Regulation and modulation of growth : insights from human and animal studies

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Chapter 2

High dose growth hormone treatment limited to the prepubertal period in young children with idiopathic short stature does not increase adult height

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European Journal of Endocrinology 2010;162(4): 653-660

Abstract

Objective: to assess the long-term effect of prepubertal high-dose growth hormone treatment on growth in children with idiopathic short stature (ISS).

Design and methods: 40 children with no signs of puberty, age at start 4-8 years (girls) or 4-10 (boys) years, height SDS<–2.0 SDS and birth length>-2.0 SDS, were randomly allocated to receive GH at a dose of 2 mg/m^2/day (equivalent to 75 µg/kg/day at start and 64 µg/kg/day at stop) until the onset of puberty for at least 2 years (preceded by two 3-month periods of treatment with low or intermediate doses of GH separated by two washout periods of 3 months) or no treatment. In 28 cases adult height (AH) was assessed at a mean (SD) age of 20.4 (2.3) years.

Results: GH-treated children (mean treatment period on high dose GH 2.3 yr (range 1.2-5.0 yr)) showed an increased mean height SDS at discontinuation of treatment compared with controls (-1.3 (0.8) SDS versus -2.6 (0.8) SDS, respectively). However, bone maturation was significantly accelerated in the GH-group compared with controls (1.6 (0.4) versus 1.0 (0.2) yr, respectively) and pubertal onset tended to advance. After an untreated interval of 3-12 yr, AH was -2.1 (0.7) and -1.9 (0.6) in the GH-treated and control group, respectively. Age was a positive predictor of adult height gain.

Conclusion: High dose GH treatment restricted to the prepubertal period in young ISS children augments height gain during treatment, but accelerates bone maturation, resulting in a similar adult height compared to untreated controls.

Introduction

Growth hormone (GH) treatment in children with idiopathic short stature (ISS) has been the subject of many clinical trials. There are essentially four outcome parameters of GH treatment that can be considered: short term growth response (1st year's height velocity), bone age advance, onset and progress of puberty, and increase in adult height (AH). As recently reviewed (1;2), almost all children with ISS respond to GH treatment with an increase in height velocity, even on a relatively low dose. The dose-response curve for the first year's height velocity appears to reach a plateau after a dose of 50 µg /kg/d. No acceleration of bone age advance and pubertal onset and progress has been observed in the dose range of 30-53 µg /kg/d and the most effective dose regimen (50 µg /kg/d) leads to approximately 7 cm adult height gain (1;2).

At the time this study was designed, there were three important issues with respect to GH treatment of children with ISS that awaited resolving. First, it was unknown what the effect would be of a further increase of the GH dosage on growth velocity, bone maturation, puberty and AH. Second, the relative contribution of GH treatment before and during puberty was unclear. Third, there was a need to gain more insight into the factors affecting the growth response, as only a modest part of the inter-individual variation can be explained (3).

In order to address these issues, we started a controlled clinical trial in children with ISS in 1993. We hypothesized that a high GH dose before puberty might be able to bring height within the population's range, as shown for a dose of 0.1 mg/kg/day (4), without undue bone maturation and advance in puberty, and without adverse effects. We limited the period of GH therapy to the years before pubertal onset, primarily to improve the cost-benefit ratio, but also based on studies showing that HSDS at the onset of puberty is a strong predictor of AH in GH deficient children (5;6) and that pubertal height gain on GH treatment was not different between GH treated children and untreated controls (7). In order to improve the predictive power of clinical and biochemical variables we included an elaborate assessment of GH sensitivity.

In three previous papers on this study we reported that high dose GH limited to the prepubertal period increased growth, but also advanced bone age maturation and pubertal development, so that the predicted adult height (PAH) did not change (8), and that biochemical (9) and *in vitro* (10) indices of GH sensitivity had little predictive power for the short-term growth response. In the present paper we report the results on adult height.

