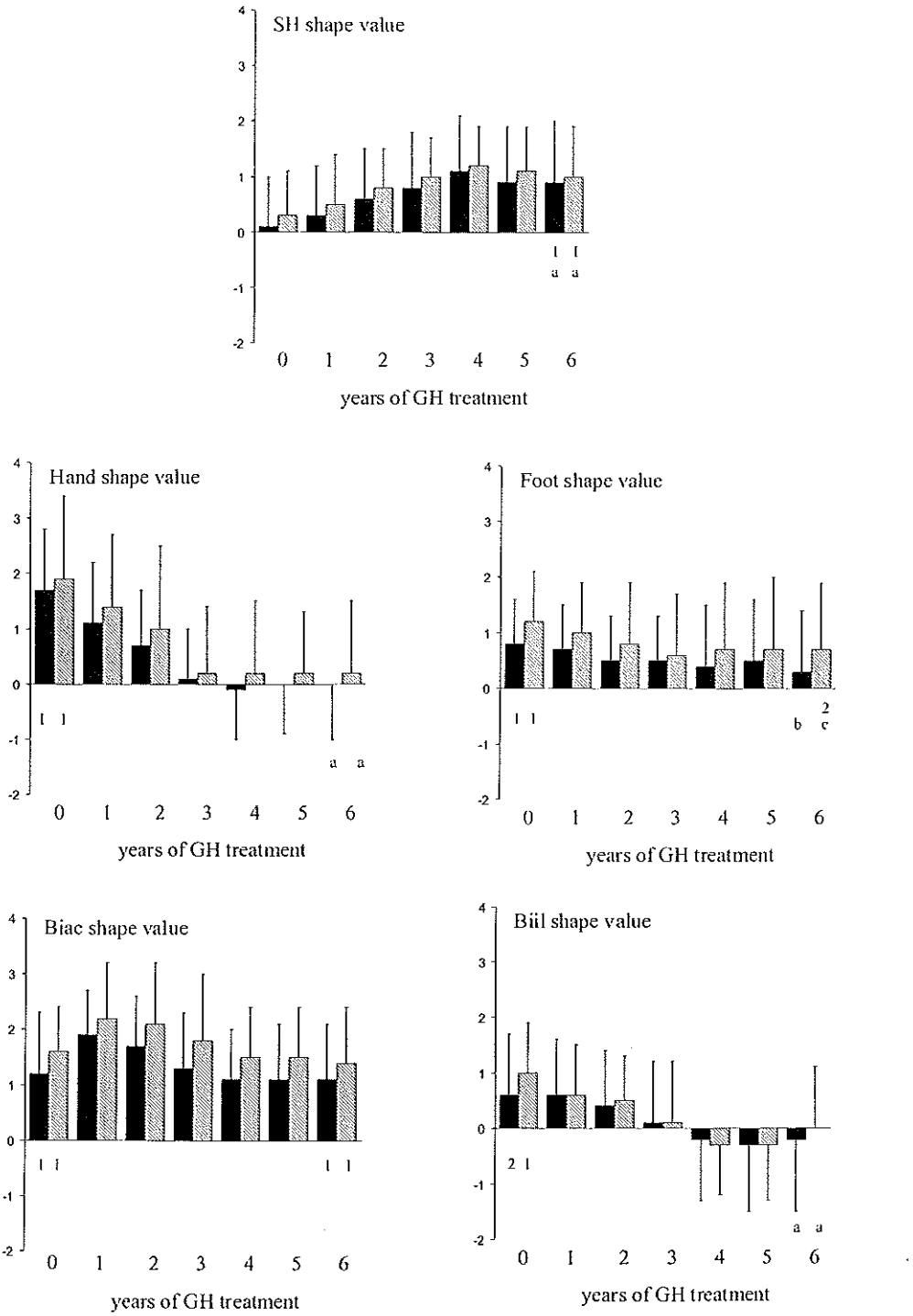

Fig. 2. Mean (SD) shape values at baseline and during 6 years of treatment for group A, (3 IU/m²/d, black bars) and group B, (6 IU/m²/d, grey bars), respectively. Significantly differences from zero (1) $p < 0.001$ and (2) $p < 0.005$, and significant changes from baseline to 6 years of GH treatment (a) $p < 0.001$, (b) $p < 0.005$, and (c) $p < 0.05$ are indicated.

Discussion

The present study shows that untreated children with short stature born SGA have small hands and feet and narrow shoulders and pelvis compared to healthy peers. Height and SH, however, are even more affected. Consequently, on average, these children have relatively large hands and feet, and relatively broad shoulders and pelvis, but a normal sitting height in proportion to height. However, all mean values and most of the individual values are within the normal range. Thus, in most untreated children with short stature born SGA the height deficit is not accompanied by an obvious disproportion. After six years of continuous GH treatment, height had significantly increased to values within the normal range, being significantly higher in group B compared to A. In a previous paper, we demonstrated that the attained height percentile after 5 years of GH treatment was comparable with the target height percentile (14). During the six years of treatment, the proportions of Hand, Foot, and Biil to height improved significantly, while that of Biac to height did not change. Since the SD-score of SH had increased more than that of height, we may conclude that the increase in height appears to be more the result of a relative better catch-up growth in sitting height than that of leg length. None of the changes in the body proportions were different between the GH dosage groups. Thus, the higher increment in height in group B than in group A is accompanied by a comparable increase in the size of the other segments of the body.

As in other GH trials in children with short stature born SGA, we did not include a randomized control group until adult height. Unfortunately, no reference values are available for body proportions in untreated short children born SGA. Therefore, we do not exactly know the natural development of the body proportions in these children and, consequently, we cannot prove whether the changes in body proportions during treatment are caused by GH. However, in the present study, we found that a higher GH dose resulted in a higher increment in height and to the same extent a higher increment in other parts of the body resulting in no differences in the change in body proportions between the GH dosage groups. Therefore, it is likely that the changes in proportions during the GH treatment are more a result of the natural development of children with short stature born after SGA or that the changes in proportions are a part of the catch-up growth in these children rather than due to a direct effect of the GH treatment.

During the six years of GH treatment most of the children entered puberty. One may speculate that in short children born SGA the velocity of the natural changes in body proportions during puberty is different than in healthy controls. As a result, the changes in body proportions during treatment might be explained by the fact that most children had entered puberty. However, the same pattern was seen in children who remained prepubertal throughout the whole study period as in the whole study group. Therefore, it is unlikely that puberty is involved in these changes.

In conclusion, untreated short children born SGA have, on average, relatively large hands and feet, and broad shoulders and pelvis, but a normal sitting height compared to height. The dose-dependent significant increase in height during six years of GH treatment is accompanied by a dose-independent improvement of the proportions of the size of hands, feet, and biiliacal diameter, respectively, to height. The increase in height appears to be more the result of a relatively greater increase in sitting height than that of leg length. The dose-independency of the changes in body proportions suggests that the changes are more a result of the natural development of proportions in short children born SGA during childhood or part of the catch-up growth rather than due to a direct effect of GH treatment. Thus, six-year continuous GH treatment with 3 or 6 IU/m^2/day in children with short stature born SGA does not negatively influence body proportions.

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CHAPTER 4

CARBOHYDRATE METABOLISM DURING LONG-TERM GROWTH HORMONE TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE

Submitted for publication 1999

CARBOHYDRATE METABOLISM DURING LONG-TERM GROWTH HORMONE TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE

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Abstract

To assess possible side-effects of long-term continuous growth hormone (GH) treatment on carbohydrate (CH) metabolism in children with short stature born small for gestational age (SGA), the data of oral glucose tolerance tests (OGTTs) and glycosylated hemoglobin (HbA_{1c}) measurements were evaluated in 78 children with short stature (height SD-score < -1.88) born SGA (birth length SD-score < -1.88). These children, being all prepubertal with a mean (SD) chronological age of 7.3 (2.2) yr before start of treatment, participate in a randomized, double-blind, dose-response multicenter trial. We report six-year data comparing two GH dosage groups (3 versus 6 IU/m² body surface/day).

Before treatment, the glucose response to oral glucose after 120 min was in 6 of the 78 children (8%) above 7.8 mmol/l but below 11.1 mmol/l, indicating impaired glucose tolerance (IGT), whereas after six years of GH treatment, IGT was found in 4% of the children. None of the children developed diabetes mellitus. Mean fasting glucose levels had increased significantly by 0.5 mmol/l after one year of GH treatment, without a further increase thereafter. The 2-hour area under the curve adjusted for fasting levels (AUCab) for glucose and the HbA_{1c} levels were lower after six years of GH treatment compared to baseline. During GH treatment, all HbA_{1c} levels were in the normal range. In contrast to the effects on glucose levels, GH treatment induced considerably higher fasting insulin levels and glucose-stimulated insulin levels. The increase in AUCab for insulin occurred particularly during the first year of treatment, whereas the fasting insulin levels showed a further increase from one to six years. As a result, the 30- and 120-minute ratio's of insulin to glucose were higher during GH treatment compared to the start of treatment. The children who remained prepubertal during the entire study period, showed similar patterns in glucose and insulin levels compared to the children who entered puberty. None of the observed changes were different between the GH dosage groups.

In conclusion, six-year continuous GH treatment in children with short stature born SGA has no adverse effects on glucose levels, even with dosages up to 6 IU/m²/day. However, as has been reported in other patient groups, GH treatment induces higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance. Because the consequences of long-term hyperinsulinism during childhood are not known, careful follow-up of these GH-treated children born SGA is required.

Introduction

Short stature in children born small for gestational age (SGA) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the SGA newborns, about 15% of these children fail to show catch-up growth resulting in short adult stature in most of the cases (1-5). Although the mechanism of the stunted postnatal growth in short children born SGA is poorly understood, studies have shown that continuous, as well as discontinuous treatment with supra-physiological growth hormone (GH) dosages accelerates growth significantly in these children (6-11).

GH modulates tissue responses to insulin. GH-deficiency increases sensitivity to insulin (12). Supra-physiological concentrations of GH in acromegalic patients (13) and in normal (14,15) and diabetic (16) adults showed a decrease in glucose sensitivity to insulin, both in liver and in extra-hepatic tissues. Diabetogenic effects only occur if compensatory mechanisms fail, e.g. when insulin secretion is deficient.

SGA has been reported to be associated with an impairment in insulin sensitivity and noninsulin-dependent diabetes mellitus in later life (17,18). Concern has been expressed regarding possible detrimental effects of GH treatment over a long period of time during childhood.

To assess the possible side-effects of long-term continuous GH treatment on carbohydrate (CH) metabolism in children with short stature born SGA, the data of oral glucose tolerance tests (OGTTs) and glycosylated hemoglobin (HbA_{1c}) measurements were analyzed in 78 children with short stature born SGA participating in a randomized, double-blind, dose-response multicenter trial (19). We now report six-year data comparing two GH dosage groups (3 versus 6 IU/m^2 body surface/day).

Subjects and Methods

Study groups

Seventy-nine prepubertal short children born SGA were included after meeting the following criteria: 1) birth length standard deviation score (SDS) below -1.88 (20), 2) chronological age (CA) between 3 and 11 years in boys and 3 and 9 years in girls at start of study, 3) height SDS for CA ($HSDS_{CA}$) below -1.88 (21), 4) height velocity SDS for CA (HVSDS) ≤ zero (21,22) to exclude children presenting spontaneous catch-up growth, 5) prepubertal, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys (23), 6) uncomplicated neonatal period, that is without signs of severe asphyxia (defined as an Apgar score below 3 after 5 minutes), without neonatal sepsis and without long-term complications of respiratory ventilation. Exclusion criteria were: endocrine or metabolic disorders, chromosomal disorders, growth failure caused by other disorders or syndromes (emotional deprivation, severe chronic illness, chondrodysplasia), and previous or present use of drugs that could interfere with GH treatment. Patients with Silver-Russell syndrome (SRS), however, were included in this study. GH-deficiency (GHD) was defined as a peak GH secretion < 20 mU/L during two GH provocation tests or during one provocation test and a 24-hour GH profile. However, GH-deficiency was not an exclusion criterium.

Four centers in the Netherlands participated in the study. The study was approved by the Ethics Committee of each participating center. Written informed consent was obtained from the parents or custodians of each child.

Study design

After stratification for spontaneous GH secretion during a 24-hour GH profile and CA, all

children were randomly and blindly assigned to either one of two GH dosage groups: group A, 3 IU/m² body surface/day, or group B, 6 IU/m² body surface/day (≈ 0.1 or 0.2 IU/kg/d, respectively) (19,24). 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 three months the total GH dose was adjusted to the calculated body surface. The study was kept double-blind by using an equal volume of a reconstituted preparation. Criteria to discontinue the GH treatment were 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.

Before start of treatment (baseline) and every three months after the start of GH treatment, all children were seen at their local hospital for a physical examination including measurements of standing height (H) and weight (W). Height was expressed as standard deviation score (HSDS) (21). Body mass index [BMI: weight (kilogram)/height (meter squared)] was expressed in standard deviation score (BMI-SDS) for sex and chronological age (21). Glycosylated hemoglobin (HbA_{1c}) was determined every 6 months. At baseline and after one year of GH treatment, all children underwent an OGTT. In addition, after six years of GH treatment, OGTTs were performed in the children of three of the four participating centers. A single team performed all OGTTs after three days of unrestricted diet supplemented with 100 g of carbohydrate (Fantomalt®)) and after overnight fasting. Glucose (2 g glucose /kg body weight (maximum 50 g) was administered orally within 5 minutes. Blood samples were collected at -15, 0, 15, 30, 60, 90, and 120 min for glucose determination and at 0, 30, 60, and 120 min for insulin determination.

To evaluate the overall responses to the oral glucose load, apart from the plasma levels at the various time-points, 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 (25): the 2-hour (2h) level > 7.8 mmol/L (140 mg/dL) and < 11.1 mmol/L (200 mg/dL). (2) The 2h area under the curve for time-concentration corrected for baseline values (2h AUCab) during the OGTT 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.

Assays

The plasma glucose level was measured at the local hospital laboratories with automatic analyzers using a hexokinase catalyzed-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 < 20 mU/L). HbA_{1c} levels were measured in one laboratory using an automatic HPLC analyzer (DIAMAT, BioRad, Edgemont, CA, USA). The upper-normal assay limit is less than 6.6%.

Statistical analyses

Since the study remains double-blind until final height, statistical analysis was performed by an independent statistician (PM) and therefore data are only expressed as mean (SD). For practical reasons, after six years of GH treatment the OGTTs were performed in the children of three of the four participating centers, resulting in a number of missing data after six years of GH treatment. To properly test the time effect (0, 1 and 6 years of GH treatment) and dose effect (3 and 6 IU/m²/d) in the total group of 79 children participating in the trial, a repeated measurement analysis of variance (rmANOVA) with maximum likelihood estimation of the coefficients was

performed. In this analysis, the outcome variable was modeled as a linear function of the following explanatory factors: a within-subject factor 'time' with two levels (1 and 6 years after start of treatment), a between-subject factor 'dose' also with two levels (3 and 6 IU/m²/d), and the interaction between these two factors. Moreover, the baseline measurement (at year 0) of the outcome variable considered was included as a continuous between-subjects covariate in the model. In the 2x2 within-subject (co)variance matrix of the two-dimensional residual term the two variances (at 1 and 6 years after start of treatment) were assumed to be the same (compound symmetry structure). A restricted maximum likelihood (REML) method was used to estimate and test the coefficients; this method properly deals with the unbalance due to missing data. Relations between insulin levels and BMI-SDS and chronological age was tested by using the partial correlation coefficient, adjusted for the randomized treatment. A p-value < 0.05 was considered significant.

Results

Table 1 lists the baseline clinical data of the 79 children. Both GH dosage groups had similar initial characteristics. Seven children had SRS. Twenty-one children were GHD. Five children dropped out of the study for the following reasons long before reaching adult height: treatment of early puberty in one child, signs of GH insensitivity and no catch-up growth in another child, problems with motivation to inject GH in three other children. As these five children were lost to follow-up after discontinuation of GH, their data were not included in the analysis.

TABLE 1. Mean (SD) baseline data for each treatment group.

	group A 3 IU GH/m²/day (n=41)	group B 6 IU GH/m²/day (n=38)
Male/Female	31/10	21/17
Gestational age (wk)	37.3 (3.2)	36.0 (4.1)
Birth length SD-score	-3.6 (1.4)	-3.7 (1.7)
Birth weight SD-score	-2.6 (1.2)	-2.6 (1.0)
Chronological Age (yr)	7.3 (2.1)	7.2 (2.4)
Height SD-score	-3.0 (0.7)	-3.1 (0.7)
Body mass index SD-score	-1.2 (1.3)	-1.3 (1.1)

At baseline and after one year of GH treatment 78 of the 79 children underwent an OGTT. Three children had discontinued GH treatment because of reaching final height before the 6th year OGTT. In 47 out of the 71 children a 6th year OGTT was performed.

Figure 1 shows the mean glucose levels during OGTT at start, after one year and after 6 years of GH treatment for group A and for group B, respectively. Before treatment, the glucose response to oral glucose after 120 min was in 6 of the 78 children (8%) above 7.8 mmol/l but below 11.1 mmol/l, indicating impaired glucose tolerance (IGT). After one year of GH treatment, in 7 of the 78 children (9%), IGT was found, whereas after six years of treatment, in 2 of the 47 children (4%) IGT was present. Only one of the children with baseline IGT, had IGT after one and after six years as well. The other children had an abnormal 120 min value just at one time-point during the study period. All children with IGT had normal HbA_{1c} values. None of the

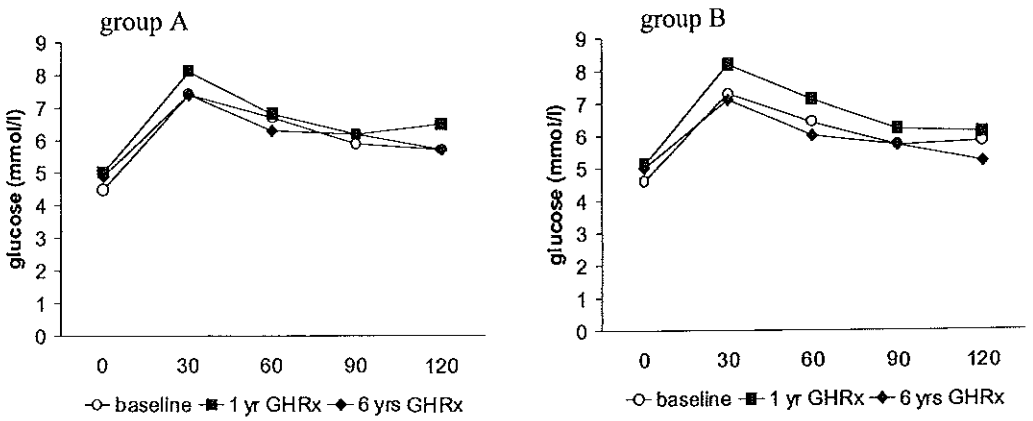

Fig. 1. Mean glucose levels during OGTT before treatment, after one year and after 6 years of GH treatment for group A and for group B, respectively.

children developed diabetes mellitus. Figure 2 shows the fasting glucose levels and the AUCab for glucose during the study period. Fasting glucose levels increased significantly during the first year in group A, as well as in group B ($p<0.001$ and $p=0.006$, respectively). After six years, fasting glucose levels were still higher than at baseline for both GH dosage groups ($p=0.013$ and $p=0.037$, respectively), but not higher than after one year of GH treatment. After six years of GH treatment, the AUCab for glucose had significantly decreased compared to baseline ($p=0.045$ and $p<0.001$ for group A and B, respectively). No significant differences in the change in fasting glucose levels or AUCab for glucose were found between the GH dosage groups.

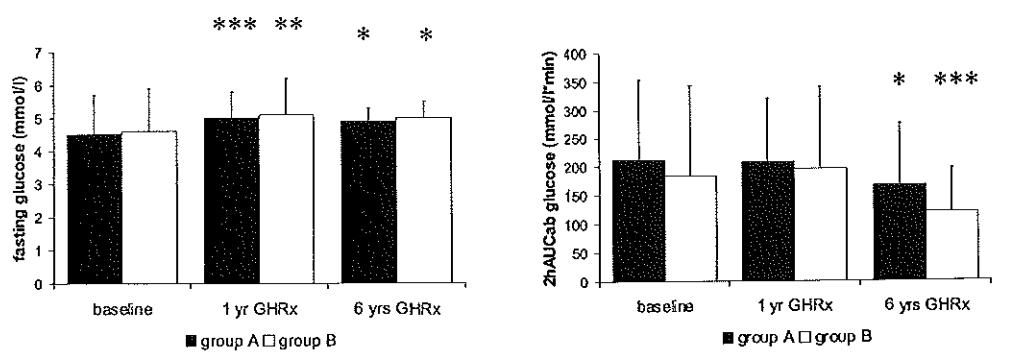

Fig. 2. Mean (+SD) fasting glucose levels (*left panel*) and 2-hour area under the curve above fasting values (2h AUCab) (*right panel*), before treatment, after one year and after 6 years of GH treatment for group A (black bars) and for group B (white bars), respectively. Significant changes from baseline (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ are indicated.

Figure 3 demonstrates the mean insulin levels during OGTT at the three time-points during the study for group A and B, respectively. Figure 4 shows the fasting insulin levels and the AUCab for insulin. Fasting insulin levels increased in the first year of treatment, however, this was only statistically significant in group B ($p=0.002$), and just not in group A ($p=0.061$). After six years of

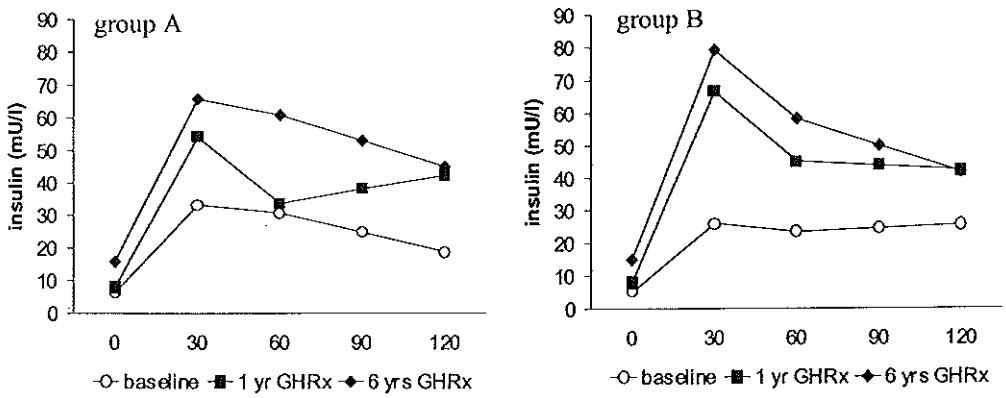

Fig. 3. Mean insulin levels during OGTT before treatment, after one year and after 6 years of GH treatment for group A and for group B, respectively.

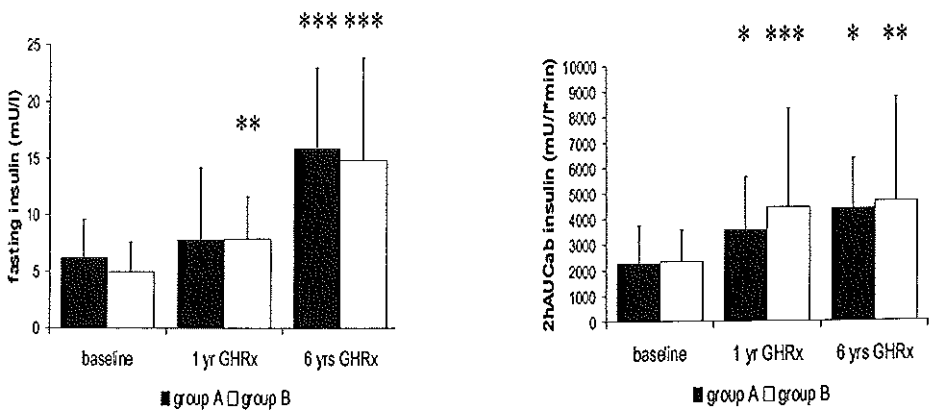

Fig. 4. Mean (+SD) fasting insulin levels (*left panel*) and 2-hour area under the curve above fasting values (2h AUCab) (*right panel*), before treatment, after one year and after 6 years of GH treatment for group A (black bars) and for group B (white bars), respectively. Significant changes from baseline (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ are indicated.

GH treatment, fasting insulin levels had increased even more compared to baseline ($p < 0.001$ for group A as well as for group B). The AUCab for insulin showed a significant increase after one year of treatment in both dosage groups ($p=0.019$ and $p<0.001$), without a further increase thereafter. The increase in fasting insulin levels and AUCab for insulin were not significantly different between the GH dosage groups.

Figure 5 shows the ratios of insulin to glucose at 30 and 120 minutes after the start of the OGTT, respectively, at the three time-points during the study. The ratio at 30' had increased significantly after one year ($p=0.016$ and $p<0.001$, for group A and B, respectively), and even more so after six years of treatment ($p<0.001$ for both GH dosage groups). The ratio at 120' had increased significantly after one year ($p=0.007$ and $p<0.001$ for group A and B, respectively) without a further significant change thereafter.

Individual HbA_{1c} levels never showed abnormal values. After six years of GH treatment the mean values had significantly decreased in both GH dosage groups (from 5.1 (0.3) to 4.8 (0.4) in

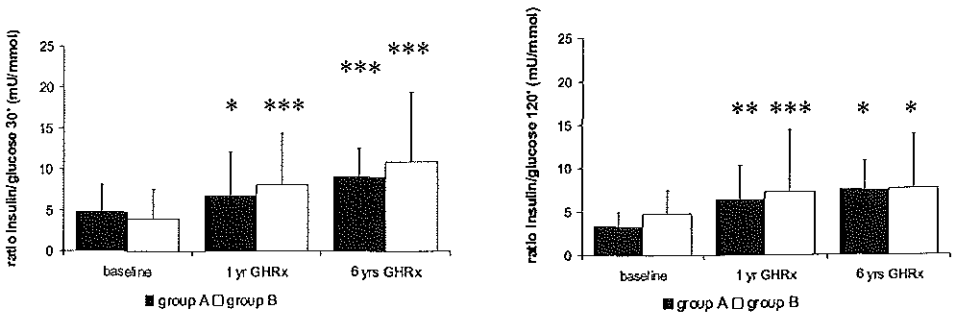

Fig. 5. Mean (+SD) ratio's of insulin to glucose at 30 minutes (*left panel*) and at 120 minutes (*right panel*) after the start of the OGTT, respectively, before treatment, after one year and after 6 years of GH treatment for group A (black bars) and for group B (white bars), respectively.
Significant changes from baseline (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$ are indicated.

group A, and from 5.1 (0.3) to 4.7 (0.4) in group B, respectively, $p < 0.001$), with no significant differences between the GH dosage groups.

After six years of GH treatment, 35 of the 47 children who underwent a 6th-year OGTT had entered puberty. To determine the influence of pubertal maturation on the observed changes in glucose and insulin levels during treatment, we listed the carbohydrate variables (CH) variables at baseline and after six years of treatment in the children who remained prepubertal throughout the whole study period (group 1) and in those who entered puberty during the study (group 2). Table 2 shows that the observed changes in glucose and insulin levels after six years of GH treatment in the prepubertal children were similar as in the children who entered puberty.

TABLE 2. Mean (SD) of insulin levels before and after 6 years of GH treatment in children who remained prepubertal throughout the entire study period (group 1) and in those who entered puberty during treatment (group 2).

	group 1		group 2	
	baseline	after 6 years of GH treatment	baseline	after 6 years of GH treatment
Fasting glucose (mmol/L)	4.4 (0.7)	4.8 (0.4)	4.6 (1.3)	5.0 (0.5)
AUCab glucose (min.mmol/L)	242 (103)	137 (98)	183 (162)	147 (96)
Fasting insulin (mU/L)	4.1 (1.9)	15.0 (9.9)	6.2 (3.2)	15.4 (7.4)
AUCab insulin (min.mU/L)	1648 (958)	5159 (5511)	2612 (1435)	4339 (1833)
ratio insulin/glucose 30' (mU/mmol)	2.7 (1.4)	11.9 (11.2)	4.9 (3.8)	9.2 (3.2)
ratio insulin/glucose 120' (mU/mmol)	3.1 (1.6)	8.2 (6.9)	4.4 (2.5)	7.4 (4.0)

To evaluate the influence of chronological age (CA) and BMI-SDS on the observed changes in the CH metabolism, we tested the correlation between the CH variables after six years

of GH treatment and CA and BMI-SDS, respectively, after adjustment for GH dose and/or the presence or absence of puberty. CA was not significantly related to the CH variables after six

years of GH treatment. BMI-SDS had significantly increased from -1.2 (1.3) to -0.3 (1.4) in group A and from -1.3 (1.1) to 0.0 (0.9) in group B after six years of GH treatment. However, no significant correlations were found between the CH variables and the BMI-SDS after six years of treatment, except for the fasting insulin levels, being significantly positively correlated with BMI-SDS (partial r=0.370, p=0.02), after adjustment for the GH dosage. The increase in BMI-SDS did not significantly correlate with the changes in the CH variables.

The 21 children with GHD showed similar baseline values of the CH variables and similar patterns in CH variables during the study period as the total group of 78 children (data not shown). Baseline values, as well as values during GH treatment were not significantly different between boys and girls.

Discussion

To our knowledge, the present study is the first study describing the effects of long-term continuous treatment with supraphysiological GH dosages on CH metabolism in short children born SGA. We showed that six years of continuous GH treatment in children with short stature born SGA does not negatively influence glucose levels, even with dosages up to 6 IU/m²/day. Although fasting glucose levels increased significantly during the first year of GH treatment, after six years of treatment no further increase was found. The increase in mean fasting glucose levels was small and none of the children developed diabetes mellitus. HbA_{1c} levels remained normal for all children during the entire study period of 6 years. Impaired glucose tolerance (defined as plasma glucose above 7.8 mmol/l but below 11.1 mmol/l after 120 min) was found at baseline in 8% of the children, but after six years of GH treatment in 4% of the children. In contrast to the changes in glucose levels, GH treatment induced considerably higher fasting insulin levels and glucose-stimulated insulin levels. The increase in AUCab for insulin appeared particulary during the first year of treatment, while only fasting insulin levels further increased after the first year. The increased insulin levels compared to glucose levels resulted in a higher 30- and 120-minute ratio of insulin to glucose during GH treatment compared to the start of treatment. Thus, during GH treatment, relative insulin resistance occurs and this is accompanied by an increase in insulin release to maintain normoglycemia. Although the stimulated insulin levels seem to be somewhat higher in the group receiving 6 IU/m²/day compared to 3 IU/m²/day, none of the observed changes were significantly different between the two GH dosage groups.

In our study, many children entered puberty during the six years of GH treatment. Puberty is associated with a marked reduction in insulin sensitivity. We found, however, similar changes in the CH variables of the children who remained prepubertal during the entire study period as in those of the children entering puberty. We can, therefore, conclude that the observed changes in the CH variables after six years of GH treatment appeared to be irrespective of the pubertal status of the children. Since our study did not include untreated children, we cannot assess the magnitude of the effect of age on the changes in CH variables. However, it is unlikely that only age is causing the changes, because no relation with age was found after six years of treatment. It is well known that many short children born SGA are very lean. It is remarkable that the normalization of height during GH treatment was accompanied by a normalization of the body mass index (BMI). Since increased body mass is related to insulin resistance, one may speculate that the increase in insulin resistance during the study period was caused by the increase in body

mass. Except for fasting insulin levels after six years of treatment, the changes in CH variables and the CH variables after six years of GH treatment were not related to the increase in BMI SD-score. Therefore, it seems unlikely that the observed changes in the CH variables are completely caused by the increase in body mass. Thus, although other factors causing insulin resistance cannot be entirely ruled out, GH treatment seems to be the main cause of the presence of the relative insulin resistance during the study period.

The absence of a control group of healthy children excluded the possibility to assess the magnitude of a relative insulin resistance in these short children born SGA before the start of treatment. Compared to the insulin levels in healthy children reported by Potau et al. (26), the pretreatment insulin levels of the children in our study seem to be quite normal. Hofman et al. reported, however, an impairment in insulin sensitivity determined by iv glucose tolerance tests in 12 untreated prepubertal children with short stature born SGA compared to their normal birth weight peers (17). It has been postulated that fetal adaptation to an adverse intrauterine environment might alter the programming of endocrine pathways, leading to permanent metabolic changes (27). In adults born SGA the prevalence of insulin resistance and non-insulin dependent diabetes appeared to be higher compared to adults born appropriate for gestational age (18). Therefore, one may speculate that SGA children may be more at risk of the diabetogenic effect of GH than other patient groups.

The increase of insulin levels during GH treatment with minimal effects on the glucose tolerance is a well known phenomenon in other patient groups being treated with supraphysiological GH dosages. In a previous study of our group, we showed that in girls with Turner syndrome and in children with renal allografts, glucose homeostasis was maintained during GH treatment, while insulin levels had increased significantly (28,29). In the present study, we show that the effects of GH treatment during childhood in short children born SGA are comparable with those found in other GH-treated patients groups.

In our opinion, it is reassuring that we found no negatively influence of long-term GH treatment on glucose levels in these children. The consequences of long-term hyperinsulinism during childhood, however, are unknown. The greater demand placed on pancreatic ß-cells to produce larger amounts of insulin to achieve normoglycemia may put the children at risk of eventual ß-cell exhaustion and its consequences. However, no human data are available to confirm these considerations. In addition, we do not yet know whether the insulin secretion will normalize after discontinuation of GH treatment in short children born SGA. Previous data showed a normalization of insulin levels after discontinuation of GH treatment in girls with Turner syndrome (30,31) and in children with idiopathic short stature (32). To assess the long-term effects of GH treatment on CH metabolism in short stature born SGA, follow-up of these children into adulthood is required. In addition, more sophisticated methods such as glucose clamp or the more recent developed minimal model method have to be performed to improve insights into the CH metabolism in these children during and after long-term GH treatment.

In conclusion, six-year continuous GH treatment in children with short stature born SGA does not negatively influence glucose levels, even with dosages up to 6 IU/m²/day. However, as has been reported in other patient groups, GH treatment induces higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance. Since the consequences of long-term hyperinsulinism are not known, follow-up of these children is required.

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CHAPTER 5

BODY COMPOSITION, BLOOD PRESSURE, AND LIPID METABOLISM BEFORE AND DURING LONG-TERM GROWTH HORMONE (GH) TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE EITHER WITH OR WITHOUT GH-DEFICIENCY

Submitted for publication 1999

BODY COMPOSITION, BLOOD PRESSURE, AND LIPID METABOLISM BEFORE AND DURING LONG-TERM GROWTH HORMONE (GH) TREATMENT IN CHILDREN WITH SHORT STATURE BORN SMALL FOR GESTATIONAL AGE EITHER WITH OR WITHOUT GH-DEFICIENCY

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Abstract

To assess the effects of long-term continuous growth hormone (GH) treatment on body composition, blood pressure (BP), and lipid metabolism in children with short stature born small for gestational age (SGA), body mass index (BMI), skinfold thickness measurements, systemic BP measurements, and levels of blood lipids were evaluated in 79 children with short stature (height SD-score < -1.88) born SGA (birthlength SD-score < -1.88). Twenty-two of the 79 children were GH-deficient (GHD) (peak GH secretion < 10 μg/L). All children participate in a randomized, double-blind, dose-response multicenter GH trial. Four- and 6-yr data were compared between 2 GH dosage groups (3 vs. 6 IU/m^2 body surface/day).

The pretreatment mean BMI SD-score (-1.3) and the mean SD-score of the sum of 4 skinfold measurements (-0.8) were significantly lower than zero. The pretreatment mean systolic BP SD-score (0.7), adjusted for height age and sex, was significantly higher than zero, whereas the diastolic BP SD-score (-0.1) was not different from zero. The mean pretreatment lipid levels, total cholesterol (TC) 4.7, low-density lipoprotein (LDL) 2.9, high density lipoprotein (HDL) 1.3 mmol/L, were comparable with healthy reference values. During GH treatment the BMI SD-score showed a sustained increase during GH treatment (to -0.2), whereas the SD-scores of the skinfold measurements had, after an initially decrease, not changed after 6 yr compared to pretreatment values. The BP SD-score decreased significantly during GH treatment: the mean systolic BP SD-score to 0.2, being not significantly different from zero and the diastolic BP SD-score to -0.8, being significantly lower than zero. TC and LDL decreased significantly to 4.3 and 2.5 mmol/l, respectively, whereas HDL remained unchanged, resulting in a significantly decreased atherogenic index. Although the mean 6-yr increase in height SD-score was significantly higher in the children receiving 6 IU GH /m^2/d (2.7) than in those receiving 3 IU GH /m^2/d (2.2), no differences in the changes in BMI, skinfold measurements, BP, and lipids were found between the GH dosage groups. The pretreatment SD-scores for BMI, skinfold, and BP, as well as the lipid levels were not significantly different between GHD and non-GHD children, but after 6 yr of GH treatment the skinfold SD-score and BP SD-score had decreased significantly more in the GHD than in the non-GHD children.

In conclusion, untreated children with short stature born SGA either with or without GHD are lean, have a higher systolic BP, but normal diastolic BP and normal lipids compared to healthy peers. During long-term continuous GH treatment with 3 or 6 IU/m^2/d, the BMI normalized without overall changes in subcutaneous fat compared to age-matched references, whereas the BP SD-score and the atherogenic index decreased significantly, indicating that GH treatment has at least up to 6 yr positive instead of negative effects on these parameters. In view of the reported higher risk of cardiovascular diseases in later life in children born SGA, further research into adulthood, however, remains warranted.

Introduction

Short stature in children born small for gestational age (SGA) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the SGA newborns, about 15% of these children fail to show catch-up growth resulting in short adult stature in most of the cases (1-5). The mechanism of the stunted postnatal growth in short children born SGA is poorly understood. It has been previously shown that disturbances in the growth hormone (GH) / insulin-like growth factor I (IGF-I) - axis may account for some of the growth retardation: up to 60 percent of the short children born SGA have GH-secretory abnormalities and/or reduced levels of IGFs (6-12). Studies have shown that continuous or discontinuous treatment with recombinant human GH in varying dosages accelerates growth significantly in short children born SGA resulting in catch-up growth to values within the normal range followed by growth along their target height percentile (7, 13-21).

SGA has been associated with increased prevalence of diabetes mellitus type II, hypertension, and hyperlipidemia at a relative young age in later life (22). All three disorders are risk factors of cardiovascular diseases. Concern has been expressed regarding possible adverse effects of long-term GH treatment during childhood. A previous paper of our group showed that in short children born SGA either with or without GH-deficiency (GHD) long-term treatment with supra-physiological GH dosages caused a relative insulin resistance (23) similar to findings in other GH-treated patient groups (24, 25). Since relative insulin resistance is associated with the development of diabetes type II, follow-up of these children during long-term GH treatment is required. Data on possible effects of GH treatment on other risk factors for cardiovascular diseases during childhood are very limited in SGA children.

To assess the body composition, blood pressure (BP), and lipid metabolism in children with short stature born SGA before and during long-term continuous GH treatment, the body mass index (BMI), skinfold measurements, systolic and diastolic BP, and levels of blood lipids were evaluated in 79 children with short stature born SGA with or without GHD, participating in a randomized, double-blind, dose-response multicenter GH trial (21). We now report 4- to 6-yr data comparing two GH dosage groups (3 versus 6 IU/m² body surface/day).

Subjects and Methods

Study groups

Seventy-nine prepubertal short children born SGA were included after meeting the following criteria: 1) birth length SD-score below -1.88 (26), 2) chronological age (CA) between 3 and 11 yr in boys and 3 and 9 yr in girls at start of study, 3) height SD-score for CA (height SD-scoreCA) below -1.88 (27), 4) height velocity SD-score for CA ≤ zero (27) to exclude children presenting spontaneous catch-up growth, 5) prepubertal, defined as Tanner breast stage I for girls and testicular volume less than 4 ml for boys (28), 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 or syndromes (emotional deprivation, severe chronic illness, chondrodysplasia), and previous or present use of drugs that could interfere with GH treatment. Patients with Silver-Russell syndrome (SRS), however, were included in this study. GH-deficiency (GHD) was defined as a peak GH secretion < 10 μg/L during 2 GH provocation tests or during one provocation test and a 24-hour GH profile. GHD was not an exclusion criterium.

Four centers in the Netherlands participated in the study. The study was approved by the

Ethics Committee of each participating center. Written informed consent was obtained from the parents or custodians of each child.

Study design

After stratification for spontaneous GH secretion during a 24-hour GH profile and CA, all children were randomly and blindly assigned to either 1 of 2 GH dosage groups: group A, 3 IU/m² body surface/day, or group B, 6 IU/m² body surface/day (≈ 0.1 or 0.2 IU/kg/d, respectively) (6,21). Biosynthetic GH (recombinant human GH 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. The study was kept double-blind by using an equal volume of a reconstituted preparation. Criteria to discontinue the GH treatment were a height velocity below 0.5 cm over the last 6 months and/or bone age ≥ 15 yr for girls and ≥ 16.5 yr for boys.

Before start of treatment and every 3 months after the start of GH treatment, all children were seen at their local hospital for a physical examination including measurements of standing height and weight. Height was expressed as SD-score (21). Body mass index [BMI: weight (kilogram)/height (meter squared)] was expressed as SD-score for sex and CA (21). The thickness of 4 skinfolds (biceps, triceps, subscapular, and supra-iliacal) were measured according to Cameron (29). The measurements of all children were performed by two trained observers (W de Waal and later on T Sas) using a Holtain skinfold caliper. Two measurements per visit were made and the mean was used for the analysis. The sum of the 4 skinfold measurements were expressed as SD-score using the references for healthy Dutch children (30). To calculate SD-scores, data of the reference population were transformed using the LMS method (31,32). This method transforms the reference data at each age to a normal distribution. Pubertal stages were assessed by the same two investigators according to Tanner (28), using an orchidometer in boys.

Every 6 months blood pressure (BP) was measured. Systolic and diastolic BP was determined with a single Dynamap Critikon 1846SX 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 (age-matched reference values; BP_{age}) (33). As described previously, a child was considered normotensive if BP was below the 90th percentile. Since body size is the most important determinant of BP in childhood and adolescence, additionally, we adjusted the pretreatment BP values and those after 6 yr of GH treatment for height-matched reference values (BP_{height}) (33).

During the first 4 yr, once a year, blood samples were collected for the determination of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol. Dutch age-matched reference values were used for TC and HDL cholesterol (34). For the other lipids our own reference values of healthy children were used (35). The atherogenic index was calculated as the ratio of TC to HDL cholesterol.

Assays

Lipid analysis was subject to the quality-assessment program of the World Health Organization Regional Lipid Reference Center (Prague, Czech Republic). The TC level was measured using an automated enzymatic method (36) with the CHOD-PAP High Performance reagent kit (Boehringer, Mannheim, Germany). HDL and LDL cholesterol were measured by the same method after precipitation, For HDL cholesterol, the phosphotungstate method of Burstein was modified (37). LDL cholesterol precipitation was performed with polyvinylsulfate

(Boehringer). The overall coefficient of variance for TC, HDL cholesterol, and LDL cholesterol was 2.9%, 3.7%, and 5.8%, respectively.

Statistical analyses

Since the study remains double-blind until final height, statistical analysis was performed by an independent statistician (PM) and therefore data are only summarized as mean and standard deviation (SD), unless indicated otherwise. The SD-scores of the pretreatment values and after 6 yr of GH treatment were compared with zero using Student's one-sample t-tests. Differences between points in time were tested by paired Student's t-tests. Differences between groups were tested using Student's two-sample t-tests. To compare pretreatment levels or changes during GH treatment between the GHD children and the non-GHD children, multiple linear regression analyses were performed with adjustment made for baseline covariables. For these analyses, pretreatment age and birth length SD-score were chosen as baseline covariables along with GHD (yes/no); the changes during treatment were, in addition, adjusted for the GH dose. A p-value < 0.05 was considered significant.

Results

Table 1 lists the pretreatment clinical data of the 79 children. Both GH dosage groups had similar initial characteristics. Seven children had SRS. Twenty-two children were GHD (Table 2). During the first 5 yr, 5 children dropped out of the study for the following reasons long before reaching adult height: treatment of early puberty (n=1), signs of GH insensitivity and no catch-up growth (n=1), problems with motivation to inject GH (n=3). These 5 children were lost to follow-up after discontinuation of GH. After 6 yr of GH treatment, in 5 adolescents GH treatment was

TABLE 1. Mean (SD) pretreatment data for each GH dosage group.

	group A 3 IU GH/m²/day (n=41)	group B 6 IU GH/m²/day (n=38)
Male/Female	31/10	21/17
Gestational age (wk)	37.3 (3.2)	36.0 (4.1)
Birth length SD-score	-3.6 (1.4)	-3.7 (1.7)
Birth weight SD-score	-2.6 (1.2)	-2.6 (1.0)
Chronological Age (yr)	7.3 (2.1)	7.2 (2.4)
Height SD-score	-3.0 (0.7)	-3.1 (0.7)
Target height SDS	-1.0 (0.9)	-0.5 (0.9)

TABLE 2. Mean (SD) pretreatment data for the GHD group as well as for the non-GHD group.

