CLINICAL STUDY

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

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Abstract

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

Design and methods: Forty children with no signs of puberty, age at start 4–8 years (girls) or 4–10 years (boys), height SDS < −2.0 SDS, and birth length > −2.0 SDS, were randomly allocated to receive GH at a dose of 2 mg/m² per day (equivalent to 75 µg/kg per day at start and 64 µg/kg per 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 (s.d.) age of 20.4 (2.3) years.

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

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Introduction

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 (first year's height velocity), bone age (BA) 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 at 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 per day. No acceleration of BA advance and pubertal onset and progress has been observed in the dose range of 30–53 µg/kg per day, and the most effective dose regimen (50 µg/kg per day) leads to ~7 cm AH 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. Secondly, the relative contribution of GH treatment before and during puberty was unclear. Thirdly, 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 per 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 BA maturation and pubertal development, so that the predicted AH (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 AH.

Subjects and methods

Patients

This report includes AH data on 28 of 40 children with ISS who originally enrolled in a multicenter study in The Netherlands from December 1993 to December 1996. Inclusion criteria were as follows: 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 $\mu\text{g/l}$ (1 mg = 2 IU, using World Health Organization International RP 66/217 as standard) after provocation (exercise, arginine, clonidine, L-dopa, or glucagon); and a normal sitting height (SH)/subischial leg length (LL) 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: one girl and one boy in the GH-treated group, and one boy in the control group. H_{start} SDS was calculated using Dutch references (-2.50 , -2.73 , and -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 metaRegister of Controlled Trials (ISRCTN52337368) of the Current Controlled Trials Ltd (London, UK).

Study design

Forty patients were randomly allocated to receive GH treatment or no treatment (Fig. 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 per day (equivalent to 19 or 38 $\mu\text{g/kg}$ per day respectively) during two periods of 3 months, separated by two washout periods of 3 months without GH treatment (Fig. 2). In the second year, long-term GH treatment with 2.0 mg/m^2 per day (75 $\mu\text{g/kg}$ per day) was started and was intended to be given for at least 2 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 years). At discontinuation of GH treatment, the dose was equivalent to 64 $\mu\text{g/kg}$ per day. 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, Pfizer Inc., New York, NY, USA) was administered subcutaneously, 7 days per week between 1800 and 2000 h. The measurements at discontinuation of treatment in the GH-treated group were compared with measurements after attaining Tanner stage 2 (B2 for girls and 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 AH analysis, six patients from the control group could not be motivated to participate. One patient from the GH-treated group could not be traced and was lost to

Figure 1 Trial design.

Figure 2 Time scheme for GH-treated and control groups. GH doses: 0.5 mg/m² per day = 19 µg/kg per day; 1.0 mg/m² per day = 38 µg/kg per day; and 2.0 mg/m² per day = 75 µg/kg per day.

follow-up. One boy stopped using high-dose GH after 1.2 years and could not be motivated to continue according to the protocol. However, his growth data are included in this report. Pubertal onset and development were not registered for one girl from the control group, and her last known auxological information at the age of 9.7 years was used for the analysis at study end.

At follow-up, we took a short medical history, performed a physical examination; assessed BA (15); and measured height, weight, and SH. 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 250 K 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: i) AH SDS; ii) AH minus height at start SDS ($AH - H_{start}$ SDS); iii) AH minus height for BA at start ($AH - H$ for BA_{start} SDS); and iv) AH minus conditional target height (cTH) SDS ($AH - cTH$ SDS). Because of the young BA of most patients at start of the intervention, the PAH according to Bayley & 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 PAH 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 body mass index (BMI) SDS were based on recent Dutch references (19). For calculation of AH SDS, the age of each patient was set at 21 years, enabling comparison of AH with the height distribution in the normal adult population. For four patients, a BA radiograph at discontinuation of treatment was not available, but BA 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 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 years, SDS values were calculated using references for 19-year-old adolescents.

Parental height SDS was calculated and corrected for the secular trend (in The Netherlands estimated at 4.5 cm/30 years) 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). cTH, which is the target height corrected for the effect of assortative mating and parent-offspring correlations, was calculated using the formula: $cTH\ SDS = 0.72 \times$ the average of father's and mother's height SDS (21).

The SH, 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 years.

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, USA). Results are expressed as mean (s.d). 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 BA delay were analyzed by means of linear regression analysis using ANOVA applied to the whole group of subjects. Possible associations between insulin-like growth factor 1 (IGF1) SDS after 3 months or 1 year of treatment with 2.0 mg/m² GH and the changes from IGF1 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 (BA 13) and one male (BA 15.5) from the control group had not reached AH, and their PAH (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 with the controls (in the original cohort

Table 1 Summary of initial and outcome variables mean (s.d.). Results are presented as mean (s.d.).

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

AH, (adult) height; BA, bone age; PBI, pediatric bone index; BMI, body mass index; cTH, conditional target height; LL, leg length; PAH, predicted adult height; SH, sitting height; SH/H, sitting height/height ratio; T2, Tanner stage 2; TH, target height.

^aStart signifies the start of the on-off scheme.

^bStop is defined as the moment of discontinuation of GH treatment in the GH-treated group and the moment of attainment of T2 in the control group.

^cTwo boys from the control group had late pubertal onset at the age of 14.2 and 16.0 years respectively.

^dBone maturation calculated for the full trial period.

^eIncludes the first year's on-off scheme.

of 40 patients, this was not significant). GH-treated children tended to have more BA delay than controls. The mean GH treatment period was 3.3 years (including the first year's on-off scheme), resulting in a mean high-dose GH treatment duration of 2.3 years (range 2.0–5.0 years,

with the exception of one 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 that elapsed between treatment discontinuation and AH analysis was 8.5 (1.7) years (range 3.2–11.7 years).