Subjects and methods

Patients

This report includes AH data on 28 out of 40 children with ISS who originally enrolled in a multicenter study in The Netherlands from December 1993 to December 1996. Inclusion criteria were: no signs of puberty (G1 in boys and B1 in girls); height at baseline (H_{start}SDS) <-2.0 SDS for Dutch references available at that time (11), age at baseline 4-8 years for girls and 4-10 years for boys; birth length >-2.0 SDS (12); maximum serum GH level more than 10 µg/liter (1 mg = 2 IU, using World Health Organization (WHO) International Reference Preparation 66/217 as standard) after provocation (exercise, arginine, clonidine, L-dopa or glucagon), and a normal sitting height/subischial leg length ratio (between P3 and P97) (13). Screening blood tests and urinalysis were normal. No organic causes of growth failure, primary bone disease, chronic illness, or dysmorphic syndrome were present. Further details of the subjects and data obtained after discontinuation of treatment have been reported previously (8;9).
Three children of Turkish origin were included in the trial, 1 girl and 1 boy in the GH-group, and 1 boy in the control group. H_{start}SDS was calculated using Dutch references (-2.50, -2.73, -3.23, respectively), and their height was also <-2 SDS for references for Turkish children that became available in 1997 (14). For further analyses, SDS values of these and all other children were calculated using references for Dutch children.
The protocol was approved by the medical ethical review boards at the three participating centers (Amsterdam, Rotterdam, and Eindhoven). Before conducting any study-related procedure, written informed consent was obtained from parents and, when appropriate, also from the participants. For AH analysis, written informed consent was obtained from the participants. This clinical trial was registered in the *meta*Register of Controlled Trials (ISRCTN52337368) of the Current Controlled Trials Ltd.

Study design

Forty patients were randomly allocated to receive GH treatment or no treatment (figure 1). Details have been reported previously (8). In short, in the GH treatment group GH responsiveness was assessed during the first year of the study by administering GH in an on/off scheme at a dose of 0.5 or 1.0 mg/m^2/d (equivalent to 19 or 38 µg/kg/day, respectively) during two periods of 3 months, separated by two wash-out periods of 3 months without GH treatment (figure 2). In the second year, long-term GH treatment with 2.0 mg/m^2/d (75 µg/kg/d)

was started and was intended to be given for at least two years. Treatment was discontinued at the first full year visit after the onset of puberty (G2 for boys and B2 for girls), which resulted in a treatment period of 2-5 years on high dose GH (mean 2.3 yr). At discontinuation of GH treatment, the dose was equivalent to 64 µg/kg/d. The GH dose per kg body weight was lower at discontinuation of treatment than at start of the high dose treatment phase due to the fact that body weight shows a larger increase with age than body surface. GH (Genotropin; Pharmacia & Upjohn, Uppsala, Sweden; now Pfizer, New York, USA) was administered subcutaneously, 7 days per week between 6.00 and 8.00 p.m. The measurements at discontinuation of treatment in the GH-group were compared with measurements after attaining Tanner stage 2 (B2 for girls, G2 for boys) in the control group.

Directly after randomization, four patients (two from each group) refused to start the treatment they were randomly allocated to receive and dropped-out (fig 1). In addition, one boy from the GH treatment group was found to have neurofibromatosis and was excluded from the study.

At adult height analysis, 6 patients from the control group could not be motivated to participate. One patient from the GH-group could not be traced and was lost to follow-up. One boy stopped using high dose GH after 1.2 years and could not be motivated to continue according to protocol. However, his growth data are included in this report. Pubertal onset and development were not registered for 1 girl from the control group and her last

Figure 1

Trial design.

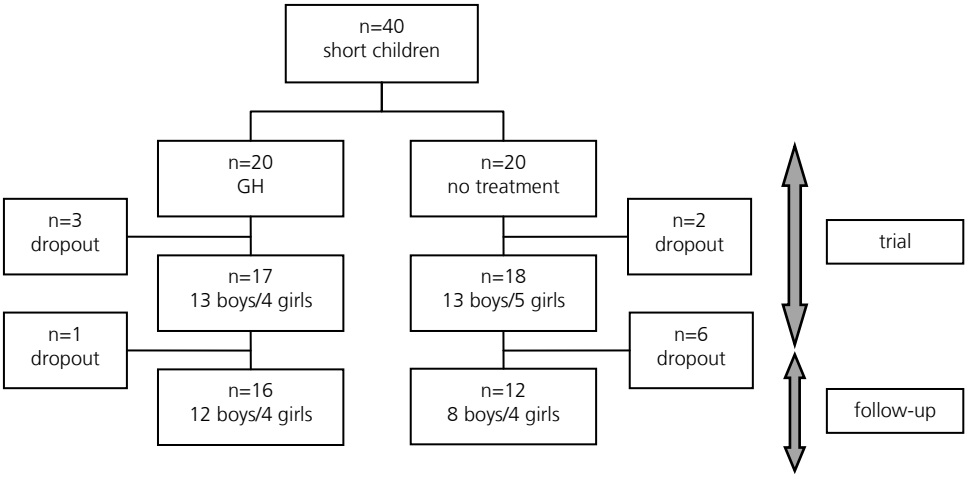
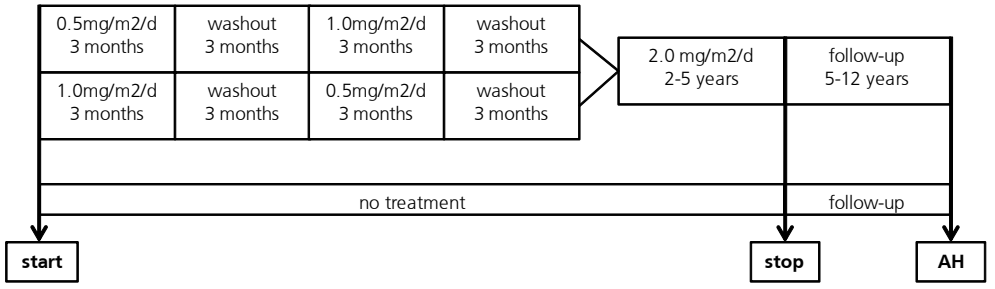