	GHD (n=22)	non-GHD (n=57)
GH dosage group A / B	13/9	28/29
Birth length SD-score	-3.1 (1.0)	-3.8 (1.6)
Birth weight SD-score	-2.4 (1.0)	-2.7 (1.1)
Chronological Age (yr)	8.1 (1.6)	7.0 (2.3)
Height SD-score	-3.0 (0.6)	-3.1 (0.7)

discontinued because (near) adult height was reached. Only data obtained during the GH treatment period were included in the analysis. After 6 yr of GH treatment, 17 children were still prepubertal.

Height, BMI, and skinfolds

The height SD-score increased significantly during GH treatment ($p < 0.001$). The mean (SD) 6-yr increment in height SD-score was significantly higher in group B compared to group A (from -3.1 (0.7) to -0.4 (1.1) in group B vs. from -3.0 (0.7) to -0.8 (0.6) in group A; $p = 0.044$). The 6-yr increment in height SD-score was not significantly different between the GHD and non-GHD children.

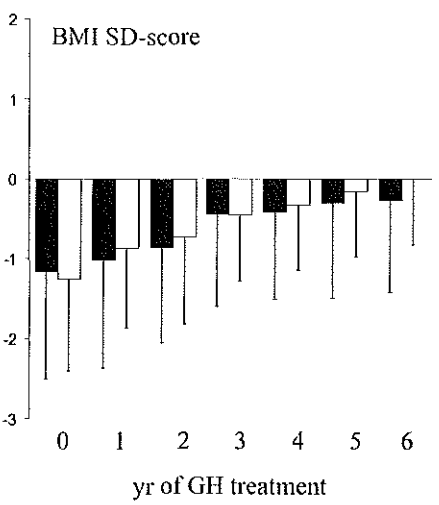

Fig. 1. Mean (SD) body mass index (BMI) SD-score before and during 6 yr of GH treatment for group A (black bars) and for group B (white bars).

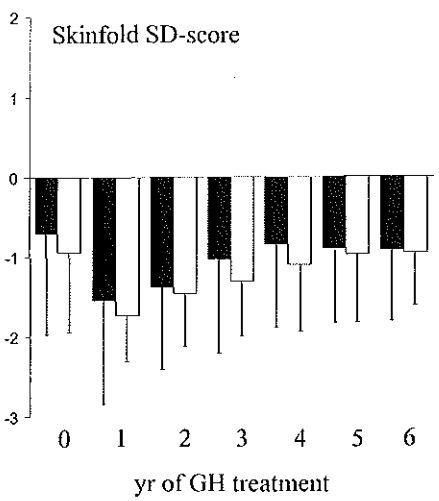

Fig. 2. Mean (SD) skinfold SD-score before and during 6 yr of GH treatment for group A (black bars) and for group B (white bars).

Figure 1 shows the BMI SD-score during 6 yr of GH treatment. The pretreatment mean BMI SD-score of the children was significantly lower than zero. During GH treatment, BMI SD-score increased significantly ($p < 0.001$) to values being not significantly different from zero. The increment in BMI SD-score was not significantly different between the GH dosage groups. Although the pretreatment BMI SD-score in the GHD group was higher than in the non-GHD group, this difference was not statistically significant. The increase in mean (SD) BMI SD-score was not significantly different between the GHD group and the non-GHD group (from -0.8 (1.4) to -0.2 (1.4) vs from -1.4 (1.2) to -0.1 (0.9)).

Figure 2 shows the SD-scores of the sum of the 4 skinfolds during 6 yr of GH treatment. In one very obese boy, it was not possible to measure the skinfolds appropriately during the study period. The data of all other children were used for this analysis. The pretreatment SD-scores were significantly lower than zero ($p < 0.001$). During the first yr, the SD-scores decreased significantly ($p < 0.001$). Thereafter, the mean SD-score increased significantly ($p < 0.001$) to a value being not significantly different from pretreatment values, but still significantly less than zero ($p < 0.001$). No differences in the changes of the SD-scores over time were found between the two GH dosage groups. Although the pretreatment mean SD-score in the GHD group was

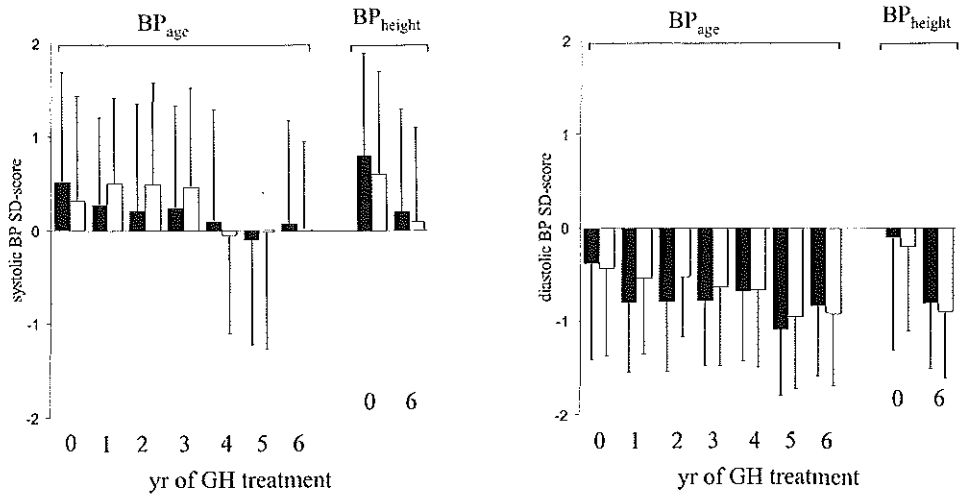

Fig. 3. Mean (SD) systolic blood pressure (BP) SD-score (left panel) and diastolic BP SD-score (right panel) using age-matched reference values (BP_{age}), before and during 6 yr of GH treatment for group A (black bars) and for group B (white bars). On the right part of each panel the BP SD-score using height-matched reference values (BP_{height}) before and after 6 yr of GH treatment.

slightly higher than in the non-GHD group, this difference was not statistically significant. In the GHD group, the mean (SD) skinfold SD-score showed a trend towards lower values after 6 yr of GH treatment (from -0.5 (1.1) to -0.9 (0.5); this change was significantly different (p=0.044) from the 6-yr change in the non-GHD group (from -1.0 (1.1) to -1.0 (0.9)).

Blood pressure

Figure 3 shows the SD-scores of BP_{age} (using age-matched reference values) before treatment and during treatment as well as the BP_{height} SD-scores (using height-matched references values) before treatment and after 6 yr of treatment. The mean pretreatment systolic BP_{age} SD-score was significantly higher than zero, whereas the mean diastolic BP_{age} SD-score was significantly lower than zero (Table 3). During 6 yr of GH treatment, systolic BP_{age} descreased significantly to values not significantly different from zero, whereas diastolic BP_{age} decreased significantly to values significantly lower than zero. The decrease in the systolic BP_{age} SD-score was particularly seen during the last 3 yr of the study period. The mean pretreatment systolic BP_{height} SD-score was even higher than that of systolic BP_{age}, whereas, in contrast to the diastolic BP_{age} SD-score, the mean diastolic BP_{height} SD-score was not significantly different from zero (Table 3). Consequently, before treatment, about a quarter of the children had a systolic BP above the 90th percentile using height-matched reference values. After 6 yr of GH treatment, a similar change was found for the SD-scores of BP_{height} as for the BP_{age} SD-scores. No differences in the BP changes were found between GH dosage groups. The pretreatment BP_{age} SD-score was not significantly different between the GHD and non-GHD children. The changes in the systolic BP SD-score as well as in the diastolic BP SD-score during GH treatment were significantly higher in the GHD group than in the non-GHD group: mean (SD) systolic BP_{age} SD-score from 0.8 (0.8) to 0.0 (1.1) vs 0.3 (1.2) to 0.1 (1.0), p=0.037; diastolic BP_{age} SD-score from -0.2 (0.9) to -1.0 (0.8) vs from -0.5 (1.0) to -0.8 (0.7), p=0.014.

TABLE 3. Mean (SD) of BP levels before and after 6 years of GH treatment

	Pretreatment			After 6 yr		
	group A	group B	group A+B	group A	group B	group A+B
Systolic BP (mm Hg)	103 (13)	101 (13)	102 (13)	109 (13)	108 (12)	109 (12)
Diastolic BP (mm Hg)	57 (10)	56 (9)	56 (10)	57 (7)	56 (7)	57 (7)
systolic BP_{age} SD-score	0.5 (1.2)	0.3 (1.1)	0.4 (1.1)*	0.1 (1.1)	0.0 (0.9)	0.0 (1.0)[1]
no (%) > P90			14 (17.7%)			4 (5.8%)
diastolic BP_{age} SD-score	-0.4 (1.0)	-0.4 (0.9)	-0.4 (1.0)*	-0.8 (0.7)	-0.9 (0.8)	-0.9 (0.8)**[3]
no (%) > P90			4 (5.1%)			1 (1.4%)
systolic BP_{height} SD-score	0.8 (1.1)	0.6 (1.1)	0.7 (1.1)**	0.2 (1.1)	0.1 (1.0)	0.2 (1.0)[2]
no (%) > P90			20 (25.3%)			5 (7.2%)
diastolic BP_{height} SD-score	-0.1 (1.2)	-0.2 (0.9)	-0.1 (1.0)	-0.8 (0.7)	-0.9 (0.7)	-0.8 (0.7)**[3]
no (%) > P90			6 (7.6%)			1 (1.4%)

BP, blood pressure; BP_{age} SD-score, standard deviation score using age-matched reference values; BP_{height} SD-score, standard deviation score using height-matched reference values; P90, 90th percentile.
Significantly different from zero: (*) $p < 0.005$, (**) $p < 0.001$.
Significantly different from pretreatment values: ([1]) $p < 0.05$, ([2]) $p < 0.01$, ([3]) $p < 0.001$.

Lipids

Figure 4 shows the levels of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol. Table 4 shows the lipid levels before and

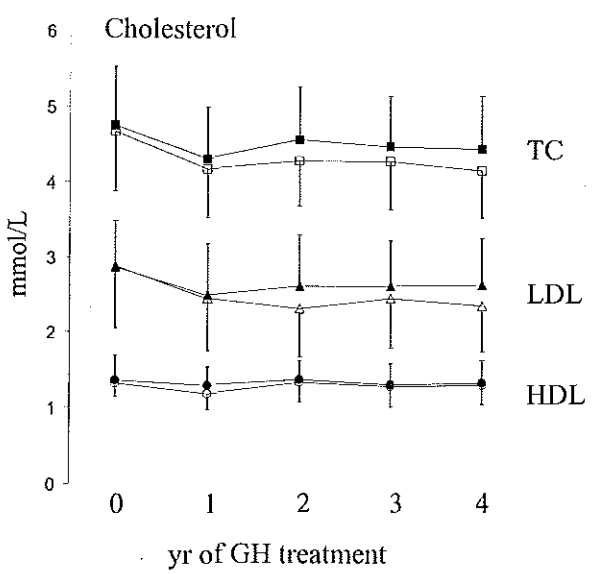

Fig 4. Mean (SD) levels of total cholesterol (TC; squares), low-density lipoprotein (LDL; triangles) cholesterol, high-density lipoprotein (HDL; circles) cholesterol before and during 4 yr of GH treatment for group A (filled symbols) and for group B (open symbols).

after 4 yr of GH treatment. The pretreatment mean levels of the lipid profiles were normal. Three children (3.8%) had TC > 6 mmol/L, 7 children (8.9%) LDL > 3.7 mmol/L, and 2 children (2.6%) HDL < 0.9 mmol/L. After 4 yr of GH treatment, none of the children had an abnormal TC, while 3 (4.1%) of the children had LDL > 3.7 mmol/L, 3 children (3.8%) HDL < 0.9 mmol/L. During 4 yr of GH treatment, TC, LDL, and the atherogenic index significantly decreased, whereas HDL remained unchanged. The changes in lipid levels were not significantly different between the GH dosage groups. In the GHD group, similar results were found as in the non-GHD group: mean (SD) TC from 4.9 (0.9) to 4.5 (0.7) mmol/L, LDL from 2.9 (0.8) to 2.6 (0.7) mmol/L, and HDL from 1.4 (0.3) to 1.4 (0.3) mmol/L vs TC from 4.6 (0.7) to 4.2 (0.6) mmol/L, LDL from 2.9 (0.7) to 2.4 (0.6) mmol/L, and HDL from 1.3 (0.3) to 1.3 (0.3) mmol/L.

TABLE 4. Mean (SD) of the lipid values before and after 4 years of GH treatment

	pretreatment			after 4 years			Reference values
	group A	group B	group A+B	group A	group B	group A+B	
Total cholesterol (mmol/L)	4.7 (0.8)	4.7 (0.8)	4.7 (0.8)	4.4 (0.7)	4.1 (6.2)	4.3 (0.7)[1]	3.2 - 6.0
Low density cholesterol (mmol/L)	2.9 (0.6)	2.9 (0.8)	2.9 (0.7)	2.6 (0.6)	2.3 (0.6)	2.5 (0.6)[1]	1.3 - 3.7
High density cholesterol (mmol/L)	1.4 (0.3)	1.3 (0.2)	1.3 (0.3)	1.3 (0.3)	1.3 (0.2)	1.3 (0.3)	0.9 - 1.6
Atherogenic index	3.6 (0.8)	3.6 (0.8)	3.6 (0.8)	3.5 (0.8)	3.3 (0.7)	3.4 (0.8)[2]	

Significantly different from pretreatment values: (1) $p < 0.001$, (2) $p = 0.018$.

Subgroups

Children who remained prepubertal throughout the whole study period showed similar patterns in BMI, skinfold thicknesses, BP, and lipids as the whole study group. The data of the children with Silver Russell syndrome were also comparable with the total study group. During GH treatment similar patterns of the results were found in boys as in girls (data not shown).

Discussion

Our study is the first demonstrating data on body composition, BP, as well as lipids before and during long-term continuous GH treatment in children with short stature born SGA either with or without GHD. In short children born SGA, height, BMI, and the thickness of the skinfolds were lower than age-matched controls. While the height SD-score and BMI SD-score increased significantly during 6 yr of GH treatment, the skinfolds measurements showed an initial decrease in SD-score, followed by an increase to pretreatment levels, thereby still remaining significantly lower than age-matched reference values. The changes in body composition during GH treatment were not significantly different between GH dosage groups. Thus, short children born SGA have a low weight compared to height and have a relatively low body fat percentage. During 6 yr of GH treatment, the catch-up in height was accompanied by an increase in body weight for height, without an overall change in body fat percentage compared to healthy controls. Before treatment, 28 percent of the patients were considered GH-deficient. A previous paper describing the 5-yr data on height, showed that the growth response on long-term GH treatment were comparable between the GHD children and the non-GHD children (21). In the present study, we found that the pretreatment BMI SD-score and the skinfold SD-score of the GHD children were not signifcantly different from the non-GHD children. During GH treatment, a similar change of the BMI SD-score was found for the GHD as for the non-GHD children, whereas the small decrease in skinfold SD-score in the GHD children over 6 yr was significant greater than in the non-GHD group.

Our data are comparable with the results of the fat and muscle measurements using magnetic resonance imaging (MRI) described by Leger et al. She reported an increase in muscle tissue cross-sectional (cs) area in 14 prepubertal short children born SGA without GHD during 3 yr of GH treatment with 0.2 IU/kg/day (≈ 6 IU/m²/d). In addition, the adipose tissue cs area showed an initial decrease during the first yr of treatment, followed by an increase in the second and third yr to values similar as to a control group of 7 healthy children (38).

Several earlier reports have demonstrated the negative relationship between birth weight and BP in childhood as well as in adulthood (22,39-41). Therefore, the SGA children may be more at risk of hypertension in later life than their healthy peers. To optimize GH treatment in children with short stature born SGA, supra-physiological GH doses are given for a long period during childhood. Since in adults, GH hypersecretion in acromegaly is associated with an increased incidence of hypertension (42), concern has been expressed regarding possible negative effects of long-term GH treatment in these children. We showed that pretreatment systolic BP was significantly higher than age-matched as well as height-matched reference data, whereas pretreatment diastolic BP was significantly lower compared to age-matched reference data, but not significantly different compared to height-matched reference data. During GH treatment, the SD-scores of systolic and diastolic BP decreased significantly. After 6 yr, systolic BP was not different from controls anymore, whereas diastolic BP was even lower than healthy age-matched and height-matched controls. The changes in the BP SD-scores were not significantly different between the GH dosage groups. The GHD children showed a significantly greater decrease in BP SD-score than the non-GHD children, resulting in similar BP SD-scores after 6 yr in these two groups. Barton et al. described that in contrast to adult subjects, treatment with a high dose of GH in short children is not associated with activation of the renin-angiotensin-aldosterone system (43). This suggests that it is unlikely that GH treatment in childhood is associated with the increased risk of hypertension seen in adults with GH hypersecretion. Our long-term data support these findings by showing even a decrease in the BP SD-scores during treatment with GH in dosage up to 6 IU/m²/day.

GHD is associated with dyslipidemia (44,45). Barker demonstrated the negative correlation between birthweight and syndrome X (hypertension, diabetes mellitus type II, and hyperlipidemia) in adult men (22). Consequently, the children of our study seem to be at risk of problems with lipid metabolism in later life. In the present study we showed that children with short stature born SGA had normal mean pretreatment lipid values. In addition, no differences in lipids were found between the GHD and the non-GHD SGA children. No previously published data are available about the effects of GH treatment on lipids in children with short stature born SGA, whereas the effects of GH treatment in GHD children are inconsistent. Some studies showed no changes in TC and HDL during short-term GH treatment (46), whereas others found a decrease in TC (47) or an increase in HDL (48). In the present study, TC, LDL, and the atherogenic index decreased significantly during the first yr of GH treatment and remained stable thereafter, while HDL did not change during GH treatment. In a study evaluating the effects of lipid profiles, the atherogenic index was the most efficient predictor of coronary heart diseases in adults (49). We found similar patterns in the GHD children as in the non-GHD children. Similar changes in lipids were seen in the children who remained prepubertal throughout the entire study period as in the total study group. Thus, the start of puberty during treatment cannot explain the changes in the lipid profiles. In healthy children, no age-related change in TC was observed between 5 and 10 yr of age, but TC decreased between 10 and 16 yr in boys, as well as in girls (34). During the first yr of GH treatment most children were younger then 10 yr of age. It is,

therefore, likely that the changes, particularly seen in the first yr of treatment, are not age-related but due to GH. Thus, GH treatment seems to have a beneficial effect on lipid metabolism in children with short stature born SGA.

Although the increment in height SD-score was higher in the children receiving 6 IU GH /m²/d compared to 3 IU GH /m²/day, no differences were found between the two GH dosages groups regarding the change in BMI, skinfold thicknesses, BP, and lipids. Before GH treatment, BMI, skinfold, and BP SD-scores, as well as the lipid levels were not significantly different between GHD and non-GHD children. During GH treatment, however, the changes in skinfold SD-score and BP SD-score were slightly, but significantly greater in the GHD than in the non-GHD children, resulting in similar values after 6 yr of GH treatment in both groups.

In our opinion, it is reassuring that our data show that long-term GH treatment does not seem to have a negative effect on BP and lipids. However, follow-up of this children into adolescence is warranted, because problems might arise later in life. Another reason for long-term follow-up is the evaluation of metabolic changes and changes in body composition after discontinuation of GH treatment, as previously described in young GHD adults (50).

In conclusion, untreated children with short stature born SGA either with or without GHD are lean, have a higher systolic BP, but normal diastolic BP and normal lipids compared to healthy peers. During long-term continuous GH treatment with 3 or 6 IU/m²/d, the BMI normalized without overall changes in subcutaneous fat compared to healthy controls, whereas the BP SD-score and the atherogenic index decreased significantly, indicating that GH treatment has at least up to 6 yr positive instead of negative effects on these parameters. In view of the reported higher risk of cardiovascular diseases in later life in children born SGA, further research into adulthood remains, however, warranted.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

part "Children with short stature born small for gestational age"

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This part of the doctoral dissertation gives the results of the randomized, double-blind dose-response study on GH treatment in 79 children with short stature born SGA either with or without abnormalities of the GH/IGF axis. This study was started in 1991 and had an inclusion period of two years. The study population consisted of patients from four centers in the Netherlands. Preliminary results of the first years of the study period were reported in the thesis of W de Waal entitled "Influencing the extremes of growth", Rotterdam 1996. The following discussion describes the significance of the presented data as such and in relation to the current literature. In addition, considerations and queries at the start of the study as listed in the Introduction (Chapter 1) are addressed, followed by recommendations for the treatment of these children and ideas about future research.

The effects of GH treatment on growth and growth factors

Growth

Our randomized, double-blind dose-response GH trial showed that five years of continuous GH treatment (3 or 6 IU/m²/day) in short children born SGA results in a normalization of height and subsequent growth along the target height percentile. After 5 years, almost every child had achieved a height well within the normal range for healthy Dutch children. The normalization of height was seen in the children who remained prepubertal, as well as in those who entered puberty during the study period. Comparing our growth results with other GH-treated patient groups receiving long-term GH treatment, the gain in height in short children born SGA is comparable with that in GHD patients using a GH dose that is slightly higher (3 IU/m²/d) than the conventional GH dose (2 IU/m²/d)[1]. Our 5-year results consolidate the previously described effects of short-term GH treatment in short children born SGA[2-9].

Five years of continuous GH treatment (3 or 6 IU/m²/day) in short children born SGA results in a normalization of height and subsequent growth along the target height percentile.

Growth hormone dose

Our 5-year results showed that the increment in height SD-score$_{CA}$ was higher in the children receiving 6 IU/m²/d than those receiving 3 IU/m²/d. However, this difference was just not statistically significant in the total group of children. Both GH dosage groups (3 and 6 IU/m²/d) reached their target height SD-score well within 5 years of GH treatment, indicating that long-term GH treatment with a lower GH dose of 3 IU/m²/d is also able to normalize height of short children born SGA.

Only in children who remained prepubertal during the study, the mean gain in height SD-score$_{CA}$ after 5 years of GH treatment was significantly greater in those treated with 6 compared to 3 IU/m²/d. In the 3-year study reported by Boguszewski *et al.*, similar daily GH dosages (3 and 6 IU/m²) have been used as in our study. Interestingly, the GH dose-dependent 3-year increase in height SD-score$_{CA}$ in prepubertal children was found to be almost identical in both studies[8]. A metanalysis of four European trials showed that the 4-year growth response was similar between continuous GH use, 3 IU/m²/d for 4 years, and discontinuous GH use, 6 IU/m²/d for 2 years

followed by 2 years without GH[9], suggesting that the cumulative GH dose received and not the daily GH dose determines the growth response.

Although the difference in height gain was statistically significant between the two GH dosage groups in the children who remained prepubertal during the entire study period (5-year increment in height SD-score 2.4 vs 3.3), the clinical relevance of the difference in 5-year height gain between 3 and 6 IU/m²/day is, in our opinion, rather limited. However, only final height data will give the answer which GH dosage is required to attain an adult height within the normal range and whether the difference in gain in adult height between the two GH dosages groups is clinically relevant. Therefore, we will continue our double-blind dose-response study until all children have attained adult height.

Our 5-year results showed that the difference in growth response of short children born SGA between those receiving 6 IU/m²/day and those receiving 3 IU/m²/day was small and only statistically significant in the children who remained prepubertal during the study. Long-term GH treatment with a dose of 3 IU/m²/d is also able to normalize height during childhood.

Final height prediction

Since data on final height are very limited, an indication of the expected final height is often given by the height SD-scoreBA or the predicted adult height according to the Tanner & Whitehouse prediction method[10] during GH treatment. In both methods, bone age is used to adjust the gain in height for the progression of bone maturation.

Some short-term studies showed that bone maturation was accelerated compared to untreated short children born SGA[5,7,9], whereas in our study we demonstrated that during 5 years of GH treatment bone maturation was faster compared to that of healthy children. However, it was remarkable that during the 5-year treatment period the bone maturation in the total group, as well as in the children who remained prepubertal during the study period, appeared to be independent of the given GH dose, whereas no progressive acceleration of the bone maturation was found. Previous reports have demonstrated a gradual increase in bone maturation during the second half of the first decade of life or a catch-up of bone age in early puberty in children with short stature born SGA[11,12]. Therefore, the acceleration of bone maturation seen in GH-treated short children born SGA may be partly explained by the effect of GH treatment, but may also be explained by the spontaneous acceleration of bone maturation observed in untreated SGA children.

We showed that height SD-scoreBA and predicted adult height had increased significantly after 5 years of GH treatment. Thus, the gain in height seems to outweigh the faster bone maturation. However, we realize that both prediction methods have limitations and therefore, data on adult height have to substantiate our results. Only two studies reported data on final height in relatively small numbers of patients[13,14]. One study reported data from a pharmaceutical registration database showing that 16 SGA patients, treated with GH at a median daily GH dose of approximately 3 IU/m² until near adult height, achieved an adult stature that was 1.0 SDS greater than the pretreatment height SD-score$_{CA}$[13]. However, in these patients the median age at start of treatment was 12.7 years. Data of the study of Albanese *et al.*, demonstrated that in 12 GH-treated children with short stature born SGA, GH treatment with approximately 4 IU/m²/day, starting at a mean age of 7.6 years, significantly improved final height. Although the height SD-scoreBA did not improve throughout the study, the height SD-score$_{CA}$ did increase from -2.9 at baseline to -1.5 at final height14.

Our five-year data show that adult height prognosis and height SD-score for bone age

increased significantly during GH treatment, despite a GH dose-independent acceleration of bone maturation which is not progressive during treatment.

Puberty

For some untreated children with short stature born SGA an earlier onset of puberty has been reported[15,16]. The question arose as to whether GH could further advance the timing of puberty and consequently reduce the growth period. In our study the mean pubertal onset seemed not to be advanced by GH treatment (girls 10.8 year, boys 11.8 year). However, longer follow-up is required to establish whether all of our children will start puberty at an appropriate age and whether the overall duration of puberty is not altered.

GH treatment does not seem to have a negative effect on the timing of puberty.

Predictive factors for growth response

The growth response on GH treatment is quite different between individuals. Therefore, it is important to find baseline factors which can predict the response on GH.

In our study, the 5-year increment in height SD-score was significantly negatively related with the pretreatment chronological age and bone age. Thus, the younger the child at the start of GH treatment, the better the 5-year increase in height SD-score. Boguszewski *et al.* described a negative relation of chronological age with 2-yr increase in height SD-score as well8. The pretreatment height velocity was not significantly related to the 5-year increase in height. Although the height after 5 years of treatment was highly related with target height, no significant relationship was found between increment in height and target height. Thus, the parental height does not significantly influence the growth response to GH treatment.

It is very interesting and of clinical importance to know whether extremely small children would benefit more from GH treatment than children who are not that small. In order to answer that question, the pretreatment height SD-score (or the target height SD-score minus the pretreatment height SD-score) is often correlated with the increase in height SD-score during the study period8. Very often a negative correlation is found: The smaller the child before start of treatment, the better the growth response. We have to realize, however, that the relationship has to be interpreted very cautiously since it is a statistical phenomenon that an initial measurement will be correlated to the change in that measurement over time even if treatment is ineffective. This phenomenon is called 'regression to the mean'[17,18] and can (partly) explain this negative relationship. Therefore, in our 5-year analysis we decided not to evaluate the relationship between the pretreatment height and the 5-year height gain. There are complex approaches for handling this phenomenon, but they may have errors as well, and therefore, the magnitude of the 'real' influence of the pretreatment height SD-score on the growth response can only be determined in a controlled trial.

Our results show a negative relationship between the age and bone age at start of treatment, respectively, and the 5-year increment in height SD-score.

Levels of growth hormone and growth factors

A previous paper showed that up to 60 percent of the children had 24-hour GH profile abnormalities and/or subnormal responses to arginine provocation, whereas the IGF-I and -II levels were significantly reduced[19]. The pretreatment height SD-score$_{CA}$ and height velocity SD-score$_{CA}$, however, did not correlate with either spontaneous or stimulated GH secretion, IGF-I or -II levels. To study the relation between the baseline parameters of GH secretion and the growth

response to GH treatment, we included, in contrast to other prospective studies, patients regardless of their GH secretion. We found that the maximum GH levels during the provocation tests before the start of the GH treatment were not significantly related to the growth response. This has been reported in two other papers as well[1,20]. In addition, our study showed that neither the pretreatment IGF-I levels, nor the pretreatment 24-hour GH levels were significantly related to the growth response.

During GH treatment, the SD-scores of IGF-I and IGFBP3 increased significantly compared to baseline levels. During the first three years, the IGF-I SD-score was significantly higher in children receiving 6 IU/m²/d compared to those receiving 3 IU/m²/day. After five years of GH treatment, the SD-scores of IGF-I and IGFBP3 were significantly higher than zero, but not significantly different between the two GH dosage groups. The absence of a statistically significant difference in 5-year growth response between the two dosage groups seems to be a reflection of the rather small differences in plasma IGF-I and IGFBP3 SD-scores between the dosage groups particularly during the last two years of the 5-year study period. Since the IGF-I levels were on the upper area of the normal range and we do not yet know the consequences of high IGF-I levels for a long period during childhood, follow-up of the children into adulthood is required.

Although the stunted growth in short stature born SGA may be partly explained by disturbances in the GH/IGF-I axis, the growth promoting effect of GH treatment at a dose of 3-6 IU/m²/d seems to be independent of the baseline GH/IGF-I status. GH treatment at a dose of 3-6 IU/m²/d induces IGF-I levels at the upper area of the normal range.

Safety of long-term GH treatment

The development of body proportions during GH treatment

We have evaluated the body proportions before and during six years of GH treatment in all children participating in the dose-response GH trial. The obtained values were compared to values of healthy Dutch children[21]. We found that untreated children with short stature born SGA had small hands and feet and narrow shoulders and pelvis compared to healthy peers. Height and SH, however, were even more affected. Consequently, on average, these children had relatively large hands and feet, and relatively broad shoulders and pelvis compared to their height, but a normal sitting height in proportion to height. All mean values and most of the individual values were, however, within the normal range. Thus, in most untreated children with short stature born SGA the height deficit is not accompanied by an obvious disproportion.

One has to realize, however, that our pretreatment results of the body proportions have to be interpreted with caution, since the normal range for body proportions particularly in extremely small children appears to be dependent on the chosen mathematical approach to combine two measurements e.g. hand length with height. One's perception of a body being disproportionate is dependent on what one is used to see and not on a chosen method to describe body proportions. Further research is, therefore, required to assess which method describes best the body proportions in very small children. After 6 years of GH treatment, most children had reached a normal height and as a result, the normal range for body proportions is far less dependent on the chosen method than before treatment.

Whereas the 5-year height increase in the total group of children, described in a previous paragraph on the effects of growth and growth factors, was just not statistically significantly

different between the two GH dosage groups, the 6-year increase in height SD-score was just statistically significantly higher in the children receiving 6 compared to those receiving 3 IU/m²/d. This increment in height was accompanied by an improvement of the size of hands, feet, and biiliacal diameter, respectively, in relation to height. The increase in height appears to be the result of the increase in sitting height as well as leg length, but the sitting height SD-score increased slightly more than leg length SD-score. The dose-independency of the changes in body proportions suggests that the changes are more the result of the natural development of proportions in short children born SGA during childhood or part of the catch-up growth rather than due to a direct effect of GH treatment.

These findings show that six-year continuous GH treatment with 3 or 6 IU/m²/day in children with short stature born SGA does not negatively influence body proportions.

GH treatment and carbohydrate metabolism

SGA has been associated with an impairment in insulin sensitivity and noninsulin-dependent diabetes mellitus in later life[22,23]. Since supra-physiological concentrations of GH induce a decrease in glucose sensitivity to insulin[24-27], concern has been expressed regarding possible adverse effects of long-term GH treatment in short children born SGA. We show that six-year continuous GH treatment in children with short stature born SGA has no adverse effects on glucose metabolism. The percentage of children with impaired glucose tolerance was decreased after 6 years of GH treatment compared to baseline levels and none of the children developed diabetes mellitus. All individual glycosylated hemoglobin levels stayed within the normal range and the mean level was significantly decreased after 6 years of treatment.

As has been reported in other patient groups, GH treatment induces higher fasting insulin levels and glucose-stimulated insulin levels, indicating relative insulin resistance. The consequences of long-term hyperinsulinism during childhood are, however, unknown. In addition, we do not know whether the insulin secretion will normalize after discontinuation of the GH treatment in short children born SGA. Previous data showed a normalization of insulin levels after discontinuation of GH treatment in girls with Turner syndrome[28,29] and in children with idiopathic short stature[30], but no data are yet available to confirm this in children with short stature born SGA.

This study showed that six-year continuous GH treatment in children with short stature born SGA does not negatively influence glucose levels, even with dosages up to 6 IU/m²/day. GH treatment induces, however, relative insulin resistance.

GH treatment and body composition, blood pressure, and lipids

SGA has also been associated with increased prevalence of hypertension and dyslipidemia at a relative young age in later life[23]. Both disorders are risk factors of cardiovascular diseases. Concern has been expressed regarding possible detrimental effects of GH treatment over a long period of time during childhood. We showed that untreated children with short stature born SGA either with or without GH-deficiency are lean, have a higher systolic blood pressure (BP), but normal diastolic BP and normal lipids compared to healthy peers. During long-term continuous GH treatment with 3 or 6 IU/m²/d, the mean body mass index normalized without overall changes in subcutaneous fat compared to healthy controls, whereas the mean BP SD-scores and the atherogenic index decreased significantly. Before GH treatment, mean BMI, skinfold thickness, and BP SD-scores, as well as the lipid levels were not significantly different between GHD and non-GHD children. During GH treatment, however, the changes in skinfold SD-score and BP SD-

score were slightly, but significantly greater in the GHD than in the non-GHD children, resulting in similar values after 6 years of GH treatment in both groups. Our data indicate that GH treatment has at least up to 6 years a rather positive instead of negative effect on these parameters. **Our data demonstrated that long-term GH treatment has no adverse effects on body composition, blood pressure, and lipids.**

Recommendations and future research.

Long-term continuous GH treatment (3 or 6 IU/m²/day) in short children born SGA results in a normalization of height and subsequent growth along the target height percentile. To assess the gain in adult height, and to determine the pretreatment factors and the GH dose leading to the most optimal gain in adult height, follow-up of these children until adult height is required. Until adequate long-term experience is gained, treatment of short children with SGA should be limited to investigational settings.

In our opinion, it is very important for children to attain a height within the normal range during childhood and to have a persistent growth in accordance with their healthy peers. We expect that normal growth during childhood will be beneficial for the rest of their lives. A two-year psychosocial evaluation in the children of our study showed a beneficial effect on behavioral and emotional problems and on the self-concept of the children[31]. Further psychosocial research on quality of life, self-perception, social functioning, etc. is required to confirm this improvement on the long-term.

To determine the effect of GH treatment on childhood growth and adult height, it is important to know the natural growth and development of untreated children with short stature born SGA. Although a large randomized trial with a control group of children receiving no GH treatment during the entire childhood would give the best opportunity to determine the GH effect on adult height, such a study has been considered unethical. Therefore, as in Turner syndrome, growth charts have to be developed, based on data of historical controls.

It is reassuring that long-term GH treatment in short children born SGA does not have adverse effects on body proportions, glucose metabolism, body composition, blood pressure, and lipid metabolism. Since SGA children may have a higher risk of an impairment in insulin sensitivity and noninsulin-dependent diabetes mellitus in later life whereas GH treatment induces relative insulin resistance, the carbohydrate metabolism needs to be followed up till final height and after discontinuation of GH treatment. In addition, more sophisticated methods such as glucose clamp or the more recently developed frequent sampling intravenous glucose tolerance test could lead to improved insights into the CH metabolism in these children during and after long-term GH treatment. To evaluate other safety aspects on the very long-term follow-up into adulthood is required. It is, however, important to compare the incidence of possible adverse events in later life with a matched control group of short individuals born SGA who did not receive GH treatment.

Since a number of children of our study were considered GH-deficient before start of GH treatment, the endogenous GH status in these children has to be reevaluated after discontinuation of GH treatment. If these individuals are considered GH-deficient in young adulthood, GH treated should be offered, as in non-SGA GH deficient adults.

It has been suggested that psychological support to learn coping with the psychosocial problems concerning their short stature would be a cheaper and less invasive alternative for GH

treatment. One has to realize, however, that very short individuals have also to deal with many practical problems in society. The adaptation of houses, cars, furniture, etc. to very short individuals is very costly as well. In addition, to date, no structural psychological programs are available that have proven to be effective and of practical use. To assess whether a 'psychological program' is an alternative for growth promoting therapy, a randomized trial has to be performed evaluating the long-term physical and psychosocial effects and the cost-benefit analyses of both therapies.

As a Dutch proverb says, "To prevent is better than to cure", preventing intrauterine growth retardation is the best way to reduce the risk of postnatal growth failure. Therefore, improvement of the insights into the mechanisms of intrauterine growth retardation is required. Only extended research may elucidate the causes of the postnatal growth failure in a substantial percentage of children born SGA.

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CHAPTER 7

SUMMARY

part "Children with short stature born small for gestational age"

SUMMARY

part "Children with short stature born small for gestational age"

Persistent short stature in children born small for gestational age (SGA) is a well known phenomenon. Although postnatal catch-up growth occurs in most of the SGA newborns, about 8-15% of these children fail to show catch-up growth resulting in short adult stature in most of the cases. Although the mechanism of the stunted postnatal growth in short children born SGA is poorly understood, short-term studies have shown that treatment with supra-physiological growth hormone (GH) dosages accelerates growth significantly in these children. In this part of the doctoral dissertation the results of a randomized, double-blind dose-response study on long-term GH treatment in 79 short children born SGA either with or without abnormalities of the GH / insulin like growth factor (IGF) axis are described.

Chapter 1 gives an overview of literature data regarding the prevalence of short stature after SGA, the few data about the natural growth and puberty in these children and the possible mechanisms underlying the postnatal growth failure. In addition, short-term results of GH treatment on height are described as well as considerations concerning possible side-effects of GH treatment in these children.

Chapter 2 describes the effects of 5 years of GH treatment on height, bone maturation, predicted final height, plasma IGF-I, and IGF-binding protein (BP) -3 levels in short children born SGA.

Five-year data show that long-term continuous GH treatment (3 or 6 IU/m²/day) in short children born SGA results in a normalization of height and a subsequent growth along the target height percentile. The increase in height appears to be independent of the baseline GH/IGF-I status. Adult height prognosis and height SDS for bone age increased significantly, despite acceleration of bone maturation. The difference in growth response between the children receiving daily 6 IU/m² and those receiving 3 IU/m² was small and only significant in the children who remained prepubertal during the study.

Chapter 3 describes the body proportions of the children before and during six years of GH treatment. Untreated short children born SGA have small hands and feet and narrow shoulders and pelvis compared to healthy peers. Height and sitting height, however, are even more affected. Consequently, on average, these children have relatively large hands and feet, and relatively broad shoulders and pelvis, but a normal sitting height in proportion to height. All mean values and most of the individual values are well within the normal range. Thus, in most untreated children with short stature born SGA the height deficit is not accompanied by a disproportion. The dose-dependent significant increase in height during six years of GH treatment is accompanied by an improvement of the proportions of the size of hands, feet, and biiliacal diameter, respectively, in relation to height. The increase in height appears to be the result of the increase in sitting height as well as leg length, but the sitting height SD-score increased slightly more than leg length SD-score. The fact that the changes in body proportions are not different between the two GH dosage groups suggests that the changes are more the result of the natural development of proportions in short children born SGA during childhood or part of the catch-up growth rather than due to a direct effect of GH treatment.

Chapter 4 presents the effect of GH treatment on carbohydrate metabolism during six years of GH treatment. GH treatment in children with short stature born SGA does not negatively influence glucose metabolism, even with dosages up to 6 IU/m²/day. However, as in other GH-

treated patients, GH treatment induces higher fasting insulin levels and glucose-stimulated insulin levels.

Chapter 5 shows the data on body composition, blood pressure, and serum lipid levels before and during long-term GH treatment. Untreated short children born SGA either with or without GHD are lean, have a higher systolic blood pressure, but normal diastolic blood pressure and normal lipids compared to healthy reference values. During long-term continuous GH treatment with either 3 or 6 IU/m²/d a GH dose-independent catch-up in body mass was found without a catch-up in body fat, whereas blood pressure and the atherogenic index decreased significantly.

Chapter 6 discusses the significance of the presented data as such and in relation to the current literature. Our final conclusions and recommendations for the treatment of children with short stature born SGA are listed. In addition, suggestions for future research are given.

SAMENVATTING

deel "Kinderen met een kleine lengte na kleine geboortelengte voor zwangerschapsduur"

De meeste kinderen die geboren worden met een te kleine lengte voor de zwangerschapsduur (in het Engels: small for gestational age, SGA) vertonen een inhaalgroei in de eerste jaren na de geboorte. Acht tot vijftien procent van de SGA kinderen haalt niet voldoende in. Het grootste deel van deze kinderen heeft als kind en als volwassene een kleine lengte. Hoewel de mechanismen die leiden tot de afwezigheid van de inhaalgroei na de geboorte nog niet goed bekend zijn, hebben korte-termijn studies laten zien dat behandeling van deze kinderen met supra-fysiologische groeihormoon (GH) doses de groei significant doet versnellen.

In dit deel van het proefschrift worden de resultaten van een gerandomiseerde, dubbel-blinde dosis-respons studie gepresenteerd betreffende lange-termijn GH behandeling in 79 SGA kinderen met een kleine lengte. Een aantal van deze kinderen hadden afwijkingen in de GH / insuline achtige groeifactor (IGF) as, terwijl andere geen duidelijke afwijkingen van de GH/IGF as vertoonden.

Hoofdstuk 1 geeft een overzicht van de literatuur betreffende de incidentie van kleine lichaamslengte bij SGA kinderen en betreffende de weinig gegevens die er zijn over de natuurlijke groei en puberteit bij deze kinderen. Vervolgens worden de mogelijke mechanismen besproken die ten grondslag liggen aan de afwezigheid van voldoende inhaalgroei na de geboorte. Bovendien worden in dit hoofdstuk de korte-termijn resultaten van GH behandeling op de lengte en de overwegingen betreffende mogelijke neveneffecten van de GH behandeling bij deze kinderen beschreven.

Hoofdstuk 2 beschrijft de effecten van 5 jaar GH behandeling op de lengte, botrijping, voorspelde volwassen lengte, plasma IGF-I en IGF bindend eiwit nummer 3 (IGFBP3) bij kleine SGA kinderen.

Vijf jaars resultaten laten zien dat lange-termijn continue GH behandeling met 3 of 6 IU/m²/dag leidde tot een normalisatie van de lengte. Dit werd gevolgd door groei langs de percentiellijn van de gecorrigeerde midden-ouder-lengte. De toename in lengte leek onafhankelijk te zijn van de GH/IGF-I status vóór de start van de behandeling. Tijdens de groeihormoon behandeling namen de voorspelde volwassen lengte en de lengte SD-score voor botleeftijd significant toe, ondanks een versnelde skeletrijping. Het verschil in groeirespons tussen de kinderen die behandeld werden met 6 IU/m²/d en de kinderen die 3 IU/m² kregen was klein en alleen statistisch significant bij de kinderen die prepubertair waren gebleven gedurende de studieperiode.

Hoofdstuk 3 beschrijft de lichaamsverhoudingen van de kinderen vóór en gedurende 6 jaar GH behandeling. Wij toonden aan dat onbehandelde kleine SGA kinderen kleine handen en voeten, smalle schouders en een smal bekken hebben vergeleken met gezonde leeftijdsgenoten. Lengte en zithoogte waren echter meer aangedaan, waardoor, gemiddeld gezien, deze kinderen relatief wat grotere handen en voeten en bredere schouders en een breder bekken hadden in verhouding tot de lengte, terwijl de verhouding van de zithoogte ten opzichte van de lengte volstrekt gemiddeld was. Alle gemiddelde waarden en de meeste individuele waarden waren ruim binnen het normale gebied. Dus bij de meeste kinderen gaat de kleine lengte niet gepaard met een disproportie van het lichaam. De significante dosis-afhankelijke toename in lengte gedurende 6 jaar GH behandeling ging gepaard met een verbetering van de verhoudingen van de grootte van handen, voeten en van de breedte van het bekken, respectievelijk, ten opzichte van de lengte. De toename in de lengte was het resultaat van een toename van zowel zithoogte als beenlengte, maar

de zithoogte SD-score leek wat meer toegenomen te zijn dan de beenlengte SD-score. Het feit dat de veranderingen van de lichaamsverhoudingen niet verschillend waren tussen de twee GH dosis groepen suggereert dat de veranderingen meer het resultaat zijn van de natuurlijke ontwikkeling van de proporties in kleine SGA kinderen gedurende de jeugd of een onderdeel zijn van de inhaalgroei dan dat het een direct effect van de GH behandeling is.

Hoofdstuk 4 laat de effecten van GH behandeling op het koolhydraatmetabolisme zien gedurende 6 jaar GH behandeling. GH behandeling in kleine SGA kinderen heeft geen negatieve invloed op het glucose metabolisme, zelfs met doses tot 6 IU/m²/d. Echter, net zoals in andere GH behandelde patiënten, induceert GH behandeling hogere nuchtere en glucose gestimuleerde insuline waarden.

Hoofdstuk 5 toont de gegevens van de lichaamssamenstelling, bloeddruk en serum lipiden, vóór en gedurende lange-termijn GH behandeling. Onbehandelde kleine SGA kinderen met of zonder GH-deficiëntie zijn mager, hebben een hogere systolische bloeddruk, maar een normale diastolische bloeddruk en normale lipiden waarden vergeleken met referentie waarden van gezonde kinderen. Gedurende lange-termijn continue GH behandeling met 3 of 6 IU/m2/d werd een GH dosis-onafhankelijke significante toename van de SD-score van het gewicht in verhouding tot de lengte gevonden, zonder dat de SD-score van het onderhuidsvet toenam, terwijl de bloeddruk en de atherogene index significant afgenomen waren.