Effect on growth, bone maturation, and puberty

At discontinuation of treatment, height SDS increased significantly in GH-treated children compared with the controls (Table 1, Fig. 3) as reported previously (8). Bone maturation in the first 2 years of treatment was faster in GH-treated children compared with the controls, both in the original cohort (3.6/2 vs 2.0/2 years) and in the cohort available for AH evaluation (3.1/2 vs 2.1/2 years). Over the full trial period, bone maturation was also significantly advanced in GH-treated subjects compared with the controls (1.6 (0.4) years/years versus 1.0 (0.2) years/years respectively, $P < 0.001$). PBI SDS was not different between the GH-treated and control groups (Table 1). Madelung's 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} SDS, and AH - cTH SDS were not significantly different between the GH-treated and control groups (Table 1), and in both groups, 50% of the participants attained an AH > -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-treated group and from 67 to 27% in the controls. The loss of growth potential after discontinuation of therapy (AH - PAH_{stop} SDS) tended to be greater in the treated group compared with the 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 s.d. higher than BMI_{start}, while there was only an increment of 0.1 s.d. in the controls ($P < 0.05$).

At baseline, treatment and control groups were found to be slightly disproportionate, with relatively short legs in comparison to SH, 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-treated group compared with the 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 the controls during the 4 years after the 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.

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).

Our analysis 5 years after inclusion demonstrated significantly earlier pubertal onset in GH-treated subjects (8). However, in our present analysis on 26 of 28 subjects (missing data on pubertal onset for one 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 years and from 9.2 to 16.0 years respectively. In girls, these ranges were 9.2–12.1 years

and 10.0–12.4 years 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 of eight controls had delayed puberty (at 14.2 and 16.0 years), while in both groups 50% of the 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 four AH outcome parameters and on $AH - PAH_{stop}$ and the baseline parameters gender, age_{start} , H_{start} , and BA delay were analyzed. Age at baseline was a predictor for the treatment effect ($GH \times age_{start}$) expressed as AH ($B=0.4$, 95% CI=0.03–0.7, $P=0.04$) and $AH - H_{start}$ ($B=0.4$, 95% 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 ($GH \times gender$) for AH ($B=-1.1$, CI= -2.3 – 0.01 , $P=0.052$), with boys showing a larger

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

increase in AH SDS than girls. Height and BA delay at start were not significant predictors for treatment effect in any of the outcome parameters.

IGF1 SDS after 3 months of treatment with 2.0 mg/m² GH was associated with growth potential loss, with higher IGF1 levels resulting in a higher loss of growth potential expressed as AH-PAH_{start} ($B = -0.6$, 95% CI = -1.2 – (-0.02) , $P = 0.045$). The change in IGF1 SDS between start and 3 months of high-dose GH treatment showed a trend toward negative interactions with treatment effect being expressed as AH SDS ($B = -0.5$, 95% CI = -1.0 – (-0.1) , $P = 0.09$) and AH- H for BA_{start} SDS ($B = -1.0$, 95% CI = -2.0 – (-0.1) , $P = 0.07$), with higher changes in IGF1 SDS over 3 months of high-dose GH treatment showing lower increases in AH SDS and AH- H for BA_{start} SDS.

Genetic analysis

Informed consent for genetic analysis was obtained from 18 of 28 patients (11 GH-treated subjects and 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 AH. 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 PAH (8). In contrast to retrospective studies (3), where the growth response was inversely associated with age at start of the treatment, in our study a younger age at start was associated with a lower AH.

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 three times higher than substitution) (23) administered to young children not only leads to faster growth but also results in faster bone maturation. There are few data on GH treatment of young children with ISS, as in virtually all studies, the average age was ~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 per day) starting at a mean age of 11 years achieve an AH gain of 1.3 s.d., which is slightly more than that achieved on 33 µg/kg per day (24). We speculate that the epiphyseal plates of young children may be more sensitive to high doses of GH and/or IGF1 than at later ages. The finding that a higher IGF1 SDS after 3 months of high-dose GH treatment was

associated with less AH gain would suggest that circulating IGF1 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 per day) titrated on circulating IGF1 levels of +2 SDS, administered to young children (age range 2.9–13.5 years, mean 7.53), no BA advance 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 IGF1 level may have been more resistant to GH. The trend toward a worse response to treatment in females may reflect the relatively strong influence of estrogens on bone maturation.

The 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, which may have primed the epiphyseal growth plate. We cannot exclude this possibility, but should consider it less likely than the first explanation. The third explanation, which 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 shown previously in children with short for gestational age, who showed attenuation of growth after discontinuation of GH while puberty (and thus skeletal maturation) was progressing (25). However, the equal PAH 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. Secondly, 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 AH gain, if treatment is started at a young age. Dose is positively associated with AH gain in the range of 25–50 µg/kg per day (2), but in young children higher doses may decrease AH 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 AH 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 ~12 h of suppression. During the peak, plasma free GH must be considerably higher than that 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 the start of the trial between the groups, the inability to reach statistical significance may well be related to the limited number of subjects who 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 with two of the eight controls. Secondly, at follow-up, the GH-treated subjects had a significantly shorter LL 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 not only serve as comparison for the GH-treated children, but also illustrate the natural history of ISS. Up to early adolescence, height SDS remained stable at -2.6 , but AH was 0.7 s.d. 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 HS/DS for BA in young children severely over-predicts AH. However, the average PAH according to Bayley and Pinneau at discontinuation of the trial was almost identical to the attained AH, 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 AH, probably caused by concomitant advance of bone maturation, and may advance pubertal onset.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

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