Figure 2

Time-scheme for GH-treatment and control groups. GH doses: 0.5 mg/m^2/d = 19 µg/kg/d; 1.0 mg/m^2/d = 38 µg/kg/d; 2.0 mg/m^2/d = 75 µg/kg/d.



known auxological information at the age of 9.7 yr was used for the analysis at stop.
At follow-up, we took a short medical history, performed a physical examination, assessed bone age (15) and measured height, weight, and sitting height (SH). Leg length (LL) was obtained by subtracting SH from height. Blood was collected for DNA extraction and single-nucleotide polymorphism genotyping (SNP-array) as described before (16) using the Affymetrix Genechip Human Mapping 250K array set. We also assessed the psychosocial status, which will be reported separately.

Outcome parameters

Four outcome parameters were used to evaluate the response to treatment: 1) Adult height SDS (AH SDS); 2) AH minus height at start SDS (AH–H_{start} SDS); 3) AH minus height for bone age at start (AH–H for BA_{start} SDS); and 4) AH minus conditional target height SDS (AH–cTH SDS). Because of the young bone age of most patients at start of the intervention, the predicted adult height (PAH) according to Bayley and Pinneau (17) could not be calculated at start.
To assess the degree of change of growth potential after discontinuation of treatment, we analyzed AH SDS minus predicted adult height at discontinuation of therapy (AH-PAH_{stop} SDS). For both groups, pubertal development at Tanner stage 2 was expressed as SDS for age and gender according to a recent technique (18).
Height and BMI SDS were based on recent Dutch references (19). For calculation of AH SDS, the age of each patient was set at 21 yr, enabling comparison of AH with the height distribution in the normal adult population. For 4 patients, a BA radiograph at discontinuation

of treatment was not available, but bone age was extrapolated from a BA determination closest to this time point (at visit x) using the formula: $BA_{stop} = (BA_{visit\ x}/CA_{visit\ x}) \times age_{stop}$.

A total of 24 out of 28 patients consented to undergo an X-ray of the left hand for automatic determination of the pediatric bone index (PBI), an index for the amount of cortical bone specifically developed for the pediatric population (20). PBI was expressed as SDS based on a Dutch reference cohort. For patients older than 19 yr, SDS values were calculated using references for 19-yr old adolescents.

Parental height SDS was calculated and corrected for the secular trend (in the Netherlands estimated at 4.5 cm/30 yr) as follows: $Height_{father}$ SDS = $[(AH_{father} + 4.5) - 184] / 7.1$ and $Height_{mother}$ SDS = $[(AH_{mother} + 4.5) - 170.6] / 6.5$ (19). Conditional target height (cTH), which is the target height corrected for the effect of assortative mating and parent-offspring correlations, was calculated using the formula: cHT SDS = 0.72 × the average of father's and mother's height SDS (21).

The SH, leg length (LL) and SH/H ratio were expressed in SDS based on Dutch references (22). For calculation of adult SH SDS, LL SDS, and SH/H SDS, the age of each patient was set at 21 yr.

Statistical analysis

The study was designed to compare the effects of high dose GH treatment with those of no treatment on AH. Statistical analyses were performed using the statistical package SPSS version 14.0 (SPSS, Chicago, IL). Results are expressed as mean (SD). Comparisons among treatment and control groups were made using Student's unpaired *t* tests. Possible interactions between the effect of GH treatment on the outcome parameters and the baseline parameters gender, age (age_{start}), height (H_{start} SDS), and bone age delay were analyzed by means of linear regression analysis using ANOVA applied to the whole group of subjects. Possible associations between IGF-I SDS after 3 months or 1 year of treatment with 2.0 mg/m^2 GH and the changes from IGF-I SDS at start of high dose GH were also tested by means of linear regression. The significance level was set at 0.05.