Hoofdstuk 6 bespreekt de resultaten van de studie in samenhang met de meest recente literatuurgegevens. Onze uiteindelijke conclusies en aanbevelingen voor de behandeling van kleine SGA kinderen worden gepresenteerd. Bovendien geven we ideeën voor toekomstig onderzoek.

LONG-TERM GROWTH HORMONE TREATMENT IN TWO GROWTH DISORDERS

- PART
"GIRLS WITH TURNER SYNDROME"

ISBN 90-75561-03-2

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- PART "GIRLS WITH TURNER SYNDROME"

LANGDURIGE GROEIHORMOONBEHANDELING
BIJ TWEE GROEISTOORNISSEN

-DEEL
"MEISJES MET HET SYNDROOM VAN TURNER"

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFCUS
PROF. DR. P.W.C. AKKERMANS M.A.
EN VOLGEND BESLUIT VAN HET COLLEGE VOOR PROMOTIES.

DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 15 DECEMBER 1999 OM 9.45 UUR

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Co-promotoren:	Dr. A.C.S. Hokken-Koelega
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The printing of this thesis and the studies decribed were financially supported by Novo Nordisk Farma B.V., The Netherlands and Novo Nordisk A/S, Denmark.

Opgedragen aan:

Jet, Marike, Minou, Pamela, Jernice, Ineke, Fleur, Marcia, Debby, Corinne, Yvette, Floor, Adriënne, Anouk, Marlijn, Rianne, Anniek, Monique, Sabrina, Gerda, Mirella, Sarah, Debbie, Sarah, Maaike, Andrea, Ngoc, Jennifer, Muge, Ester, Marleen, Soraya, Esther, Ilse, Krista, Suzanne, Tamara, Eline, Natasja, Jacqeline, Eva, Femke, Jonneke, Malou, Anne, Wendy, Cindy, Eveline, Lianne, Leonie, Michelle, Nadja, Maaike, Marieke, Esma, Sophie, Linda, Marlijn, Sylvia, Fanny, Annemijn, Charlotte, Manon, Karin, Ilona, Debbie, Songul, Suzanne, Marian, Yvonne, Jessica, Mariska, Wai Ling, Kim, Willie, Francisca, Ada, Gaytri, Linda, Marleen, Ayse, Loan, Marijke, Sharon, Elzeline, Trees en Merel.

CONTENTS

PART
"GIRLS WITH TURNER SYNDROME"

CHAPTER 1

INTRODUCTION

part "Girls with Turner syndrome"

INTRODUCTION

Turner syndrome

In the year 1938, the American physician Henry H Turner described a disorder in 7 women which was characterized by certain physical features including short stature, absence of female secondary sexual characteristics, webbing of the neck, low posterior hairline, and increased angle of the elbow[1]. This syndrome is now called Turner syndrome (TS), although the German pediatrician Otto Ullrich had already described patients with similar physical characteristics in 1930[2]. Therefore, German studies called this syndrome Ullrich-Turner syndrome.

The cause of TS is the total of or partial absence of one of the two X chromosomes in some or all of the body cells. An entire X chromosome can be lost due to non-disjunction during either of the two meiotic divisions of gametogenesis or during the first mitotic divisions of the zygote. These all result in numerical abnormalities, 45,X (60% of the cases). Structural abnormalities with partial loss of X chromosomal material may be due to transverse meiotic division of the chromosome around the centromere leading to isochromosomes. Furthermore, the long or short arm of the X chromosome may be missing (in part), but also small fragments can be lost. In addition, an X chromosome may have formed a ring with the loss of chromosomal material at the point of fusion. If an X chromosome is lost by faulty distribution during later cell divisions, then a mixture of cell lines with both normal and reduced chromosome counts develops, described as mosaics. Research on mapping of the X-chromosome has led to improved insights into the relationship between clinical features and special regions of the X-chromosome[3-5]. It has been suggested that short stature and skeletal abnormalities in TS are caused by a haploinsufficiency of a pseudoautosomal growth gene on the X-chromosome (the short stature homeobox containing gene on the X and on the Y chromosome, SHOX-gene)[6]. Many questions about the genetic etiologies and mechanisms leading to the clinical features remain, however, yet to be unanswered.

TS occurs in approximately 1:2500 female births. Table 1 shows that short stature and gonadal dysgenesis are almost invariably present in TS[7]. Since the scope of the studies in this thesis is related to growth-promoting therapies, only some aspects of gonadal dysgenesis and the natural growth pattern are described here.

Ovarian dysgenesis

In TS the ovaries develop apparently normally during the first three months in utero[8]. Thereafter, oocytes are rapidly lost and connective tissue transformation takes place. However, there is wide variation in the loss of germ cells in girls with TS, such that 5-10% retain sufficient ovarian function for puberty to commence spontaneously, though in most girls incompletely. Only in a few cases does spontaneous menstrual bleeding occur, and in most of these it persist for only a short period of time[9]. Occasionally, reports of 'spontaneous' pregnancies have been reported[10].

To induce puberty, synthetic estrogens or the natural estrogen 17ß-estradiol have to be given to girls with TS. However, the optimal age to start estrogen therapy is a point of discussion. Although it has been shown that lower doses may stimulate growth[11], it has been suggested to postpone estrogen therapy to delay closure of the epiphysial growth plates and, consequently, to prolong the growth phase. However, delay of pubertal development may have serious psychosocial consequences. Therefore, it is important to maximize the growth before higher levels of estrogens, required for a complete pubertal development, will result in epiphysial fusion.

For the adult woman with TS in vitro fertilization using oocyte donation can be offered.

Natural growth pattern in Turner syndrome

Growth is reduced in virtually all of the patients. Ranke *et al.* was the first one describing the pattern of natural growth in a large number of girls with TS, who had not received any growth promoting therapy[12]. In this thesis, the effects of treatment on growth are compared with the Dutch-Swedish-Danish reference values for TS which are based on data from a large multinational study and comprise data of a relatively large group of Dutch girls with TS. The results of that study are described by Karlberg *et al* (Figure 1)[13].

Newborns with TS delivered at term are smaller than average girls, body length by about 3 cm, and the body weight by about 500 g. Postnatal growth rate appears to be in the normal range during the first 2 to 3 years of life. Thereafter, height velocity shows a gradual decrease compared with healthy girls. There is a lack of the pubertal growth spurt because the ovaries are non-functional. Due to the delayed epiphyseal fusion most untreated girls continue to grow until their late teens or beyond[14]. The mean adult height of women with TS in Northwestern Europe is about 148 cm, that is about 20 cm smaller than the mean of the normal female population[13]. Convincing evidence that patients with a 45,X karyotype differ from those with another chromosomal pattern has not been documented[7].

Pathophysiology of the stunted growth

Although the absence of (or a part of) the entire X-chromosome results in the typical features of Turner syndrome, such as short stature, the mechanisms leading to the stunted growth in girls with TS are poorly understood. Physiological and stimulated plasma growth hormone (GH) levels are normal[15-20], IGF-I plasma levels are in the (sub)normal range[21-23], and binding protein levels of GH and IGF-I are thought to be normal[21]. It has been reported that an increased proportion of non-22-kDa GH isoforms (in controls 70-75% 22-kDa) were found in girls with TS. These non-22-kDa GH isoforms may interact as weak agonists or antagonists of the GH-receptor[24]. Since the structure and biochemical composition of epiphysial cartilage are normal[25], it seems to be unlikely that the growth failure is due to a form of skeletal dysplasia. It has been suggested that girls with TS have a relative end-organ insensitivity to growth factors[26].

GH treatment for short stature

Studies in the early 1970s with small numbers of older girls with TS were disappointing probably due to the low dose and frequency of GH administration (2-3 times weekly)[27]. With the availability of recombinant human GH after 1985, greater numbers of girls with TS were treated in standardized studies. An earlier GH treatment study of girls with TS in the Netherlands showed a doubling of the height velocity (HV) in the first year of treatment with 4 IU/m^2/day compared with pretreatment values[28]. However, this increase could not be maintained during the subsequent years of treatment. In GHD patients, a similar effect was observed, which can be overcome by a 2- to 3-fold increase of the GH dose[29,30]. In addition, previous studies showed that the growth response after the first years in younger girls was better than in older girls with TS[28,31,32]. To optimize GH treatment in TS, two randomized multicenter studies were started in the Netherlands: In 1989 a GH *dose-response study* was initiated in 68 relatively young girls with TS. In addition, in 1990, a *frequency-response study* on GH treatment in 19 relatively older girls with TS was started to evaluate whether a twice daily GH injection regimen results in better growth response than one daily GH injections, by mimicking more the natural pulsatile GH secretion. Four-year results of the *dose-response study* showed that a stepwise increase in GH dose reduced the waning effect of the growth response without undue bone maturation. In addition, the initiation of GH

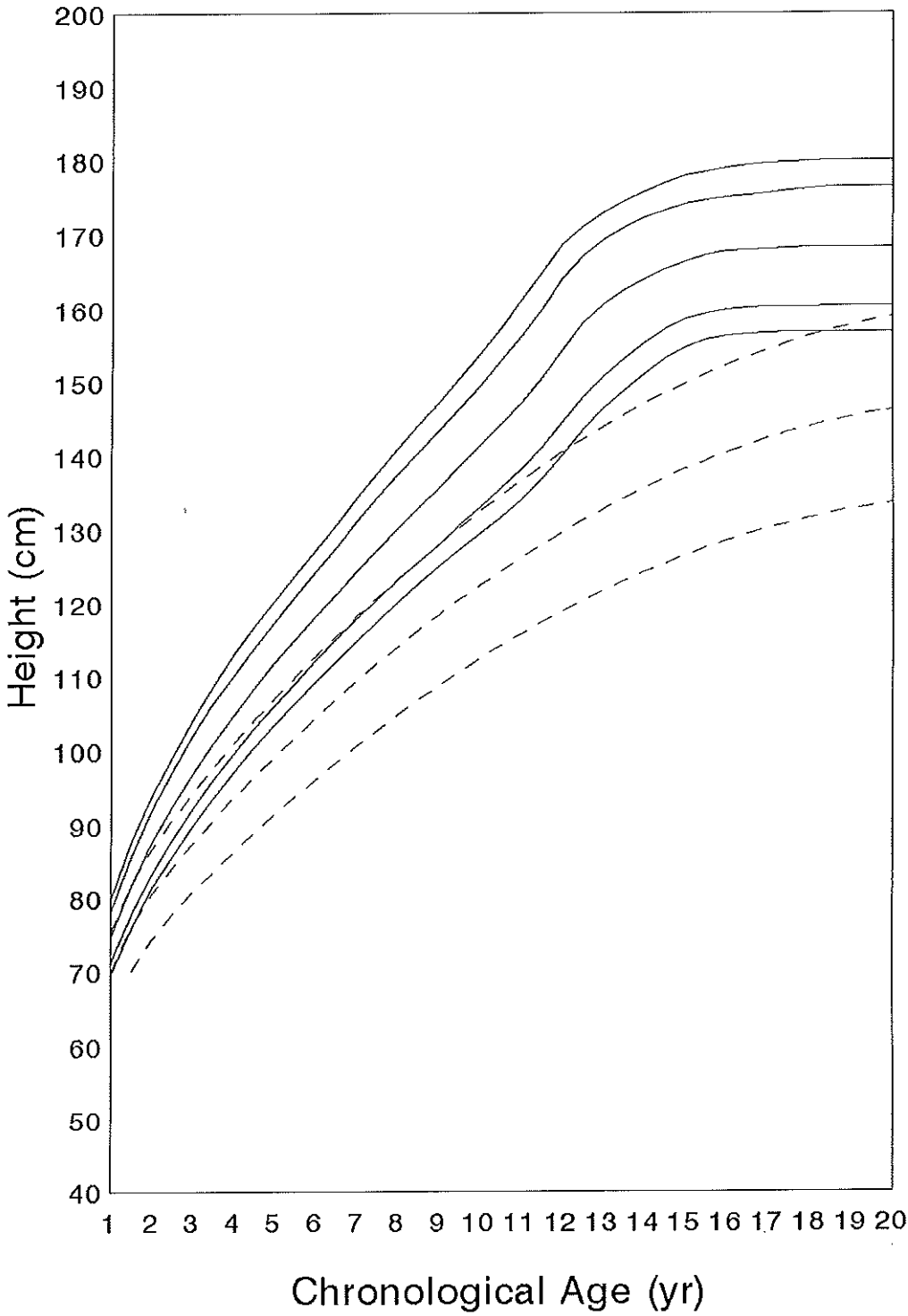

Figure 1. Reference curve for healthy girls (3rd, 10th, 50th, 90th, and 97th percentile) and for untreated girls with Turner syndrome (North European references; 3rd, 50th, and 97th percentile dotted lines)

Table 1. Frequency distribution for physical features and aberrations of internal organs in girls with Turner syndrome (adapted from M.B. Ranke[7])

Present in 80-100% of the cases:	
Growth	Small for dates at birth
	Growth retardation after birth
Ovaries	Gonadal dysgenesis
Present in 60-79% of the cases:	
Mouth and jaw	High arched palate
	Small or backward rotated lower jaw
	Defective dental development
Neck	Short, thick ('webbed') neck
	Low neck hair line
	Pterygium colli
Chest	Scutiform thorax (apparent wide nipples)
	Inverted nipples
Skin (appendages)	Lymphedema of hands and feet
	Increased number of pigmented naevi
	Increased body hair growth
	Dysplasia of finger-nail and toe-nail
	Increased skin ridge patterns
	Alopecia
	Vitiligo
Present in 40-59% of the cases:	
Skeleton	Wide angle of arm (cubitus valgus)
	Short metacarpal bones (e.g. 4th)
	Spongiose bone structure
	Scoliosis
Heart and vessels	Stenosis of aortic isthmus
	Bicuspid aortic valve
	Aortic dilatation/aneurysm
Kidneys	Renal malformation (e.g. horseshoe)
	Renal aplasia
	Changes in renal pelvis and ureters
	Vessel abnormalities
Ears	Deformed auricles
	Recurrent otitis media
	Impaired hearing
Present in 20-39% of the cases:	
Eyes	Ptosis
	Epicanthus
	Myopia
	Strabismus
	Nystagmus

treatment at a younger age was beneficial in terms of the four-year increase in predicted final height[33]. Two-year results of the *frequency-response study* demonstrated that the growth response was not significantly different between the OD and the BID daily GH injection regimens[34].

Possible side effects of GH treatment in girls with TS

TS is associated with congenital cardiac abnormalities[35,36] (Table 1) and hypertension[36-38]. Previous studies showed that GH has, at least in adults, an anabolic effect on the myocardium and that high levels of GH in adults with acromegaly are associated with hypertension[39-43]. Therefore, concern has been expressed regarding possible adverse effects of long-term GH treatment on the cardiovascular status of the girls with TS.

Insulin resistance and carbohydrate (CH) intolerance have been reported in untreated girls with TS[44-46]. In addition, in adults with TS who had not received GH treatment in childhood, glucose intolerance, non-insulin- and insulin dependent diabetes mellitus are more common than in healthy women[38,47]. Since supra-physiological concentrations of GH in acromegalic patients[48] and in normal adults[49,50] resulted in a decrease in glucose sensitivity to insulin in liver and in extra-hepatic tissues, one may suggest that long-term treatment with supra-physiological GH dosages could have detrimental effects on the CH metabolism in girls with TS.

The general clinical impression of body proportions of untreated girls with TS is that these girls have a more coarse and stocky figure compared to healthy girls. This is confirmed by earlier studies describing relatively short lower extremities and relatively broad shoulders and pelvis[51-53]. Little is known about the effects of supraphysiological GH dosages given for a long time during childhood.

Despite only limited reports of a greater number of fractures during childhood[54] or adulthood[38,55], osteoporosis historically has been described as a feature in TS, because of the frequent observation of radiographic osteopenia and the coarse trabecular pattern of the carpal bones on radiographs[56]. An intrinsic bone defect, as well as oestrogen deficiency may explain these findings. Since during GH administration bone formation is enhanced preferentially to bone resorption[57], one may suggest that GH treatment for short stature in girls with TS may have positive effects on bone mineral density as well. However, no data are available on longitudinal measurements of the volumetric bone mineral density in girls with TS during long-term GH treatment either with or without estrogen therapy.

Considerations, questions, and aims of the studies

Efficacy of GH treatment

Although short-term data showed an increase in height velocity and final height prediction in girls with TS, we wondered if long-term GH treatment results in a sustained growth during childhood and, finally, gain in attained adult height. Furthermore, we wanted to assess the most optimal GH dose, injection frequency, and the optimal age to start GH treatment. Moreover, we wanted to know whether starting low dose estrogen therapy at a normal pubertal age would result in pubertal development without an obvious interference on the growth promoting effect of GH treatment. Therefore, we evaluated the effects of 7 years of GH treatment on **growth during childhood** in 68 girls with TS, age 2-11 years, receiving 4, 4->6, or 4->6->8 IU/m²/day in combination with low dose 17ß-estradiol ≥ 12 yr of age. Furthermore, we assessed the effect of GH treatment on adult height in those girls who had reached **adult height** until recently (*dose-*

response study). In addition, we assessed the effects of GH treatment on **adult height** in 19 girls with TS, age ≥ 11 years, receiving 6 IU GH /m²/day divided over once (OD) or twice daily (BID) injections, in combination with low dose ethinyl estradiol (*frequency-response study*).

Safety of GH treatment

Using supraphysiological GH dosages for a long period during childhood, we wanted to know if GH treatment may have side-effects. We, therefore, evaluated the **body proportions, cardiac left ventricular dimensions** and **blood pressure** during long-term GH treatment in girls with TS participating in the dose-response study. In addition, we determined the effects of GH treatment on **carbohydrate metabolism** during GH treatment and after discontinuation of GH treatment in the girls of the dose-response study, as well as in those of the frequency-response study. Furthermore, the effects of GH treatment either with or without estrogen therapy on **bone mineral density** were evaluated in the dose-response study and in the frequency-response study.

Outline of the thesis, part "Girls with Turner syndrome"

This part of the **doctoral dissertation** gives the results of the randomized, multicenter, *dose-response study* on GH treatment in 68 young girls with Turner syndrome. This dose-response study was started in 1989 and is still ongoing. In addition, the results of the randomized, multicenter, *frequency-response study* on GH treatment in 19 relatively older girls with TS are described. This frequency-response study was started in 1990 and all girls have yet reached adult height. Four-year results of the dose-response study and two-year results of the frequency-response study were reported in the thesis of A. van Teunenbroek entitled "Growth hormone treatment modalities in girls with Turner syndrome" , Rotterdam 1996. **Chapter 2** describes the results of the dose-response study on height and bone maturation during 7 years in childhood, as well as on adult height in those girls who have reached (near) final height before the end of August 1998. In Chapter 2, the final height results of the frequency-response study are presented as well. **Chapter 3** describes the body proportions of the girls of the dose-response study before and during 7 years of GH treatment and in those who have reached (near) adult height. **Chapter 4** presents the 7-year data on the effects of GH treatment on cardiac left ventricular dimensions and blood pressure in the dose-response study. **Chapter 5** demonstrates the effects of GH treatment on carbohydrate metabolism during GH treatment and after discontinuation of GH treatment in the girls of the dose-response study, as well as in those of the frequency-response study. **Chapter 6** describes the 7-year data of the dose-response study on bone mineral density (BMD). In addition, the BMD data of the frequency-response study during GH treatment, as well as three years after discontinuation of treatment are described in this chapter. **Chapter 7** discusses the significance of the presented data as such and in relation to the current literature. Our final conclusions are listed and recommendations for future research are given. Finally, **Chapter 8** summarizes this part of the dissertation in English as well as in Dutch.

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CHAPTER 2

THE EFFECTS OF GROWTH HORMONE TREATMENT ON GROWTH DURING CHILDHOOD AND ON ADULT HEIGHT IN GIRLS WITH TURNER SYNDROME

CHAPTER 2.1

NORMALIZATION OF HEIGHT IN GIRLS WITH TURNER SYNDROME AFTER LONG-TERM GROWTH HORMONE TREATMENT: RESULTS OF A RANDOMIZED DOSE-RESPONSE TRIAL

Adapted from Journal of Clinical Endocrinology and Metabolism, in press.

NORMALIZATION OF HEIGHT IN GIRLS WITH TURNER SYNDROME AFTER LONG-TERM GROWTH HORMONE TREATMENT: RESULTS OF A RANDOMIZED DOSE-RESPONSE TRIAL

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Abstract

Short stature and ovarian failure are the main features in Turner syndrome (TS). To optimize GH and estrogen treatment, we studied 68 previously untreated girls with TS, age 2-11 yr, who were randomly assigned to 1 of 3 GH dosage groups: group A, 4 IU/m²/day (≈0.045 mg/kg/d); group B, first yr 4, thereafter 6 IU/m²/d (≈0.0675 mg/kg/d); group C, first yr 4, second yr 6, thereafter 8 IU/m²/d (≈0.090 mg/kg/d). In the first 4 yr of GH treatment, no estrogens for pubertal induction were given to the girls. Thereafter, girls started with 17ß-estradiol (5 μg/kg bw/d, orally) when they had reached the age of 12 yr. Subjects were followed up until attainment of adult height or until cessation of treatment because of satisfaction with the height achieved.

Seven-year data of all girls were evaluated to compare the growth promoting effects of 3 GH dosages during childhood. After 7 yr 85% of the girls had reached a height within the normal range for healthy Dutch girls. The 7-yr increment in height SD-score was significantly higher in group B and C than in group A. In addition, we evaluated the data of 32 of the 68 girls who had completed the trial after a mean duration of treatment of 7.3 (range: 5.0 - 8.75) yr. Mean (SD) height was 158.8 (7.1), 161.0 (6.8), and 162.3 (6.1) cm in group A, B, and C, respectively. The mean (SD) difference between predicted adult height before treatment and achieved height was 12.5 (2.1), 14.5 (4.0), and 16.0 (4.1) cm for group A, B, and C, respectively, being significantly different between group A and group C. GH treatment was well tolerated in all 3 GH dosage groups.

In conclusion, GH treatment starting in relatively young girls with TS results in normalization of height during childhood as well as of adult height in most of the individuals. With this GH and estrogen treatment regimen, most girls with TS can grow and develop much more in conformity with their healthy peers.

Introduction

Short stature and ovarian failure are the main features in Turner syndrome (TS). The median adult height of North European girls with TS is 146.9 cm, being on average approximately 20 cm less than their healthy peers[1,2]. Although these girls are not GH deficient[3], GH

administration accelerates growth in a dose-dependent way[4,5]. In many countries, TS is an accepted indication for GH treatment, although the effect of GH on adult height is inconsistent[6-11]. In most girls with TS, puberty has to be induced by estrogen therapy. The optimal age to start estrogen therapy is still a point of discussion. It has been suggested to postpone estrogen therapy to delay closure of the epiphysial growth plates and, consequently, to prolong the growth phase[9-11]. However, delay of pubertal development may have serious psychosocial consequences.

To optimize GH and estrogen treatment, in 1989 we started a randomized dose-response study in 68 girls with TS. Four-yr results were described earlier[5]. We now report 7-yr results to compare the long-term growth promoting effect of GH in childhood between the 3 dosage groups. In addition, growth data of the girls who had completed the trial before the end of August 1998 have been evaluated.

Study Subjects

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH dose-response study. The diagnosis was confirmed by lymphocyte chromosomal analysis. Three 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[13], 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[14]. 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 center.

Methods

Study Design

After stratification for chronological age (CA) and height SD-score for CA girls were randomly assigned to group:

A (n=23) receiving 4 IU/m² body surface/day (equivalent to 0.045 mg/kg),
B (n=23) receiving 4 IU/m²/d in the first yr, followed by 6 IU/m²/d (≈0.0675 mg/kg/d), or
C (n=22) receiving 4 IU/m²/d in the first yr, 6 IU/m²/d in the second yr, and thereafter 8 IU/m²/d (≈0.090 mg/kg/d).

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 stopped when subjects had grown less than 1 cm over 6 months. However, when girls were satisfied with their height achieved, they elected to stop GH treatment before study criteria for the termination of treatment had been reached. In the first 4 yr of GH treatment, no estrogens for pubertal induction were given to the girls. After 4 yr of GH treatment, estrogen therapy was started in the girls who were older than 12.0 yr of age; the younger girls started estrogen therapy at a yearly visit after reaching the age of 12 yr. Five μg 17ß-estradiol/kg body weight/day, orally, were given in the first 2 yr, 7.5 μg/kg/d in the third yr and 10 μg/kg/d thereafter. Cyclic progestagen therapy (Duphaston 5 mg/d in the first 14 d of the month) was added after 2 yr of estrogen therapy. If puberty had developed spontaneously (Tanner breast stage ≥ 2) during the study period and before start of estrogens, no estrogens were given.

Height was measured at baseline and subsequently every 3 months using a Harpenden stadiometer. Four measurements per visit were made by 2 trained observers (A van Teunenbroek,

and subsequently T Sas) and the mean was used for the analysis. For the adult height evaluation, adult height was defined as the most recent available height after discontinuation of GH treatment. Height was expressed as SD-score using the references for healthy Dutch girls[13] or the references for North European untreated girls with TS2. At adult height, the height SD-score for TS was calculated using the reference data of 21 yr of age. Target height was adapted from Dutch reference data with addition of 3 cm for secular trend: TH = ½ x (H_{mother} + H_{father} - 12 cm) + 3 cm[13]. Target range was defined as the TH ± 8 cm[15]. During GH treatment pubertal stages were assessed according to Tanner[14]. Bone age was determined by the same 2 observers according to the Tanner & Whitehouse radius, ulna, short-bones score[16]. Bone maturation was expressed as the ratio of the change in BA to the change in CA (△BA/△CA). Predicted adult height was calculated with the modified projected adult height method, using the equation of Lyon, adapted to North European untreated girls with TS[2,17,18]. To assess the gain in adult height, the attained adult height was compared with the modified projected adult height before treatment. Blood samples were taken at start of the study and subsequently every yr for determination of the glycosylated hemoglobin.

Biochemical parameters and hormone assays

Blood samples were taken at start of the study and subsequently every year for determination of the hemoglobin A1c levels. IGF-I was determined at start of the study and after 6, 18, 30, 48, 60, 72, and 84 months of GH treatment. After centrifugation, all samples were frozen (-20°C) until assayed. The RIA measurements of IGF-I were performed as described previously[19-22]. All measurements were performed in the same laboratories. Since levels of IGF-I are dependent on age and sex, values were transformed to SD scores using reference values for healthy children determined in the same laboratory[23].

Statistical Analysis

Results were expressed as mean (SD), unless indicated otherwise. Differences between the dosage groups were first tested by a linear trend test. In case of a significant result, this was followed by comparisons with Student's t-tests. Differences between points in time were tested by paired Student's t-tests.

Results

Clinical data and 7-yr results

The trial started in November 1989. During the first 3 yr, 3 girls, one in each dosage group, dropped out of the study because of noncompliance and were lost to follow-up. In October 1997 the remaining 65 girls had been followed for 7 years. Table 1 lists the baseline clinical data of these girls. The 3 dosage groups had similar initial characteristics.

Figure 1 shows the individual heights of the 65 girls at start of GH treatment, as well as after 7 yr. Twelve girls had completed the trial during the 7-yr study period. After 7 yr of GH treatment, 55 of the 65 girls (85%) had a height within the normal range for healthy Dutch girls, while only 10 girls (15%) had a height just below the 3rd percentile. Figure 2 shows the height SD-score of the 65 girls using references of healthy Dutch girls (lower panel) and the height SD-score using Turner references (upper panel) during the 7-yr study period. At baseline, the girls in all 3 groups have a mean height which is normal for untreated North European girls with TS. After 7 yr of GH treatment the Turner height SD-score in all 3 groups has increased significantly ($p < 0.001$). The change in SD-score was significantly higher in group B and C compared to group A (95% CI: 0.08, 0.95; $p = 0.02$ and 95% CI: 0.38, 1.27; $p = 0.001$, respectively). However,

the difference in SD-score between group B and C was not statistically significant (95% CI: -0.19, 0.81, p = 0.22). Expressed in centimeters: the height after 7 yr was 16.5 (3.5) cm in group A, 19.6 (4.5) cm in group B, and 21.2 (4.3) cm in group C greater than the expected height assuming that these girls would follow their height percentile when they were not treated with GH. Compared to healthy Dutch girls, the mean baseline height of the girls was far below normal (lower panel figure 2). After 7 yr, the mean height SD-score in all 3 groups had increased to values within the normal range for healthy girls.

Table 1. Mean (SD) baseline data for each treatment group. Karyotype (45,X; other) is expressed in numbers (percentage) of patients

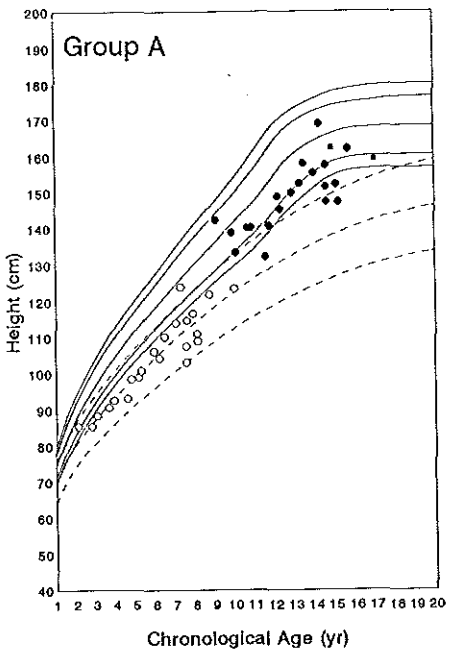
	Group A	Group B	Group C
Number of girls	22	22	21
Baseline chronological age (yr)	6.1 (2.1)	6.7 (2.4)	6.5 (2.4)
Baseline bone age (yr)	5.5 (2.2)	6.0 (2.5)	5.8 (2.4)
Baseline SD-score for height (references healthy Dutch girls)	-2.7 (0.9)	-2.4 (1.0)	-2.6 (1.0)
Baseline SD-score for height (references girls with Turner syndrome)	0.06 (1.03)	0.42 (1.05)	0.18 (1.06)
Baseline modified projected adult height (cm)	146.0 (5.5)	147.9 (5.6)	146.6 (5.6)
Target height (cm)	168.8 (6.3)	170.1 (6.1)	169.5 (5.6)
Karyotype: 45,X	18 (82%)	21 (96%)	16 (76%)
Karyotype: other	4 (18%)	1 (4%)	5 (24%)

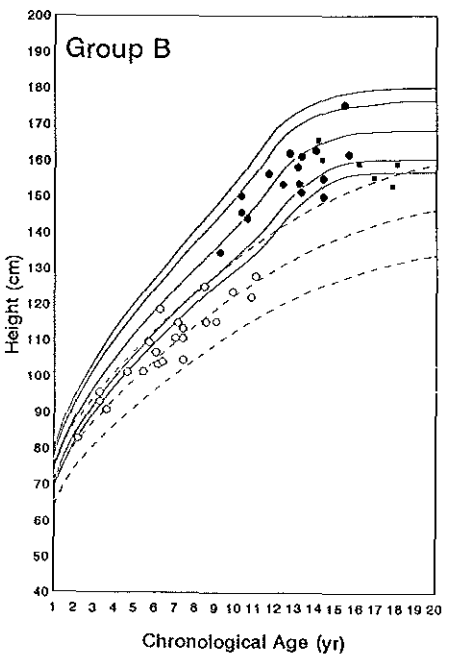
To compare skeletal maturation during 7 yr of GH treatment between the 3 GH dosage groups one can determine the bone maturation of all girls still receiving GH treatment. However, to avoid selection bias, only girls with a chronological age ≤ 14 yr after 7 yr of GH treatment were included in this analysis (group A, B and C, n= 12, 11, 12, respectively). The mean ratio ☐ bone age (yr) / ☐ chronological age (yr) over 7 yr of GH treatment was 1.17 (0.14), 1.24 (0.16) and 1.20 (0.14) in group A, B, and C, respectively, being significantly higher than 1 ($p < 0.005$ for all 3 GH dosage groups). These data indicate an acceleration of bone maturation compared to healthy children. However, no significant differences in bone maturation was found between the 3 GH dosage groups.

After 7 yr of GH treatment, 35 girls had started estrogen therapy at a mean (SD) chronological age of 12.9 (0.8) yr and a bone age of 13.2 (0.6) yr. Twelve of the 35 girls started estrogen therapy between the chronological age of 13 and 15 yr, because in the first 4-yr study period no estrogens were given even if these girls were older than 12 yr of age. The other 23 of the 35 girls started estrogen therapy after reaching the age of 12 yr. The distribution of the 35 girls over the Tanner breast stages was: 11 girls M2, 14 girls M3, 6 girls M4, and 4 girls M5 after a mean (SD) duration of estrogen therapy of 1.8 (0.8) years. Five girls did not receive estrogens because of starting spontaneous puberty during GH treatment (at the end of 7-yr study period: 1 girl had Tanner breast stage M2, 2 girls M3, 1 girl M4, and 1 girl M5).

Adult height results

At the end of August 1998, 32 of the 65 girls (group A, B, and C, n = 10, 10, 12, respectively) had completed the study after a mean duration of treatment of 7.3 (1.1) (range:5.0 - 8.75) yr. Twenty girls had discontinued GH treatment because of a height velocity less than 1 cm





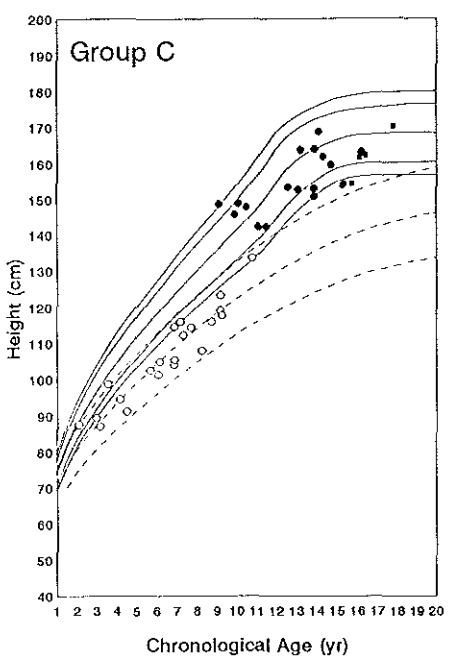


Figure 1. Individual heights at start of the study (open circles) and after 7 yr of GH treatment (filled circles) in group A, B, and C, respectively. Twelve girls had completed the trial during the 7-yr study period (filled squares). Reference curves for healthy Dutch girls (3rd, 10th, 50th, 90th and 97th percentile) and for untreated girls with Turner syndrome (North European references; 3rd, 50th, 97th percentile) are given.

over 6 months, while twelve girls stopped GH treatment because they were satisfied with their attained height. Table 2 lists the clinical data of these 32 patients. Except for age, the baseline clinical data of these 32 girls were comparable with the baseline data of the 65 girls. Mean height was 158.8 cm (SD 7.1, range 148.3 to 172.4 cm) in group A, 161.0 cm (SD 6.8, range 152.8 to 176.2 cm) in group B, and 162.3 cm (SD 6.1, range 154.3 to 171.2 cm) in group C, respectively. Except one girl, all girls (31/32) had a height of more than 150 cm. More than half of the girls (17/32) had a height above 160 cm and 5 of them exceeded 170 cm. The mean difference between the target height and the attained height was 10.9 (5.1) cm for group A, 9.5 (4.1) cm for group B,

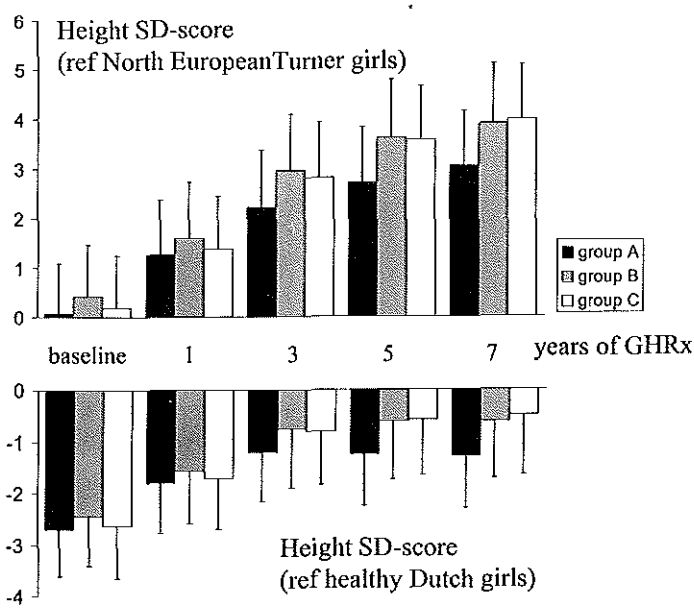


Figure 2. Height SD-score for chronological age during 7 yr of GH treatment for group A (n=22), B (n=22), and C (n=21), respectively. Upper panel of figure: height compared to references for North European girls with Turner syndrome. Lower panel of figure: height compared to references for healthy Dutch girls.

Table 2. Mean (SD) baseline and adult height data in 32 girls who have completed the study.

	Group A	Group B	Group C
Number of girls	10	10	12
Baseline age (yr)	7.9 (0.9)	8.6 (1.6)	8.1 (1.4)
Baseline SD-score for height (references healthy Dutch girls)	-2.81 (1.17)	-2.79 (0.72)	-2.84 (0.82)
Baseline SD-score for height (references girls with Turner syndrome)	0.09 (1.42)	0.17 (0.85)	0.10 (1.01)
Baseline modified projected adult height (cm)	146.2 (7.5)	146.6 (4.5)	146.2 (5.3)
Target height (cm)	169.7 (4.6)	170.5 (5.0)	169.0 (4.5)
Duration growth hormone treatment (months)	93.3 (8.5)	81.3 (14.5)	87.0 (14.8)
Age start 17ß-estradiol	12.7 (0.6)	13.3 (1.1)	12.9 (0.8)
Last age (yr)	16.0 (0.8)	15.8 (1.0)	15.7 (0.9)
Last height (cm)	158.8 (7.1)	161.0 (6.8)	162.3 (6.1)

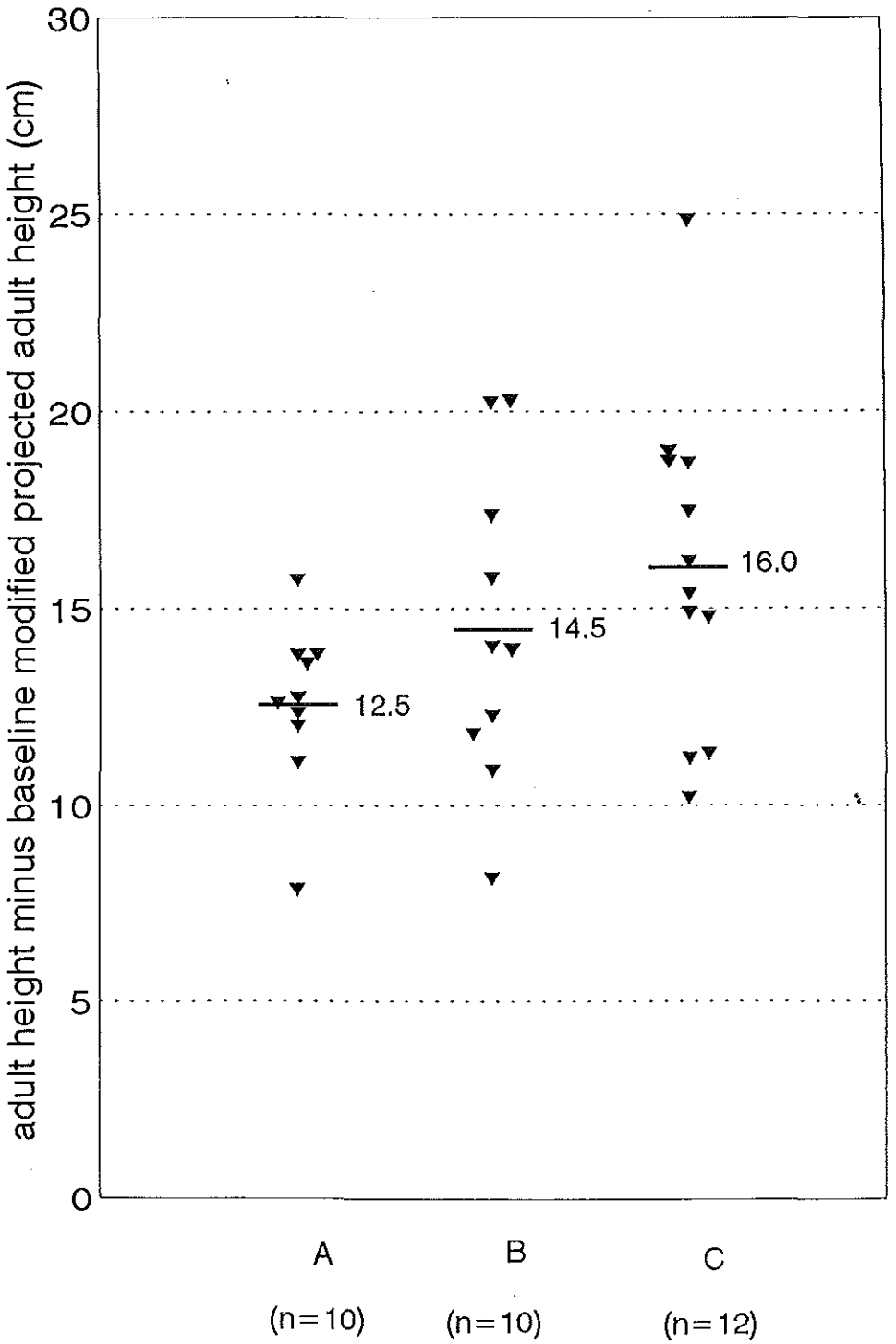


Figure 3. The most recent height of each subject completing GH treatment relative to each subject's modified projected adult height for group A (n=10), B (n=10), and C (n=12), respectively. Mean increment in height (in cm) relative to modified projected adult height is indicated.

and 6.7 (4.6) cm for group C. The target range was reached in 4/10 girls of group A, 4/10 girls of group B, and 9/12 girls of group C. Figure 3 shows the attained height of each subject relative to each subject's modified projected adult height. The mean increment in height was 12.5 cm (SD 2.1, range 7.8 to 15.7 cm) for group A, 14.5 cm (SD 4.0, range 8.1 to 20.3 cm) for group B, and 16.0 cm (SD 4.1, range 10.2 to 24.8 cm) for group C, respectively, being significantly different from baseline ($p < 0.001$). The linear trend test showed a significant positive trend towards a higher gain in height (=attained height minus baseline modified projected adult height) in the dosage groups receiving higher GH dose ($p = 0.027$). The gain in height was significantly higher in group C compared to group A ($p = 0.024$), but without significant differences between group A and B and group B and C, respectively.

IGF-I levels

Figure 4 shows the IGF-I SD-scores before and during 7 years of GH treatment. Before start of GH treatment, the mean IGF-I SD-score was significantly lower than zero ($p < 0.001$). During GH treatment the mean IGF-I SD-score was significantly higher than zero and baseline levels at each point in time ($p < 0.001$). The IGF-I SD-score after 7 years of GH treatment was significantly higher in group B and C (2.5 (0.9) $p = 0.02$ and 2.7 (0.9) $p = 0.003$, respectively) than in group A (1.8 (0.8)), without a significant difference between group B and C.

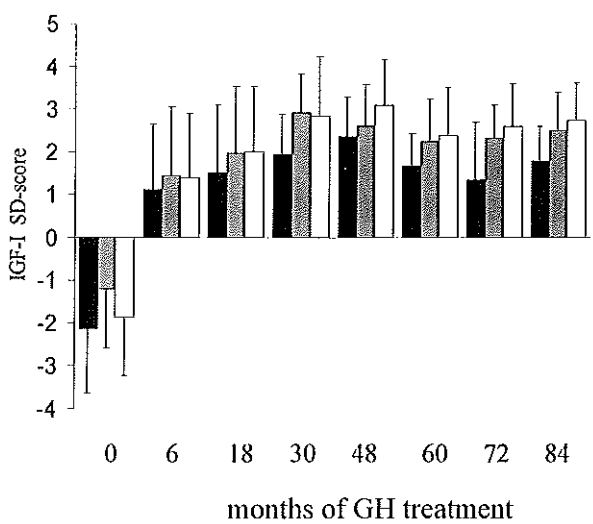


Figure 4. The mean (SD) IGF-I SD-score during 7 yr of GH treatment for group A (n=22) (black bars), B (n=22) (grey bars), and C (n=21) (white bars), respectively.

Safety aspects

Treatment was well tolerated and no adverse events were detected that were considered to be drug related. During the study period, glycosylated hemoglobin levels remained within the normal range. In addition, none of the girls developed diabetes mellitus.