Results

A complete analysis was carried out for the remaining 28 of 40 originally included patients (70%). One female (bone age 13) and one male (bone age 15.5) from the control group had not reached AH, and their predicted AH (17) was used for further analysis. Patient characteristics at start, at stop, and at follow-up are listed in table 1. Age and BMI SDS at

baseline were higher in the GH-treated subjects compared to controls (in the original cohort of 40 patients this was not significant). GH-treated children tended to have more bone age delay than controls. The mean GH treatment period was 3.3 yr (including the first year's on/off scheme), resulting in a mean high dose GH treatment duration of 2.3 yr (range 2.0-5.0 yr, with the exception of 1 boy who stopped after 1.2 years). Children in the control group were seen for a period of 4.9 (1.9) years. AH data were collected at a mean age of 20.4 (2.3) years. The mean period elapsed between treatment discontinuation and AH analysis was 8.5 (1.7) yr (range 3.2-11.7 yr).

Effect on growth, bone maturation and puberty

At discontinuation of treatment, height SDS significantly increased in GH treated children compared to controls (table 1, figure 3), as reported previously (8). Bone maturation in the first two years of treatment was faster in GH treated children compared to controls, both in the original cohort (3.6 yr/2 yrs versus 2.0 yr/2 yrs) and in the cohort available for adult height evaluation (3.1 yr/2 yrs vs. 2.1 yr/2yrs). Over the full trial period bone maturation was also significantly advanced in GH-treated subjects compared with controls (1.6 (0.4) yr/yr versus 1.0 (0.2) yr/yr, respectively, $p<0.001$). PBI SDS was not different between the GH- and control groups (table 1). Madelung deformities or other apparent anatomical anomalies were not detected on the hand X-rays.

AH SDS, AH–H_{start} SDS, AH–H for $BA_{start,}$ and AH-cTH were not significantly different between the GH-treated and control group (table 1), and in both groups 50% of the participants attained an adult height >-2.0 SDS. The percentage of individuals with a height below the target range (cTH SDS – 1.6) decreased from 75% at start to 44% at follow up in the GH-group and from 67% to 27% in controls. The loss of growth potential after discontinuation of therapy (AH–PAH_{stop} SDS) tended to be greater in the treated group compared with controls, but the difference did not reach statistical significance ($p=0.1$).

BMI was significantly higher in the GH group compared with the control group at all stages of the trial. At follow up, BMI in GH-treated subjects was 1.0 SD higher than BMI_{start}, while there was only an increment of 0.1 SD in controls ($p<0.05$).

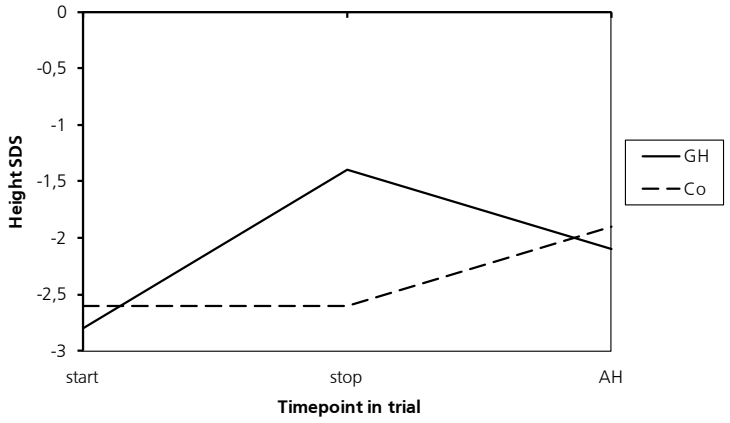
Table 1. Summary of initial and outcome variables (mean (SD)