Discussion

This study shows that in girls with TS treatment with biosynthetic GH, even with the

'standard' dose of 4 IU/m²/day (≈0.045 mg/kg/d), results in a normalization of height during childhood and a normalization of adult height in most of the girls. The ideal study design to assess the growth promoting effect of GH is a randomized controlled trial with an untreated study group until adult height. Since at the start of the present study, GH treatment in girls with TS was an accepted indication in the Netherlands, such a trial was not possible. Therefore, a randomized dose-response design was chosen to assess the effect of GH treatment on growth. To determine the effect of GH on adult height, the attained adult height was compared to the individually predicted adult height using the modified projected adult height method based on own references for untreated Dutch girls with TS[2,18]. However, in such a growth analysis, secular trend and errors in adult height prediction have to be taken into account. Remarkably, the growth promoting effect of GH found in the present study exceeds the effects of secular trend and prediction errors. After 7 yr of GH treatment, most girls had a height within the normal range for healthy Dutch girls. In addition, in the 32 girls who had reached adult height, the mean height in the 3 dosage groups was approximately 160 cm. Baseline predicted adult height was exceeded in all subjects and the mean gain in adult height was well above 10 cm, even in the group receiving the standard GH dose of 4 IU/m²/d. Moreover, in more than half of the 32 girls, the attained height was within the target range.

As in the study of Rosenfeld *et al.*[9], some girls discontinued the GH treatment earlier because they were satisfied with their attained height. Although the maximal growth promoting effect could not be obtained in these girls, such a precocious termination of GH treatment is the consequence of the good growth response of the treatment.

The results of the present study contrast with reports stating that GH treatment in girls with TS results only in modest increments or have no positive effect at all[6,24,25]. In these studies the age of start of GH treatment was considerably older than in our study. In the study of Rosenfeld *et al.*, however, girls were started at a younger age (9.1 yr) and showed a mean gain in adult height (8.4 cm) being more comparable with our results[9]. The girls who have reached adult height in our study had a mean age at baseline of 8.2 yr, being even younger than in the study of Rosenfeld *et al.* Therefore, in our opinion, the most important reason of our better results is the fact that GH treatment was started at a younger age compared to earlier studies. Since, the girls who are still receiving GH are even younger than the girls who have already attained their adult height, we expect at least a similar gain in adult height in these younger girls.

In our view, it is very important for the psychosocial well-being of the girls to induce the pubertal development in conformity with their healthy peers. Therefore, in the present study, estrogen therapy was started at a pubertal age. However, when estrogen therapy is started before the end of the growth phase one runs the risk of earlier epiphysial closure, and consequently a lower gain in adult height. From the results of the present study we can state that low dose estrogens at a pubertal age does not interfere with the capability of GH treatment to normalize adult height in most of the girls with TS. Thus, a major outcome of our study is that with these GH and estrogen treatment regimens, most girls with TS can grow and develop much more in conformity with their healthy peers.

Seven-year data demonstrated a higher increment in height in the GH dosage groups receiving 6 or 8 IU/m²/d compared to 4 IU/m²/d. In the 32 girls who had attained their adult stature, the gain in adult height over the baseline predicted adult height was higher with a GH dose of 8 compared to 4 IU/m²/d (16.0 *vs.* 12.5 cm). However, this difference in gain in adult height is quite small in proportion to the difference in the GH dose between these 2 groups. In contrast, Carel *et al.* found an increment in adult height which was twice as high in a study group

who received an increasing GH dose (dependent on the height velocity, up to 9 IU/m²/d) compared to a fixed GH dose (approximately 3.9 IU/m²/d) group (10.6 vs 5.2 cm)[26]. However, these increments in adult height in both study groups were even lower compared to the results of our dosage group receiving 4 IU/m²/d, probably due to the higher baseline age of the girls in that study compared to our study.

In conformity with the study of Carel *et al.*[26] in which even higher GH dosages were used than in our study, tolerance to all 3 GH regimens was good. Our 4-yr results of the effects of GH treatment on lipid metabolism showed no unwanted side-effects[27]. As described in other studies, GH treatment had no adverse effect on glucose metabolism, but the insulin levels had increased significantly after 4 yr of GH treatment compared to baseline levels[27,28,29]. In the present study, we showed that during 7 yr of treatment glycosylated hemoglobin levels stayed within the normal range; an extensive evaluation of the effects of long-term GH treatment on carbohydrate metabolism is described elsewhere[30]. In a previous paper, we showed that 7 yr of GH treatment does not have adverse effects on left ventricular heart dimensions or blood pressure[31]. Since the mean IGF-I levels were on the upper area of the normal range in group A and higher in group B and C, whereas the consequences of high IGF-I levels during childhood are not yet known, follow-up of the girls into adulthood is required.

If higher GH dosages have proven to be safe on the very long-term and result in a clinically significant higher increment in adult height compared to lower GH dosages, cost-benefit evaluations have to be performed. Furthermore, psychological studies are required to evaluate whether the (early) normalization of height is accompanied by an improvement of the psychosocial functioning in childhood as well as in adulthood.

In conclusion, GH treatment starting in relatively young girls with TS results in normalization of height during childhood and normalization of adult height in most of the girls, even using the 'standard' GH dose of 4 IU/m²/day (≈0.045 mg/kg/d), and without unwanted side-effects. Higher GH doses may be more effective, but the efficacy on adult height and safety in the very long-term have still to be proven. Induction of puberty with low dose estrogens can be started at normal pubertal age without interference with the capability of GH treatment to normalize adult height in most of the girls with TS.

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CHAPTER 2.2

FINAL HEIGHT IN GIRLS WITH TURNER'S SYNDROME TREATED WITH ONCE OR TWICE DAILY GROWTH HORMONE INJECTIONS

Adapted from Archives of Disease in Childhood 1999;80:36-41

FINAL HEIGHT IN GIRLS WITH TURNER SYNDROME TREATED WITH ONCE OR TWICE DAILY GROWTH HORMONE INJECTIONS.

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Abstract

Objectives- To study final height (FH) in girls with Turner syndrome (TS) treated with once or twice daily injections of GH in combination with low dose ethinyl estradiol.

Design- In this study until FH the effect of fractionated twice daily (BID) was compared to once daily (OD) s.c. injections of a total GH dose of 6 IU GH/m²/day. BID injections were given as one-third in the morning and two-thirds at bedtime (BID). All girls concurrently received low dose estradiol (0.05 μg ethinyl estradiol/kg body weight/day, increased to 0.10 μg/kg/day after 2.25 years).

Patients- Nineteen girls with TS aged 11 years or over (mean 13.6, SD 1.7 yr).

Measurements- To determine FH gain, we assessed the difference between the attained FH and the FH predictions at start of treatment. These FH predictions were calculated by using the Bayley-Pinneau prediction (BP) method, the modified projected adult height (mPAH), the modified index of potential height ($mIPH_{RUS}$), and the Turner-specific prediction method (PTS_{RUS}).

Results- The gain in FH (Mean(SD)) was not significantly different between the OD and the BID GH injection regimens (7.6 (2.3) *vs.* 5.1 (3.2) cm). All girls exceeded their adult height prediction (range: 1.6;12.3 cm). Thirteen of the 19 girls had a FH gain > 5.0 cm. Mean attained FH was 155.5 (SD 5.4). A "younger BA" at baseline and a higher increase in HSDS for chronological age (Dutch-Swedish-Danish references) in the first year of GH treatment predict a higher FH gain after GH treatment.

Conclusions- Division of the total daily GH dose (6 IU GH/m²/day) into 2/3 in the evening and 1/3 in the morning is not advantageous over the once daily GH regimen with respect to FH gain. Treatment with a GH dose of 6 IU/m²/day in combination with low dose estrogens can result in a significant increase in adult height in Turner girls, even if they start GH therapy at a relatively late age.

Introduction

Growth failure and subsequently short adult stature is one of the main features in Turner syndrome (TS)[1,2]. It has been shown that short stature, independent of etiology, can be associated with psychosocial problems[3]. GH therapy improves height velocity and adult height in most girls

with TS[4-12]. Studies in patients with TS have shown that the initial growth response to GH treatment is dependent on the dose and frequency of administration[4-6 13 14]. In order to improve the growth response intramuscular injections three times a week have been replaced by a once daily subcutaneous regimen[13 14]. However, just as in normal growing girls[15], spontaneous GH secretion in TS is characterized by a large peak soon after falling asleep and the occurrence of several other peaks during the course of a 24-hour period[16-21]. A more frequent injection regimen might thus improve the growth response, as has also been suggested by a study in GH-deficient patients[22].

In this study, an attempt was made to mimic the normal pulsatile GH secretion pattern more closely. The total GH dose of 6 IU/m² body surface/day was divided in two-thirds in the evening and one-third in the morning in one group of patients (BID). The other group received the same total GH dose once daily (OD), in the evening. A total dose of 6 IU/m²/day was chosen instead of the more commonly used 4 IU/m²/day since an earlier study has shown that on the latter dose the growth response in a somewhat older subgroup of girls with TS was poorer than in younger girls[14]. Two year results were described earlier by our group[23]. We now report follow-up until final height comparing the effects of BID versus OD GH administration in 19 girls with TS aged 11 years or over concurrently receiving low dose ethinyl estradiol.

Patients and methods

Study group

We studied 19 previously untreated girls with TS, confirmed by lymphocyte chromosomal analysis. Prior to treatment, ten girls were enrolled in a 10-week (cross-over design) 24-hour GH-profile study, as described earlier[24]. In brief, they started taking ethinyl estradiol (0.05 microgram/kg/day) 4 weeks before they were randomly divided into OD or BID GH injection groups. GH was then administered for 2 weeks. Following a washout interval of 2 weeks, GH therapy was resumed for another period of 2 weeks using the alternative injection frequency. The additional nine girls followed the same schedule without 24-hour GH-profile testing. After a second randomization, which was carried out independently from the first randomisation, all 19 girls entered the present study immediately after completion of the 10-week design. At start of the cross-over study all girls had Tanner puberty stage B1[25], were aged 11 years or over, and had a Tanner and Whitehouse RUS bone age (RUS BA)[26] of less than 13.5 years. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, and previous use of drugs that may interfere with GH therapy. Written informed consent was obtained from the girls and their parents. The study protocol was approved by the Ethics Committee of each participating centre.

Study design

After stratification for RUS BA and height standard deviation score for chronological age ($HSDS_{CA}$), the girls were randomly divided into two GH injection frequency groups. One group (n=9) received 6 IU GH/m² body surface once daily (OD), in the evening. A second group (n=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime (BID). GH (r-hGH Norditropin®) was injected by a pen injection system (Nordiject®24 Novo Nordisk A/S, Denmark). Compliance was carefully monitored. GH treatment was stopped when height velocity (HV) had decreased to less than 1 cm / 6 months. The figure for final height was recorded 6 months after cessation of therapy. All girls received, from the start of the 10-week cross-over study 0.05 μg ethinyl estradiol(EE2)/kg body weight/day, once daily. After the first 2,25 years of GH treatment, the dose of ethinyl estradiol was increased to 0.10 μg/kg/d and cyclic

progestagen therapy was added.

Growth evaluation

Height (H) was measured at baseline and three monthly till final height. Heights were determined according to Cameron[27] using a Harpenden stadiometer; four measurements per visit were made by two trained observers (AvT and later on ThS). Height was expressed as SD-score for chronological age using the Roede en van Wieringen references for healthy Dutch girls ($HSDS_{CA}$ (RvW))[28] and the Dutch Swedish Danish Turner references ($HSDS_{CA}$ (DSD))[2]. At FH, $HSDS_{CA}$ (DSD) was calculated by using the mean height (146.95 cm) and the standard deviation (6.37 cm) of untreated girls with Turner syndrome (DSD references) of 21 years of age. Target height (TH) was adapted from Dutch reference data[28] with addition of 3 cm for secular trend: TH = 1/2 * (H_{mother} + H_{father} -12 cm) + 3 cm. Pubertal stages were assessed according to Tanner[25]. Bone age (BA) was determined by the same two investigators (AvT and later on ThS) according to Tanner & Whitehouse RUS-score (RUS BA)[26] and to Greulich and Pyle (GP BA)[29]. To determine FH gain, we assessed the difference between the attained FH and the FH predictions at start of treatment. These FH predictions were calculated by using the Bayley-Pinneau (BP) prediction method[30], the modified projected adult height (mPAH)[31 32], the modified index of potential height ($mIPH_{RUS}$)[32 33], and the recently developed Turner-specific prediction method (PTS_{RUS}) based on regression coefficients for H, CA, and RUS BA[32]. The latter three methods are based on Dutch Turner references, as described previously[32].

Prior to any treatment all girls underwent a GH provocation test by infusion of arginine (0.5 g/kg body weight in 30 min). In addition, blood was taken for the determination of IGF-I, IGFBP-3, and GHBP at the start of the study (pretreatment) and subsequently at 6 and 18 months after the start of the present study. The methods and the results of these measurements were described previously[23 24 34-37].

Statistics

Results are expressed as mean (standard deviation (SD)), unless indicated otherwise. Student's t-test or the Chi-square test was used to test differences between groups. Student's paired sample test was used for comparing means within groups. Correlations were tested with Pearson's linear correlation coefficient. To compare FH between the OD en BID group, a multiple linear regression analysis, adjusted for baseline variables was performed. For this analysis, RUS BA and the average of the four FH predictions were chosen as baseline variables. In the search for determinants of treatment success (FH gain = attained FH minus predicted FH), multiple linear regression analyses, adjusted for treatment group, were done. For each possible predictive factor, seperate analyses were performed. Possible predictive factors for FH gain after GH treatment were at baseline: CA, RUS BA, $HSDS_{CA}$(DSD), TH, levels of IGF-I, IGFBP-3, IGF-I/IGFBP-3 ratio, GHBP, and the maximal GH value during a provocation test; after the first six months of GH therapy: the change from baseline of IGF-I, IGFBP-3, IGF-I/IGFBP-3 ratio and GHBP levels; and in the first year of GH treatment: the change in $HSDS_{CA}$(DSD). A p-value < 0.05 was considered significant.

Results

Growth evaluation

Table 1 lists the baseline (after the second randomization) clinical data of the girls. As described in a previous paper[23], at baseline, there were no relevant differences between the two

groups for any of the variables. Prior to the treatment the mean CA, BA, and $HSDS_{CA}$ of the girls in the BID group were slightly (not significantly) higher compared with those in the OD group.

Table 1. Pretreatment clinical data, expressed as mean (SD)

	OD Group	*BID group*
Number of girls	9	10
CA (yr)	13.3 (1.7)	13.8 (1.8)
RUS BA (yr)	12.2 (1.0)	12.7 (0.9)
GP BA (yr)	11.4 (0.6)	11.8 (0.8)
Height (cm)	134.3 (5.1)	140.8 (7.9)
$HSDS_{CA}$ (RvW)	-3.7 (1.3)	-3.1 (1.2)
$HSDS_{CA}$ (DSD)	0.2 (1.1)	1.1 (1.3)
TH (cm)	166.5 (4.5)	168.3 (6.3)
Karyotype:		
45,X	6	8
other	3	2

OD: once daily GH injections; BID: twice daily GH injections; CA: chronological age; RUS BA: bone age, RUS score; GP BA: bone age, Greulich & Pyle; $HSDS_{CA}$: height standard deviation score for chronological age; RvW: Roede van Wieringen references for healthy Dutch girls; DSD: Dutch-Swedish-Danish references for girls with Turner syndrome; TH: target height

Table 2 shows the mean predicted adult height according to the four different prediction methods. For boths groups the differences between the four predictions were small and not significantly different from zero. Therefore we used for further analyses the average of the four predictions as the predicted adult height.

Table 2. Predictions of adult height at start of GH treatment, expressed as mean (SD)

	OD group	*BID group*
Number of girls	9	10
Prediction method		
mPAH (cm)	146.7 (6.0)	151.5 (6.9)
$mIPH_{RUS}$ (cm)	146.9 (3.7)	151.2 (5.6)
BP pred.(cm)	145.8 (5.2)	151.5 (6.9)
PTS_{RUS} (cm)	147.4 (5.7)	151.4 (6.4)
Average of the 4 predictions	146.7 (4.9)	151.4 (6.3)

OD: once daily GH injections; BID: twice daily GH injections; mPAH: modified projected adult height; $mIPH_{RUS}$: modified index of potential height, including RUS bone age; BP pred.: Bayley & Pinneau prediction of adult height; PTS_{RUS}: Turner-specific FH prediction method, including RUS bone age.

Table 3 shows the clinical data at FH. The difference in FH between the two treatment groups was 2.2 cm in favour of the BID group, but this difference was not significant. However,

after adjustment for baseline variables (RUS BA and the predicted adult height), the mean FH of the BID group minus the mean FH of the OD group was -1.2 cm (95% confidence interval: -3.8 ; 1.4). Thus, the OD group had a slightly better growth response on GH treatment until FH compared to the BID group, but this difference was not significant. For both treatment groups, FH was significantly higher than the predicted adult height at start ($p < 0.001$). Sixteen of the 19 girls have reached a FH > 150 cm, while five of them even had a FH > 160 cm.

Table 3. Clinical data at final height, expressed as mean (SD)

Treatment group	n	Final height (cm)	Predicted adult height (cm)	Months of GH therapy	increase HSDS ref. RvW	increase HSDS ref. DSD	FH gain (cm)
OD	9	154.3 (5.2)	146.7 (4.9)	48.0 (6.7)	1.5 (0.6)	1.0 (0.5)	7.6 (2.3)
BID	10	156.5 (5.6)	151.4 (6.3)	38.4 (8.1)	1.2 (0.6)	0.4 (0.7)	5.1 (3.2)
Total	19	155.5 (5.4)	149.2 (6.0)	42.9 (8.8)	1.3 (0.6)	0.8 (0.7)	6.3 (3.0)

OD: once daily GH injections; BID: twice daily GH injections; $HSDS_{CA}$: height standard deviation score for chronological age; RvW: Roede van Wieringen references for healthy Dutch girls; DSD: Dutch-Swedish-Danish references for girls with Turner syndrome; FH gain: final height minus predicted adult height.

The duration of GH treatment (from start GHRx till HV < 1 cm/6 mos) was significantly shorter in the BID group compared to the OD group, even after adjustment for RUS BA at baseline ($p < 0.02$). No significant difference in bone maturation from start of GH therapy until final height was found between the OD and BID group: BA/CA ratio (yr/yr) 0.7 vs 0.8.

At FH, HSDS for healthy Dutch girls (RvW) and for untreated Turner girls (DSD) were significantly increased compared to baseline in the OD group ($p < 0.001$). In the BID group the HSDS for healthy Dutch girls (RvW) as well as for untreated Turner girls (DSD) were increased compared to baseline, but only the increase in HSDS (RvW) was statistically significant ($p < 0.001$) . The increase in $HSDS_{CA}$ (RvW and DSD) was higher in the OD group compared to the BID group, but this difference was not statistically significant. All girls exceeded their adult height prediction (FH gain range: 1.6;12.3 cm). Thirteen of the 19 girls had a FH gain > 5.0 cm.

Relations with growth response

Multiple linear regression analyses of the FH gain after adjustment for treatment group, revealed a significant negative correlation with baseline RUS BA (ß=-1.6, p=0.021) (see Figure 1) and a significant positive correlation with increase in $HSDS_{CA}$(DSD) in the first year of GH treatment (ß=7.8, p=<0.0001) (see Figure 2). FH gain was not significantly related to pretreatment CA, $HSDS_{CA}$(DSD), TH, plasma IGF-I, IGFBP3, IGF-I/IGFBP3 ratio, GHBP and maximum GH levels after provocation, nor to the change after six months of GH treatment in plasma IGF-I, IGFBP3, IGF-I/IGFBP3 ratio, and GHBP.

Pubertal development

Tanner breast stage development was not significantly different between the OD and BID

group: at the end of GH treatment the distribution of the number of girls with Tanner stages B1 to B5 was 1-0-3-3-2 and 0-2-2-4-2, respectively. At the end of GH treatment, six girls had had their menarche.

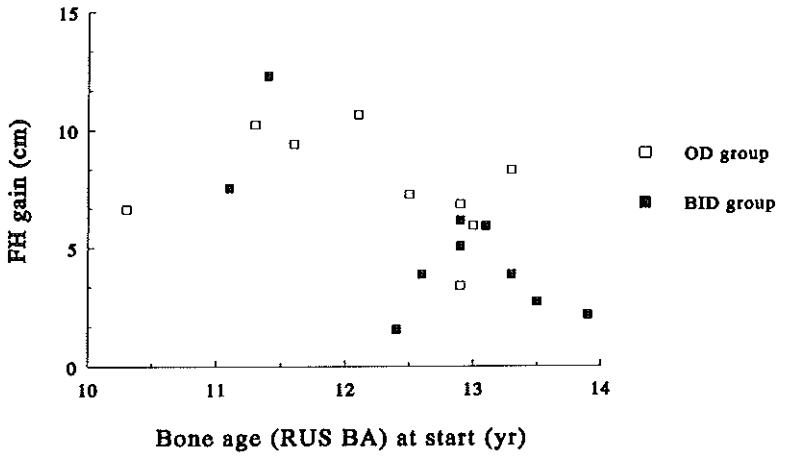

Figure 1. Relation between the final height gain (cm) and the bone age (RUS BA) (yrs) at start of the GH treatment (ß=-1.6, p=0.021).

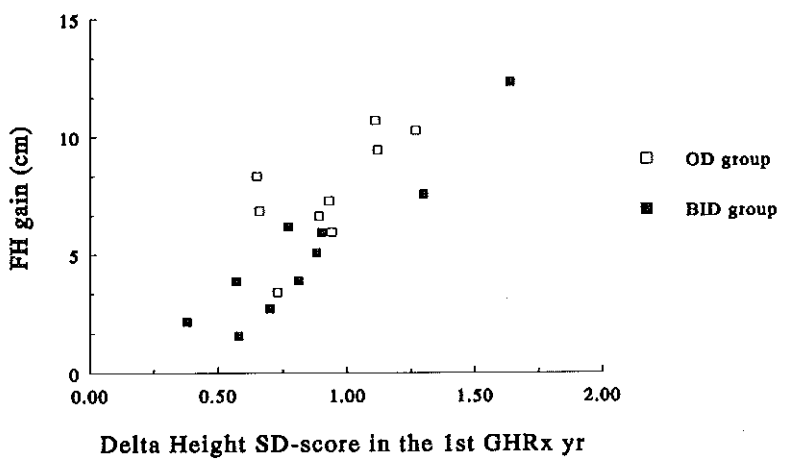

Figure 2. Relation between the final height gain (cm) and the increase in height SD-score(DSD) in the first year of GH treatment (ß=7.8, p<0.0001).

Side effects

No clinically relevant side effects attributable to GH were noted.

Discussion

Division of the total daily GH dose (6 IU GH/m²/day) into 2/3 in the evening and 1/3 in the morning is not advantageous over the OD GH regimen with respect to FH gain. FH tended towards a higher mean value with OD GH compared to BID GH after correction for baseline

variables, but a significant difference between the two groups was not found. The mean corrected difference in FH (BID minus OD) was -1.2 cm with a 95% confidence interval between -3.8 and +1.4 cm in favour of the OD group. The absence of a statistically significant difference in FH between the OD and BID group might be caused by lack of power due to the relatively small numbers of girls in both groups. However, we have proved that the effect of GH treatment of more than 1.4 cm in favour of the BID group can be excluded with 95% confidence.

The present FH data of the OD group compared to the BID group are in line with the two year results[23]. The growth response after two years GH on a total daily dose of 6 IU/m² in combination with 0.05 μg ethinyl estradiol/kg bw/day was not significantly different between the OD and the BID daily GH injection regimens. However, HV in the second year of the study and the change in $HSDS_{CA}$ during the second year and after two years of study was higher in the OD group compared with the BID group. Thus a tendency in favour of OD injections could be observed already in the first two years of therapy.

Table 4. Final height in girls with Turner syndrome treated with growth hormone

Author	n	GH		Ox	EE2		FH gain over (mean)	
		age start (years)	dose (IU/m²/wk)	dose (mg/kg/day)	age start (years)	dose (ng/kg/day)	BP (cm)	(m)PAH (cm)
Massa [1995]	45	<12 >12	24 2 yrs GH: 24 >2 yrs GH:36		>12	100	4.7	2.6
v.d.Broeck[1995]	78	mean 11.7-14.6	~21-27		mean 14.5	2-5 μg/d	3.3	3.0
Taback[1996]	17	median 12.4	~29		median 13.0	5-20 μg/d	-	-0.2
	14		no GH		median 13.3	5-20 μg/d	-	-3.3
Nilsson[1996]	44:	mean 12.2						
	1a) 6		1 yr: no GH >1 yr: ~21	0.05	mean ~15.3	in 4 yrs from 100 to 400	-	7.4
	1b) 7		1 yr: no GH >1 yr: ~21	0.05	mean ~13.3	100	-	4.3
	2) 16		~21	0.05	mean ~15.1	in 4 yrs from 100 to 400	-	8.5
	3) 15		~21	0.05	mean 12.3 mean BA 10.7	100	-	3.0
Rosenfeld [1992]	30:	9.3	~31.5	0.0625	BA≥14	-		10.4
	-		~31.5	no Ox			-	5.6
Haeusler [1996]	20	7.3-16.4	0.9-2.5 yrs: 12-18 thereafter: 18	0.0625 Ox or 5 mg testosteron/ 2 wk i.m.	Mean BA 13.3	in 18 mos from 50 to 200	-	9.3

Ox: Oxandrolone; EE2: ethinyl estradiol ; BP: Bayley Pinneau prediction method; (m)PAH: (modified) projected adult height

The 24h GH profiles in ten of these girls showed that the mean Area Under the Curve (AUC) was 3.1 times higher for the night-time period compared with the day-time period in the girls receiving BID GH treatment, although the GH dose at bedtime was only 2 times higher than the GH dose in the morning. In addition, the mean AUC values for the night-time period were 1.9 times higher with the OD than with BID GH treatment, while the GH dose at bedtime was only 1.5 times higher in the OD group[24]. These results suggest a difference in GH bioavailability and are in line with the trend towards a better FH gain in the OD group.

To our knowledge, this study is the only one in which girls with Turner syndrome have received twice daily injections of GH until FH. In a study in girls with TS, comparing an OD regimen with a BID regimen in which an equal division of a daily GH dose (25 IU/m²/wk) was made, the change in HV in the first year of treatment was somewhat higher in the OD group (3.5 ± 1.3 cm/yr) compared to the BID (2.7 ± 1.8 cm/yr), but also in this study the difference was not significant[35].

Besides the small and statistically non-significant difference in FH gain between the OD and BID group, the duration of the GH treatment in our study was significantly longer in the OD group than in the BID group, even after adjustment for bone age at baseline. Apart from the possibility that these differences are due to a type I error, one can speculate that the effect of the BID GH regimen is comparable with the effect of a lower GH dose (for example, due to lower GH bioavailability). As a result, this causes a worse growth response than the OD GH regimen. Consequently, this may cause an earlier decrease in HV, resulting in an earlier discontinuation of GH therapy and a lower FH gain. Another explanation might be a more pronounced effect of BID regimen on bone maturation than on HV compared to the OD regimen. However, no significant difference in bone maturation between the two groups was found.

The FH gain was, independent of treatment group, negatively related with baseline RUS BA. Therefore, the large interindividual differences in FH gain could partly be explained by the interindividual difference in RUS BA at baseline. From a statistical point of view, it is important to mention that baseline RUS BA was only moderately and not significantly correlated (r=0.26; p=0.278) with the adult height prediction at start. Accordingly, this relationship with FH gain cannot be explained by regression to the mean. In addition, FH gain was positively related with the increase in $HSDS_{CA}$(DSD) in the first year of GH treatment. In conclusion, a "younger BA" at baseline and a higher increase in $HSDS_{CA}$(DSD) in the first year of GH treatment predict a higher FH gain after GH treatment.

Previous publications on the effect of GH treatment on FH in girls with Turner syndrome are summarized in table 4. The only other study in which girls with TS used a GH dose comparable with the dose in our study is described by Massa *et al.* The lower FH gain in that study[10] compared to our results can be explained by the lower GH injection frequency and/or total GH dose per week in the first two years of the study. Other studies in which "relatively old" girls were treated with lower GH doses (without oxandrolone (Ox)) are quite disappointing[7,11]. In the published studies in which girls with TS started GH treatment at a younger age and/or were treated with GH in combination with Ox, the gain in height was comparable with or better than that of our study [8,9,12]. The effect of a relatively short period of higher dose GH treatment on FH gain seems to be comparable with the effect of long-term lower GH dose treatment or GH combined with Ox treatment. In addition, higher GH doses do not seem to have negative side-effects[39,40]. These promising data support the use of higher GH doses in girls with Turner syndrome. However, the higher costs of higher GH doses compared to the lower costs of Ox should be taken into consideration when the use of higher GH doses is discussed.

Although estrogen therapy in girls with Turner syndrome can result in an acceleration of bone maturation if initiated too early [41,42], the effect of low dose estrogen starting within the normal range of age of puberty on final height is not clear. Nilsson *et al.* found a significant lower FH gain in the girls who started at a mean CA of 12.3 yr with 100 ng EE2/kg/d compared to the FH gain of the girls who started EE2 at a mean CA of at least 15.1 yr. However, the difference in FH gain between these groups seems to be caused by a decreased HV in the second and subsequent years of the treatment in the "young starting EE2 group" and not by a significant acceleration of the bone maturation in the first three years of GH treatment[8]. Other GH (with or without Ox) studies in which the induction of puberty was delayed until the epiphysial plates were almost closed[7,9,12], showed no obvious better FH gain than that of our study in which low dose estrogens were combined with a relatively high GH dose. Despite the low estrogen dose, apart from one girl who was still prepubertal, all girls had breast development at the end of GH therapy. Sixteen out of 19 girls had breast stage 3 or more and six girls had experienced their menarche. We consider it of utmost importance for the psychological well-being of the girls to have their pubertal development in conformity with their peers.

Conclusions

(1) Division of the total daily GH dose of 6 IU GH/m²/day into 2/3 in the evening and 1/3 in the morning is not advantageous over the once daily GH regimen with respect to FH gain. (2) Treatment with a GH dose of 6 IU/m²/day in combination with low dose estrogens can result in a significant increase in adult height in Turner girls, even if they start GH therapy at a relatively late age.

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CHAPTER 3

BODY PROPORTIONS DURING LONG-TERM GH TREATMENT IN GIRLS WITH TURNER SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE TRIAL

Journal of Clinical Endocrinology and Metabolism, in press.

BODY PROPORTIONS DURING LONG-TERM GH TREATMENT IN GIRLS WITH TURNER SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE TRIAL

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Abstract

To assess body proportions in girls with Turner syndrome (TS) during long-term growth hormone (GH) treatment, height, sitting height (SH), hand (Hand) and foot length (Foot), and biacromial (Biac) and biiliacal diameter (Biil) were measured in 68 girls with TS participating in a GH dose-response trial. These previously untreated girls with TS, age 2-11 years, were randomly assigned to one of three GH dosage groups: group A, 4 IU/m²/day; group B, first year 4, thereafter 6 IU/m²/d; group C, first year 4, second year 6, thereafter 8 IU/m²/d. Seven-year data were evaluated to assess the effect of GH treatment on body proportions during childhood. In addition, data of all girls who had reached adult height were evaluated to determine the effect on the adult body proportions. All results were adjusted for age and sex, and expressed as SD-scores (SDS) using reference values of healthy Dutch girls. To describe the proportions of SH, Hand, Foot, Biac, and Biil to height, these values were adjusted for the SDS of height and were expressed as shape values, using the formula, e.g. for SH: shape SH = (SH SDS - height SDS) / $\sqrt{}$(2-2 x correlation coefficient between SH and height in the reference population). Furthermore, SD-scores using references of untreated girls with TS were calculated for height and SH. Values being less than -2 or more than +2 were considered outside the normal range.

At baseline, the shape values of all measurements were significantly higher than zero, but most mean shape values were still within the normal range. Seven-year data of 64 girls and adult height data of 32 girls showed that an increase in height was accompanied by an even higher increase in size of Foot resulting in mean SD-scores above zero and shape values of +2 and higher. The increase in the shape value of Foot was significantly higher in group B and C compared to A after 7 years of GH treatment, however, without significant differences between the GH dosage groups in the girls who had reached adult height. The shape values of SH had decreased to values closer to zero after reaching adult height, especially in group A. A similar pattern in proportion of SH to height was seen using references of girls with TS. No significant changes in the other proportions were found after reaching adult height.

In conclusion, on average, untreated girls with TS have relatively large trunk, hands and feet, and broad shoulders and pelvis compared to height. The increase in height after long-term GH treatment is accompanied by an even higher increase in the size of feet and a moderate improvement of the disproportion between height and sitting height. Recently published reference data of untreated adults with TS and results of a different patient group receiving a comparable GH dosage, suggest that the disproportionate growth of feet has to be considered a part of the natural development in TS, but might be influenced by higher GH dosages. The development of

large feet can play a role in the decision of the girl to discontinue GH treatment in the last phase of growth.

Introduction

Growth failure and subsequently short adult stature is one of the main features in Turner syndrome (TS) (1,2). Although these girls are not clearly GH-deficient (3), GH administration accelerates growth in a dose-dependent way (4-6). In addition, recent papers reported a considerable increase in adult height in girls who started GH treatment at a relatively young age (7-9).

The general clinical impression of body proportions of untreated girls with TS is that these girls have a more coarse and stocky figure compared to healthy girls. This is confirmed by earlier studies describing relatively short lower extremities and relatively broad shoulders and pelvis (10-12). Little is known about the effects of supraphysiological GH dosages given for a long time during childhood. The most remarkable reported changes in body proportions in TS after two years of GH treatment were an increase in pelvic width and an increased length and breadth of hands and feet compared to the increase in height (10,13). Four-year data on height and sitting height were described by Rongen *et al.*, demonstrating no change in proportion (14).

To determine the body proportions before, during and after long-term GH treatment, we measured height, sitting height, hand length, foot length, biacromial diameter and biiliacal diameter in 68 girls with TS participating in a randomized dose-response study. The effect on height was described earlier (15). We now report the results of the body proportions during childhood comparing 7-year data between 3 GH dosage groups. In addition, data of all girls who have reached adult height have been evaluated.

Subjects and Methods

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH dose-response study. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: a chronological age between 2 and 11 years, height below the 50th percentile for healthy Dutch girls (16), and a 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 GH treatment, and spontaneous puberty (17). 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 center.

Sixty-eight girls were approached and after the randomization all girls started GH treatment. After stratification for chronological age and height standard deviation score for chronological age (HSDS) girls were randomly assigned to group:

A (n=23) receiving 4 IU/m²/body surface/day (equivalent to 0.045 mg/kg),
B (n=23) receiving 4 IU/m²/d in the first year, followed by 6 IU/m²/d, or
C (n=22) receiving 4 IU/m²/d in the first year, 6 IU/m²/d in the second year, and thereafter 8 IU/m²/d.

Biosynthetic-hGH (Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime using a pen injection system. Every three months the total GH dose was adjusted to the calculated body surface. According to study protocol, treatment was stopped when subjects had grown less than 1 cm over 6 months. After the first 4 years of the study period, girls started estrogen therapy when they had reached the age of 12 years. In the first 2 years of estrogen

therapy, the girls received 5 μg 17ß-estradiol/kg body weight/day, in the 3rd year 7.5 μg/kg/d and thereafter 10 μg/kg/d.

Height, sitting height (SH), left hand length (Hand), left foot length (Foot), biacromial diameter (Biac) and biiliacal diameter (Biil) were measured at baseline and subsequently every six months by two trained observers. Measurements were taken according to Cameron (18). Height and SH were obtained using a Harpenden stadiometer and sitting height table. The other measurements were taken with the Harpenden anthropometer. Two measurements per visit were made and the mean was used for the analysis. The results of each measurement were adjusted for age and sex, and expressed as a SD-score. When GH treatment was stopped the last available measurements at discontinuation of GH treatment were considered the definite values assuming that no change in body proportions will take place after stop of GH treatment. For the evaluation of body proportions in the girls after reaching adult height, the reference values at the age of 18 years were used for calculating the SD-scores. The data of the Oosterwolde Study were used as references for healthy Dutch girls (19). This reference population consisted of 1,093 Dutch girls, ranging from birth to 18 years of age. To calculate SD-scores, data of the reference population were transformed using the LMS method (20,21). This method transforms the reference data at each age to a normal distribution. In addition, the SD-scores of the measurements of SH, Hand, Foot, Biac and Biil were adjusted for the height SD-score and expressed as the shape value. The shape value, in this example defined as the SH SD-score adjusted for the height SD-score, was calculated using the formula (10,22),

$$\text{shape SH} = (\text{SH SDS} - \text{height SDS}) / \sqrt{(2-2r)}$$

in which r is the correlation coefficient between height and SH in the reference population. Values less than -2 or more than +2 were considered outside the normal range.

Unfortunately, no reference values of untreated girls with TS were available, except for height (Dutch-Swedish-Danish Turner references) (2) and SH (Dutch Turner references) (12). Because the reference populations for height and SH were not totally identical and the correlation coefficient between height and SH in girls with TS was not known, only SD-scores of both measurements were calculated using the reported means and SD's per year. For the evaluation of the body proportions in the girls after reaching adult height, the Turner reference values at the maximum age of 21 and 23 years were used for calculating the Turner SD-scores of height and SH, respectively. Seven girls were of non-Caucasian origin (3 in group A, 3 in group B and 1 in group C). Although references values of body proportions of non-Caucasian girls might be somewhat different compared to our Caucasian references, all girls were included in the statistical analysis. Pubertal stages were assessed according to Tanner (17).

Results were expressed as mean (SD), unless indicated otherwise. At baseline, after 7 years, and after reaching adult height, SD-scores and shape values were compared with zero using Student's one sample t-tests. Differences between SD-scores or shape values after 7 years of GH treatment compared to baseline were tested by paired Student's t-tests. Differences in change in shape values between the GH dosage groups were first tested by a linear trend test to assess a possible dose-dependent effect. In case of a significant result, this was followed by comparisons with Student's two-sample t-tests. An intention to treat analysis was performed based on all available data. A p-value < 0.05 was considered significant.

Results

The trial started in 1989. In October of 1997, all girls had been followed for 7 years. At the end of August 1998, thirty-two patients had discontinued GH treatment. In each group one girl

dropped out of the study long before reaching adult height for the following reasons : non-compliance, presumed increase in muscle mass and decline in school performance, and desire to initiate estrogen therapy before the 4th year of GH treatment. In another girl of group B no measurements of body proportions were available after 4 years of treatment. Therefore, analysis of body proportions during long-term GH treatment was not possible in these four girls. Table 1 lists the baseline clinical data of the remaining 64 girls as well as of the 32 girls who had discontinued GH treatment after reaching (near) adult height. The three groups of patients had similar initial characteristics.

TABLE 1. Mean (SD) baseline data for each treatment group. Karyotype (45,X; other) is expressed in number (percentage) of patients.

	Group A	Group B	Group C
Total group of girls			
Number of girls	22	21	21
Chronological Age (yr)	6.1 (2.1)	6.7 (2.5)	6.5 (2.4)
Height SD-score	-2.9 (0.9)	-2.8 (0.8)	-2.7 (1.0)
Karyotype			
45,X	18 (82%)	20 (95%)	16 (76%)
other	4 (18%)	1 (5%)	5 (24%)
32 girls who had reached (near) adult height			
Number of girls	10	10	12
Chronological Age (yr)	7.9 (0.9)	8.6 (1.6)	8.1 (1.4)
Height SD-score	-2.9 (1.0)	-3.0 (0.7)	-3.0 (0.7)

Seven-year evaluation

Figure 1 shows the SD-scores of the measurements at baseline and during 7 years of GH treatment in group A, B, and C, respectively. At start of GH treatment, all mean SD-scores were significantly lower than zero, indicating a stunted growth in several body parts. However, the baseline SD-score of height was lower than the SD-scores of the other measurements. After 7 years of GH treatment all SD-scores had increased significantly compared to baseline. The SD-scores of height, SH, Biac and Biil showed an increase, especially in the first four years of treatment, while the SD-scores of Hand and Foot demonstrated a sustained increase throughout the whole study period up to values higher than zero in the higher GH dosage groups.

The SD-scores of height and SH using the Turner references are presented in figure 2. At start, height and SH were not different from Turner controls, except SH in group B. After 7 years, height and SH showed a similar increase, suggesting no obvious change in body proportion between height and SH.

Figure 3 shows the shape values at baseline and during 7 years of treatment for group A, B, and C. At start of the treatment all mean shape values were significantly higher than zero, indicating a relatively large trunk, relatively large hands and feet, and relatively broad shoulders and pelvis compared to height. However, most shape values were within the normal range. While the shape values of SH and Biac had not significantly changed over the 7 years, the shape value of Biil had significantly decreased. The mean shape values of Hand showed a decrease in the first years of the study followed by an increase up to values around +2, being, however, only statistically significant from baseline in group C. The shape values of Foot increased significantly

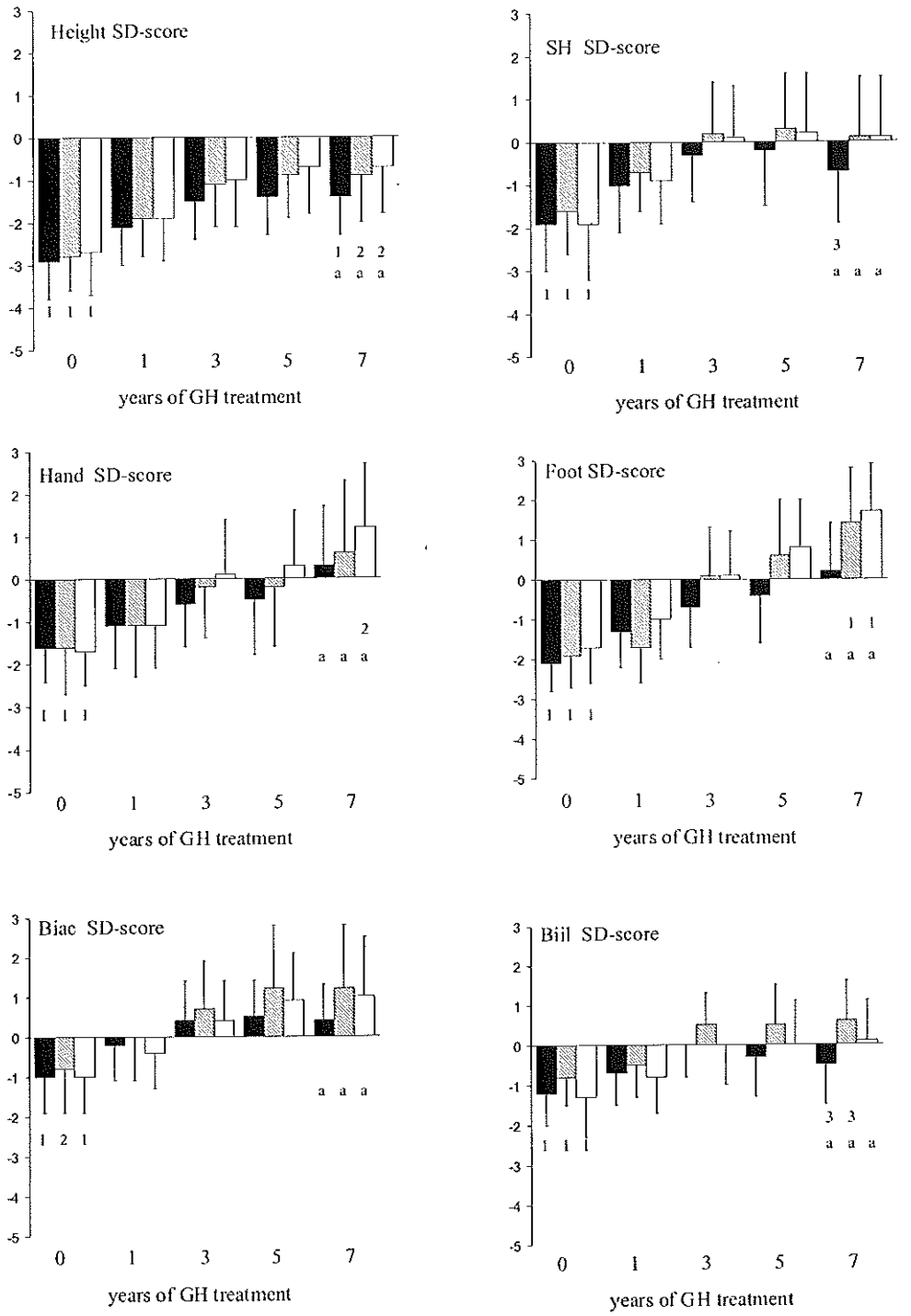

Fig. 1. Mean (SD) SD-scores using reference values of healthy Dutch girls at baseline and during 7 years of GH treatment in group A (black bars), B (grey bars), and C (white bars), respectively. Significant differences from zero (1) $p < 0.001$, (2) $p < 0.005$, and (3) $p < 0.05$ and significant changes from baseline to 7 years of GH treatment (a) $p < 0.001$ are indicated.

during treatment up to values ≥ +2 in all three GH dosage groups. Only the increase in shape value of Foot was significantly higher in group B and C compared to A, without a significant difference between group B and C.

Girls who remained prepubertal throughout the whole study period (n=24) showed similar patterns in shape values as the whole study group (data not shown).

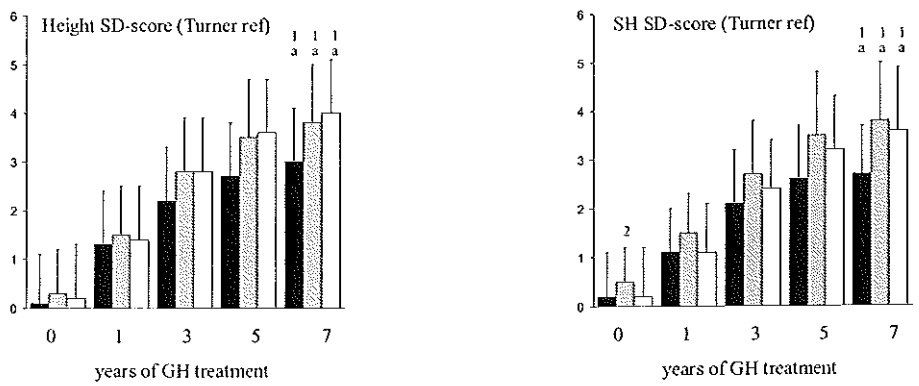

Fig. 2. Mean (SD) SD-scores of height and SH using the Turner references at baseline and during 7 years of GH treatment in group A (black bars), B (grey bars), and C (white bars), respectively. Significant differences from zero (1) $p < 0.001$ and significant changes from baseline to 7 years of GH treatment (a) $p < 0.001$ are indicated.

Body proportions after reaching adult height

Thirty-two patients (group A, B, and C, n = 10, 10, 12, respectively) have discontinued GH treatment after a mean duration of treatment of 7.3 (1.1) (range: 5.0 - 8.5) years. Twenty girls had discontinued GH treatment because of a height velocity less than 1 cm over 6 months, while twelve girls stopped GH treatment in the last phase of growth because they were satisfied with their attained height.

The baseline clinical data of these thirty-two girls were, except for age, comparable with the baseline data of the total group of sixty-five girls (Table 1). At the last measurements, the mean (SD) age was 15.6 (0.7) yr in group A, 15.2 (1.3) yr in group B, and 15.2 (0.9) yr in group C. Although, the duration of the GH treatment (from baseline to the last measurement) seemed to be shorter in group B (6.7 (1.3)) and C (7.1 (1.2) than in group A (7.6 (0.7)), the linear trend test showed no significant dose-dependent effect.

Table 2 shows the SD-scores and shape values at start of treatment and at discontinuation of GH treatment. Baseline values of these 32 girls were similar to those of the total group of 64 girls. Except the SD-score of SH in group A, all SD-scores had significantly increased compared to baseline. The largest increase in SD-scores was seen in Hand and Foot. In group C the length of feet had increased to mean values significantly higher compared to healthy women. The shape values of Biac and Biil showed no change compared to baseline, while the shape value of SH had significantly decreased. The decrease in the shape value of SH was significant less in group B and C compared to A. The mean shape values of Hand deviated more towards abnormal high values, however these changes were not statistically significant. The shape value of Foot had increased significantly to values outside the normal range, without significant differences between the GH dosage groups.

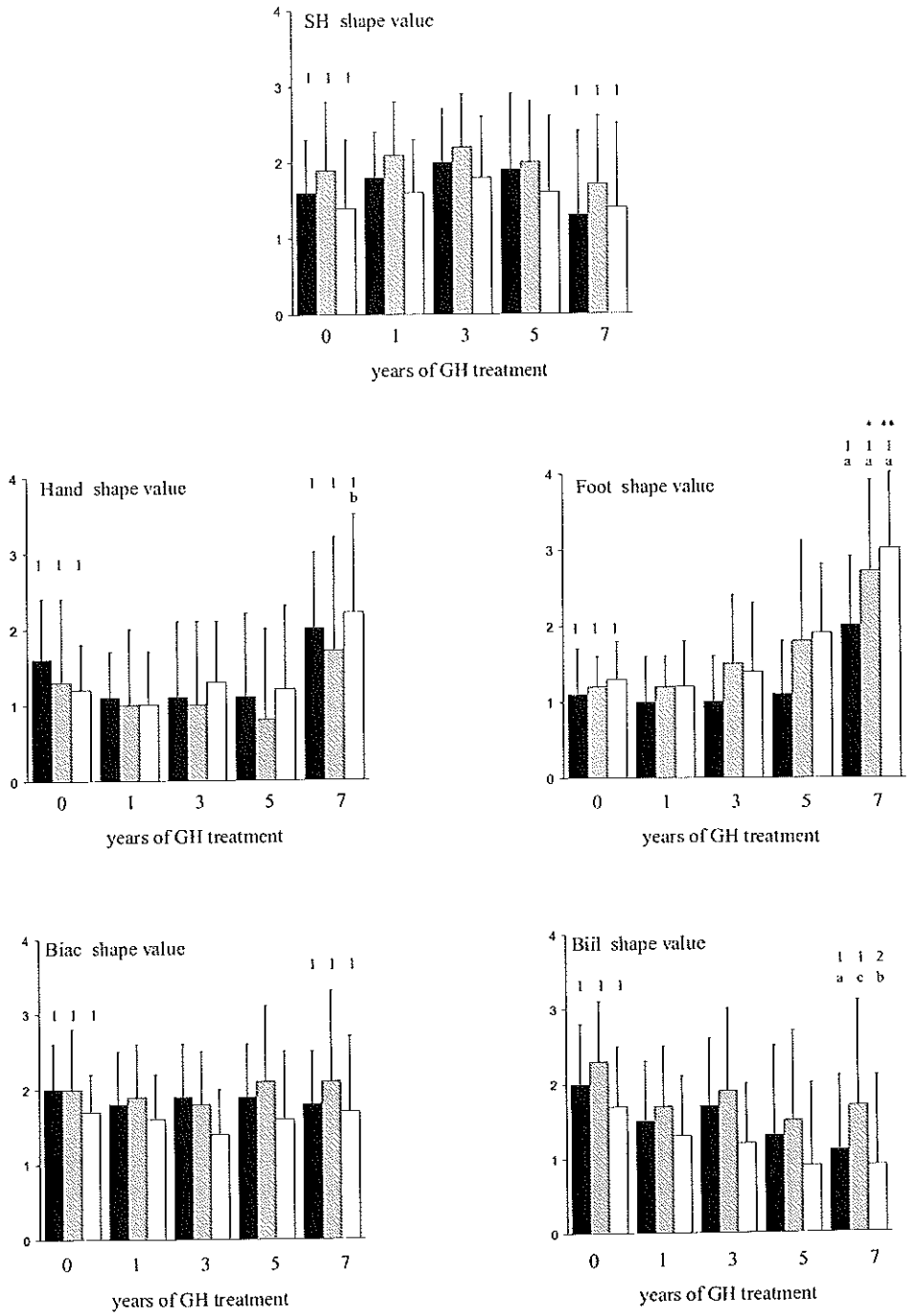

Fig. 3. Mean (SD) shape values at baseline and during 7 years of treatment for group A (black bars), B (grey bars), and C (white bars), respectively. Significantly differences from zero (1) $p < 0.001$ and (2) $p < 0.005$, and significant changes from baseline to 7 years of GH treatment (a) $p < 0.001$, (b) $p < 0.005$, and (c) $p < 0.05$ are indicated. Significant change in shape value from baseline to 7 years of GH treatment in this GH dosage group compared to group A were indicated by (*) $p < 0.05$, (**) $p < 0.005$.

TABLE 2. Mean (SD) SD-scores and shape values at baseline and after reaching adult height in 32 girls who have reached adult height.

	At baseline			At stop GH treatment		
	Group A	Group B	Group C	Group A	Group B	Group C
SD-score						
H	-2.9 (1.0)[1]	-3.0 (0.7)[1]	-3.0 (0.7)[1]	-2.0 (1.0)[1a]	-1.8 (1.0)[1a]	-1.6 (0.9)[1a]
SH	-1.9 (1.3)[2]	-1.6 (0.8)[1]	-2.0 (1.2)[1]	-2.3 (1.5)[2]	-1.1 (1.2)[3c]	-1.2 (1.4)[2c]
Hand	-1.8 (0.9)[1]	-2.0 (1.1)[1]	-1.9 (0.8)[1]	-0.1 (1.9)[b]	-0.6 (1.9)[b]	0.3 (2.2)[b]
Foot	-2.1 (0.8)[1]	-2.2 (0.7)[1]	-1.9 (0.7)[1]	0.2 (1.4)[a]	0.9 (1.4)[a]	1.4 (1.3)[2a]
Biac	-1.3 (1.1)[2]	-1.0 (1.0)[3]	-1.2 (0.9)[2]	-0.2 (1.1)[a]	0.0 (1.3)[c]	0.2 (1.3)[b]
Biil	-1.4 (1.0)[2]	-0.8 (0.7)[2]	-1.4 (0.8)[1]	-0.4 (0.5)[b]	0.4 (0.6)[3b]	0.0 (1.0)[b]
shape value						
SH	1.7 (0.6)[1]	2.4 (0.7)[1]	1.6 (1.0)[1]	-0.4 (1.1)[a]	1.2 (1.2)[3b*]	0.7 (1.4)[c*]
Hand	1.4 (0.5)[1]	1.2 (1.2)[3]	1.3 (0.6)[1]	2.2 (1.2)[1]	1.5 (1.5)[3]	2.2 (1.8)[2]
Foot	1.0 (0.6)[2]	1.0 (0.3)[1]	1.3 (0.5)[1]	2.7 (1.0)[1a]	3.2 (0.9)[1a]	3.6 (1.0)[1a]
Biac	1.6 (0.5)[1]	2.0 (0.8)[1]	1.8 (0.3)[1]	1.8 (0.9)[1]	1.8 (1.0)[1]	1.8 (1.2)[1]
Biil	1.7 (0.9)[1]	2.5 (0.7)[1]	1.7 (0.9)[1]	1.9 (0.9)[1]	2.6 (1.0)[1]	1.8 (1.2)[1]

Significantly different from zero: (1) $p < 0.001$, (2) $p < 0.005$, and (3) $p < 0.05$.
Significantly different from baseline: (a) $p < 0.001$, (b) $p < 0.005$, and (c) $p < 0.05$.
Significant difference in the change in shape value from baseline to adulthood compared to that of group A: (*) $p < 0.05$.
NOTE: The differences between the GH dosage groups were only tested for the change in shape values.

The SD-scores of height using the Turner references had significantly increased by 1.7 (0.5) in group A, 1.9 (0.6) in group B, and 2.2 (0.7) in group C compared to baseline ($p < 0.001$). The increase in SD-score of SH using Turner references increased with 0.6 (0.5) in group A, 0.9 (0.4) in group B, and 1.3 (0.7) in group C compared to baseline ($p = 0.004$ for group A, $p < 0.001$ for group B and C). Thus, the increase in Turner SD-scores for height seems to be higher than those for SH.

Discussion

The present study showed that untreated girls with TS with an age between 2 and 11 years are short and have smaller hands and feet and narrow shoulders and pelvis compared to healthy peers. Since height is more affected than other parts of the body, untreated girls have, on average, a relatively large trunk, relatively large hands and feet, and relatively broad shoulders and pelvis compared to height. Most shape values, however, were within the normal range. Our data are in line with the results of Gerver *et al.*, describing also higher mean shape values in somewhat older girls (6-19 yrs) with TS (10). Zellner *et al.* demonstrated also higher SD-scores of Hand and Foot than that of height, indicating relatively large hands and feet (13). Rongen *et al.* reported that especially younger girls with TS have relatively short legs compared to trunk height (12).

During GH treatment all measured parts of the body increased in size to values being more close to those of healthy peers, or even higher. The seven-year data showed that the increase in SH and Biac was comparable with that of height, resulting in no change in shape values. Although we do not have shape values for SH based on Turner references, our 7-year Turner SD-scores also suggest no change in the proportion of SH to height. The shape value of the Biil had decreased during the seven years to values more appropriate for height. Hand and Foot showed a sustained

increase in size throughout the whole study period resulting in mean SD-scores above zero and shape values around +2 or higher. Only the increase in shape value of Foot was dose-dependent. Other studies in girls with TS also reported a higher increase in SD-score of hand and foot than that of height after 2 years of GH treatment (10,13). Rongen *et al.* demonstrated, as we did, no change in the preexisting disproportion between height and sitting height during childhood after 4 years of GH treatment (14). However, the biggest change in body proportions in the study of Gerver *et al.* was the increased biiliacal diameter compared to height after 2 years of GH treatment (10). In our study, the shape Biil decreased during 7 years of GH treatment and was not changed in the subgroup of girls who had reached adult height. The discrepancy between the previous published data and our data concerning Biil compared to height, have to be found in the good catch-up in height and the less increase in Biil. We have to realize that compared to the other measurements, the measurement of the Biil is higher influenced by the increase in body fat which is especially seen in adolescents and adults with TS.

Thirty-two girls had reached their adult stature after long-term GH treatment. For the present study, we made the assumption that no change in body proportions will take place after stop of GH treatment. Our clinical impression is that only some additional millimeters to a centimeter in height can be achieved in the years after discontinuation of treatment after reaching (near) final height. However, we assumed that the possible additional millimeters in height will be accompanied by a similar increase in size of other parts of the body after stop of GH treatment. Preliminary data of older girls with TS obtained by our group seem to confirm this assumption, however, follow-up of the girls of the present study is required to substantiate this. The data of the 32 girls showed no change in shape values of Biac and Biil. Although the shape value of Hand had deviated more from normal compared to baseline, the change was not significant. In contrast, Foot had increased to mean values equal to or higher than the mean value for healthy women and shape values outside the normal range. However, no significant differences between the GH dosages groups were found. The shape value of SH showed a decrease to values more close to zero, especially in group A. The SD-scores for height and SH using Turner references showed the same pattern: a higher increase in height than in SH. Thus, in the girls who had reached adult height after long-term GH treatment, the disproportion between SH and height had improved, but the proportion of Foot to height had worsened.

The cause and mechanism of the change in body proportions after long-term GH treatment in girls with TS are not clear-cut. In our study, girls of group A received the usual GH dose, but higher GH doses were given in group B and C. The 7-year increase in shape value of Foot was dose-dependent, suggesting a GH-induced abnormality. In adults excessive GH secretion can lead to acromegalic features including big hands and feet which is caused by thickening of the bone and surrounding soft tissue. Less is known about the effects of high GH levels during childhood. Although, we did not include systematic radiographic measurements of bone length and soft tissue in our study, we saw no obvious soft tissue thickening on the standardized X-rays of the hand. This suggests that in girls with TS the increase in the size of the hands are caused by longitudinal growth of bone and does not seem to be part of acromegalic changes. In addition, it has been reported that high GH levels in children during the growth phase (gigantism; most common cause is an autonomously functioning pituitary adenoma) result in proportional growth in length and width of the bones (23). As in most other studies, we did not include a randomized control group receiving no GH treatment until adult height. Unfortunately, no reference values for TS were available for Hand, Foot, Biac, and Biil during the growth phase. Therefore, we do not know the natural development of those body proportions in untreated girls with TS during

childhood. However, recent reference values were reported of adult women with TS, who had received no GH therapy in childhood (24). To compare those data with ours, we calculated the mean shape values from the SD-scores of that study, since the same reference population was used as in our study. It was shown that untreated adult women with TS have no disproportion between height and SH, but shape values of Hand were even higher than that of our 32 girls having reached adult height. The mean shape value of Foot of these women was as high as that of group A, however, seemed to be lower than that of group B and C in our analysis on adult data. The shape values for Biac and Biil were considerably higher than our adult shape values. These reference data suggest that the decrease in shape SH and the increase in shape Hand and Feet found in our study is partly due to the natural development of these body proportions in girls with TS. As observed in forms of skeletal dysplasia, body proportions may change during childhood. One might speculate that GH exaggerates this naturally occurring disproportionate growth in girls with TS while growing within the normal range of height. Remarkably, our data of a different patient group receiving 6 IU GH/m²/day showed no abnormal growth of hands and feet (25). Because the increments in shape values of Hand and Foot appeared mainly after the fourth year, estrogen therapy was thought to be one of the causes of the abnormal development of Hand and Foot. However, the same pattern was seen in the girls without pubertal signs and estrogen therapy throughout the study. Therefore, the changes in body proportions could not be attributed to puberty or estrogen therapy.

As described in previous papers, long-term GH treatment results in a height within the normal range in most girls with TS (7,15). In the present study, we showed that the increase of height is accompanied by an abnormal growth of feet, resulting in relatively large feet for height, but also for age. Especially in the last phase of growth, some girls of our study complained about big feet. The possibility of a further growth of feet may have influenced their decision to discontinue GH treatment before adult height was reached.

In conclusion, girls with TS have on average relatively large trunk, hands and feet, and broad shoulders and pelvis compared to height. The increase of height after long-term GH treatment is accompanied by an even higher increase in the size of feet and a moderate improvement of the disproportion between height and sitting height. Recent published reference data of adults with TS and results of a different GH-treated patient group, suggest that the disproportionate growth of feet is part of the natural development in TS, but might be influenced by higher GH dosages.

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CHAPTER 4

THE EFFECTS OF LONG-TERM GROWTH HORMONE TREATMENT ON CARDIAC LEFT VENTRICULAR DIMENSIONS AND BLOOD PRESSURE IN GIRLS WITH TURNER'S SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE STUDY.

Adapted from Journal of Pediatrics 1999 Oct, 135 (4): 470-476

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Summary

Objective: To assess the effects of long-term growth hormone (GH) treatment on left ventricular (LV) dimensions and systemic blood pressure (BP) in girls with Turner's syndrome (TS) without clinically relevant cardiac abnormalities and receiving GH for short stature.

Study design: Echocardiographically measured LV dimensions and systemic BP were assessed before and during 7 years of GH treatment in 68 girls with TS participating in a randomized dose-response study. These previously untreated girls, age 2-11 years, were randomly assigned to one of three GH dosage groups: group A, 4 IU/m²/day; group B, first year 4, thereafter 6 IU/m²/d; group C, first year 4, second year 6, thereafter 8 IU/m²/d. After the first 4 years, girls ≥ 12 years of age started with 17ß-estradiol 5 μg/kg bw/d for induction of puberty.

Results: At baseline the LV dimensions of almost every girl were within the normal range and the mean SD-scores were close to zero. During 7 years of GH treatment the growth of the LV was comparable with that of healthy girls. No signs of LV hypertrophy were found. Before start of GH treatment, mean BP was within the normal range but significantly higher than in healthy controls. Diastolic or systolic blood pressure was above the 90th percentile in 23% and 28% of the girls, respectively. After 7 years of treatment these percentages were 14% and 36%, respectively, being not significantly different from baseline. The SD-score of the diastolic BP showed a small decrease after 7 years of treatment. The growth of the LV and the development of the BP were not different between the GH dosage groups.

Conclusions: Long-term GH treatment, even at dosages up to 8 IU/m²/day, does not result in LV hypertrophy or hypertension in girls with TS. Continued observation into adulthood is recommended to follow the further development of the relatively high BP and to ensure that GH treatment has no negative effect on the heart on the very long-term.

Introduction

Growth failure and subsequently short adult stature is one of the main features in Turner's syndrome (TS) [1,2]. Although these girls are not clearly growth hormone-deficient[3], growth hormone (GH) administration accelerates growth in a dose-dependent way[4-6]. In addition, recent papers reported a considerable increase in adult height in girls with TS who started GH treatment at a relatively young age and/or were treated with supra-physiological GH dosages[6-9].

Previous studies suggest that GH treatment has, at least in adults, an anabolic effect on the myocardium as well[10,11]. Moreover, in patients with acromegaly GH hypersecretion is associated

with an increased morbidity and mortality from cardiovascular disease[12]. An increase in left ventricular (LV) mass and hypertension have been reported in acromegalic patients[13-15]. To optimize GH treatment in girls with TS, supra-physiological GH doses are given for a long period during childhood. Although previous (short-term) studies in small numbers of girls reported no obvious negative cardiovascular effects[16,17], little is known about the consequences of high levels of GH over a long period during childhood on the morphology of the heart and the blood pressure (BP).

To determine the possible cardiovascular side-effects of long-term GH treatment, echocardiographically measured LV dimensions and systemic BP were evaluated in 68 girls with TS participating in a randomized dose-response study. The effect on height was described earlier[18]. We now report the results of the cardiovascular evaluation comparing 7-year data between 3 GH dosage groups.

Methods

Study design

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH dose-response study in the Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: a chronological age between 2 and 11 years, height below the 50th percentile for healthy Dutch girls[19], and a 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 GH treatment, and spontaneous puberty[20]. Girls with previous coarctation repair, but without a residual gradient and without LV hypertrophy or hypertension and girls with a non-stenotic abnormal aortic valve were included. 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 standard deviation score for chronological age girls were randomly assigned to group:

A (n=23) receiving 4 IU/m²/day (equivalent to 0.045 mg/kg/day),
B (n=23) receiving 4 IU/m²/day in the first year, followed by 6 IU/m²/day, or
C (n=22) receiving 4 IU/m²/day in the first year, 6 IU/m²/day in the second year, and thereafter 8 IU/m²/day.

Biosynthetic-hGH (Norditropin®, Novo Nordisk A/S, Denmark) was given subcutaneously once daily at bedtime using a pen injection system. Every three months the total GH dose was adjusted to the calculated body surface. According to the study protocol, treatment was stopped when subjects had grown less than 1 cm over 6 months. However, when girls were satisfied with their height achieved, they elected to stop GH treatment before study criteria for the termination of treatment had been reached. After the first 4-year study period, girls started estrogen therapy when they had reached the age of 12 years. In the first 2 years of estrogen therapy, the girls received 5 μg 17ß-estradiol/kg body weight/day, in the 3rd year 7.5 μg/kg/d and thereafter 10 μg/kg/d.

Measurements

Before the start of treatment (baseline) and subsequently every three months all girls were seen at their local hospital for measurements of height and weight. Height was expressed as SD-score using Dutch references of healthy Dutch girls[19]. Every six months blood pressure was measured. BP was determined with the girls in sitting position using a cuff size corresponding to

the size of their arm. Only the second to fourth readings were used to calculate the mean systolic and diastolic BP. BP was expressed as SD-score, using age and sex specific reference values[21]. Once a year, the echocardiographic measurements were performed by an experienced technician or pediatric cardiologist. All echocardiographic tracings were evaluated by a single pediatric cardiologist (AC). M-mode echocardiography was used to assess end-diastolic interventricular septal thickness, end-diastolic left ventricular posterior wall thickness, and end-diastolic and end-systolic left ventricular internal diameter according to Gutgesell[22]. Standard deviation scores for IVSed, LVPWed, LVIDed and LVIDes were calculated using equations based on the LV dimensions and weight values of healthy Dutch children[23]. The end-diastolic relative wall thickness (RWT), an index of the thickness of the left ventricular wall compared to the internal diameter, was calculated using the formula: 2 (LVPWed) / LVIDed[24]. The RWT data were compared with the previously reported values of 62 healthy children[24].

Statistical analysis

Results are expressed as mean (SD), unless indicated otherwise. Differences in continuous variables between baseline values and values after 7 years of GH treatment were tested by paired Student's t-tests. Differences between zero and SD-scores at baseline or after 7 years were tested by one-sample Student's t-tests. Differences in the change in values between the GH dosage groups were first tested by a linear trend test to assess a possible dose-dependent effect. In case of a significant result, this was followed by comparisons between two groups with Student's t-tests. The McNemar's test was used to test paired dichotomous variables. A p-value < 0.05 was considered significant.

Results

The trial started in November 1989 with an inclusion period of one year. Four girls dropped out of the study long before reaching adult height mainly because of non-compliance. In all four cases no cardiac problems were involved in the decision to discontinue the treatment. Data of these four girls were not used for the analysis of long-term GH treatment. Two other girls of group B with a congenital abnormal aortic and mitral valve, respectively, developed significant valve stenosis during the study. Data of both girls were excluded from the analysis since it became impossible to separate a GH effect from the hemodynamic effects of valve stenosis on the LV architecture.

Table 1. Baseline clinical data.

	group A	group B	group C
Number of girls	22	19	21
Chronological age	6.1 (2.1)	6.9 (2.4)	6.5 (2.4)
Height SD-score	-2.7 (0.9)	-2.6 (0.8)	-2.6 (1.0)
Karyotype: 45,X	18 (82%)	18 (95%)	16 (76%)
Karyotype: other	4 (18%)	1 (5%)	5 (24%)

Height SD-score: height standard deviation-score, references healthy Dutch girls.

Table 1 lists the baseline clinical data. The three groups had similar initial characteristics. During 7 years of GH treatment, in 2 girls in group A, in 6 girls in group B, and in 4 girls in

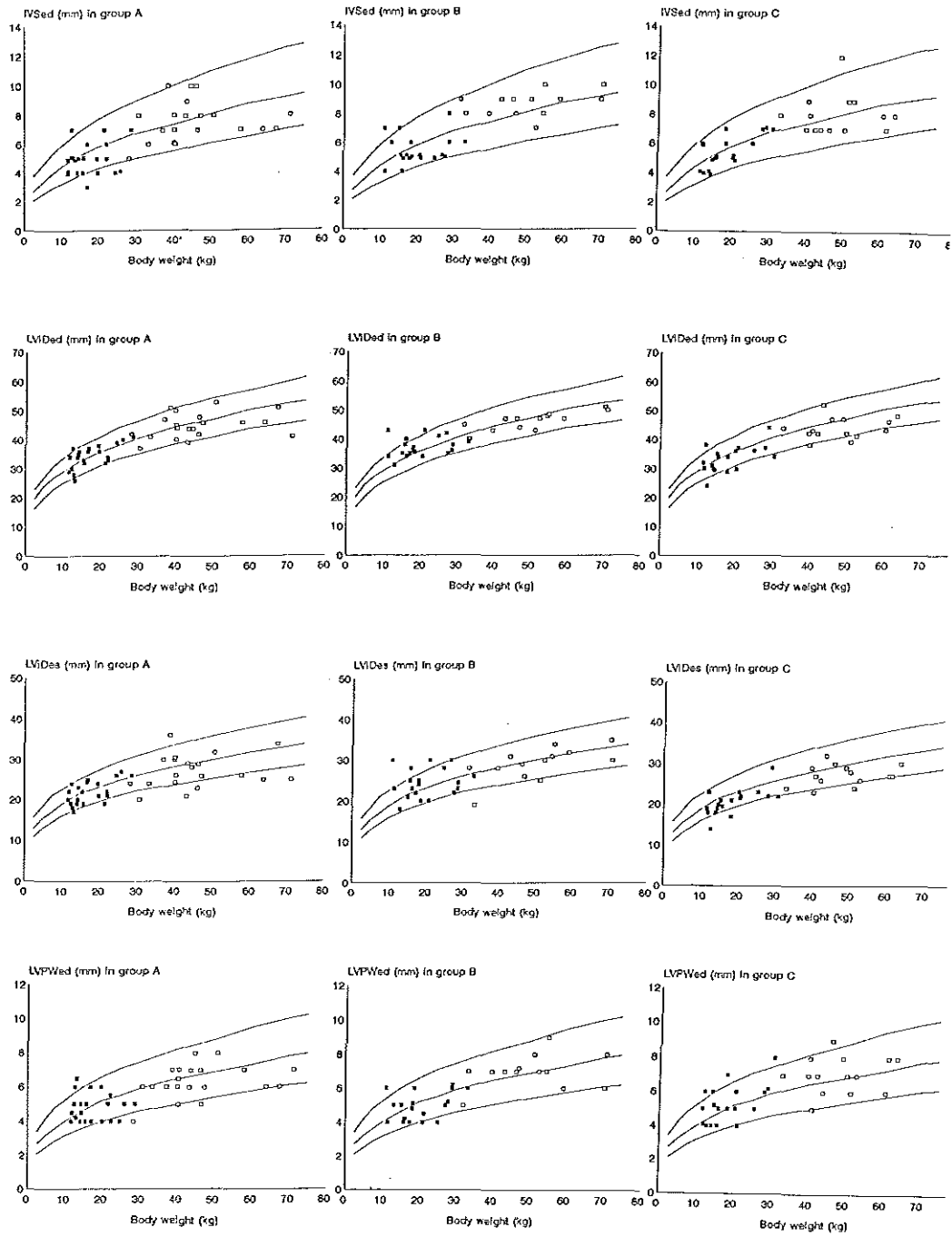

Figure 1. Normal Dutch femal percentiles (P5, P50, and P95) for IVSed, LVIDed, LVIDes, and LVPWed. The individual values before treatment (filled squares) and after 7 years of GH treatment (open squares) in group A, B and C, respectively.

group C, GH treatment was discontinued because of reaching adult height or satisfaction with height achieved. Only data of the period during GH treatment were available for the 7-year analysis.

Figure 1 shows the individual IVSed, LVIDed, LVIDes, and LVPWed before and after 7 years of GH treatment in group A, B and C, respectively. The values were well distributed around the P50 and most values were within the normal range (between P5 and P95).

Table 2 shows the SD-scores for IVSed, LVIDed, LVIDes, and LVPWed before and after 7 years of GH treatment. At baseline, mean SD-scores of the four measurements were close to zero and well within the normal range. Only the SD-score of IVSed was significantly lower than zero, however, the difference was small. After 7 years of GH treatment, the SD-scores of the IVSed and the LVPWed had not significantly changed. The SD-scores of LVIDed and LVIDes showed a significant decrease to values lower than zero, however, well within the normal range. No differences in changes in SD-scores between the GH dosage groups were found.

Table 2. Mean (SD) SD-scores of the echocardiographic data before and after 7 years of GH treatment

	before start of GH treatment				after 7 years of GH treatment			
	group A	group B	group C	group A+B+C	group A	group B	group C	group A+B+C
SD-score								
IVSed	-0.7 (1.3)	-0.3 (1.2)	-0.2 (0.9)	-0.4 (1.2)[1]	-0.2 (1.1)	0.4 (0.7)	0.0 (1.0)	0.0 (1.0)
LVIDed	0.0 (1.1)	0.6 (1.4)	-0.5 (1.4)	0.0 (1.3)	-0.2 (1.2)	-0.2 (0.6)	-0.7 (1.1)	-0.4 (1.0)[1a]
LVIDes	-0.2 (1.1)	0.5 (1.5)	-0.8 (1.1)	-0.2 (1.3)	-0.8 (1.4)	-0.4 (1.2)	-0.9 (0.9)	-0.7 (1.2)[2a]
LVPWed	-0.2 (1.2)	-0.3 (1.2)	0.4 (1.2)	-0.1 (1.2)	-0.4 (1.0)	0.1 (1.0)	0.2 (1.1)	-0.1 (1.0)

Significant differences from zero (1) $p < 0.05$, (2) $p < 0.001$, and significant changes from baseline, (a) $p = 0.005$, are indicated.
SD-score: standard deviation-score, references healthy Dutch girls; IVSed, interventricular septum (end-diastolic); LVIDed, left ventricular internal diameter (end-diastolic); LVIDes, left ventricular internal diameter (end-diastolic); LVPWed, left ventricular posterior wall (end-diastolic).

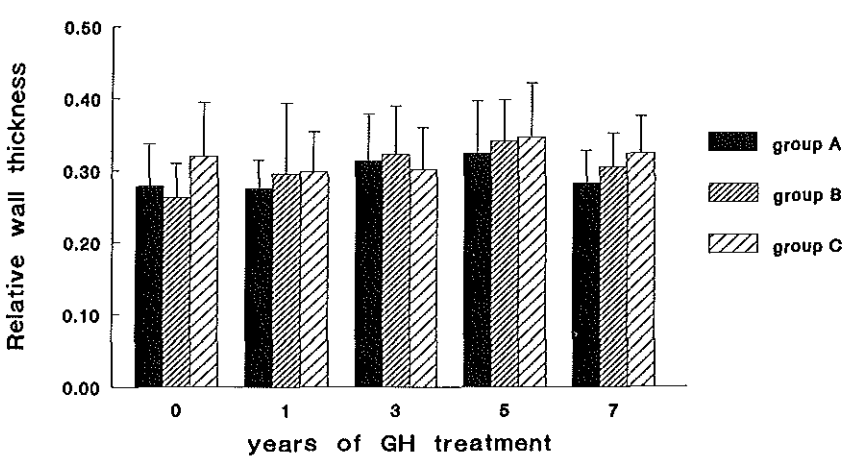

Figure 2. The mean (SD) end-diastolic relative wall thickness (RWT) during 7 years of GH treatment in group A, B, and C, respectively.

Figure 2 shows the RWT in group A, B, and C, respectively. After 7 years of treatment, mean (SD) RWT had not significantly changed from 0.29 (0.07) to 0.30 (0.05)), being comparable with those of healthy controls (0.33 (0.08))[24]. No significant differences in the change in RWT between the GH dosage groups were found.

Table 3 shows the blood pressure (BP) and the SD-scores of the BP before and after 7 years of treatment. At baseline, mean diastolic and systolic BP were within the normal range but significantly higher than in healthy controls. After 7 years, the SD-scores of diastolic BP had significantly decreased, being, however, still significantly higher than zero. The SD-score of systolic BP showed no change after 7 years. No differences in changes in BP were found between GH dosage groups. At baseline diastolic BP or systolic BP was above the 90th age-specific percentile in 23 % and in 28 % of the girls, respectively. After 7 years the percentages were 14 % and 36 %, being not significantly different from baseline values.

Table 3. Mean (SD) blood pressure and SD-scores before and after 7 years of GH treatment

	before start of GH treatment				after 7 years of GH treatment			
	group A	group B	group C	group A+B+C	group A	group B	group C	group A+B+C
Blood pressure								
Diastolic (mm Hg)	68 (8)	70 (8)	64 (11)	67 (9)	71 (8)	71 (12)	65 (9)	69 (10)
Systolic (mm Hg)	110 (14)	106 (11)	101 (15)	106 (14)	116 (15)	117 (22)	112 (14)	115 (17)
SD-score diastolic	0.9 (0.8)	1.0 (0.8)	0.5 (1.1)	0.8 (1.0)[2]	0.5 (0.7)	0.5 (1.1)	-0.1 (0.9)	0.3 (0.9)[1a]
SD-score systolic	1.2 (1.3)	0.8 (0.9)	0.4 (1.4)	0.8 (1.3)[2]	0.8 (1.3)	0.9 (1.8)	0.5 (1.4)	0.7 (1.5)[2]

Significant differences from zero (1) $p < 0.05$, (2) $p < 0.001$, and significant changes from baseline, (a) $p = 0.001$, are indicated.

Discussion

In the present study, we showed that untreated girls with TS without clinically relevant cardiac abnormalities or without a residual gradient after coarctation repair had similar left ventricular dimensions as healthy girls. In contrast, in many girls diastolic as well as systolic blood pressure was higher than the mean values of healthy peers and a considerable percentage of the girls showed values above the 90th percentile. During 7 years of GH treatment, growth of the left ventricle was comparable with that of healthy girls. In addition, the thickness of the LV wall in relation to the internal diameter remained unchanged during GH treatment and no differences between the GH dosage groups were found. Thus, long-term GH treatment did not result in left ventricular hypertrophy, even at higher GH dosages up to 8 IU/m²/day. The SD-score of systolic blood pressure did not change during GH treatment, while the SD-score of diastolic BP showed a decrease. However, after 7 years a considerable percentage of the girls still had a relatively high diastolic or systolic blood pressure.

To our knowledge, only two papers reported the effect of GH treatment on the heart in TS. De Schepper *et al.* described only a small increase in diastolic LVID, being still in the normal range, after one year of GH treatment in a dosage of 25 IU/m²/week[16]. Saenger *et al.* evaluated mixed cross-sectional and longitudinal data of 12 girls with TS who had received ± 1.1 IU GH /kg/week for prolonged periods up to 7 years with or without oxandrolone. He found no signs of left ventricular hypertrophy[17]. Thus, the results of both earlier studies are confirmed by our longitudinal study in a large group of girls receiving GH dosages up to 8 IU/m²/day over a long period of time. In children with idiopathic short stature without GH deficiency using GH doses up

to 40 IU/m²/week similar results were found[25,26]. Thus, treatment with supraphysiological GH dosages does not seem to result in left ventricular hypertrophy during childhood. In contrast, an increased left ventricular wall thickness to values higher than healthy controls was found in GH-deficient adults who were treated with GH[10]. The authors concluded that this disproportionate increase might be an effect of an excessive substitution dose. In addition, preliminary results in adults with dilated cardiomyopathy who were treated with GH for three months showed an increase in myocardial mass and a decrease in the size of the left ventricular chamber[11]. In acromegalic patients, the high GH and IGF-I levels cause a biventricular concentric hypertrophy, which is reversible by correcting GH hypersecretion[27]. Thus, it appears that in contrast to the effects in children, supraphysiological GH dosages seem to have hypertrophic effects on the heart in adults.

In TS an elevated blood pressure during childhood as well as in adulthood has been reported earlier[28-30]. The cause and the mechanism of the elevated BP in girls with TS are not clear, but do not seem to be related to the congenital renal or cardiac structural malformations, with the exception of coarctation of the aorta[28]. Previous short-term studies reported no effect of GH treatment in girls with TS on blood pressure[16,31]. Barton *et al.* described that in contrast to adult subjects, treatment with a high dose of GH in short children is not associated with activation of the renin-angiotensin-aldosterone system. And, as a result, GH therapy in childhood is unlikely to be associated with the increased risk of hypertension seen in adults with GH hypersecretion[32]. These results are in line with our long-term data, showing an elevated blood pressure compared to age-specific reference values before GH treatment and no important change during treatment. Follow-up of these girls is required because data of untreated adults with TS suggest that these girls have also other risk factors for cardiovascular diseases in later life[29].

In our study, left ventricular dimensions and blood pressure were compared with reference values of healthy girls, because no untreated control group was included in this study and no reference values were available for untreated girls with TS. It is unlikely that reference data will become available in the future because in many countries GH treatment is considered the treatment of choice for short stature in TS. Although our baseline values give some information about cardiac dimensions and blood pressure in untreated young girls with TS, we do not exactly know how these values would develop during childhood in untreated girls with TS. Nevertheless, our study showed that long-term GH treatment has no negative effect on the cardiovascular status.

In conclusion, untreated girls with TS without clinically relevant cardiac abnormalities have similar left ventricular dimensions as healthy girls, however, diastolic and systolic blood pressure are moderately elevated. During 7 years of GH treatment growth of the left ventricle is comparable with that of healthy girls. Long-term GH treatment does not result in left ventricular hypertrophy or worsening of the pre-existing relatively high blood pressure in TS, even at higher GH dosages up to 8 IU/m²/day. Continued observation into adulthood is recommended to follow the development of the relatively high blood pressure and to ensure that GH treatment has no negative effect on the heart on the very long-term.

Abbreviations

TS, Turner's syndrome; GH, growth hormone; LV, left ventricular/ventricle; BP, blood pressure; SD, standard deviation; ed, end-diastolic; es, end-systolic; IVS, interventricular septal thickness; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall thickness; RWT, relative wall thickness.

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CHAPTER 5

THE EFFECTS OF GROWTH HORMONE TREATMENT ON CARBOHYDRATE METABOLISM IN GIRLS WITH TURNER SYNDROME

CHAPTER 5.1

CARBOHYDRATE METABOLISM DURING LONG-TERM GROWTH HORMONE TREATMENT AND AFTER DISCONTINUATION OF GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE STUDY

Journal of Clinical Endocrinology and Metabolism, in press.

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Abstract

To assess possible side-effects of growth hormone (GH) treatment with supraphysiological dosages on carbohydrate (CH) metabolism in girls with Turner syndrome (TS) during long-term GH treatment and after discontinuation of GH treatment, the results of oral glucose tolerance tests (OGTTs) and HbA1c measurements were analyzed in 68 girls with TS participating in a randomized dose-response trial. These previously untreated girls, age 2-11 yr, were randomly assigned to 1 of 3 GH dosage groups: group A, 4 IU/m²/day (≈0.045 mg/kg/d); group B, first yr 4, thereafter 6 IU/m²/d (≈0.0675 mg/kg/d); group C, first yr 4, second yr 6, thereafter 8 IU/m²/d (≈0.090 mg/kg/d). After the first 4 yr, girls ≥ 12 yr of age started with 17ß-estradiol 5 μg/kg bw/d for induction of puberty. To assess the effects of long-term high dose GH treatment on CH metabolism, the 7-yr data of the OGTTs in 9 girls of group C were evaluated (group C1). To determine whether the changes in CH metabolism during GH treatment would persist after discontinuation of GH treatment, the data of 28 girls who had reached adult height (group A n=9, group B n=10, group C n=9) were evaluated at baseline, after 4 yr of GH treatment, and 6 months after discontinuation of GH.

Seven-year data of group C1 showed that glucose levels did not significantly change during GH treatment, whereas fasting insulin levels as well as glucose-induced insulin levels increased significantly. The data of the 28 girls who were treated with GH for a mean (SD) period of 85.3 (13.3) months demonstrated that the GH-induced higher insulin levels decreased to values close to or equal to pretreatment values after discontinuation of GH treatment. Changes in CH variables were not significantly related to the GH dose. HbA1c levels never showed an abnormal value. The prevalence of impaired glucose tolerance was low and none of the girls developed diabetes mellitus.

In conclusion, long-term GH treatment with dosages up to 8 IU/m²/d in girls with TS has no adverse effects on glucose levels, but induced higher levels of insulin, indicating relative insulin resistance. The increased insulin levels during long-term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values. Although the reversibility of the effects of long-term GH is reassuring, the consequence of long-term hyperinsulinism is still unknown.

Introduction

Short stature is one of the main features in Turner syndrome (TS) (1,2). Although these girls are not clearly growth hormone-deficient (3), growth hormone (GH) administration

accelerates growth in a dose-dependent way (4-6). In addition, recent papers reported a considerable increase in adult height in girls with TS who started GH treatment at a relatively young age and/or who were treated with supra-physiological GH dosages (6-9).

Insulin resistance and carbohydrate (CH) intolerance have been reported in untreated girls with Turner syndrome (10-12). In addition, in adults with TS who had not received GH treatment in childhood, glucose intolerance, non-insulin- and insulin dependent diabetes mellitus are more common than in healthy women (13,14). Since supra-physiological concentrations of GH in acromegalic patients (15) and in normal adults (16,17) resulted in a decrease in glucose sensitivity to insulin in liver and in extra-hepatic tissues, concern has been expressed regarding possible detrimental effects of long-term treatment with supra-physiological GH dosages in girls with TS. In our previous paper, the 4-yr results of oral glucose tolerance tests (OGTTs) were analyzed in 68 girls with TS participating in a randomized dose-response multicenter trial receiving 4, 6, or 8 IU/m²/day. We showed that 4 yr of GH treatment did not negatively influence glucose levels, whereas insulin levels increased significantly compared to baseline (18). Since these relatively young girls are being treated for many years until adult height is reached, the question arose as to whether the observed GH-induced insulin-resistance would show a further increase during prolonged GH treatment. In addition, it is unknown know whether the insulin-resistance in girls with TS during long-term treatment with supra-physiological GH dosages would decrease to a pretreatment level after discontinuation of GH treatment.

The results of GH treatment on growth were described earlier by our group (19). In the present study, we investigated the effects of GH treatment on CH metabolism in a subgroup receiving GH in a dose up to 8 IU/m²/day during 7 yr of GH treatment. In addition, in girls who had been treated with 4, 6, or 8 IU GH/m²/d and had reached adult height, changes in CH metabolism were evaluated during GH treatment as well as after discontinuation of GH treatment.

Subjects and Methods

Study group and treatment regimens

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH dose-response study in the Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: a chronological age between 2 and 11 yr, height below the 50th percentile for healthy Dutch girls (20), and a 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 GH treatment, and spontaneous puberty (21). 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 center.

After stratification for chronological age and height SD-score for chronological age girls were randomly assigned to group:

A (n=23) receiving 4 IU/m² body surface/day (equivalent to 0.045 mg/kg/d),
B (n=23) receiving 4 IU/m²/d in the first yr, followed by 6 IU/m²/d (≈0.0675 mg/kg/d), or
C (n=22) receiving 4 IU/m²/d in the first yr, 6 IU/m²/d in the second yr, and thereafter 8 IU/m²/d (≈0.090 mg/kg/d).

Biosynthetic human GH (Norditropin®, Novo Nordisk A/S, 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, treatment was stopped

when subjects had grown less than 1 cm over 6 months. However, when girls were satisfied with their height achieved, they elected to stop GH treatment before study criteria for the termination of treatment had been reached. In the first 4 yr of GH treatment, no estrogens for pubertal induction were given to the girls. After 4 yr of GH treatment, estrogen therapy was immediately started in the girls who were older than 12.0 yr of age. The younger girls started estrogen therapy at a yearly visit after reaching the age of 12 yr. In the first 2 yr of estrogen therapy, the girls received 5 μg 17ß-estradiol/kg body weight/day (orally), in the 3rd yr 7.5 μg/kg/d and thereafter 10 μg/kg/d. After discontinuation of GH treatment the dosage of estrogens was further increased depending on the clinical signs of breast development.

Study protocol

Before start of treatment (baseline) and every 3 months after the start of GH treatment, all children were seen at their local hospital for a physical examination including measurements of standing height (H) and weight (W). Height was expressed as SD-score, using references for healthy Dutch girls ($HSDS_{CA}$ (RvW)) (20), as well as references for Dutch-Swedish-Danish references for girls with TS ($HSDS_{CA}$ (DSD)) (2). Body mass index [BMI: weight (kilogram)/height (meter squared)] was expressed as SD-score for sex and chronological age (20).

Glycosylated hemoglobin (HbA_{1c}) was determined yearly. The girls of all 3 GH dosage groups who had reached adult height had undergone an oral glucose tolerance test (OGTT) at baseline, after 4 yr of GH treatment, and 6 months after discontinuation of GH treatment. Additional OGTTs were performed in a random sample of 10 girls from group C (group C1) at 6, 18, 30, 60, and 84 months after start of GH treatment. A single team performed all OGTTs after 3 days of unrestricted diet supplemented with 100 g of carbohydrate (Fantomalt®)) and after overnight fasting. Glucose (1.75 g glucose /kg body weight (maximum 50 g)) was administered orally within 5 minutes. Blood samples were collected at 0, 30, 60, 90, 120, 150, and 180 min and plasma glucose and insulin levels were measured.