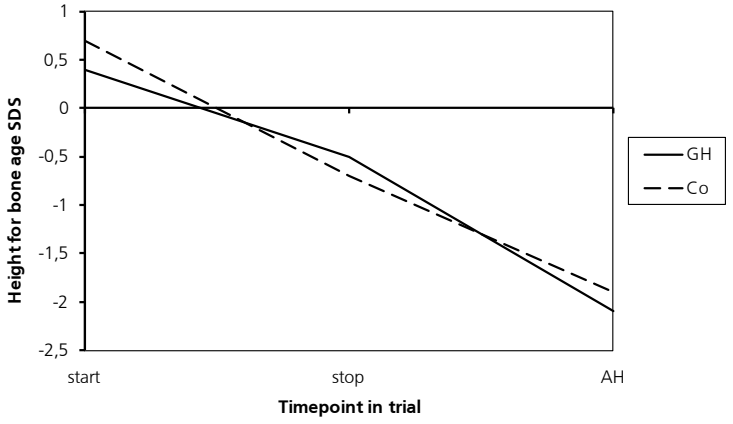
Parameter	GH (n=16)	Control (n=12)	Treatment *vs.* control
Boys/girls	12/4	8/4	
Start treatment			
Age (yr)[a]	8.7 (1.4)	7.0 (1.7)	p=0.009
Bone age delay (yr)	3.0 (1.1)	2.2 (1.3)	p=0.07
H (cm)	118.4 (8.5)	111.5 (10.6)	p=0.06
H SDS	-2.9 (0.6)	-2.5 (0.3)	p=0.09
H for BA SDS	0.4 (1.2)	0.7 (2.2)	p=0.7
cTH SDS	-0.7 (0.5)	-0.8 (0.6)	p=0.8
BMI SDS	-0.6 (0.8)	-1.1 (0.4)	p=0.04
SH SDS	-1.8 (1.0)	-1.9 (0.5)	p=0.1
Leg length SDS	-2.9 (0.7)	-2.3 (0.3)	p=0.007
SH/H SDS	1.7 (2.1)	0.8 (0.9)	p=0.2
Discontinuation of treatment (stop[b])			
Age (yr)	12.0 (1.0)	11.9 (2.0)	p=0.9
Age at T2 (yr)	11.6 (1.2)	12.1 (2.0)	p=0.5
age at B2 (girls)	10.7 (1.2)	11.5 (1.3)	p=0.5
age at G2 (boys)[c]	12.0 (1.0)	12.3 (2.2)	p=0.7
T2 SDS	0.3 (0.7)	-0.2 (1.2)	p=0.2
H (cm)	144.5 (5.6)	135.8 (6.4)	p=0.001
H SDS	-1.3 (0.8)	-2.6 (0.8)	p=0.001
H for BA SDS	-0.5 (0.6)	-0.7 (1.3)	p=0.7
H for BA SDS (stop-start)	-1.2 (0.8)	-1.3 (1.5)	p=0.04
Bone maturation[d]	1.6 (0.4)	1.0 (0.2)	p=0.000
PAH SDS	-1.3 (0.9)	-1.7 (1.1)	p=0.2
BMI SDS	-0.2 (1.0)	-1.4 (0.7)	p=0.003
SH SDS	-0.6 (1.0)	-1.7 (0.6)	p=0.02
Leg length SDS	-1.8 (0.8)	-2.2 (0.6)	p=0.3
SH/H SDS	1.4 (1.0)	1.1 (1.4)	p=0.6
Years from start to stop[e]	3.3 (0.9)	4.9 (1.9)	p=0.02
At adult height			
Age (yr)	21.0 (2.1)	19.6 (2.4)	p=0.1
AH in males (cm)	169.7 (4.2)	168.8 (3.8)	p=0.6
AH in females (cm)	154.6 (5.0)	160.8 (4.5)	p=0.1
AH SDS	-2.1 (0.7)	-1.9 (0.6)	p=0.6
[AH-H at start] SDS	0.7 (0.6)	0.7 (0.6)	p=0.8
[AH-H for BA at start] SDS	-1.6 (1.0)	-1.3 (1.1)	p=0.5
[AH-cTH] SDS	-1.4 (0.8)	-1.1 (0.4)	p=0.4
[AH-PAH at stop] SDS	-0.8 (0.9)	-0.1 (1.3)	p=0.1
BMISDS	0.6 (1.0)	-1.0 (1.2)	p=0.001
PBI SDS	0.5 (0.9)	0.2 (0.7)	p=0.4
SH SDS	-1.2 (1.2)	-1.7 (1.0)	p=0.2
Leg length SDS	-2.1 (0.6)	-1.4 (1.1)	p=0.05
SH/H SDS	1.5 (0.9)	0.4 (1.7)	p=0.04
Untreated interval (yr)	9.0 (1.5)	7.8 (2.1)	p=0.07