To evaluate the overall responses to the oral glucose load, apart from the glucose and insulin levels at the various time-points, 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 (22): the 2-hour 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 corrected for fasting levels during the OGTT (AUCab) 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.

Assays

The plasma glucose level was measured at the local hospital laboratories with automatic analyzers using a hexokinase catalyzed-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 < 20 mU/L). HbA_{1c} levels were measured in one laboratory using an automatic HPLC analyzer (DIAMAT, BioRad, Edgemont, CA, USA). The upper-normal assay limit is less than 6.6%.

Statistical analyses

Results are expressed as mean (SD), unless indicated otherwise. Differences in variables

between time-points were tested by paired Student's t-tests. Differences in the change in values between the GH dosage groups were first tested by a linear trend test to assess a possible dose-dependent effect. In case of a significant result, this was followed by comparisons between two groups with Student's t-tests. Correlations between variables were assessed with Pearson's correlation coefficient. A p-value < 0.05 was considered significant.

Results

Four girls (group A n=1, group B n=2, and group C n=1) dropped out of the study long before reaching adult height because of non-compliance. In all four cases no problems with the CH tolerance were involved in the decision to discontinue the treatment. Data of these four girls were not used for the analysis of long-term GH treatment. Table 1 lists the baseline clinical data of the remaining 9 girls of group C1 which have been followed for 7 yr, as well as those of the 28 girls who have reached adult height. None of these 28 girls belonged to the subgroup C1. While it is obvious that the 9 girls of group C1 were younger than the other 28 girls, just caused by sampling variability, there were no relevant differences in baseline variables between the groups.

Table 1. Mean (SD) baseline data for each treatment group. Karyotype (45,X; other) is expressed in numbers of patients.

	group C1	group A	group B	group C
Number of girls	9	9	10	9
Baseline age (yr)	4.4 (1.7)	8.0 (1.0)	8.6 (1.6)	8.5 (1.3)
Baseline $HSDS_{CA}$(RvW)	-2.3 (1.1)	-2.9 (1.2)	-2.8 (0.7)	-2.8 (0.8)
Baseline $HSDS_{CA}$(DSD)	0.4 (1.1)	0.0 (1.5)	0.2 (0.8)	0.1 (1.0)
Karyotype: 45,X	8	6	9	7
Karyotype: other	1	3	1	2

$HSDS_{CA}$: height standard deviation score for chronological age; RvW: Roede van Wieringen references for healthy Dutch girls; DSD: Dutch-Swedish-Danish references for girls with Turner syndrome.

OGTTs during 7 yr of GH treatment in group C1

At baseline, none of the girls had an abnormal glucose response during OGTT. After 6 and after 18 months of treatment, in one girl impaired glucose tolerance (IGT) was found (glucose levels after 120 min was 8.4 and 9.8 mmol/l, respectively). Thereafter, she had normal OGTTs. None of the girls developed diabetes mellitus. Figure 1 shows the mean glucose and insulin levels during the OGTTs, respectively, at baseline, after 4 yr, and after 7 yr of GH treatment. Table 2 lists the CH variables at the 3 time-points during the study period. Fasting glucose levels and the AUCab for glucose did not significantly change during treatment. Fasting insulin levels increased significantly during the first 4 yr of GH treatment compared to baseline levels (p=0.031), and had increased further after 7 yr of GH treatment resulting in levels significantly higher than after 4 yr of GH treatment (p=0.014). The AUCab for insulin had increased significantly in the first 4 yr of GH treatment (p<0.001) without a further increase thereafter. The ratio insulin/glucose at 30 minutes and 120 minutes, respectively, increased significantly during the first 4 yr of GH treatment (p= 0.003 and p= 0.001, respectively), without significant changes thereafter.

One of the girls started estrogen therapy after 6 yr of GH treatment and another girl had some spontaneous breast development (Tanner breast stage II) after 57 months of GH treatment. Excluding these 2 girls from the analysis, similar patterns in the development in CH variables

during GH treatment were found in this prepubertal group as in the total group of nine girls (data not shown).

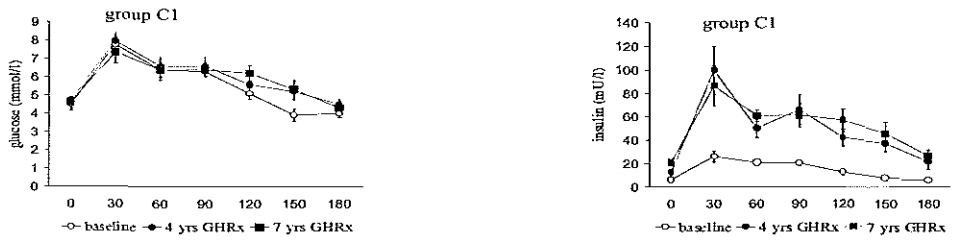

Figure 1. Mean (SE) glucose levels (left panel) and insulin levels (right panel) during the OGTTs, at baseline, after 4 yr of GH treatment, and after 7 yr of GH treatment for group C1.

Table 2. CH variables of the 9 girls of subgroup C1 during 7 yr of GH treatment (0, 4, and 7 yr).

	baseline	4 yr	7 yr
fasting glucose (mmol/L)	4.5 (1.0)	4.7 (0.3)	4.6 (0.9)
AUCab glucose (mmol/L * min)	207 (185)	266 (161)	253 (107)
fasting insulin (mU/L)	6.2 (5.6)	12.3 (6.1)*	20.7 (7.5)*@
AUCab insulin (mU/L * min)	1617 (1115)	7107 (2849)*	6431 (2449)*
ratio insulin/glucose 30' (mU/mmol)	3.2 (1.5)	12.5 (7.8)*	11.7 (6.0)*
ratio insulin/glucose 120' (mU/mmol)	2.3 (1.3)	7.2 (2.8)*	8.8 (3.7)*

Values are give as mean (SD). AUCab, 3-hour area under the curve above fasting levels; * = significant different from baseline levels, $p < 0.05$; @ = significantly different from values at 4 yr of GH treatment, $p < 0.05$.

OGTTs at baseline, after 4 yr, and after discontinuation of GH in 28 girls of group A, B, and C

Figure 2 shows the mean glucose levels during the OGTTs at baseline, after 4 yr of GH treatment, and 6 months after discontinuation of GH treatment in group A (n=9), B (n=10), and C (n=9), respectively. These 28 girls have reached adult height after a mean (SD) duration of GH

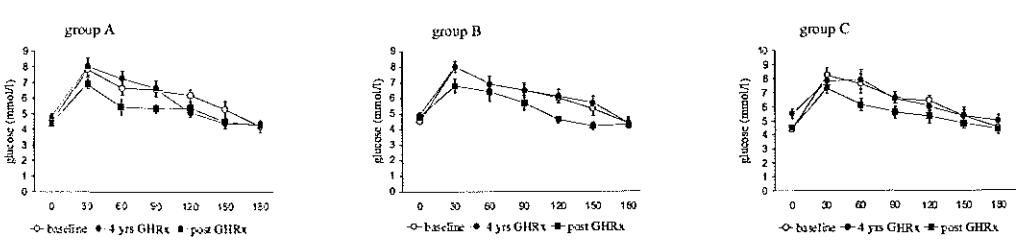

Figure 2. Mean (SE) glucose levels during the OGTTs, at baseline, after 4 yr of GH treatment, and after discontinuation of GH treatment (post GHRx), for group A, B, and C, respectively.

treatment of 85.3 (13.3) months. Before start of treatment, in 2 girls of group C IGT was found (glucose levels after 120 min, 7.9 and 8.0 mmol/l, respectively). Both girls had no IGT during GH treatment, but after discontinuation of treatment, IGT was found again in one of these girls (glucose level after 120 min, 8.0 mmol/l). After 4 yr of GH treatment, another girl of group B had IGT (glucose level after 120 min, 8.4 mmol/l), however, after discontinuation of GH treatment the values of the OGTT of this girl were normal. None of the girls developed diabetes mellitus.

Figure 3 shows the fasting glucose levels and the AUCab for glucose during the study period in group A, B, and C. Fasting glucose levels had significantly increased after 4 yr of GH treatment (p=0.003) and had significantly decreased after discontinuation of GH treatment to baseline levels (p=0.002). The AUCab for glucose was significantly decreased compared to baseline (p=0.013) after 4 yr of GH treatment, without a further significant change after discontinuation of GH treatment. No significant differences in the change in fasting glucose levels or AUCab for glucose between the GH dosage groups were found.

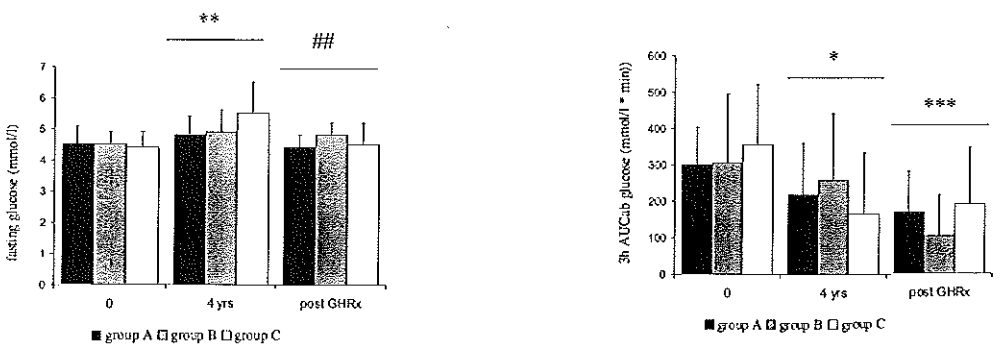

Figure 3. Mean (SD) fasting glucose levels (left panel) and 3-hour area under the curve for glucose (AUCab) (right panel), at baseline, after 4 yr of GH treatment, and after discontinuation of GH treatment (post GHRx). Differences between time-points were tested by combining the values of the 3 GH dosage groups, indicated by "___". Significant differences from baseline (*) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$, and significant changes compared to the values after 4 yr of GH treatment (##) $p < 0.01$ are indicated.

Figure 4 demonstrated the mean insulin levels during the OGTTs for group A, B, and C, respectively. Figure 5 shows that the fasting insulin levels were increased after 4 yr of GH treatment (p<0.001) and decreased after discontinuation of GH treatment (p=0.002). However, fasting insulin levels after discontinuation of GH treatment were still significantly higher than baseline levels (p<0.001). Although there seems to be a trend towards higher fasting insulin levels with higher GH dosages after 4 yr of GH treatment, this was not statistically significant. The AUCab for insulin was significantly increased after 4 yr of treatment (p=0.003) and significantly

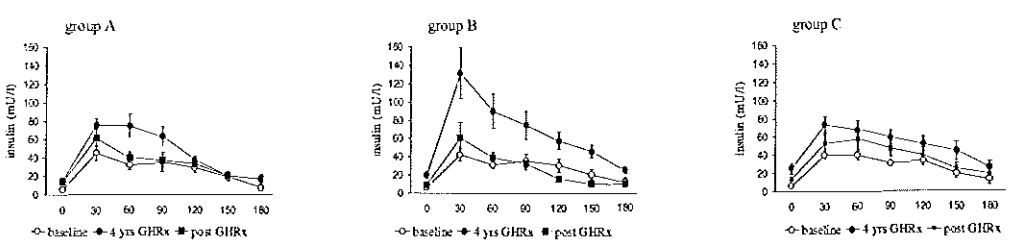

Figure 4. Mean (SE) insulin levels during the OGTTs, at baseline, after 4 yr of GH treatment, and after discontinuation of GH treatment (post GHRx), for group A, B, and C, respectively.

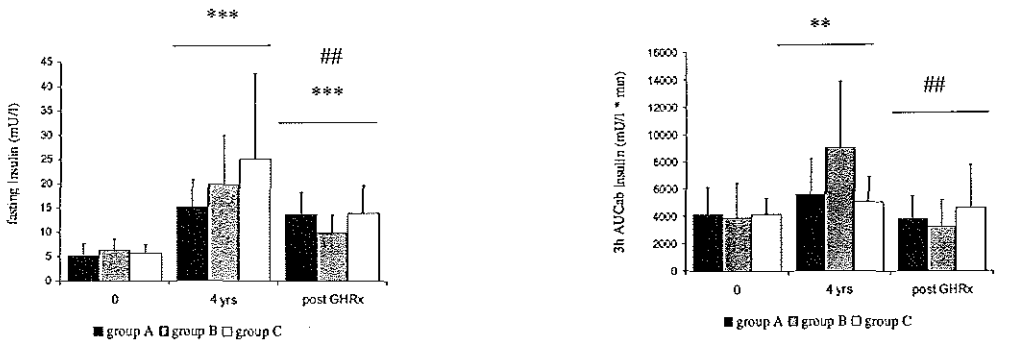

Figure 5. Mean (SD) fasting insulin levels (left panel) and 3-hour area under the curve for glucose (AUCab) (right panel), at baseline, after 4 yr of GH treatment, and after discontinuation of GH treatment (post GHRx). Differences between time-points were tested by combining the values of the 3 GH dosage groups, indicated by "___". Significant differences from baseline (**) $p < 0.01$, and (***) $p < 0.001$, and significant changes compared to the values after 4 yr of GH treatment (##) $p < 0.01$ are indicated.

decreased after discontinuation of GH treatment (p=0.001) to pretreatment levels. No significant differences in the change in AUCab for insulin between the GH dosage groups were found.

Figure 6 shows the ratio's of insulin to glucose at 30 and 120 minutes after the start of the OGTT, respectively. The ratio at 30' had significantly increased during GH treatment (p<0.001) and was followed by a significant decrease after discontinuation of GH treatment (p=0.009). However, the ratio at 30' after discontinuation of GH treatment were still significantly higher than baseline levels (p=0.010). The ratio at 120' was significantly increased after 4 yr of GH treatment compared to baseline (p<0.001), and was followed by a significant decrease after discontinuation of GH treatment to baseline levels (p=0.001). None of the changes in the ratio's were significantly different between the GH dosage groups.

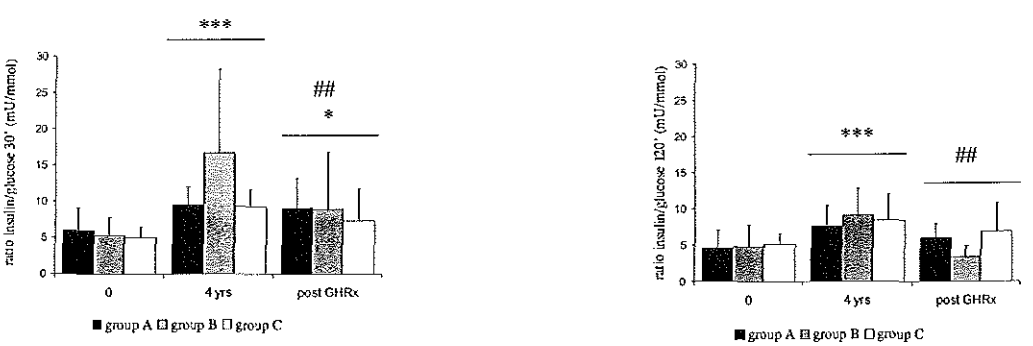

Figure 6. Mean (SD) ratio insulin/glucose levels at 30 min (left panel) and at 120 min (right panel) during the OGTTs, at baseline, after 4 yr of GH treatment, and after discontinuation of GH treatment (post GHRx). Differences between time-points were tested by combining the values of the 3 GH dosage groups, indicated by "___". Significant differences from baseline (*) $p < 0.05$, and (***) $p < 0.001$, and significant changes compared to the values after 4 yr of GH treatment (##) $p < 0.01$ are indicated.

Since obesity is associated with insulin resistance, we analyzed the development of the SD-scores of the body mass index (BMI SD-score) in the 9 girls of group C1 during 7 yr of GH treatment and in the 28 girls of group A, B, and C during and after discontinuation of GH

treatment (Table 3). In the 9 girls of group C1, baseline BMI SD-score was not significantly different from zero. After 4 yr of GH treatment, BMI SD-score had increased significantly (p=0.003) to values higher than zero (p=0.036), without obvious changes thereafter. However, the increment in insulin levels during GH treatment were not correlated with the increment in BMI SD-score. In the 28 girls, at baseline BMI SD-score was significantly higher than zero (p=0.002) and had increased significantly during GH treatment (p=0.043). After discontinuation of GH treatment, the BMI SD-score was significantly higher than after 4 yr of GH treatment (p=0.002). Thus, the BMI SD-score showed a different pattern than the insulin levels during GH treatment and after discontinuation of GH treatment.

Table 3. Mean (SD) BMI-SDS of the 28 girls during and after discontinuation of GH treatment, as well as of the nine girls of group C during 7 yr of GH treatment (0, 4, and 7 yr).

BMI SD-score	group C1	group A	group B	group C
baseline	-0.5 (0.8)	0.6 (1.2)	0.8 (1.0)	0.6 (1.0)
4 yr	0.5 (0.6)	1.0 (1.0)	1.0 (1.0)	0.8 (0.8)
7 yr	0.6 (0.7)	-	-	-
6 mos after stop GHRx	-	1.4 (1.0)	1.3 (0.8)	1.3 (0.9)

BMI, body mass index; GHRx, growth hormone treatment.

HbA_{1c}

All individual HbA_{1c} levels of the total group of 65 girls were within the normal range. HbA_{1c} levels showed an overall significant decrease during 7 yr of GH treatment compared to baseline levels (mean (SD), from 4.8 (0.5) to 4.6 (0.5), p<0.001), without differences between GH dosage groups. In the girls who had discontinued GH treatment, the mean (SD) HbA_{1c} level in the last yr of GH treatment (4.8 (0.4)) was not significantly different from baseline (5.0 (0.4)). Compared to values in the last yr of GH treatment, the HbA_{1c} levels had decreased significantly after discontinuation of GH treatment (from 4.8 (0.4) to 4.4 (0.4), p<0.001).

Discussion

To our knowledge, this is the first paper, describing the CH metabolism during long-term GH treatment, as well as after discontinuation of GH treatment in girls with TS, treated with dosages up to 8 IU/m²/day. Previous studies reported increased insulin levels with or without changes in glucose levels after relatively short periods of GH treatment (23,24). To optimize the GH treatment in girls with TS, in the present study, treatment was started at a younger age and a part of the group of girls received a higher GH dosage than described in other studies. In a previous paper, we demonstrated that in most of these girls, GH treatment resulted in a normalization of height during childhood, as well as a normalization of adult height. Although the growth response was higher in the groups receiving higher GH dosages, the difference in growth response was quite small in proportion to the difference in given GH dose (19). The present study showed that 7 yr of treatment with a high GH dose in these girls did not negatively influence glucose levels, but induced higher levels of insulin. While fasting insulin levels showed a sustained increase during GH treatment, the glucose-induced increase of insulin showed no further increase after 4 yr of treatment. The increase of insulin levels during GH treatment without marked changes in glucose levels indicated relative insulin resistance.

Since our study did not include untreated girls with TS, other factors causing insulin resistance cannot be entirely ruled out. One may speculate that increment in insulin levels were due to estrogens. However, similar patterns in insulin levels were seen in girls who remained prepubertal during the entire study period. Since increased body mass is related to insulin resistance, one may suggest that the increase in insulin resistance during the study period could be explained by the increase in body mass. However, no relationships were found between the increase in insulin levels during GH treatment and the increment in body mass index SD-score. Therefore, we can conclude that GH treatment is the main cause of the observed relative insulin resistance.

In addition, our study demonstrated that the increased insulin levels during long-term GH treatment with 4, 6, or 8 IU/m²/day in 28 girls with TS decreased after discontinuation of GH treatment to values close or equal to pretreatment values. These results are in line with reported preliminary short term data (25,26). In a previous study of our group, GH treatment with 6 IU/m²/day in 18 relatively older girls with TS showed that the increased insulin levels during the entire GH treatment period had decreased significantly at six months after discontinuation of GH treatment (25). Saenger et al, found similar results in nine girls with TS (26).

Although fasting insulin levels decreased after discontinuation of GH treatment, these levels were still significantly higher than pretreatment levels. One can speculate that this is due to a partial irreversibility of the GH-induced insulin resistance. However, compared to the insulin levels during OGTTs in healthy girls reported in a previous paper by Potau *et al.* (27), the insulin levels after discontinuation of GH treatment in the girls in our study are quite comparable. Therefore, it is more likely that these higher fasting insulin levels after discontinuation of GH treatment compared to pretreatment levels may be explained by the normal increase in insulin levels during childhood. Moreover, estrogen treatment and obesity are associated with insulin-resistance. In the present study, at the start of GH treatment and after 4 yr of GH treatment, none of the girls received estrogens, while after discontinuation of GH treatment, all girls were on estrogen treatment. Furthermore, the BMI SD-score after discontinuation of GH treatment was considerably higher than before the start of treatment. Therefore, rather than the GH treatment itself, estrogen treatment and the increased body mass may explain the higher fasting insulin levels after discontinuation of GH treatment compared to pretreatment levels.

Although it is reassuring that the effects of long-term GH treatment on CH metabolism are reversible, the consequences of hyperinsulinism during most of the childhood period on the risk of CH intolerance in adulthood is unknown. Therefore, long-term follow-up of these women is required. In addition, more sophisticated methods such as glucose clamp or the more recent developed minimal model method could led to improved insights into the CH metabolism in these girls during and after long-term GH treatment.

In conclusion, long-term GH treatment with dosages up to 8 IU/m²/day in girls with TS has no adverse effects on glucose metabolism, but induced higher levels of insulin, indicating relative insulin resistance. The increased insulin levels during long-term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values. Although the reversibility of the effects of long-term GH treatment is reassuring, the long-term consequences of hyperinsulinism during childhood are still unknown.

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CHAPTER 5.2

CARBOHYDRATE METABOLISM DURING GROWTH HORMONE TREATMENT AND AFTER DISCONTINUATION OF GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER SYNDROME TREATED WITH ONCE OR TWICE DAILY GROWTH HORMONE INJECTIONS.

Submitted for publication, 1999

CARBOHYDRATE METABOLISM DURING GROWTH HORMONE TREATMENT AND AFTER DISCONTINUATION OF GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER SYNDROME TREATED WITH ONCE OR TWICE DAILY GROWTH HORMONE INJECTIONS.

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Summary

OBJECTIVE To assess possible side-effects of treatment with supraphysiological GH dosages on carbohydrate (CH) metabolism in girls with Turner syndrome (TS) during GH treatment until adult height is reached as well as after discontinuation of GH treatment.

DESIGN In a prospective, randomized injection frequency-response study, the effect of GH treatment in combination with low dose ethinyl oestradiol on CH metabolism was evaluated, comparing twice daily (BID) with once daily (OD) injections of a total GH dose of 6 IU/m²/day until adult height was reached.

PATIENTS Nineteen untreated girls with TS, mean baseline pretreatment age 13.3 (1.7) (range 11.0-17.6) year.

MEASUREMENTS Glucose and insulin concentrations during oral glucose tolerance tests (OGTT) were measured before and during GH treatment, as well as at 6 months after discontinuation of GH treatment.

RESULTS GH treatment was discontinued after a mean duration of treatment of 43 (range: 27 - 57) months. Before treatment, during GH treatment, as well as after discontinuation of GH treatment, in 1 of the 19 girls, but in a different girl at each time point, the glucose response to OGTT after 120 min was above 7.8 mmol/l but below 11.1 mmol/l, indicating impaired glucose tolerance. None of the girls developed diabetes mellitus. Fasting glucose levels did not significantly change during GH treatment, nor after discontinuation of treatment. The 3-hour area under the curve for time-concentration adjusted for fasting levels during the OGTT for glucose showed a significant decrease during GH treatment. In contrast to the glucose levels, GH treatment induced considerably higher insulin levels compared to pretreatment values. After discontinuation of the GH treatment insulin levels decreased to values comparable with pre-treatment levels. None of these observed changes were different between the GH injection frequency groups. The changes in CH variables during and after discontinuation of GH treatment were not related to changes in body mass index.

CONCLUSIONS GH treatment with 6 IU/m²/day in combination with low dose ethinyl oestradiol in girls with TS, age ≥ 11 years, did not negatively influence glucose levels, but induced higher levels of insulin, indicating relative insulin resistance. These changes in insulin levels were independent of the frequency of the administration of the GH injections (once vs twice daily). After discontinuation of GH treatment, insulin values decreased to baseline levels.

Introduction

Growth failure and subsequently short adult stature is one of the main features in Turner

syndrome (TS) (Ranke *et al.*, 1983, Karlberg *et al.*, 1993). Although these girls are not clearly growth hormone-deficient (Wit *et al.*, 1992), growth hormone (GH) administration accelerates growth in a dose-dependent way (Stahnke *et al.*, 1993; Van Teunenbroek *et al.*, 1996; Carel *et al.*, 1998). In addition, recent papers reported a considerable increase in adult height in girls with TS who started GH treatment at a relatively young age and/or who were treated with supra-physiological GH dosages (Haeusler *et al.*, 1996; Nilsson *et al.*, 1996; Carel *et al.*, 1998; Rosenfeld *et al.*, 1998).

GH modulates tissue response to insulin. GH-deficiency increases sensitivity to insulin (Pearson *et al.*, 1973). Supra-physiological concentrations of GH in acromegalic patients (Hansen *et al.*, 1986) and in normal (Bratusch-Marrain *et al.*, 1982, Rizza *et al.*, 1982) and diabetic (Press *et al.*, 1984) adults showed a decrease in glucose sensitivity to insulin both in liver and in extra-hepatic tissues. Diabetogenic effects only occur if compensatory mechanisms fail, e.g. when insulin secretion is deficient.

Previous papers have reported insulin resistance and CH intolerance in untreated girls with Turner syndrome (Polychronakos *et al.*, 1980; Cicognani *et al.*, 1988; Caprio *et al.*, 1991). In addition, in adults with TS who had not received GH treatment in childhood, glucose intolerance, non-insulin- and insulin dependent diabetes mellitus are more common than in healthy women (Gravholt *et al.*, 1998a; Gravholt *et al.*, 1998b). Concern has been expressed regarding possible detrimental effects of treatment with supra-physiological GH dosages on CH metabolism. We published the results of oral glucose tolerance tests (OGTTs) in 19 girls with TS participating in a randomized frequency-response trial receiving 6 IU/m²/day, divided over once or twice daily injections, in combination with low dose ethinyl oestradiol (Van Teunenbroek *et al.*, 1999). We showed that two years of GH treatment did not negatively influence glucose levels. Although the glucose-stimulated increase in insulin levels during the OGTTs above the fasting levels did not significantly change during two years of GH treatment, GH treatment induced higher fasting insulin levels (Sas *et al.*, 1997). Four years of GH treatment in a younger patient group showed increased glucose-stimulated insulin levels as well (Van Teunenbroek *et al.*, 1999). Little is known about changes in the GH-induced insulin-resistance after discontinuation of GH treatment.

The results of GH treatment on final height were described earlier by our group (Sas *et al.*, 1999a). In the present paper, we evaluated possible effects of supraphysiological GH dosages on CH metabolism in these girls during treatment until final height achieved and after discontinuation of GH treatment.

Patients and Methods

Nineteen previously untreated girls with TS were enrolled in a GH frequency-response study in the Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: chronological age of 11 years or older, a bone age of less than 13.5 years, using the Tanner & Whitehouse radius, ulna, short-bones score BA_{RUS} (Tanner *et al.*, 1983), and a Tanner puberty stage B1 (Tanner & Whitehouse *et al.*, 1976). Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, and previous use of drugs that may interfere with GH therapy. Immediately prior to this study, ten girls were enrolled in a randomized 10-week (cross-over design) 24-hour GH-profile study, as described earlier (van Teunenbroek *et al.*, 1993). After a second randomisation, which was carried out independently from the first randomisation, all 19 girls entered the present study immediately after completion of the 10-week design. Written informed consent was obtained from the girls and their parents. The study protocol was approved

by the Ethics Committee of each participating centre.

After stratification for BA_{RUS} and height standard deviation score (SDS) for chronological age ($HSDS_{CA}$ (RvW)) (Roede & Van Wieringen, 1985), the girls were randomly divided into two GH injection frequency groups: the OD group (n=9) received 6 IU GH/m^2 body surface once daily (OD), in the evening, the BID group (n=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime (BID). GH (r-hGH Norditropin®) was injected by a pen injection system (Nordiject®24 Novo Nordisk A/S, Denmark). Compliance was carefully monitored. GH treatment was stopped when height velocity (HV) had decreased to less than 1 cm / 6 months. All girls received, from the start of the 10-week cross-over study 0.05 μg ethinyl oestradiol/kg body weight/day, once daily. After the first 2.25 years of GH treatment, the dose of ethinyl oestradiol was increased to 0.10 μg/kg/d and cyclic progestagen therapy was added. After discontinuation of GH treatment, the dosage of oestrogens was further increased depending on the clinical signs of breast development.

Before start of treatment (baseline) and every three months after the start of GH treatment, all children were seen at their local hospital for a physical examination including measurements of standing height (H) and weight (W). Height was expressed as SDS, using references for healthy Dutch girls ($HSDS_{CA}$ (RvW)) (Roede & Van Wieringen, 1985), as well as references for Dutch-Swedish-Danish references for girls with Turner syndrome ($HSDS_{CA}$ (DSD)) (Karlberg *et al.*, 1993). Body mass index [BMI: weight (kilogram)/height (meter squared)] was expressed in standard deviation score (BMI-SDS) for sex and chronological age (Roede & Van Wieringen, 1985).

At baseline (before start of GH and EE2 treatment), all girls underwent an OGTT. During GH treatment, OGTTs were performed once a year. In addition, before discontinuation of GH treatment, an OGTT was performed if the yearly OGTT was more than 6 months ago. Six months after discontinuation of the GH treatment, the girls underwent an OGTT again. A single team performed all OGTTs after three days of unrestricted diet supplemented with 100 g of carbohydrate (Fantomalt®)) and after overnight fasting. Glucose (1.75 g glucose /kg body weight (maximum 50 g)) was administered orally within 5 minutes. Blood samples were collected at 0, 30, 60, 90, 120, 150, and 180 min and plasma glucose and insulin levels were measured.

To evaluate the overall responses to the oral glucose load, apart from the glucose and insulin levels at the various time-points, 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 (1997): the 2-hour plasma 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 corrected for fasting levels during the OGTT (AUCab) 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.

The plasma glucose level was measured at the local hospital laboratories with automatic analyzers 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 < 20 mU/L).

Results are expressed as mean (SD), unless indicated otherwise. Differences in variables between time-points (pretreatment, in the last year of GH treatment, and 6 months after discontinuation of GH treatment) were tested by paired Student's t-tests. Differences in the change in values between the GH frequency groups were tested by Student's two-sample t-tests. A p-value

< 0.05 was considered significant.

Results

Table 1 lists the baseline clinical data of the 19 girls. There were no relevant differences between the two groups for any of the variables. GH was discontinued after a mean (SD) duration of GH treatment of 42.9 (8.8) (range: 27-57) months.

Table 1. Pretreatment clinical data, expressed as mean (SD).

	OD Group	BID group
Number of girls	9	10
CA (yr)	13.1 (1.7)	13.6 (1.8)
$HSDS_{CA}$ (RvW)	-3.7 (1.3)	-3.1 (1.2)
$HSDS_{CA}$ (DSD)	0.2 (1.1)	1.1 (1.3)
Karyotype:		
45,X	6	8
other	3	2

OD, once daily GH injections; BID, twice daily GH injections; CA, chronological age; $HSDS_{CA}$, height standard deviation score for chronological age; RvW, Roede van Wieringen references for healthy Dutch girls; DSD, Dutch-Swedish-Danish references for girls with Turner syndrome.

Figure 1 shows the mean glucose levels during the OGTTs, at baseline, in the last year of GH treatment, and after discontinuation of GH treatment for the OD group and for the BID group, respectively. In one girl of the BID group, at baseline, as well as after discontinuation of GH treatment, but not during GH treatment, the glucose response to OGTT after 120 min was above 7.8 mmol/l but below 11.1 mmol/l, indicating impaired glucose tolerance (IGT). Three girls (one of the OD group, two of the BID group), at each time-point another girl, showed IGT during GH treatment, whereas after discontinuation of GH treatment no IGT was found in these girls. None of the girls developed diabetes mellitus. Figure 2 shows the fasting glucose levels and the AUCab for glucose during the study period. Fasting glucose levels did not change significantly during and after discontinuation of GH treatment compared to baseline values. During GH treatment, the AUCab for glucose had significantly decreased compared to baseline ($p = 0.020$), without a further change after discontinuation of GH treatment. No significant differences in the change in fasting glucose levels or AUCab for glucose between the GH frequency groups were found.

Figure 3 demonstrates the mean insulin levels during the OGTTs, before, in the last year of GH treatment and after discontinuation of GH treatment for the OD group and for the BID group, respectively. Figure 4 shows the fasting insulin levels and the AUCab for insulin. The fasting insulin levels increased significantly during GH treatment compared to baseline ($p = 0.016$). After discontinuation of GH treatment fasting insulin levels decreased significantly ($p = 0.019$) to values being not significantly different from baseline. The AUCab for insulin showed a statistically non-significant increase during GH treatment, followed by a significant decrease after discontinuation of GH treatment ($p = 0.021$). The increase in AUCab for insulin during GH treatment was due to the increase in the BID group and not in the OD group. All changes in fasting insulin levels and the AUCab for insulin were, however, not significantly different between the GH frequency groups.

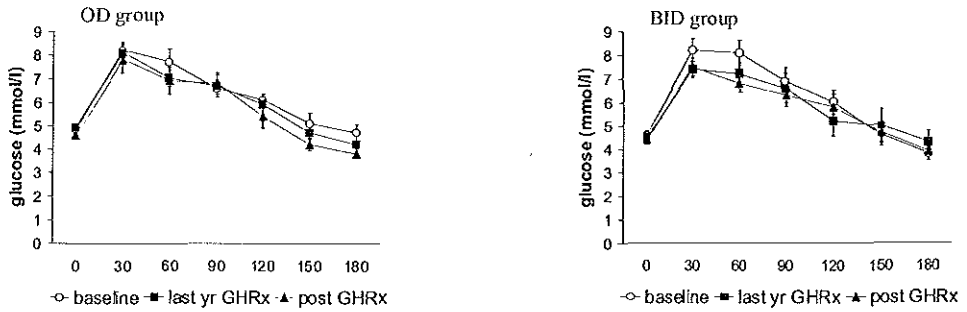

Figure 1. Mean (±SE) glucose levels during the OGTTs, at baseline, in the last year of GH treatment, and after discontinuation of GH treatment (post GHRx), for the OD group and for the BID group, respectively.

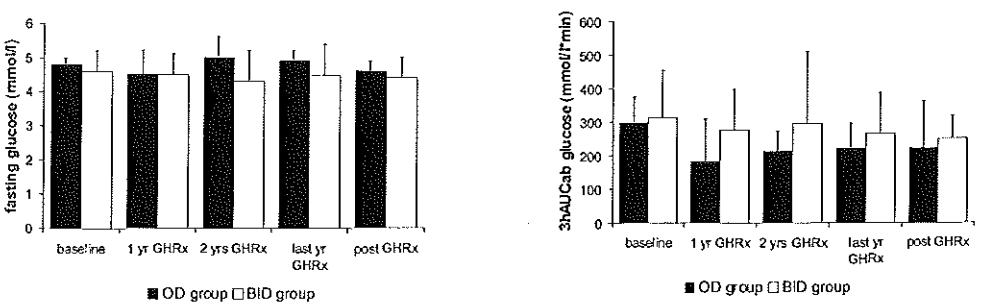

Figure 2. Mean (+SD) fasting glucose levels (left panel) and 3-hour area under the curve for glucose (AUCab) (right panel), at baseline, during GH treatment, and after discontinuation of GH treatment (post GHRx), for the OD group and for the BID group, respectively.

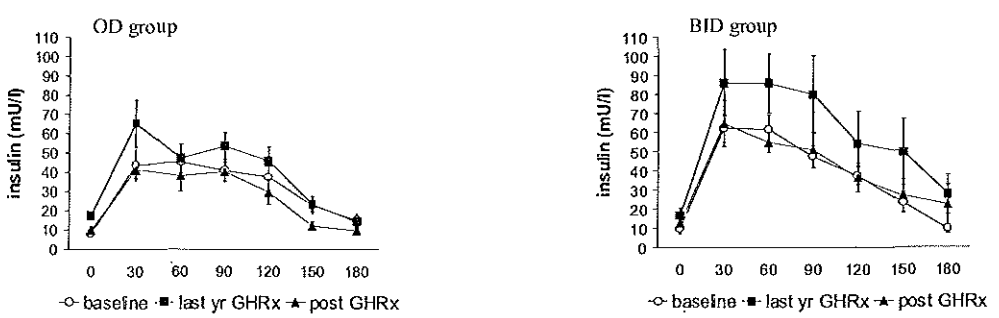

Figure 3. Mean insulin (±SE) levels during the OGTTs, at baseline, in the last year of GH treatment, and after discontinuation of GH treatment (post GHRx), for the OD group and for the BID group, respectively.

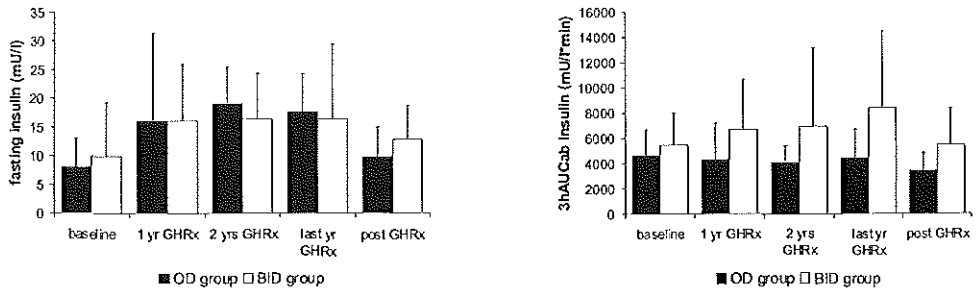

Figure 4. Mean (+SD) fasting insulin levels (left panel) and 3-hour area under the curve for insulin (AUCab) (right panel), at baseline, during GH treatment, and after discontinuation of GH treatment (post GHRx), for the OD group and for the BID group, respectively.

Figure 5 shows the ratio's of insulin to glucose at 30 and 120 minutes after the start of the OGTT, respectively. The ratio at 30' had significantly increased during GH treatment ($p = 0.010$). After discontinuation of GH treatment the ratio at 30' decreased significantly ($p = 0.048$) to values being not significantly different from baseline. The ratio at 120' showed a statistically non-significant increase during GH treatment, and was followed by a significant decrease after discontinuation of GH treatment ($p = 0.006$). None of the changes in the ratio's were significant different between the GH frequency groups.

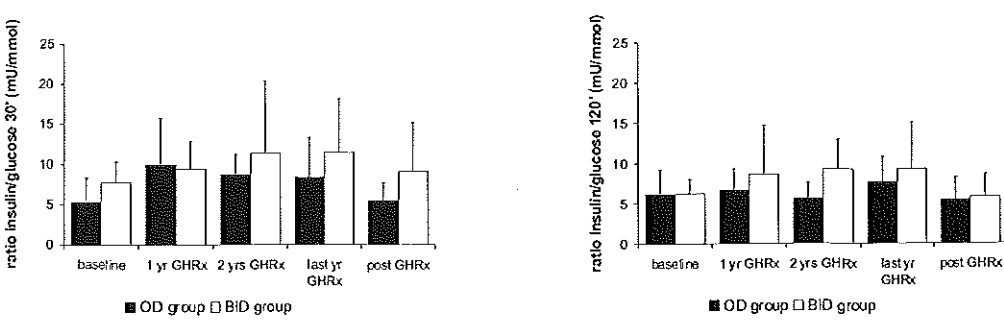

Figure 5. Mean (+SD) ratio's of insulin to glucose at 30 (left panel) and 120 minutes (right panel) after the start of the OGTT, respectively during the study period, for the OD group and for the BID group, respectively.

Since obesity is related to insulin resistance, we analysed the development of the BMI-SDS during the study period (Table 2). At baseline, BMI-SDS was significantly higher than zero. During the first year of GH treatment, BMI-SDS decreased significantly ($p=0.002$). However, thereafter, the BMI-SDS increased significantly ($p< 0.001$) to values in the last year of GH treatment being not significantly different from baseline. After discontinuation of GH treatment, the BMI-SDS showed a significant further increase ($p=0.007$). Thus, the BMI-SDS showed a different pattern than the levels of the CH variables during GH treatment and after discontinuation of GH treatment.

Table 2. Mean (SD) values of the body mass index (BMI) standard deviation-score.

	OD Group	BID group
BMI SD-score		
baseline	1.2 (0.9)	1.1 (1.4)
1 yr GHRx	0.4 (0.9)	0.8 (1.2)
2 yrs GHRx	0.7 (1.0)	0.9 (1.5)
last yr GHRx	1.3 (1.2)	1.1 (1.5)
post GHRx	1.5 (1.1)	1.2 (1.6)

Discussion

Our study showed that GH treatment with 6 IU/m²/day in combination with low dose ethinyl oestradiol in girls with TS, age ≥ 11 years, did not negatively influence glucose levels, but induced higher levels of insulin, indicating relative insulin resistance. After discontinuation of GH treatment, insulin values decreased to baseline levels. The changes in insulin levels were independent of the frequency of the administration of the GH injections (once vs twice daily).

To our knowledge, this is the first paper, describing the CH metabolism before and during treatment with supra-physiological GH dosages, as well as after discontinuation of GH treatment in girls with TS. Some previous papers have reported insulin resistance and CH intolerance in untreated girls with TS (Polychronakos *et al.*, 1980; Cicognani *et al.*, 1988; Caprio *et al.*, 1991), whereas another group did not find abnormalities in CH metabolism in girls with TS (Monti *et al.*, 1997). Due to the absence of a control group of healthy girls, we could not assess the magnitude of the possible insulin resistance in the Turner girls prior to GH treatment and after discontinuation of treatment. The insulin levels in the girls of our study were, however, quite comparable with those of healthy children reported by Potau *et al.* (Potau *et al.*, 1997). In addition, at baseline as well as after discontinuation of treatment, the prevalence of IGT was low, and none of the girls developed diabetes mellitus. Previous studies reported increased insulin levels with or without changes in glucose levels during GH treatment, indicating relative insulin resistance (van Teunenbroek *et al.*, 1999; Wilson *et al.*, 1988). Our present study shows that the increased insulin levels after a mean duration of treatment of 3½ years with a supraphysiological GH dose, decreased to baseline levels after discontinuation of the treatment. Only preliminary data of 9 girls with TS were reported by Saenger *et al.* He found similar results as in our study (Saenger, *et al.*, 1996).

In the present study, all girls started low-dose oestrogens to induce pubertal development. It is known that oestrogen replacement can aggravate preexistent CH intolerance in TS (Polychronakos *et al.*, 1980). Although the low dose of oestrogens resulted in breast development, the dose of oestrogens was far below the adult replacement dose (Sas *et al.*, 1999). Thus, all the OGTTs were performed during low dose oestrogen treatment. Therefore, it is unlikely that the increase in insulin levels during GH treatment and the decrease after discontinuation of GH treatment are caused by the introduction or increase in oestrogen dosage. Besides the effects of oestrogens on CH metabolism, obesity is associated with insulin resistance as well. At baseline, in the last year of GH treatment, as well as after discontinuation of GH treatment, the girls in the present study had a mean BMI-SDS which was significantly higher than zero. However, during GH treatment, no overall increase in BMI-SDS was found. In addition, after discontinuation of GH treatment, the BMI-SDS was significantly higher than during GH treatment. Thus, the BMI-SDS showed a different pattern than the levels of the CH variables during GH treatment and after

discontinuation of GH treatment. Therefore, we can conclude that the changes in CH variables are not related to changes in BMI-SDS.

To optimise the GH treatment in girls with TS, treatment is often started at a younger age than the girls in the present study. A recent paper of our group showed that GH treatment started at an age between 2 and 11 years resulted in a considerable increase in adult height to values within the range for healthy Dutch girls (Sas *et al.*, 1999b). Four years of GH treatment in those girls resulted in a sustained increase in insulin levels during the treatment (Van Teunenbroek *et al.*, 1999). These girls will receive GH treatment until adult height is reached. The question arises as to whether the effects of GH treatment on CH metabolism after such a long period will be reversible as well. In addition, the consequences of long-term hyperinsulinism in younger girls with TS, who will be treated with GH for most of their childhood period, are not known. Although it is reassuring that GH treatment does not negatively influence glucose levels and that the effect on insulin sensitivity is reversible, follow-up of those younger girls are required. More sophisticated methods such as glucose clamp or the more recent developed minimal model method could led to improve insights into the CH metabolism in these girls during and after long-term GH treatment.

In conclusion, GH treatment with 6 IU/m²/day in combination with low dose ethinyl oestradiol in girls with TS, age ≥ 11 years, did not negatively influence glucose metabolism. However, higher levels of insulin were found, indicating relative insulin resistance. After discontinuation of GH treatment, insulin values decreased to baseline levels. The changes in insulin levels were independent of the frequency of the administration of the GH injections (once vs twice daily) or changes in body mass index.