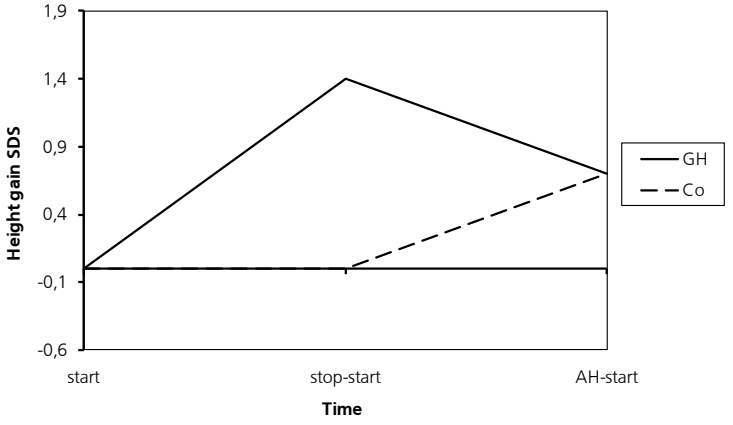
Results are presented as mean (SD). (A)H, (adult) height; BA, bone age; PBI, pediatric bone index; BMI, body mass index; cTH, conditional target height; GH, growth hormone; LL, leg length; PAH, predicted adult height; SDS, standard deviation score; SH, sitting height; SH/H, sitting height/height ratio; T2, tanner stadium 2; TH, target height. [a]Start signifies the start of the on-off scheme. [b]Stop is defined as the moment of discontinuation of GH treatment in the GH-group and the moment of attainment of T2 in the control group. [c]Two boys from the control group had late pubertal onset at the age of 14.2 and 16.0 yr, respectively. [d]Bone maturation calculated for the full trial period. [e]Includes the first year's on-off scheme.

Figure 3

Height SDS, height for bone age SDS, and height gain SDS at start and discontinuation of the intervention, and after reaching adult height (AH).







At baseline, treatment and control groups were found to be slightly disproportionate, with relatively short legs in comparison to sitting height, resulting in a positive SH/H SDS in both groups. At the end of the trial phase, SH/H SDS was similar, but at follow up it was significantly higher in the GH-group compared with controls (p=0.04). Figure 4 shows SH SDS, LL SDS, and SH/H SDS at start and at follow up. GH-treated patients displayed an increased growth of trunk and legs compared with controls during the 4 years after start of the trial phase, whereas controls had more or less stable SH SDS and LL SDS which increased after (more than) 4 years. The GH-treated group had a longer trunk, but shorter legs than controls at follow up.

Our analysis 5 years after inclusion demonstrated significantly earlier pubertal onset in GH-treated subjects (8). However, in our present analysis on 26 out of 28 subjects (missing data on pubertal onset for 1 patient from each group) the difference did not reach statistical significance (p=0.5) (table 1). In boys in the treatment and control groups pubertal onset ranged from 10.3 to 13.6 yr and 9.2 to 16.0 yr, respectively. In girls these ranges were 9.2-12.1 yr and 10.0-12.4 yr, respectively. At the attainment of Tanner stage 2, mean pubertal stage SDS for age (in boys and girls) was 0.3 (0.7) and -0.2 (1.2) in the treatment and control groups, respectively (p=0.2). None of the 12 GH-treated subjects and two boys out of 8 controls had delayed puberty (at 14.2 and 16.0 yrs), while in both groups 50% of patients had at least one parent (most often the father) with a reported late onset of puberty.

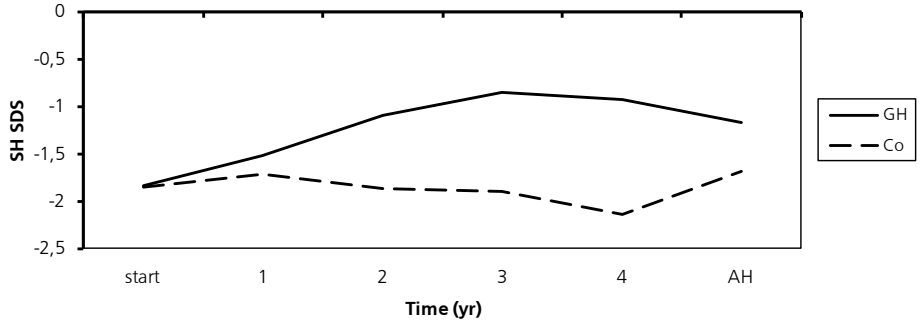
Linear regression analysis of predictors for growth response