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CHAPTER 6

BONE MINERAL DENSITY AND GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER SYNDROME

CHAPTER 6.1

BONE MINERAL DENSITY BEFORE AND DURING LONG-TERM GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER'S SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE STUDY

Submitted for publication, 1999

BONE MINERAL DENSITY BEFORE AND DURING LONG-TERM GROWTH HORMONE TREATMENT IN GIRLS WITH TURNER'S SYNDROME PARTICIPATING IN A RANDOMIZED DOSE-RESPONSE STUDY.

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Abstract

To assess bone mineral density (BMD) in girls with Turner's syndrome (TS) before and during long-term treatment with growth hormone (GH), longitudinal measurements using phalangeal radiographic absorptiometry were performed in 68 girls with TS, participating in a randomized, dose-response trial. These previously untreated girls, age 2-11 yr, were randomly assigned to 1 of 3 GH dosage groups: group A, 4 IU/m²/day (≈0.045 mg/kg/d); group B, first yr 4, thereafter 6 IU/m²/d (≈0.0675 mg/kg/d); group C, first yr 4, second yr 6, thereafter 8 IU/m²/d (≈0.090 mg/kg/d). In the first 4 yr of GH treatment, no estrogens for pubertal induction were prescribed to the girls. Thereafter, girls started with 17ß-estradiol (5 μg/kg bw/d, orally) when they had reached the age of 12 yr. BMD results were adjusted for bone age and sex, and expressed as SD-scores using reference values of healthy Dutch girls.

At baseline, almost every individual BMD value of cortical bone, as well as of trabecular bone was within the normal range of healthy girls. During 7 yr of GH treatment, BMD SD-score showed a significantly increase to values significantly higher than zero. The increment in BMD SD-score of the cortical bone was significantly higher in group C compared to that of the other two GH dosage groups, whereas no differences between the groups were found for the increment in BMD SD-score of the trabecular bone. The pretreatment bone age was significantly negatively related to the increment in BMD SD-score: the younger the child at baseline, the higher the 7-yr increase in BMD SD-score. We found no significant influence of spontaneous puberty or the use of low dose estrogens in the last 3 yr of the study period on the increment in BMD SD-score during 7 yr of GH treatment.

In conclusion, most untreated young girls with TS have a normal volumetric BMD of the cortical as well as of the trabecular bone compared to healthy girls. During 7 yr of GH treatment with 4, 6, or 8 IU/m²/day, the BMD SD-score increases significantly. Only for the cortical bone was the 7-yr increment in BMD significantly higher in the girls receiving GH doses up to 8 IU/m²/day. Spontaneous puberty or low dose estrogen therapy in the last 3 yr of the 7-yr study period in girls ≥ 12 yr of age does not contribute to the increase in BMD.

Introduction

Short adult stature and ovarian failure are the main features in Turner's syndrome (TS) (1,2). Although girls with TS are not growth hormone-deficient (3), many patients now receive growth hormone (GH) treatment in order to increase adult height. As most girls fail to initiate or progress through puberty, estrogen replacement is required. Our previous paper showed that

starting GH treatment at a relatively young age and beginning low dose estrogen therapy from the age of 12 yr, result in a normalization of height in most girls and pubertal development in conformity with their healthy peers (4).

Despite limited reports of a greater number of fractures during childhood (5) or adulthood (6,7), osteoporosis historically has been described as a feature in TS, because of the frequent observation of radiographic osteopenia and the coarse trabecular pattern of the carpal bones on radiographs (8). An intrinsic bone defect as well as estrogen deficiency may explain these findings. Although more refined methods, such as single- and dual photon absorptiometry and dual energy x-ray absorptiometry (DEXA) has led to improved insights into bone mineral density (BMD) status in TS, these projections methods report areal and not true volumetric BMD, resulting in an underestimation of the true BMD in subjects with short stature. Thus, it would be preferable to measure the actual BMD e.g. by means of quantitative computed tomography (QCT). However, this is an irradiating method and difficult to use in pediatric practice. In contrast, phalangeal radiographic absorptiometry (RA) determines the BMD from a radiograph of the hand, which is already often made for the assessment of bone age (9,10). Several studies in adults have shown that the RA method, using an aluminium wedge in the field of view to correct for differences in exposure and processing variables, is as good in predicting fracture risk as DEXA (11-13).

To assess the volumetric BMD in young girls with TS before and during long-term treatment with recombinant human GH, longitudinal measurements using phalangeal radiographic absorptiometry were performed in 68 girls with TS, participating in a randomized, dose-response trial. The effect on height was described earlier (4). We now report the results of the BMD data over 7 years among 3 GH dosage groups.

Methods

Patients and study design

Sixty-eight previously untreated girls with TS were enrolled in a multicenter GH dose-response study in the Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: a chronological age between 2 and 11 yr, height below the 50th percentile for healthy Dutch girls (14), and a 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 GH treatment, and spontaneous puberty (15). 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 center.

After stratification for chronological age and height standard deviation score for chronological age girls were randomly assigned to group:

A (n=23) receiving 4 IU/m²/day (equivalent to 0.045 mg/kg/day),

B (n=23) receiving 4 IU/m²/d in the first yr, followed by 6 IU/m²/d (≈0.0675 mg/kg/d), or

C (n=22) receiving 4 IU/m²/d in the first yr, 6 IU/m²/day in the second yr, and thereafter 8 IU/m²/d (≈0.090 mg/kg/d).

Recombinant human GH (Norditropin®, Novo Nordisk A/S, 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, treatment was stopped when subjects had grown less than 1 cm over 6 months. However, when girls were satisfied with their height achieved, they elected to stop GH treatment before study criteria for the termination

of treatment had been reached. In the first 4 yr of GH treatment, no estrogens for pubertal induction were prescribed to the girls. After 4 yr of GH treatment, estrogen therapy was started in the girls who were older than 12.0 yr of age; the younger girls started estrogen therapy at a yearly visit after reaching the age of 12 yr. Five μg 17ß-estradiol/kg body weight/day, orally, were given in the first 2 yr, 7.5 μg/kg/d in the third yr and 10 μg/kg/d thereafter. Cyclic progestagen therapy (Duphaston 5 mg/d in the first 14 d of the month) was added after 2 yr of estrogen therapy. If puberty had developed spontaneously (Tanner breast stage $\geq$ 2) during the study period and before start of estrogens, no estrogens were given.

Before the start of treatment (baseline) and subsequently every three months all girls were seen at their local hospital for measurements of height. Height was expressed as SD-score using references of healthy Dutch girls (14). Pubertal stages were assessed according to the method of Tanner (15). Before the start of the GH treatment (baseline), and subsequently once a year, standardized radiographs of the left hand were taken. Bone age was determined by two trained observers according to the Greulich and Pyle method (16).

Radiographic absorptiometry

The standardized radiographs of the left hand (postero-anteriorly, PA) and the left index finger (lateral, LAT) were taken, on a single film using a dedicated cassette (Imation GTU film, α-II Trimax intensifying screens, small 0.6 mm focus, film-focus distance 1.5 m, 45 kV, 16 mAs). Using these two projections it is possible to adjust for soft tissue surrounding the middle phalanx and to assess the volume of this phalanx. Consequently, the BMD as a 'true' volumetric density can be determined. With this technique two measurement sites (region of interest; ROI) are used, the first ROI is at the mid-point of the line between the distal and proximal edge of the phalanx ($BMD_{50\%}$) consisting mainly of cortical bone (approximately 80%), the second ROI is located at the proximal quarter-point of this line ($BMD_{25\%}$), consisting mainly of trabecular bone (approximately 60%) (17). This technique has a short-term in-vivo coefficient of variance of less than 1%, being slightly better than precision figures for the other RA techniques (11,18).

For the application of this technique, two identical linear aluminium wedges were placed within the field of view of each exposure, i.e. one for the hand (PA) and one for the index finger (LAT), all X-rays were obtained in a strict standardized fashion. In-house developed interactive software was used to analyze all films, using a PC equipped with a modular frame grabber (Imaging Technology) in combination with a CCD camera (SWK-31, DIFA, pixel size 37.5 μm x 37.5 μm). Analysis, performed by one operator (AA), consisted of several steps: first the system was calibrated on a daily basis, by scanning a standard radiograph containing an image of the aluminium wedge, to correct for influences of variances in the transmitted light intensity. The second step involved scanning of the aluminium wedges (thickness 0-12 mm), in order to calibrate the intensity-values in the image. Then, the predefined ROIs were semi-automatically scanned in order to determine the distal and proximal edge of the second phalanx on the digitised X-ray image. The software then automatically determined the length and the outer contours of the phalanx and defined the two ROIs being 3mm wide areas. These ROIs were placed on both the PA and LAT films. The software combined a volumetric BMD relative to the aluminium wedge.

Since chronological age may not be indicative of biological development, especially in children whose disorder affects bone maturation, all measured values of $BMD_{50\%}$ and of $BMD_{25\%}$ were adjusted for bone age (16), and expressed as SD-scores (SDS) using reference values of healthy Dutch girls. These reference values were previously described using a piece-wise linear regression analysis ('broken stick' technique). Reference values were only available for girls with

a bone age equal or higher than 5.5 years (10).

Statistical analysis

Results are expressed as mean and standard deviation (SD) or standard error (SE), unless indicated otherwise. Differences between zero and the mean SD-scores at baseline or after 7 yr were tested by one-sample Student's t-tests. Since reference data were only available for girls with a bone age ≥ 5.5 yr, it was not possible to calculate SD-scores of the youngest girls at baseline and in the first years of GH treatment, resulting in missing data. In addition, a number of girls discontinued GH treatment within the 7-yr study period because of reaching adult height causing missing values. Moreover, during the 7-yr study period, for practical and technical reasons we could not obtain standardized x-rays in a few centers anymore. To estimate and test the effect of time, GH dose and other variables on the changes of $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score during the 7-yr study period, we employed repeated measurement analysis of variance (RMANOVA) methods that used all available data (SAS Proc Mixed). In these models, the yearly increase in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score was the dependent variable. To investigate the differences between the 3 treatment groups, the GH dose was entered as a time-dependent categorical independent variable. To test the effect of pretreatment bone age on the changes in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score, pretreatment bone age was added to the model. To assess the influence of spontaneous puberty and the influence of the use of estrogens on the changes in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score, these factors were entered as time-dependent covariates in the RMANOVA model. To investigate the association between the yearly increase in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score with the yearly increase in height SD-score, the latter was entered as a time-dependent covariate in the model as well. A p-value < 0.05 was considered significant.

Results

Three girls, one in each GH dosage group, dropped out of the study long before reaching adult height mainly because of non-compliance. In all three cases no problems with BMD were involved in the decision to discontinue the treatment. Data of these three girls were not used for the analysis of long-term GH treatment.

Table 1. Mean (SD) baseline data for each treatment group. Karyotype (45,X; other) is expressed in number (percentage) of patients.

	group A	group B	group C
Number of girls	22	22	21
Chronological age	6.1 (2.1)	6.7 (2.4)	6.5 (2.4)
Bone age (GP)	5.0 (2.1)	5.5 (2.2)	5.5 (2.3)
Height SD-score	-2.7 (0.9)	-2.4 (1.0)	-2.6 (1.0)
Karyotype: 45,X	18 (82%)	21 (96%)	16 (76%)
Karyotype: other	4 (18%)	1 (4%)	5 (24%)

Height SD-score, height standard deviation-score, references healthy Dutch girls; Bone age (GP): bone age according to the Greulich and Pyle method.

Table 1 lists the baseline clinical data of the remaining 65 girls. The three groups had similar initial characteristics. After 7 yr of GH treatment, 2 girls in group A, 6 girls in group B,

and 4 girls in group C, had discontinued GH treatment because of reaching adult height or satisfaction with height achieved. For these girls, only data of the period during GH treatment were available for the 7-yr analysis. In addition, in 14 girls (n=4 in group A, n=5 in group B, and n=5 in group C) there were missing values in the 7-yr longitudinal data, because of the inability to obtain standardized x-rays in a few centers. Thus, BMD data of 39 girls were available after 7 yr of GH treatment.

After the first 4 yr of GH treatment, 35 of the 65 girls started with estrogen therapy during the subsequent 3 yr, whereas 5 girls did not receive estrogens because of spontaneous puberty. Twenty-five girls were prepubertal during the entire study period and had not started estrogen therapy because they were younger than 12 yr of age.

Figure 1 shows the individual values of $BMD_{50\%}$ and $BMD_{25\%}$, respectively, before and after 7 yr of GH treatment. At baseline, in 30 of the 65 girls, bone age was too low to compare with reference values. Most baseline $BMD_{50\%}$ and $BMD_{25\%}$ values of the remaining 35 girls were within

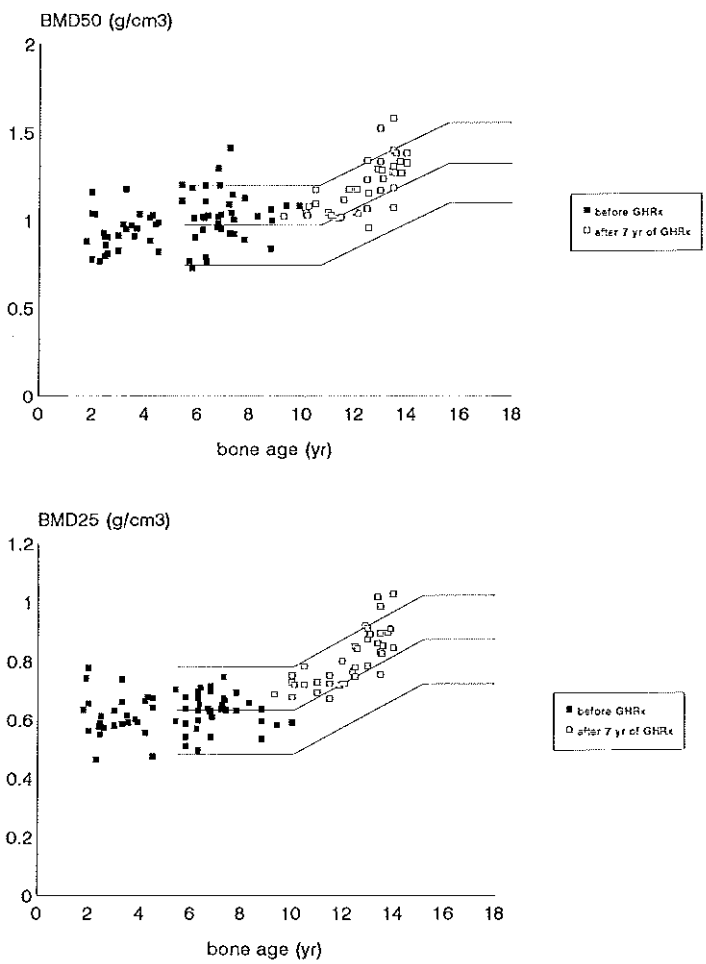

Figure 1. The individual $BMD_{50\%}$ values (upper panel) and the $BMD_{25\%}$ values (lower panel) before (filled squares) and after 7 years of GH treatment (open squares). The mean and range (-2SD to +2SD) of the reference data for healthy girls are indicated.

the normal range and rather well distributed around the mean of healthy girls. Mean (SE) $BMD_{50\%}$ and $BMD_{25\%}$ SD-score were 0.38 (0.22) and -0.04 (0.13), respectively, being not significantly different from zero. After 7 yr of GH treatment, all 39 girls with a BMD assessment had a bone age higher than the required minimal value to compare with the reference data. None of the $BMD_{50\%}$ and BMD25% values after 7 yr were below -2 SD, and most values were higher than the mean of healthy girls. The mean SD-score of the $BMD_{50\%}$, as well as that of the $BMD_{25\%}$ was significantly higher than zero (0.87 (0.15) and 0.95 (0.14), respectively, $p<0.001$).

The 7-yr change in BMD SD-score of the total group of 65 girls was calculated using the repeated measurements analysis. During 7 yr of GH treatment, the mean (SE) SD-score of the $BMD_{50\%}$, as well as that of the $BMD_{25\%}$ had increased significantly compared to baseline values (0.53 (0.16) ($p=0.001$) and 1.08 (0.15) ($p < 0.001$), respectively). The increment in $BMD_{50\%}$ SD-score was significantly higher in group C (0.82) compared to group A (0.45) and group B (0.04) ($p=0.025$), without significant differences between group A and B. No significant differences in the 7-yr increment in $BMD_{25\%}$ SD-score were found between the GH dosage groups (1.04 in group A, 0.99 in group B, and 1.30 in group C, $p=0.46$). Pretreatment bone age was significantly negatively related to the 7-yr increase in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score (ß=-0.200, $p<0.001$ and ß=-0.238, $p=0.001$, respectively). Neither the presence of spontaneous puberty or the use of estrogens, nor the 7-yr increase in height SD-score were significantly related with the yearly increase in $BMD_{50\%}$ SD-score and $BMD_{25\%}$ SD-score during 7 yr of GH treatment.

A total of 11 of 65 girls (3 in group A, 6 in group B, and 2 in group C) had experienced a fracture during the study period : humerus (n=1), wrist (n=6), hand (n=1), leg (n=1), and foot (n=2). The annual incidence rate for fractures of girls with TS was comparable with the published annual incidence of a reference population of healthy girls less than 20 yr of age (25 in 1000 *vs.* 19 in 1000) (19). The mean (SE) SD-score of $BMD_{50\%}$ and $BMD_{25\%}$ obtained on the visit close to the moment of fracture was 0.44 (0.23) and 0.74 (0.23), respectively. None of the values were below -2 SD.

Discussion

In this longitudinal study, volumetric BMD was assessed in relatively young girls with TS before and during long-term treatment with supraphysiological GH dosages. We show that most untreated young girls with TS have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. Our pretreatment data are in line with the data of Ross *et al.* She found normal BMD values of the wrist (mainly cortical bone) and the spine (mainly trabecular bone) assessed by single photon absorptiometry and dual photon absorptiometry, respectively, after adjustment for height age in girls with TS with an age between 4 and 13 years (5). Thus, although skeletal abnormalities suggest an intrinsic bone defect in girls with TS, this defect does not negatively influence BMD during childhood. In addition, the estrogen deficiency during childhood being present in most of the girls, does not result in a low BMD during childhood.

During 7 yr of GH treatment, BMD SD-score showed a significantly increment to values significantly higher than zero. The pretreatment bone age was significantly negatively related to the increment in BMD SD-score: the younger the child at baseline, the better the 7-yr increase in BMD SD-score. We found no significant influence of spontaneous puberty or the use of low dose estrogens, nor the increase in height SD-score on the increment in BMD SD-score during 7 yr of GH treatment. As in most GH trials in TS, in our study no control group of untreated girls with TS was included. Consequently, it is unknown whether the girls would have attained comparable

BMD values after 7 yr if they had not received GH treatment. GH has both direct and indirect actions on bone. In animal models, GH stimulates osteoblast number and function and the production of various bone matrix factors (20). Paracrine activity of osteoblasts stimulates osteoclasts. Bone formation is enhanced preferentially to bone resorption during GH administration (20). In GH-deficient children, an increment in volumetric bone mineral density was found after long-term GH treatment (21,22). Lanes *et al.* assessed the BMD by DEXA in prepubertal girls with TS while on GH treatment. The BMD of the lumbar spine (predominantly trabecular bone), was significant greater than in the control group of healthy children paired for bone age and height, whereas no differences in the BMD of the total body or femoral neck (predominantly cortical bone) were found (23). Neely *et al.* reported BMD in prepubertal and pubertal girls with TS receiving GH treatment. Both lumbar and total body BMD and bone mineral apparent density were greater in the group with TS than in the control subjects matched for height (24). Shaw *et al.* described the BMD of the lumbar spine measured by DEXA in 14 girls with TS who had received GH and/or estradiol or no treatment. These girls had a normal baseline BMD, but a reduction in their BMD SD-score after 2.5 yr. No advantage was found for any form of treatment in optimizing bone mineralization (25). In the present study, we showed that the BMD of the cortical as well as that of the trabecular bone increased significantly during GH treatment. Since we do not know the development of the BMD in untreated girls with TS, we cannot prove that the increment in BMD during treatment is due to GH administration. However, the higher increment in BMD SD-score of the cortical bone in the girls receiving the highest GH dose compared to the other two GH dosage groups suggests that GH treatment may have a positive effect on BMD.

Most girls with TS are estrogen-deficient during both their pre- and postpubertal years. It is well known that estrogens are required to attain a normal peak bone mass which is a major determinant of osteoporosis later in life. Davies *et al.* described the BMD in women with TS and in women with other causes of primary amenorrhoea, using estrogen replacement therapy in most of the cases. Both groups had severe osteopenia compared with healthy controls, after correction for weight and height. Since in most of these women estrogen therapy was not started until young adulthood, it was concluded that estrogen therapy should be instituted from an early age in patients with TS (7). In the present paper we show that low dose estrogens in the 5th, 6th, and/or 7th yr of GH treatment in 35 of the 64 girls did not significantly attribute to the increment in BMD SD-score. A previous study on the effects of increasing estrogen dosages up to adult levels after discontinuation of GH treatment showed, however, that prolonged treatment with higher estrogen dosages than used in the present study does have a beneficial effect on BMD in girls with TS resulting in a normal adult BMD (26).

Only Ross *et al.* did report a higher incidence of wrist fractures compared to healthy girls in a group of untreated prepubertal girls with TS with normal BMD (Ross *et al.*, 1991). In the present study, we showed that the annual incidence rate for fractures in the girls with TS was comparable with that for a reference population of healthy girls. More data are available about the incidence of osteoporotic fractures in adult with TS. Davies *et al.* showed a significantly higher frequency of fractures in women with TS than in a control group. Half of the fractures occurred at typical osteoporotic sites (wrist, spine, femoral neck) (7). In addition, on the basis of data from a large database of Turner women, Gravholt *et al.* reported an increased incidence of osteoporosis and fractures (6). Although insufficient estrogen therapy has a role in osteopenia, other factors, such as an intrinsic bone defect, may contribute to the increased risk of fractures in later life.

In conclusion, most untreated young girls with TS have a normal volumetric BMD of the

cortical, as well as of the trabecular bone compared to healthy girls. During 7 yr of GH treatment with 4, 6, or 8 IU/m²/day, the BMD SD-score increases significantly. Only for the cortical bone was the 7-yr increment in BMD significantly higher in the girls receiving GH doses up to 8 IU/m²/day. Spontaneous puberty or low dose estrogen therapy in the last 3 yr of the 7-yr study period in girls ≥ 12 yr of age did not significantly contribute to the increase in BMD. Since these girls have not attained their peak bone mass yet, follow-up of these girls into adulthood is required.

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CHAPTER 6.2

A LONGITUDINAL STUDY ON BONE MINERAL DENSITY UNTIL ADULTHOOD IN GIRLS WITH TURNER'S SYNDROME PARTICIPATING IN A GROWTH HORMONE INJECTION FREQUENCY-RESPONSE TRIAL

Submitted for publication, 1999

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Summary

OBJECTIVE The aim of this study was to assess the volumetric bone mineral density (BMD) in girls with Turner's syndrome (TS) before and during growth hormone (GH) treatment in combination with low dose oestrogens as well as three years after discontinuation of GH treatment.

DESIGN In a prospective, randomized injection frequency-response study, the effect of GH treatment in combination with low dose ethinyl oestradiol (starting with 0.05 μg /kg/day), on BMD was evaluated, comparing twice daily (BID) with once daily (OD) injections of a total GH dose of 6 IU/m²/day until adult height was reached. After discontinuation of GH treatment, the dosage of oestrogens was further increased to adult supplementation levels.

PATIENTS Nineteen untreated girls with TS, mean (SD) baseline pretreatment age 13.3 (1.7) (range 11.0-17.6) year.

MEASUREMENTS Before and during GH treatment, measurements of volumetric BMD were performed using phalangeal radiographic absorptiometry. In addition, the BMD measurements were repeated three years after discontinuation of GH treatment. BMD results were adjusted for bone age and sex, and expressed as SD-scores (SDS) using reference values of healthy Dutch girls.

RESULTS At baseline, most individual BMD values of cortical bone as well as those of trabecular bone were within the normal range of healthy girls. However, the mean BMD SDS of the trabecular bone was significantly lower than zero. During treatment, the BMD SDS showed a significantly increment to values equal or higher than zero after mean (SD) GH treatment period of 36.6 (7.5) months. The increase in BMD of the cortical bone was significantly higher in the OD group than in the BID group. The BMD SDS in the last year of GH treatment was not significant different between the two injection frequency groups. Three years after discontinuation of GH treatment, the BMD values had increased further similar as in healthy girls, resulting in BMD values all within normal range or even higher.

CONCLUSIONS Most untreated girls with TS, age ≥ 11 years, have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. During GH treatment with 6 IU/m²/day in combination with low dose oestrogens, the BMD SDS increases significantly. After discontinuation of GH treatment and the use of oestrogens in an adult dosage, the BMD was as high as in young healthy women.

Introduction

Short adult stature and ovarian failure are the main features in Turner's syndrome (TS)

(Ranke et al, 1983; Karlberg *et al.*, 1993). Although girls with TS are not clearly growth hormone-deficient (Wit *et al.*, 1992), many patients now receive growth hormone (GH) treatment in order to increase adult height. As most girls fail to initiate or progress through puberty, oestrogen replacement for induction of puberty is usually started between 12 and 16 years of age.

Despite only limited reports of a greater number of fractures during childhood (Ross *et al.*, 1991) or adulthood (Davies *et al.*, 1995; Gravholt *et al.*, 1998), osteoporosis historically has been described as a feature in TS, because of the frequent observation of radiographic osteopenia and the coarse trabecular pattern of the carpal bones on radiographs (Bercu *et al.*, 1976). An intrinsic bone defect, as well as oestrogen deficiency may explain these findings.

Although more refined methods, such as single- and dual photon absorptiometry and dual energy x-ray absorptiometry (DEXA) has led to improved insights into bone mineral density (BMD) status in TS, these projections methods report areal and not true volumetric BMD, resulting in an underestimation of the true BMD in subjects with short stature. Thus, it would be preferable to measure the actual BMD e.g. by means of quantitative computed tomography (QCT). However, this is an irradiating method and difficult to use in paediatric practice. In contrast, phalangeal radiographic absorptiometry (RA) determines the BMD from a radiograph of the hand, which is already often made for the assessment of bone age (Trouerbach *et al.*, 1987; van Rijn *et al.*, 1999). Several studies in adults have shown that the RA method, using an aluminium wedge in the field of view to correct for differences in exposure and processing variables, is as good in predicting fracture risk as DEXA. (Ravn *et al.*, 1996; Grampp *et al.*, 1997; Takada *et al.*, 1997).

We earlier described the results of GH treatment on final height in 19 girls with TS, receiving 6 IU recombinant human GH/m²/day, divided over once (OD) or twice daily (BID) injections, in combination with low dose ethinyl oestradiol (Sas *et al.*, 1999). In the present paper, we evaluated the longitudinal measurements of the volumetric BMD in these girls before and during treatment using phalangeal RA. In addition, the BMD measurements were repeated three years after discontinuation of GH treatment, while these girls receive an adult oestrogen supplementation dose.

Methods

Patients and study design

Nineteen previously untreated girls with TS were enrolled in a GH frequency-response study in the Netherlands. The diagnosis was confirmed by lymphocyte chromosomal analysis. Inclusion criteria were: chronological age of 11 years or older, a bone age of less than 13.5 years, using the Tanner & Whitehouse RUS bone age (BA_{RUS}) (Tanner *et al.*, 1983), and a Tanner puberty stage B1 (Tanner & Whitehouse, 1976). Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure due to other disorders or emotional deprivation, hydrocephalus, and previous use of drugs that may interfere with GH therapy. Immediately prior to this study, ten girls were enrolled in a randomized 10-week (cross-over design) 24-hour GH-profile study, as described earlier (van Teunenbroek *et al.*, 1993). After a second randomisation, which was carried out independently from the first randomisation, all 19 girls entered the present study immediately after completion of the 10-week design. Written informed consent was obtained from the girls and their parents. The study protocol was approved by the Ethics Committee of each participating centre.

After stratification for BA_{RUS} and height standard deviation score (SDS) for chronological age (Roede & Van Wieringen, 1985), the girls were randomly divided into two GH injection

frequency groups: the OD group (n=9) received 6 IU GH/m² body surface once daily (OD), in the evening, the BID group (n=10) received the same total GH dose divided in one-third in the morning and two-thirds at bedtime (BID). GH (recombinant-human GH, Norditropin®) was injected by a pen injection system (Nordiject®24 Novo Nordisk A/S, Denmark). Compliance was carefully monitored. GH treatment was stopped when height velocity had decreased to less than 1 cm / 6 months. All girls received, from the start of the 10-week cross-over study 0.05 μg ethinyl oestradiol/kg body weight/day, once daily. After the first 2.25 years of GH treatment, the dose of ethinyl oestradiol was increased to 0.10 μg/kg/d and cyclic progestagen therapy was added. After discontinuation of GH treatment the dosage of oestrogens was further increased to adult levels.

Before the start of treatment (baseline) and subsequently every three months all girls were seen at their local hospital for measurements of height and weight. Height was expressed as SDS using references of healthy Dutch girls (Roede & Van Wieringen, 1985). Pubertal stages were assessed according to Tanner (Tanner & Whitehouse, 1976). Bone age was determined by two trained observers according to the Greulich and Pyle method ((Greulich & Pyle, 1959) as well as to the Tanner & Whitehouse RUS bone age method (Tanner *et al.*, 1983). Before start of oestrogen or GH treatment ('baseline'), and subsequently once a year, standardized radiographs of the left hand were taken, until GH treatment was discontinued. After discontinuation of GH treatment, the most recent obtained yearly radiograph during GH treatment was considered the BMD measurement in the last year of GH treatment ('last year of GHRx'). Between two and three years after discontinuation of GH treatment a standardized radiograph of the left hand was taken again ('3 years post GHRx').

Radiographic absorptiometry

The standardized radiographs of the left hand (postero-anteriorly, PA) and the left index finger (lateral, LAT) were taken, on a single film using a dedicated cassette (Imation GTU film, α-II Trimax intensifying screens, small 0.6 mm focus, film-focus distance 1.5 m, 45 kV, 16 mAs). Using these two projections it is possible to adjust for soft tissue surrounding the middle phalanx and to assess the volume of this phalanx. Consequently, the BMD as a 'true' volumetric density can be determined. With this technique two measurement sites (region of interest; ROI) are used, the first ROI is at the mid-point of the line between the distal and proximal edge of the phalanx ($BMD_{50\%}$) consisting mainly of cortical bone (approximately 80%), the second ROI is located at the proximal quarter-point of this line ($BMD_{25\%}$), consisting mainly of trabecular bone (approximately 60%)(Trouerbach *et al.*, 1991). This technique has a short-term in-vivo coefficient of variance of less than 1%, being slightly better than precision figures for the other RA techniques (Yates *et al.*, 1995; Ravn *et al.*, 1996) .

For the application of this technique, two identical linear aluminium wedges were placed within the field of view of each exposure, i.e. one for the hand (PA) and one for the index finger (LAT), all X-rays were obtained in a strict standardized fashion. In-house developed interactive software was used to analyse all films, using a PC equipped with a modular frame grabber (Imaging Technology) in combination with a CCD camera (SWK-31, DIFA, pixel size 37.5 μm x 37.5 μm). Analysis, performed by one operator (AA), consisted of several steps: first the system was calibrated on a daily basis, by scanning a standard radiograph containing an image of the aluminium wedge, to correct for influences of variances in the transmitted light intensity. The second step involved scanning of the aluminium wedges (thickness 0-12 mm), in order to calibrate the intensity-values in the image. Then, the predefined ROIs were semi-automatically scanned in order to determine the distal and proximal edge of the second phalanx on the digitised

X-ray image. The software then automatically determined the length and the outer contours of the phalanx and defined the two ROIs being 3mm wide areas. These ROIs were placed on both the PA and LAT films. The software combined a volumetric BMD relative to the aluminium wedge.

Since chronological age may not be indicative of biological development, especially in children whose disorder affects bone maturation, all measured values of $BMD_{50\%}$ and of $BMD_{25\%}$ were adjusted for bone age (Greulich & Pyle, 1959), and expressed as SDS using reference values of healthy Dutch girls. These reference values were previously described using a piece-wise linear regression analysis ('broken stick' technique) (Van Rijn *et al.*, 1999).

Statistical analysis

Results are expressed as mean (SD), unless indicated otherwise. Differences in variables between baseline values and last year of GHRx values or 3 years post GHRx values were tested by paired Student's t-tests. SD-scores were compared with zero by one-sample Student's t-tests. Differences in the change in values between the GH frequency groups were tested by Student's t-tests. Correlations between variables were assessed with Pearson's correlation coefficient. A p-value < 0.05 was considered significant.

Results

Table 1 lists the baseline clinical data of the 19 girls. There were no relevant differences between the two groups for any of the variables. GH was discontinued after a mean (SD) duration of GH treatment of 42.9 (8.8) (range: 27-57) months, but the last yearly BMD measurement during GH treatment was done after a mean (SD) duration of GH treatment of 36.6 (7.5) (range: 24-48) months. At 3 years post GHRx, the standardized radiographs of 15 (n=7 in the OD group, n=8 in the BID group) of the 19 girls were available for the determination of the BMD.

Table 1 Pretreatment clinical data, expressed as mean (SD).

	OD Group	BID group
Number of girls	9	10
Chronological age	13.1 (1.7)	13.6 (1.8)
Bone age (GP)	11.4 (0.6)	11.7 (1.0)
Height SD-score	-3.7 (1.3)	-3.1 (1.2)
Karyotype:		
45,X	6	8
other	3	2

OD: once daily GH injections; BID: twice daily GH injections; Bone age (GP): bone age according to the Greulich and Pyle method; Height SD-score, height standard deviation-score, references healthy Dutch girls.

Figure 1 shows all individual values of $BMD_{50\%}$ and $BMD_{25\%}$, respectively, before GH and oestrogen treatment (baseline), in the last year of GHRx, and at 3 years post GHRx (OD and BID group combined). Most baseline $BMD_{50\%}$ values were within the normal range and rather well distributed around the mean values of healthy girls. Although most baseline $BMD_{25\%}$ values were within the normal range as well, 13 of the 19 girls had a $BMD_{25\%}$ below the mean of the reference values of healthy girls. In the last year of GH treatment, the $BMD_{50\%}$ and $BMD_{25\%}$ values had increased and at 3 years post GHRx, all values were above -2 SD.

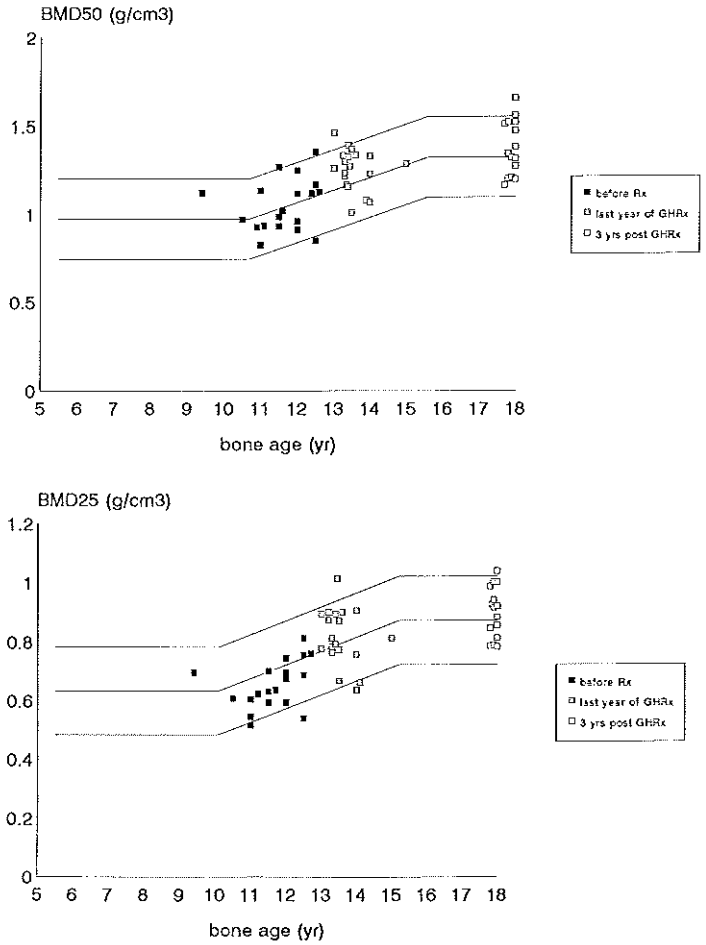

Figure 1. The individual $BMD_{50\%}$ values (upper panel) and the $BMD_{25\%}$ values (lower panel) for bone age, before (filled squares), in the last year of GH treatment (grey squares), and 3 years after discontinuation of GH treatment (open squares). The mean and range (-2SD to +2SD) of the reference data for healthy girls are indicated.

Table 2 shows the SD-scores (SDS) for $BMD_{50\%}$ and $BMD_{25\%}$ at baseline (n=19), in the last year of GHRx (n=19), and 3 years post GHRx (n=15). At baseline, only the $BMD_{25\%}$ SDS was significantly lower than zero. In the last year of GHRx, the $BMD_{50\%}$ SDS, as well as the $BMD_{25\%}$ SDS had increased significantly compared with baseline values ($p = 0.001$ and $p < 0.001$, respectively). The increment in $BMD_{50\%}$ SDS during GH treatment was significantly higher in the OD group than in the BID group ($p = 0.021$), whereas the $BMD_{50\%}$ SDS in the last year of GHRx was not significant different between the two injection frequency groups. In the last year of GHRx, $BMD_{50\%}$ SDS was significantly higher than zero ($p = 0.018$), whereas the $BMD_{25\%}$ SDS was not significantly different from zero. At 3 years post GHRx, the $BMD_{25\%}$ SDS had significantly increased ($p=0.007$), whereas the $BMD_{50\%}$ SDS had not significantly changed compared to the values in last year of GHRx. The $BMD_{50\%}$ SDS, as well as $BMD_{25\%}$ SDS were both not significant different from zero, but significantly higher than baseline values ($p < 0.001$ and $p = 0.04$, respectively). No differences in BMD SDS were found between the OD and BID

group at 3 years post GHRx. The increase in BMD SDS during GH treatment was not significantly correlated with baseline bone age nor with the duration of GH treatment.

Table 2. Mean (SD) SD-scores of the $BMD_{50\%}$ and $BMD_{25\%}$.

	before start of GH and E2 Rx			last year of GHRx			3 years post GHRx		
	OD group	BID group	group OD+BID	OD group	BID group	group OD+BID	OD group	BID group	group OD+BID
No. of girls	9	10	19	9	10	19	7	8	15
SD-score									
$BMD_{50\%}$	-0.1 (1.1)	0.2 (1.4)	0.1 (1.2)	0.7 (1.1)	0.6 (1.2)[a]	0.7 (1.1)[2a]	0.5 (1.1)	0.4 (1.6)	0.5 (1.3)[b]
$BMD_{25\%}$	-1.0 (0.7)	-0.4 (1.2)	-0.7 (1.0)[1]	0.2 (1.3)	0.4 (1.6)	0.3 (1.4)[*]	0.4 (0.9)	0.4 (1.4)	0.4 (1.1)[2*]

Significant differences from zero: (1) $p = 0.007$, (2) $p = 0.018$; significant changes from baseline: (a) $p \leq 0.001$, (b) $p = 0.04$; significant changes compared to the last year of GH treatment: (*) $p = 0.007$; significant difference in the change from baseline between the OD and BID group: (&) $p = 0.021$. Rx, treatment; SD-score: standard deviation-score, references healthy Dutch girls; BMD, bone mineral density; $BMD_{50\%}$, bone mineral density at the measurement site at the mid-point of the line between the distal and proximal edge of the phalanx consisting mainly of cortical bone (approx. 80%); $BMD_{25\%}$; bone mineral density at the measurement site at the proximal quarter-point of this line, consisting mainly of trabecular bone (approx. 60%).

Discussion

In this study, we assessed volumetric BMD in girls with TS before and during GH treatment with 6 IU/m²/day in combination with low dose oestrogens, as well as in young adulthood receiving estrogen supplementation only. We show that most untreated girls with TS have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. However, the mean BMD SDS of the trabecular bone was significantly lower than zero. Ross *et al.* found normal BMD values of the wrist (mainly cortical bone) and the spine (mainly trabecular bone) assessed by single photon absorptiometry and dual photon absorptiometry, respectively, after adjustment for height age in girls with TS with an age between 4 and 13 years (Ross *et al.*, 1991). Compared to those girls, the girls of our study were older at the start of treatment, age 11.0-17.6 year. Bone turnover is higher in trabecular bone than in cortical bone (Dempster & Lindsay, 1993). In addition, in postmenopausal women the change in BMD is faster in trabecular bone than in cortical bone (Riggs *et al.*, 1981). Therefore, the lower pretreatment BMD of the trabecular bone of the girls in our study might be explained by oestrogen deficiency at a pubertal age.

During GH treatment in combination with low dose oestrogens, the BMD SDS increased significantly. The increase in BMD of the cortical bone during the treatment was higher in the OD group than in the BID group, whereas no differences between the two GH injection frequency groups were found in the last BMD measurement during GH treatment. After 3 years post GHRx, in which the dose of oestrogens was increased to adult levels, BMD had increased markedly and was not significantly different compared to healthy controls and still higher compared to the values before the start of GH and oestrogens.

Since a control group of girls with TS without GH treatment was not included in our study, it is unknown whether the girls would have attained comparable BMD values if they had not received GH treatment. GH has both direct and indirect actions on bone. In animal models, GH stimulates osteoblast number and function and the production of various bone matrix factors

(Inzucchi & Robbins, 1994). Paracrine activity of osteoblasts stimulates osteoclasts. Bone formation is enhanced preferentially to bone resorption during GH administration (Inzucchi & Robbins, 1994). In GH-deficient children, an increment in volumetric BMD was found after long-term GH treatment (Saggese, *et al.* 1996, Boot *et al.*, 1997). Lanes *et al.* assessed the BMD by DEXA in prepubertal girls with TS while on GH treatment. The BMD of the lumbar spine (predominantly trabecular bone), was significant greater than in the control group of healthy children paired for bone age and height, whereas no differences in the BMD of the total body or femoral neck (predominantly cortical bone) were found (Lanes *et al.*, 1995). Neely *et al.* measured BMD in prepubertal and pubertal girls with TS receiving GH treatment. Both lumbar and total body BMD and bone mineral apparent density were greater in the group with TS than in the control subjects matched for height (Neely *et al.*, 1993). Shaw *et al.* described the BMD of the lumbar spine measured by DEXA in 14 girls with TS who had received GH and/or oestradiol or no treatment. These girls had a normal baseline BMD, but a reduction in their BMD SDS after 2.5 years. No advantage was found for any form of treatment in optimizing bone mineralisation (Shaw *et al.* 1997).

Most girls with TS are oestrogen-deficient during both their pre- and postpubertal years. It is well known that oestrogens are required to attain a normal peak bone mass which is a major determinant of osteoporosis later in life. Davies *et al.* described the BMD in women with TS and in women with other causes of primary amenorrhoea, using oestrogen replacement therapy in most of the cases. Both groups had severe osteopenia compared with healthy controls, after correction for weight and height. Since in most of these women oestrogen therapy was not started until young adulthood, it was concluded that oestrogen therapy should be instituted from an early age on (Davies *et al.*, 1995). In the present study with relatively older girls with TS, low dose oestrogens was started at the beginning of the study period to induce pubertal development. Since oestrogen therapy was accompanied by GH treatment, the effect of low-dose oestrogens on BMD could not be distinguished from the effect of GH treatment. However, a large part of the increment in BMD from childhood to adulthood took place after discontinuation of GH treatment in the period in which the oestrogen dose was increased to adult levels. Thus, it appears that oestrogens do have a beneficial effect on BMD in girls with TS resulting in a normal adult BMD.

Only Ross *et al.* did report a higher incidence of wrist fractures compared to healthy girls in a group of untreated prepubertal girls with TS with normal BMD (Ross *et al.*, 1991). More data are available about the incidence of osteoporotic fractures in adults with TS. Davies *et al.* showed a significantly higher frequency of fractures in women with TS than in a control group. Half of the fractures occurred at typical osteoporotic sites (wrist, spine, femoral neck) (Davies *et al.*, 1995). In addition, from a large database of Turner women, Gravholt *et al.* reported an increased incidence of osteoporosis and fractures (Gravholt *et al.*, 1998). Although insufficient oestrogen therapy have a role in osteopenia, other factors, such as an intrinsic bone defect, may contribute to the increased risk of fractures in later life.

In conclusion, most untreated girls with TS, age ≥ 11 years, have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. During GH treatment with 6 IU/m²/day in combination with low dose oestrogens, the BMD SDS increased significantly. After discontinuation of GH treatment and the use of oestrogens in an adult dosage, the BMD had increased further to values as high as in young healthy women.

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CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

part "Girls with Turner syndrome"

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This part of the doctoral dissertation gives the results of two multicenter studies on growth hormone (GH) treatment in girls with Turner syndrome (TS): a dose-response study in 68 young girls, using 4, 6, or 8 IU GH/m²/day, and an injection frequency-response study in 19 relatively older girls using 6 IU/m²/day. The study population consisted of patients from 12 pediatric centers in the Netherlands. Four-year results of the dose-response study and two-year results of the frequency-response study were reported in the thesis of A. van Teunenbroek entitled "Growth hormone treatment modalities in girls with Turner syndrome" , Rotterdam 1996. The following discussion describes the findings as such and in in relation to current literature data. In addition, considerations and queries at the start of the study as listed in the Introduction (Chapter 1) are addressed, followed by recommendations for the treatment of these girls and ideas about future research.