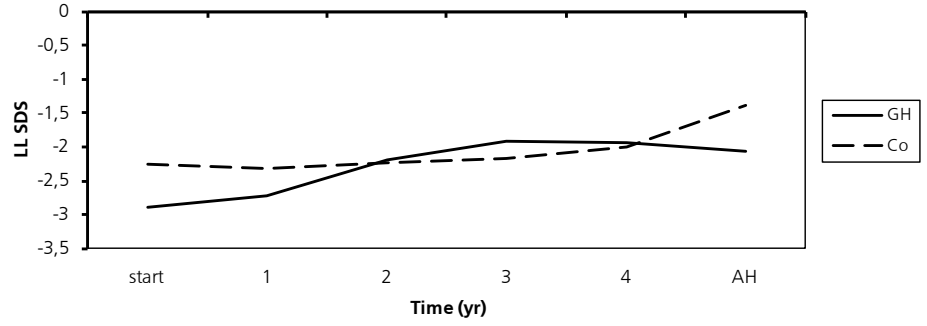
Possible interactions between the effect of GH treatment on the 4 AH outcome parameters and on AH-PAH_{stop} and the baseline parameters gender, age_{start}, H_{start}, bone age delay were analyzed. Age at baseline was a predictor for the treatment effect (GH x age_{start}) expressed as AH (B=0.4, CI=0.03-0.7, p=0.04) and AH–H_{start} (B=0.4, CI=0.06-0.7, p=0.02), but not for the other outcome parameters, thus older children had a better response to treatment. Gender showed a negative interaction with treatment effect (GH × gender) for AH (B=-1.1, CI=-2.3-0.01, p=0.052), with boys showing a larger increase in AH SDS than girls. Height and bone age delay at start were no significant predictors for treatment effect in any of the outcome parameters.

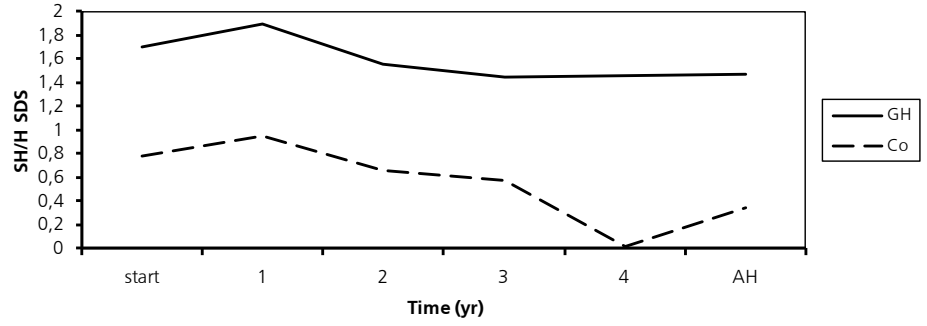
IGF-I SDS after three months of treatment with 2.0 mg/m2 GH was associated with growth potential loss, with higher IGF-I levels resulting in a higher loss of growth potential expressed as AH-PAH_{start} (B=-0.6, CI=-1.2 – (-0.02), p=0.045). The change in IGF-I SDS between start and 3 months of high dose GH treatment showed a trend towards negative interactions with treatment effect expressed as AH SDS (B=-0.5, CI=-1.0-(-0.1), p=0.09) and [AH-$HforBA_{start}$] SDS (B=-1.0, CI=-2.0-(-0.1), p=0.07), with higher changes in IGF-I SDS over 3 months of high dose GH treatment showing lower increases in AH SDS and [AH-$HforBA_{start}$] SDS.

Figure 4

Development of SH SDS, LL SDS, and SH/H SDS during four years after onset of the trial phase and during follow-up until AH. At start and on AH, data of 100% of patients are represented. After 1, 2, 3, or 4 whole years after start of the trial phase, data of 96%, 89%, 89%, and 61% of patients are shown.







Genetic analysis

Informed consent for genetic analysis was obtained from 18 out of 28 patients (11 GH-treated, 8 controls). SNP-array did not detect insertions, deletions or duplications explaining short stature. Mutational analysis was not performed.

Discussion

The long-term results of this first randomized controlled study on the effect of high dose GH treatment restricted to the prepubertal period show that this regimen does not lead to an increased adult height. This confirms our earlier findings after discontinuation of treatment, where we showed that the positive growth response significantly accelerated skeletal maturation and advanced the onset of pubertal development, and did not improve predicted adult height (8). In contrast to retrospective studies (3), where the growth response was inversely associated with age at start of treatment, in our study a younger age at start was associated with a lower adult height.

The lack of effect of this therapeutic regimen can be explained in at least three ways. The most likely explanation is that a high GH dose (approximately 3 times higher than substitution) (23) administered to young children not only leads to faster growth but also results in faster bone maturation. There are only few data on GH treatment of young children with ISS, as in virtually all studies the average age was approximately 11 years. It seems unlikely that the high dose *per se* causes the lack of effect, because children treated with a high GH dosage (67 µg/kg.day) starting at a mean age of 11 years achieve an adult height gain of 1.3 SD, which is slightly more than on 33 µg/kg/day (24). We speculate that the epiphyseal plates of young children may be more sensitive to high doses of GH and/or IGF-I than at later ages. The finding that a higher IGF-I SDS after 3 months of high dose GH treatment was associated with less adult height gain would suggest that circulating IGF-I plays a role in advancing epiphyseal maturation. The report by Cohen et al (2007) that even on a high GH dose (median 98, range 20-346 µg/kg/d) titrated on circulating IGF-I levels of +2SDS, administered to young children (age range 2.9-13.5, mean 7.53), no bone age advance was observed is not necessarily in contradiction with our findings, as the dose range in that study was large, and the children who needed high GH doses to reach the aimed IGF-I level may have been more resistant to GH. The trend towards a worse response to treatment in females may reflect the relatively strong influence of estrogens on bone maturation.

A second explanation is that the effect on bone maturation may be caused by the on-off GH treatment scheme employed during the first year of the trial, that may have primed the epiphyseal growth plate. We cannot exclude this possibility, but consider it less likely than the first explanation. The third possibility, that also cannot be ruled out with certainty, is that the poor result may be due to discontinuation of GH in puberty. The discontinuation of GH may have led to a 'catch-down' phenomenon, as was previously shown in children with SGA, who showed attenuation of growth after discontinuation of GH while puberty (and thus skeletal maturation) was progressing (25). However, the equal predicted adult height in the GH-treated and control groups at discontinuation of the trial phase argues against this hypothesis.

There are two noteworthy limitations of our study. First, the long diagnostic phase that may have been a confounder of the effect of long-term GH therapy. Second, the small size of the cohort. With respect to the latter limitation, we believe that even in this small study group the absence of any effect of treatment makes it very unlikely that this is a false negative result.

Our results imply that there may be an inverse U-shaped relationship between GH dose and adult height gain, if treatment is started at a young age. Dose is positively associated with adult height gain in the range of 25-50 µg/kg/d (2), but in young children higher doses may decrease adult height gain due to accelerated maturation of the epiphyseal plates and possibly also of the GnRH regulatory center, while the effect on growth has reached a plateau. This observation appears in contradiction to the overgrowth and tall adult height of children with pituitary gigantism, but in that condition plasma GH levels are characterized by an elevated baseline without high peaks, while the GH profile on a high GH dose shows one very high peak per day, followed by approximately 12 hours of suppression. During the peak plasma free GH must be considerably higher than in children with pituitary gigantism. Furthermore, the different GH profiles may also have different biological effects, similarly to observations in rodents (26).

The effect of a high GH dosage on pubertal onset in young children is less clear. In the final analysis on 26 children using a novel technique for expressing pubertal stage in SDS (correcting for age and gender) (18), we found a trend ($p=0.2$), but no statistically significant difference between the groups at Tanner stage 2. While this technique enables appropriate correction for the (statistically significant) age difference at start of the trial between the groups, the inability to reach statistical significance may well be related to the limited number of subjects that could be studied at follow up. In the larger group of 35 subjects studied 5 years after inclusion, the age difference at start did not reach significance, some patients had not yet entered puberty at the moment of analysis, and another method (cumulative proportions of patients having entered puberty, and calculation of relative risk) was used. In that analysis the relative risk for early puberty, adjusted for age and sex, was 4.7 (1.4-15.8, $p=0.012$) (8). There are two other observations that can serve as indirect evidence for an effect on puberty onset. First, the

observation that none of the 12 males in the GH treatment group entered puberty late, compared to 2 of the 8 controls. Second, at follow-up the GH-treated subjects had a significantly shorter leg length than controls and a higher SH/H SDS, suggesting earlier exposure to sex steroids. The higher SH/H ratio may also explain the increase in BMI SDS observed in the GH-treated children (27). Unfortunately, the study design during follow-up did not allow for the collection of sufficient data on the progression of puberty.

The untreated controls do not only serve as comparison with the GH treated children, but also to illustrate the natural history of ISS. Up to early adolescence, height SDS remained stable at -2.6, but adult height was 0.7 SD higher than height SDS at start, presumably due to a rather delayed and possibly protracted puberty. A similar pattern was seen for SH and LL SDS. This result confirms our and others' earlier findings (28;29). It also shows that HSDS for BA in young children severely over-predicts adult height. However, the average predicted adult height according to Bayley and Pinneau at discontinuation of the trial was almost identical with the attained adult height, consistent with our previous report (30).

In conclusion, high-dose GH treatment limited to the prepubertal period in young children with ISS has no effect on adult height, probably caused by concomitant advance of bone maturation, and may advance pubertal onset.

Acknowledgements

We greatly appreciate the contributions of H.Visser-van Balen and J. Looij in performing measurements, and the financial support of Pfizer (New York, USA) for this study. We express our gratitude to Hans van der Horst, Annelies van Gool, Simone Sanders, and Anna Rompen from Justus Medical Center (Eindhoven, The Netherlands) for facilitating our study.

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