The effects of GH treatment on growth during childhood and on final height

Growth during childhood

Our dose-response study showed that 7 years of GH treatment starting in relatively young girls with TS results in normalization of height during childhood. After 7 years of GH treatment 55/65 girls had a height within the normal range for healthy Dutch girls, while only 10 girls had a height just below the 3rd percentile. The increase in height was accompanied with an acceleration of bone maturation, without significant differences between the GH dosages groups.

GH treatment, even using the 'standard' GH dose of 4 IU/m²/day, started in relatively young girls with TS results in a normalization of height.

Final height

Our dose-response study showed that long-term GH treatment (7.3 (5 - 8.5) years) in 28 girls with TS (mean pretreatment age 8.2 yr) results in a normalization of adult height in most of the girls, even using the 'standard' growth hormone dose of 4 IU/m²/day. The mean adult height over the 3 GH dosage groups was approximately 160 cm. Baseline predicted adult height was exceeded in all subjects and the mean gain in adult height was well above 10 cm (12.5-16.0 cm). Moreover, in more than half of the 32 girls, adult height was within the target range.

Our frequency-response study showed that treatment with a GH dose of 6 IU/m²/day in combination with low dose estrogens for mean duration of 3.5 years in relatively older girls with TS (mean baseline age 13.6 yr) results in an increase in adult height of 6.3 cm above the predicted adult height.

To assess the effects of GH treatment on final height, the ideal study design is a randomized controlled trial with an untreated study group until adult height. Since at the start of our studies, GH treatment in girls with TS was an accepted indication in the Netherlands, such a trial was not possible. To determine the effect of GH on adult stature, in our both studies the attained adult height was compared to the individually predicted adult height using the prediction methods based on own references for untreated Dutch girls with TS. We realize that, in such a growth analysis, errors in adult height prediction have to be taken into account. Van Teunenbroek

et al. showed that the Turner-specific prediction method and the simpler modified projected adult height method gave the smallest mean prediction errors and were remarkably good at most ages[1]. Although, individual values of predicted adult height may have, particularly in young girls, big uncertainties, the mean errors of the prediction methods which we have used ranged just from -2.1 to 2.6 cm. In both studies, however, particularly in the dose-response study, the gain in adult height exceeds the effects of prediction errors and secular trend.

The results of the dose-response study contrast with reports stating that GH treatment in girls with TS results only in modest increments or have no positive effect at all[2-4]. The study of Rosenfeld *et al.*, however, in which GH treatment was started at a younger age (9.1 years), showed a mean gain in adult height of 8.4 cm being more comparable with the results of our study[5], in which the girls started GH treatment at an even younger age (8.2 years). Therefore, in our opinion, the most important reason of our better results is the fact that GH treatment was started at a younger age compared to earlier studies.

Long-term GH treatment (± 7 years) starting at a young age results in a normalization of adult height and a gain in adult height of more than 10 cm in most of the girls with TS, even using the 'standard' GH dose of 4 IU/m²/day. GH treatment in relatively older girls with TS increases adult height as well, however, far less than in younger girls.

GH dose and injection frequency

Seven-year data demonstrated a higher increment in height in the GH dosage groups receiving up to 6 or 8 IU/m²/d compared to 4 IU/m²/d, without significant differences between 6 and 8 IU/m²/d. The gain in adult height was significantly higher in group C compared to group A (16.0 vs 12.5 cm), but without significant differences between group A and B and group B and C, respectively. This difference in gain in adult height is quite small in proportion to the difference in given GH dose between group A and C. This suggests that near-maximal GH effects in girls with TS are reached in this dose-range. In contrast, Carel *et al.* found an increment in adult height which was twice as high in a study group who received an increasing GH dose (dependent on the height velocity, up to 9 IU/m²/d) compared to a fixed GH dose (approximately 3.9 IU/m²/d) group (10.6 vs 5.2 cm)[6]. However, these increments in adult height in both study groups were even lower compared to the results of our dosage group receiving 4 IU/m²/d, probably due to the higher baseline age of the girls in that study compared to our study. One may speculate that a higher GH dose should be used when the duration of GH treatment will be shorter. These ideas are in line with the results of continuous vs discontinuous treatment with GH in children with short stature born SGA, suggesting that cumulative GH dose received determines the growth response[7-9].

The frequency-response study showed that division of the total daily GH dose of 6 IU GH/m²/day into 2/3 in the evening and 1/3 in the morning is not advantageous over the once daily GH regimen with respect to FH gain. Thus, although twice daily GH injections mimics more the natural pulsatile GH secretion, this does not result in better growth response.

Long-term GH treatment resulted in a dose-dependent increase in height during childhood, as well as an increase in adult height, however the differences are small in proportion to the difference in given GH dose. The effect of GH on adult height does not seem to be better with more frequent injections than one per day.

Predictive factors of growth response

The growth response on GH treatment is quite different between individuals. Therefore, it is important to find pretreatment factors which can predict the response on GH. In the most

optimal situation, one can predict how much a girl will benefit from the GH treatment and consequently, one can for example, decide to treat or not to treat a girl with GH, or to wait for a few years, etc.

In the frequency-response study, in which all girls had reached adult height, the gain in adult height was significantly negatively related with the pretreatment bone age (range: 11.1-13.9 yr). Preliminary results of the dose-response study, showed a similar relationship between the pretreatment bone age (range: 4.1 - 10.5) and the gain in adult height. Thus, the younger the bone age before the start of GH treatment, the better the effect of GH treatment on adult height. However, we do not know whether the gain in adult height will be even higher in the girls who are younger than the girls who have yet reached adult height. One may suggest that this relationship is not linear and/or that starting GH treatment under a certain minimal age will not result in relevant additional gain in adult height. Therefore, adult height data of all girls of the dose-response study are required to obtain this relationship over an even wider range of pretreatment bone age. Although the relationship between chronological age and gain in adult height is negative as well, this relationship was not statistically significant. In our opinion, this is caused by the fact that chronological age is less indicative of biological development and thereby of the duration of the rest of the growth phase than bone age.

Due to a better growth response in the girls who have started GH treatment at a younger (bone) age, it is not unlikely that more younger girls will discontinue GH treatment because of satisfaction with height achieved compared to the older girls of which most of them have been treated until the height velocity had decreased to 1 cm per 6 months. Although there may be a strong linear relation between pretreatment (bone) age and the possible gain in adult height, one may speculate that due to the good growth response on GH more younger girls 'do not have to use' their entire growth phase to reach a normal height. They will decide to discontinue their GH treatment before the height velocity have decreased to 1 cm per 6 months and they will attain a 'submaximum' adult height. Consequently, the predictive factor "pretreatment (bone) age" may become less important.

Although final height is highly related with target height, no significant relationship was found between gain in adult height and target height. Thus, the parental height does not significantly influence the growth response on GH treatment. In addition, the frequency response study showed that pretreatment GH and IGF-I levels were not related to the gain in adult height.

It is very interesting and of clinical importance to know whether extremely small girls would benefit more from GH treatment than girls who are not that small. In order to answer that question, the pretreatment height SD-score (or the target height minus the pretreatment height SD-score) is often correlated with the increase in height SD-score during the study period[10]. Very often a negative correlation is found: The smaller the girl before the start of treatment, the better the growth response. We have to realize, however, that the relationship has to be interpreted very cautiously since it is a statistical phenomenon that an initial measurement will be correlated to the change in that measurement over time even if treatment is ineffective. This phenomenon is called 'regression to the mean'[11,12] and can (partly) explain this negative relationship. Therefore, in our analysis we decided not to evaluate the relationship between the pretreatment height and the height gain. There are complex approaches for handling this phenomenon, but they may have errors as well, and therefore, the magnitude of the 'real' influence of the pretreatment height SD-score on the growth response can only be determined in a controlled trial.

Our data show a negative relationship between the gain in adult height and the pretreatment bone age.

Induction of puberty

Because most girls fail to initiate or progress through puberty, estrogen replacement for the purpose of secondary sexual development is usually begun at 12-16 yr of age. It has been suggested to postpone estrogen therapy to delay closure of the epiphysial growth plates and, consequently, to prolong the growth phase. However, delay of pubertal development may have serious psychosocial consequences. Therefore, in both of our studies estrogen therapy was started at a more or less normal pubertal age.

Our dose-response study showed that starting GH treatment at a relatively young age and beginning low dose estrogen therapy from the age of 12 yr, result in a normalization of height in most girls and pubertal development in conformity with their healthy peers. We were only able to evaluate preliminary data on the short-term effect of pubertal induction with estradiol on skeletal maturation, by assessing the ratio Δbone age (year) / Δchronological age (year) over the year before the start of estradiol compared with the ratio over the year after start of estradiol. We found no significant change in bone maturation. In addition, our frequency-response study demonstrated that receiving GH treatment simultaneously with low dose estrogens for mean duration of 3.5 years in relatively older girls with TS results in pubertal development and an increment in adult height. To avoid the influence of estrogen therapy on the assessment of differences in the growth promoting effect of GH treatment between dosage or injection frequency groups, the start age and dose (per kg) of estrogens were similar for all girls between treatment groups per study. Therefore, we cannot obtain the effect of estrogens on final height and we do not know whether the gain in adult height would be even better if estrogen therapy was started at a later age. We can only state that there are no short-term effects of low-dose estrogens on bone maturation, and that, "despite" estrogen therapy at a normal pubertal age, GH treatment has a great positive effect on adult height.

We conclude that low dose estrogens at a normal pubertal age does not interfere with the capability of GH treatment to normalize adult height in girls with TS.

Levels of insulin-like growth factor I

Before start of GH treatment, in the girls of the dose-response study, aged 2-11 years, the mean IGF-I SD-score was significantly lower than zero. During GH treatment the mean IGF-I SD-score was significantly higher than zero and higher than baseline levels at each point in time. The increase in IGF-I SD-score was particularly seen in the first years of GH treatment without a further increase thereafter. After 7 years of GH treatment, the IGF-I SD-score was significantly higher in group B and C than in group A, without a significant difference between group B and C. The mean IGF-I levels were within the upper part of the normal range in group A (1.8) and higher than the normal range in group B and C (2.5 and 2.7, respectively).

GH treatment at a dose of 4 IU/m²/d induces mean IGF-I levels on the upper area of the normal range, whereas treatment with 6 or 8 IU/m²/d results in mean levels being even higher than the normal range. Since the consequences of high IGF-I levels during childhood are not yet known, follow-up of the girls into adulthood is required.

Safety of long-term GH treatment

The development of body proportions during GH treatment

To determine the body proportions before, during and after long-term GH treatment, we

measured height, sitting height, hand length, foot length, biacromial diameter and biiliacal diameter in the girls of the dose-response study. We evaluated the 7-year results of the body proportions during childhood and the data of all girls who have reached adult height. Our study showed that untreated girls with TS with an age between 2 and 11 years are short and have smaller hands and feet and narrow shoulders and pelvis compared to healthy peers. Since height is more affected than other parts of the body, untreated girls have, on average, a relatively large trunk, relatively large hands and feet, and relatively broad shoulders and pelvis compared to height. However, the body proportions of most girls were still within the normal range.

One has to realize, however, that our pretreatment results of the body proportions have to be interpreted with caution, since the normal range for body proportions particularly in extremely small children appears to be dependent on the chosen mathematical approach to combine two measurements e.g. hand length with height. One's perception of a body being disproportionate is dependent on what one is used to see and not on a chosen method to describe body proportions. Further research is, therefore, required to assess which method describes best the body proportions in very small children. After long-term GH treatment, most girls of our study had reached a normal height and as a result, the normal range for body proportions is far less dependent on the chosen method than before treatment.

The most important findings of the evaluation of 7-year data of 64 girls and adult height data of 32 girls was that the increase in height was accompanied by an even higher increase in size of feet, resulting in abnormal proportions of feet compared to height. The increase in the size of feet in proportion to the increase in height was significantly higher in group B and C compared to A after 7 years of GH treatment, however, without significant differences between the GH dosage groups in the girls who had reached adult height. In addition, a moderate improvement of the disproportion between height and sitting height was found. Reference data of adults with TS who were not treated with GH suggest that the increase in the disproportion of feet to height found in our study is partly due to the natural development of these body proportions in girls with TS, since this disproportion was found in the 'untreated' adult women with TS as well[13]. As observed in forms of skeletal dysplasia, body proportions may change during childhood. One might speculate that GH exaggerates this naturally occurring disproportionate growth in girls with TS while growing within the normal height range. Remarkably, our data of children with short stature born SGA group receiving 6 IU GH/m²/day showed no abnormal growth of feet at all[14]. **Untreated girls with TS have relatively large trunk, hands and feet, and broad shoulders and pelvis compared to height. The increase in height after long-term GH treatment is accompanied by an even higher increase in the size of feet and a moderate improvement of the disproportion between height and sitting height. Recently published reference data of untreated adults with TS and results of a short children born SGA receiving a comparable GH dosage, suggest that the disproportionate growth of feet has to be considered a part of the natural development in TS, but might be influenced by higher GH dosages. The development of large feet can play a role in the decision of the girl to discontinue GH treatment in the last phase of growth.**

Cardiac left ventricular dimensions and blood pressure during GH treatment

Since previous studies suggest that GH has, at least in adults, an anabolic effect on the myocardium and that high levels of GH in adults with acromegaly are associated with hypertension[15-19], we evaluated the consequences of high levels of GH over a long period during childhood on the morphology of the heart and the blood pressure (BP) in the dose-response study.

We found that untreated girls with TS without clinically relevant cardiac abnormalities (which could influence the analysis on the effect of GH) have similar left ventricular dimensions as healthy girls, however, diastolic and systolic BP are moderately elevated. During 7 years of GH treatment growth of the left ventricle is comparable with that of healthy girls. Long-term GH treatment does not result in left ventricular hypertrophy or worsening of the pre-existing relatively high BP in TS, even at higher GH dosages up to 8 IU/m²/day.

Seven years of GH treatment using dosages up to 8 IU/m²/day has no unwanted cardiovascular side-effects.

Carbohydrate metabolism during GH treatment and after discontinuation of GH treatment

Insulin resistance and carbohydrate (CH) intolerance have been reported in untreated girls with TS[20-22]. In addition, in adults with TS who had not received GH treatment in childhood, glucose intolerance, non-insulin- and insulin dependent diabetes mellitus are more common than in healthy women[23-24]. Since supra-physiological concentrations of GH in acromegalic patients[25] and in normal[26,27] adults showed a decrease in glucose sensitivity to insulin in liver and in extra-hepatic tissues, concern has been expressed regarding possible detrimental effects of long-term treatment with supra-physiological GH dosages in girls with TS.

Our dose-response study and frequency-response study show that long-term GH treatment with dosages up to 8 IU/m²/day in girls with TS has no adverse effects on glucose metabolism, but induced higher levels of insulin, indicating relative insulin resistance. This insulin resistance cannot be explained by changes in body mass index or estrogens. The increased insulin levels during long-term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values.

Long-term treatment with supraphysiological GH dosages has no adverse effects on glucose metabolism, but induces hyperinsulinism, indicating relative insulin resistance. After discontinuation of GH treatment insulin levels decrease to values close to or equal to pretreatment values.

Bone mineral density before and after GH treatment

Despite only limited reports of a greater number of fractures during childhood[28] or adulthood[23,29], osteoporosis historically has been described as a feature in TS, because of the frequent observation of radiographic osteopenia and the coarse trabecular pattern of the carpal bones on radiographs[30]. An intrinsic bone defect, as well as the estrogen deficiency may explain these findings.

In the dose-response study as well as in the frequency-response study, the measurements of volumetric BMD were performed using phalangeal radiographic absorptiometry. The volumetric BMD measurement is preferable to an areal BMD measurement, since no adjustments for height are required in children with short stature.

The dose-response study showed that most untreated young girls with TS have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. During 7 years of GH treatment with 4, 4->6, or 4->6->8 IU/m²/day, the BMD SD-score increased significantly. Only for the cortical bone was the 7-year increment in BMD significantly higher in the girls receiving GH doses up to 8 IU/m²/day. Spontaneous puberty or low dose estrogen therapy in the last three years of the 7-year study period in girl ≥ 12 years of age did not contribute to the increase in BMD.

The frequency-response study demonstrated that most untreated girls with TS, age ≥ 11

years, had a normal volumetric BMD of the cortical bone, as well as of the trabecular bone compared to healthy girls, as well. However, the mean BMD SD-score of the trabecular bone was significantly lower than zero. During GH treatment with 6 IU/m²/day in combination with low dose estrogens, the BMD SD-score increased significantly. After discontinuation of GH treatment and the use of estrogens in an adult dosage, the BMD was as high as in young healthy women.

In the frequency-response study, the effect of low dose estrogens cannot be distinguished from the effect of GH treatment, but it is likely that the further increase in BMD after discontinuation of GH treatment is due to the increased dosages of estrogens. In the dose-response study, we found that low dose estrogens does not contribute to the increase in BMD. Therefore, we can conclude that GH treatment is associated with an increase in BMD and that low dose estrogens does not seem to influence BMD. In contrast, adult estrogen dosages can increase the BMD to normal adult values after discontinuation of GH treatment.

In conclusion, most girls with TS have a normal volumetric BMD during childhood. During GH treatment BMD increases significantly, without obvious effects of low dose estrogens. After discontinuation of GH treatment and the increase of estrogen dosages to adult levels, the BMD reaches adult values.

Recommendations and future research.

The results of our studies showed that if GH treatment is started at approximately 8 years of age, a normalization of height during childhood and an adult height within the normal range can be achieved in most girls with TS. When girls with TS are older, GH treatment is still able to increase adult height, however, to a lesser extent. We showed, in addition, that the normalization of adult height can be reached with a dosage of 4 IU/m²/day. However, a higher GH dosage might be required in very short girls or if GH treatment is started at a later age. Furthermore, our studies showed that the induction of puberty does not have to be delayed until the end of the growth phase to optimize the effect of GH if GH treatment is started at a relatively young age. Follow-up of all girls participating in the dose-response is required to assess whether an even earlier start of GH and/or the use of higher GH dosages will result in a clinically significantly higher adult height. Furthermore, when all girls will have reached adult height, an extended analysis on other predictive factors for gain in adult height should be performed. When the most important factors influencing the growth promoting effect of GH treatment are more clear, an individual approach in the treatment of short stature and pubertal induction should be followed.

It is important to know that during GH treatment girls with TS will develop, on average, big feet in proportion to height. Although GH treatment does not seem to be the main cause of the increase in this disproportion, the absolute size of the feet as well as height is, dependent on the growth promoting effect of GH treatment. In contrast, sitting height in proportion to standing height will show, on average, a moderate improvement after reaching adult height.

It is reassuring that long-term GH treatment does not have adverse effects on left ventricular dimensions of the heart, blood pressure, and glucose metabolism in girls with TS. In addition, it is reassuring that the GH-induced relative insulin resistance decreased after discontinuation of GH treatment. However, follow-up of these girls into adulthood is required to assess possible side-effects on the very long-term.

Our clinical impression is that girls with TS benefit from the normalization of height. However, psychosocial research on quality of life, self-perception, and social functioning is

required to confirm this improvement.

GH treatment is an expensive therapy, particularly when GH treatment is started at an earlier age and higher GH dosages are given. When a girl with TS started GH treatment at 6 years of age and is treated until adult height is reached using the 'standard' GH dose of 4 IU/m²/day, the costs of GH are approximately Dfl. 500,000,=. To reduce the costs of the treatment, it has been proposed to discontinue the GH treatment when a certain height is reached. In our study, some girls, particularly with tall parents, had attained an adult height above 170 cm. One may suggest to stop GH treatment when the lower limit of the normal height range has been reached (3th or 10th percentile). However, these girls will, consequently, still end up as one of the smallest people of a society. One can, therefore, propose to discontinue the GH treatment when for example the 25th height percentile and/or target range has been reached. Another possibility to shorten the GH treatment is to start GH treatment at later age. The disadvantage of starting later is that most girls will be very short during childhood and that the magnitude of the growth response is yet unpredictable in an individual girl. Consequently, one runs the risk of starting too late with GH treatment to attain a normal adult height. The use of the cheaper anabolic steroid oxandrolone at a low dose in combination with GH is still under investigation in another multicenter study in the Netherlands. When an additional effect of oxandrolone has proven to be effective and safe, further research on use of a lower GH dosage in combination with oxandrolone can be started.

It has been suggested that psychological support to learn coping with the psychosocial problems concerning their short stature would be a cheaper and less invasive alternative for GH treatment. One has to realize, however, that very short individuals have also to deal with many practical problems in society. The adaptation of houses, cars, furniture, etc. to very short individuals is very costly as well. In addition, to date, no structural psychological programs are available that have proven to be effective and of practical use. To assess whether a 'psychological program' is an alternative for growth promoting therapy, a randomized trial has to be performed evaluating the long-term physical and psychosocial effects and the cost-benefit analyses of both therapies.

Whereas the scope of this thesis is the treatment of short stature and the induction of puberty in TS, it should be realized that girls with TS and their parents often have to face various other psychosocial and medical problems. Therefore, effective management of girls with TS is required: a timely diagnosis, awareness of the potential physical disorders and the psychosocial burden of the syndrome, as well as counselling and support.

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CHAPTER 8

SUMMARY

part "Girls with Turner syndrome"

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Growth failure and subsequently short adult stature is one of the main features in Turner syndrome (TS). The median adult height of North European girls with TS is 146.9 cm, being on average approximately 20 cm less than their healthy peers. The cause of the stunted growth in TS is poorly understood. Although these girls are not growth hormone (GH) deficient, GH administration accelerates growth in a dose-dependent way. This part of the doctoral dissertation presents the results of two prospective randomized, multicenter clinical trials in previously untreated girls with TS. In the first trial, we studied 68 girls with TS, age 2-11 years, who were randomly assigned to one of three GH dosage groups: group A, 4 IU/m²/day; group B, first year 4, thereafter 6 IU/m²/d; group C, first year 4, second year 6, thereafter 8 IU/m²/d. Girls ≥ 12 years of age started with 17ß-oestradiol 5 µg/kg bw/d for induction of puberty (dose-response study). In the second trial with 19 girls, aged 11 years or older, the relationship between the injection frequency and the effect on adult height was studied comparing fractionated twice daily (BID) injections with once daily (OD) in a total GH dose of 6 IU/m²/day, concurrently receiving low dose ethinyl estradiol (frequency-response study).

Chapter 1 gives an overview of literature data regarding the characteristic features, the incidence, and the genetics in TS. Subsequently, the ovarian dysgenesis, the natural growth pattern, and the pathophysiology of the stunted growth are described. Furthermore, the results of short-term GH treatment on growth and considerations concerning possible side-effects of GH treatment in TS are discussed.

Chapter 2 describes the results of the dose-response study on growth during 7 years of treatment in childhood, as well as on adult height in those girls who have reached final height. GH treatment starting in relatively young girls with TS results in normalization of height during childhood and normalization of adult height in most of the girls, even using the 'standard' GH dose of 4 IU/m²/day, and without unwanted side-effects. Higher GH doses may be more effective, but the efficacy on adult height and safety in the very long-term have still to be proven. Induction of puberty with a low dose of natural estrogens at a normal pubertal age does not interfere with the capability of GH treatment to normalize adult height in girls with TS.

Chapter 2 also describes the final height results of the frequency-response study. Treatment with a GH dose of 6 IU/m²/day in combination with low dose estrogens can result in a significant increase in adult height in relatively older girls with TS. However, the gain in adult height is far less than in younger girls. Division of the total daily GH dose (6 IU GH/m²/day) into 2/3 in the evening and 1/3 in the morning is not advantageous over the once daily GH regimen with respect to FH gain.

Chapter 3 describes the body proportions before, during and after long-term GH treatment in the dose-response study. Untreated girls with TS have, on average, relatively large trunk, hands and feet, and broad shoulders and pelvis compared to height. The increase in height after long-term GH treatment is accompanied by an even higher increase in the size of feet and a moderate improvement of the disproportion between height and sitting height. Recently published reference data of untreated adults with TS and results of children with short stature born SGA receiving a comparable GH dosage, suggest that the disproportionate growth of feet has to be considered a part of the natural development in TS, but might be influenced by higher GH dosages. The development of large feet can play a role in the decision of the girl to discontinue GH treatment in the last phase of growth.

Chapter 4 presents the 7-year data on the effects of GH treatment on cardiac left ventricular dimensions and blood pressure (BP) in the dose-response study. Untreated girls with TS without clinically relevant cardiac abnormalities have similar left ventricular dimensions as healthy girls, however, diastolic and systolic BP are moderately elevated. During 7 years of GH treatment growth of the left ventricle is comparable with that of healthy girls. Long-term GH treatment does not result in left ventricular hypertrophy or worsening of the pre-existing relatively high BP in TS, even at higher GH dosages up to 8 IU/m²/day.

Chapter 5 demonstrated the effects of GH treatment on carbohydrate metabolism during GH treatment and after discontinuation of GH treatment in the girls of the dose-response study, as well as in those of the frequency-response study. Both studies show that long-term GH treatment with dosages up to 8 IU/m²/day in girls with TS has no adverse effects on glucose metabolism, but induced higher levels of insulin, indicating relative insulin resistance. The increased insulin levels during long-term GH treatment decreased after discontinuation of GH treatment to values close to or equal to pretreatment values.

Chapter 6 describes the volumetric bone mineral density (BMD) before and during 7 years of GH treatment in the dose-response study. In addition, the volumetric BMD data of the frequency-response study before and during GH treatment, as well as three years after discontinuation of treatment are described in this chapter. The BMD measurements were performed using phalangeal radiographic absorptiometry. The dose-response study showed that most untreated young girls with TS have a normal volumetric BMD of the cortical, as well as of the trabecular bone compared to healthy girls. During 7 years of GH treatment, the BMD SD-score increased significantly. Only for the cortical bone was the 7-year increment in BMD significantly higher in the girls receiving GH doses up to 8 IU/m²/day. Spontaneous puberty or low dose estrogen therapy in the last three years of the 7-year study period in girls ≥ 12 years of age does not contribute significantly to the increase in BMD. The frequency-response study demonstrated that most untreated girls with TS, age ≥ 11 years, have a normal BMD of the cortical and trabecular bone compared to healthy girls, as well. However, the mean BMD SD-score of the trabecular bone was significantly lower than zero. During GH treatment with 6 IU/m²/day in combination with low dose estrogens, the BMD SD-score increases significantly. After discontinuation of GH treatment and the use of estrogens in an adult dosage, the BMD was as high as in young healthy women.

Chapter 7 discusses the results of our studies in the context of the most recent literature data. Our final conclusions and recommendations for the treatment of girls with TS are listed. In addition, suggestions for future research are given.

SAMENVATTING

deel "Meisjes met het syndroom van Turner"

Eén van de meest voorkomende kenmerken van het syndroom van Turner (TS) is een groeistoornis die resulteert in een kleine volwassen lengte. De mediane volwassen lengte van Noord-Europese vrouwen met TS is 146.9 cm; dat is ongeveer 20 cm minder dan de gemiddelde lengte van gezonde volwassen vrouwen. De oorzaak van de groeistoornis bij meisjes met TS is nog niet goed bekend. Hoewel deze meisjes niet groeihormoon (GH) deficiënt zijn, versnelt toediening van recombinant humaan GH de groei op een dosis-afhankelijke manier.

In dit deel van het proefschrift worden de resultaten gepresenteerd van twee prospectieve gerandomiseerde onderzoeken naar de effecten van GH behandeling bij meisjes met TS. In de eerste studie, de dosis-respons studie, werden 68 meisjes met TS met een leeftijd tussen de 2 en 11 jaar verdeeld over drie GH dosis groepen: groep A werd behandeld met 4 IU/m² lichaams oppervlakte/dag; groep B in het eerste jaar met 4 en daarna met 6 IU/m²/d; groep C in het eerste jaar met 4, het tweede jaar met 6 en daarna met 8 IU/m²/d. Bij meisjes met een leeftijd ≥ 12 jaar werd gestart met 17ß-oestradiol (5 µg/kg/d) om de puberteit te induceren. Op dit moment hebben nog niet alle meisjes die deelnemen aan deze studie hun volwassenlengte bereikt. In de tweede studie, de frequentie-respons studie, werd de relatie tussen de frequentie van de GH injecties en het effect op volwassen lengte bestudeerd bij 19 meisjes met TS met een leeftijd van 11 jaar of ouder. De helft van de meisjes diende zich éénmaal daags 6 IU/m²/d toe en de andere helft van de meisjes verdeelde de 6 IU/m²/d over 2 dagelijkse injecties. Tijdens de GH behandeling werd tegelijkertijd een lage dosis ethinyl oestradiol gegeven om de puberteit te induceren. Deze studie is afgerond.

Hoofdstuk 1 geeft een overzicht van de literatuur betreffende de klinische verschijnselen en de incidentie van TS en de genetische aspecten betreffende dit syndroom. Vervolgens worden de ovariële dysgenesie, het natuurlijke groeipatroon en de pathofysiologie van de groeistoornis beschreven. In dit hoofdstuk worden bovendien zowel de groeiresultaten van korte-termijn GH behandeling besproken als de overwegingen betreffende mogelijke neveneffecten van de GH behandeling bij meisjes met TS.

Hoofdstuk 2 beschrijft de effecten van de behandeling met 3 verschillende GH doses op zowel de groei tijdens de jeugd in de totale groep als op volwassen lengte in de helft van de meisjes binnen de dosis-respons studie. Wij toonden aan dat GH behandeling bij relatief jonge meisjes met TS leidde tot een normalisatie van de lengte tijdens de jeugd en een normalisatie van de volwassen lengte bij de meeste meisjes, zelfs als de 'standaard' GH dosis van 4 IU/m²/d werd gebruikt. Er traden geen ongewenste neveneffecten op. Hogere GH doses zouden effectiever kunnen zijn, maar de effectiviteit en de veiligheid op de nog langere termijn moeten nog bewezen worden. De inductie van de puberteit met een lage dosis oestrogenen op een normale pubertaire leeftijd interfereert niet met het vermogen van de GH behandeling de volwassen lengte te normaliseren bij meisjes met TS.

Hoofdstuk 2 presenteert ook de resultaten van GH behandeling op de volwassen lengte bij de meisjes die deelnamen aan de frequentie-respons studie. GH behandeling met 6 IU/m²/d in combinatie met een lage dosis oestrogenen leidde tot een toename in volwassen lengte bij deze relatief oudere meisjes met TS. De lengtewinst was echter wel veel lager dan bij de jongere meisjes van de dosis-respons studie. Het verdelen van de dagelijkse totale GH dosis (6 IU/m²/d) over 2/3 in de avond en 1/3 in de ochtend resulteerde niet in een betere lengtewinst vergeleken met het éénmaal daags toedienen van 6 IU/m²/d.

Hoofdstuk 3 beschrijft de lichaamsverhoudingen vóór, gedurende en na lange termijn GH behandeling in de dosis-response studie. Onbehandelde meisjes met TS hadden gemiddeld een relatief lang bovenlichaam, grotere handen en voeten, en bredere schouders en een breder bekken in verhouding tot de lengte. De toename in lengte na langdurige GH behandeling ging gepaard met een sterkere toename in de grootte van de voeten en een matige verbetering van de disproportie van de zithoogte ten opzichte van de lengte. Recent gepubliceerde referentie data van onbehandelde volwassen vrouwen met TS en resultaten van kleine kinderen na een te kleine geboortlengte die met een vergelijkbare GH dosis werden behandeld, suggereren dat de gedisproportioneerde groei van de voeten beschouwd moeten worden als een onderdeel van de natuurlijke ontwikkeling bij TS, maar dat dit misschien door hogere GH doses beïnvloed zou kunnen worden. De ontwikkeling van grote voeten kunnen een rol spelen bij de beslissing van het meisje om in de laatste fase van de groei te stoppen met de GH behandeling.

Hoofdstuk 4 presenteert de 7 jaars gegevens van de dosis-respons studie betreffende de effecten van GH behandeling op de afmetingen van het linker ventrikel van het hart en de bloeddruk. Onbehandelde meisjes met TS zonder klinisch relevante hartafwijkingen hadden vergelijkbare afmetingen van het linker ventrikel als gezonde meisjes, maar de diastolische en systolische bloeddruk waren matig verhoogd. Gedurende de 7 jaar GH behandeling was de groei van het linker ventrikel vergelijkbaar met die van gezonde meisjes. Langdurige GH behandeling leidde niet tot linker ventrikel hypertrofie of verslechtering van de pre-existente relatief hoge bloeddruk bij meisjes met TS, zelfs bij hogere GH doses tot 8 IU/m²/d.

Hoofdstuk 5 beschrijft de effecten van GH behandeling op het koolhydraatmetabolisme gedurende de GH behandeling en na het staken van de GH behandeling bij zowel de meisjes van de dosis-respons studie als de meisjes van de frequentie-respons studie. Beide studies laten zien dat langdurige GH behandeling met doses tot 8 IU/m²/d geen negatieve effecten heeft op het glucose metabolisme, maar dat GH hogere insuline waarden induceert wat wijst op relatieve insuline resistentie. De toegenomen insuline waarden gedurende de GH behandeling namen na het staken van de GH behandeling af tot waarden vergelijkbaar met die van vóór de start van de GH behandeling.

Hoofdstuk 6 toont de resultaten van de metingen van de volumetrische botdichtheid (BMD) middels falangeale microdensitometrie vóór en tijdens 7 jaar GH behandeling bij de meisjes van de dosis-response studie. Bovendien bespreekt dit hoofdstuk de resultaten van de frequentie-respons studie betreffende de BMD metingen vóór en gedurende GH behandeling, en drie jaar na het staken van de GH behandeling. De BMD werd gemeten door middel van radiografische absorptiometrie van de falanx. De dosis-respons studie liet zien dat de meeste onbehandelde jonge meisjes met TS een normale volumetrische BMD van zowel het corticale als het trabeculaire bot hebben vergeleken met gezonde meisjes. Gedurende 7 jaar GH behandeling nam de BMD SD-score significant toe. Alleen voor het corticale bot was de 7 jaars toename in BMD significant hoger bij de meisjes die behandeld waren met een GH dosis tot 8 IU/m²/d vergeleken met de lagere doses. Spontane puberteit of een lage dosis oestrogenen in de laatste drie jaar van de 7 jaar studie periode bij meisjes ≥ 12 jaar droeg niet significant bij aan de toename in BMD. De frequentie-respons studie liet zien dat ook de meeste onbehandelde meisjes met TS ≥ 11 jaar een normale botdichtheid hadden van zowel het corticale als het trabeculaire bot vergeleken met gezonde meisjes. Echter de gemiddelde BMD SD-score van het trabeculaire bot was significant lager dan nul. Gedurende de GH behandeling met 6 IU/m²/d in combinatie met een lage dosis oestrogenen nam de BMD SD-score significant toe. Na het staken van de GH behandeling en tijdens het gebruik van oestrogenen in een volwassen dosering was de BMD even

hoog als bij jonge gezonde vrouwen.

Hoofdstuk 7 bespreekt de resultaten van onze studies in samenhang met de meest recente literatuurgegevens. Onze uiteindelijke conclusies en aanbevelingen voor de behandeling van meisjes met TS worden gepresenteerd. Bovendien geven we ideeën voor toekomstig onderzoek.

DANKWOORD

Op deze bladzijden wil ik iedereen, die in de afgelopen jaren op een of andere manier aan dit proefschrift heeft bijgedragen, zeer hartelijk bedanken. Een aantal mensen wil ik op deze plaats in het bijzonder noemen.

Beste kinderen en ouders, jullie wil ik heel hartelijk bedanken voor jullie deelname gedurende vele jaren aan het onderzoek. Een aantal van jullie is nu volwassen en al jaren geleden gestopt met de groeihormoonbehandeling terwijl anderen nog een paar jaar voor de boeg hebben. Mede door jullie bereidwilligheid telkens het ziekenhuis te bezoeken en mee te werken aan alle metingen, zijn het heel unieke studies geworden op het gebied van groeihormoonbehandeling. Ik wens jullie het allerbeste toe in jullie verdere leven.

Beste Arne van Teunenbroek en Wouter de Waal, door de kinderen te includeren in de studies en te starten met de groeihormoonbehandeling in respectievelijk 1989 en 1991, hebben jullie de basis gelegd voor dit proefschrift. Ik wil jullie hier hartelijk voor bedanken.

Beste Anne van de Wiel en Janneke van Nieuwkasteele, ik ben jullie dankbaar voor jullie geduld om mij met name in de eerste jaren van mijn onderzoeksperiode de weg te wijzen in de verschillende protocollen die er tegelijkertijd liepen. Beste Ingrid van Slobbe, erg bedankt voor het zo snel overnemen van een studie die al zoveel jaren liep en waar consequent gebruik was gemaakt van onbegrijpelijke visitnummers. Beste Ingrid en Janneke nogmaals, jullie ben ik zeer dankbaar voor jullie hulp bij het verzamelen en verwerken van de gigantische hoeveelheid gegevens en jullie aanwezigheid in mijn auto (en het aangeven van vele boterhammen) gedurende de lange files op de weg.

Dr. S.M.P.F. de Muinck Keizer-Schrama en Dr. A.C.S. Hokken-Koelega, co-promotoren, beste Sabine en Anita, graag wil ik jullie bedanken voor de goede samenwerking, jullie enthousiasme en betrokkenheid bij de studies en de vele uren die jullie hebben besteed aan het telkens verbeteren van de artikelen.

Prof. Dr. S.L.S. Drop, beste Sten, ik wil je bedanken voor de mogelijkheid die ik heb gekregen om klinisch onderzoek te kunnen doen binnen jouw afdeling kinderendocrinologie en voor je beoordelingen van de verschillende manuscripten. Nogmaals hartelijk dank hiervoor.

Beste kamergenoten, Janneke van Nieuwkasteele, Annemieke Boot, Ingrid van Slobbe, Anneke Saarloos, Lydia Velt, Dick Mul, Inge van der Sluis en Yvonne van Pareren, bedankt voor alle bekers koffie maar vooral voor de gezelligheid en voor een luisterend oor. Nicolette en Esther, jullie zitten wel wat verder weg, maar horen er natuurlijk ook bij. Beste Lydia Velt, jou wil ik bedanken voor het twee maal overnemen van het werk van Janneke tijdens haar zwangerschapsverlof. Je deed het als of je niet anders gewend was. Beste Inge en Nicolet, ik hoop dat jullie de wijze lessen statistiek van een oude AIO gewaardeerd hebben. Beste Dick, bedankt voor je steun die ik heb gekregen in de ook mindere gemakkelijke tijden. Ik wens je veel succes, ook als man, in de vrouwenwereld van onze kamer. Annemie Boehmer, een beetje het buitenbeentje van de endocrinologie, bedankt voor je gezellige, sappige verhalen en je adviezen. Yvonne, jou wil ik bedanken voor het vlot overnemen van mijn studies, zodat ik kon beginnen aan het schrijven van dit boekje. Ik wens je heel veel succes en plezier bij het uitvoeren en uiteindelijk afronden van het onderzoek.

Prof. Dr. Th. Stijnen, beste Theo, ik wil je hartelijk bedanken voor de goede en prettige samenwerking. Jij bent in staat enorm

ingewikkelde statistiek eenvoudig aan mij uit te leggen. Ik heb het zeer gewaardeerd dat je intensief met mij hebt meegedacht over de aanpak van de analyse van de lichaamsverhoudingen. (Het is alleen wel lastig dat je dezelfde initialen hebt als ik.)

Dr. P.G.H. Mulder, beste Paul, zeer bedankt voor het nauwgezet uitvoeren van alle statistische testen voor de SGA studie. Jij hebt gezorgd dat ik alle gegevens voor de artikelen kreeg zonder dat ik mijn 'blindheid' voor de studie verloor.

Dr. A.H. Cromme, beste Adri, jou wil ik bedanken voor het meebeoordelen van de cardiologische evaluaties van de Turner studie en de prettige samenwerking die heeft geleid tot de publicatie van ons artikel. Ook wil ik hierbij de kindercardiologen en echografisten in alle academische ziekenhuizen bedanken voor hun inzet en tijd die ze hebben besteed aan dit onderzoek.

Prof. Dr. B.C.J.M. Fauser, Prof. Dr. F. de Zegher, Prof. Dr. H.A. Büller, Prof. Dr. H.A. Delemarre-van de Waal en Prof. Dr. Th. Stijnen wil ik naast mijn promotor en co-promotoren van harte bedanken voor de toezegging 15 december 1999 lid te zullen zijn van de promotie commissie.

Wibeke van Leeuwen, Anjalie Asarfi, Rick van Rijn, Andries Zwamborn, jullie wil ik bedanken voor jullie inzet en medewerking om de resultaten van de botdichtheidmetingen te kunnen gaan publiceren. Aansluitend wil ik hierbij ook de afdelingen (kinder-) radiologie van alle academische ziekenhuizen bedanken voor de medewerking aan het verkrijgen van alle duizenden handfoto's voor de studies.

Dr. H.J. Aanstoot, beste Henk-Jan, ik wil je bedanken voor je deskundige beoordeling van de manuscripten betreffende het koolhydraatmetabolisme.

Mijn dank gaat uit naar alle leden van de Adviesgroep Groeihormoon en met name naar de kinderarts-endocrinologen die meegewerkt hebben aan de drie studies. Dr. M. Jansen, Dr. B.J. Otten, Drs. J.J.G. Hoorweg-Nijman, Dr. T. Vulsma, Dr. G.G. Massa, Drs. C.W. Rouwé, Dr. H.M. Reeser, Dr. W-J. Gerver, Drs. J.J. Gosen, Dr. C. Rongen-Westerlaken, Dr. J.J.J. Waelkens, Prof. Dr. H.A. Delemarre-van de Waal en Drs. E.C.A.M. Houdijk wil ik hartelijk bedanken voor de gastvrijheid in hun ziekenhuis, de samenwerking op de polikliniek en het beoordelen van de manuscripten.

Ook alle (ex-)onderzoekers, verpleegkundigen en analisten van het Sophia die ik in afgelopen jaren heb leren kennen wil ik hartelijk bedanken voor de samenwerking en gezelligheid tijdens en naast het werk.

Graag wil ik ook al diegenen in de verschillende ziekenhuizen bedanken die behulpzaam zijn geweest bij de organisatie en uitvoer van de studies: de mensen van het medisch archief, van de medische administratie, van de poliklinieken en secretariaten, van de dagbehandeling en van de verschillende laboratoria. Allen erg bedankt voor de samenwerking. In het bijzonder wil ik Erik Herdes bedanken voor het vlot en vrolijk zoeken naar oplossingen op de dagbehandeling van het VU ziekenhuis.

Dr. F. Slijper, beste Froukje, jou wil ik bedanken voor alle adviezen rondom de 'psychosociale zorg' van 'onze' kinderen.

Dr. W. Hackeng, beste Wil, ik wil je graag bedanken voor alle insulinebepalingen die je hebt verricht voor de studies.

De vele mensen van Novo Nordisk A/S Denemarken en Novo Nordisk Farma BV Nederland dank ik hartelijk voor de samenwerking en de financiële ondersteuning van de studies en dit boekje.

Jan van den Broeck, jou wil ik bedanken voor je adviezen en het meedenken over verschillende aspecten betreffende groei en 'regression to the mean'.

Dini Seip, heel erg bedankt voor het doorgeven van 'leuke post' en de faxen naar New York op de maandagochtend.

Jacqeline de Vogel, Riet Visser en Marijke Knijnenburg ben ik zeer erkentelijk voor alle hulp bij het regelen van de financiële kant van de studies.

De firma Grafitel, mijn dank voor de lay-out en het laten drukken van dit boekje.

Beste Inge en Dick, jullie dank ik dat jullie mijn paranimfen willen zijn. De praktische maar ook geestelijke steun in de periode voor mijn promotie waardeer ik zeer.

Alle vrienden en familie, die in de afgelopen jaren met mij mee hebben geleefd, wil ik hierbij ook bedanken.

Lieve ouders, mijn grote dank gaat uit naar jullie omdat jullie mij gestimuleerd hebben om te studeren en mij vrij hebben gelaten in mijn keuzes.

Lieve Carine, het proefschrift is af! Wie had dat een paar jaar geleden gedacht ? Ik wil je heel hartelijk bedanken voor je steun en je vertrouwen in mij. De leuke vrije dagen en vakanties met jou hebben er mede voor gezorgd dat ik daarna weer vol energie aan het proefschrift kon werken.

About the author

Theo C.J. Sas was born in Deventer, the Netherlands, on July 9, 1969. He passed grammar school in 1987 and started medical school at the University of Rotterdam. From January 1991 until May 1992, during his medical training, he performed research on the effects of PCBs and dioxins on the immunological status of the infants. This study was part of a larger PhD study of Dr. C Koopman-Esseboom, department of Pediatrics, division of Neonatology (head: Prof. Dr. P.J.J. Sauer), Sophia Children's Hospital, Rotterdam. In December 1994 he obtained his medical degree. In December 1994 he was working on research on the effects of growth hormone treatment in girls with Turner syndrome and children with short stature born small for gestational age at the department of Pediatrics, division of Endocrinology (head: Prof. Dr. S.L.S. Drop), Sophia Children's Hospital, Rotterdam. Since October 1999, he has started his residency in Pediatrics at the Sophia Children's Hospital, Rotterdam (head: Prof. Dr. H.A. Büller). In his spare time, he plays volleyball and he likes to play the piano and accordeon. He is married to Carine de Visser, teacher.