Final height in girls with Turner's syndrome treated with once or twice daily growth hormone injections

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Abstract
Objectives—To study final height in girls with Turner's syndrome treated with once or twice daily injections of growth hormone (GH) in combination with low dose ethinyl oestradiol.
Design—Until final height was reached, the effect of fractionated subcutaneous injections given twice daily was compared with once daily injections of a total GH dose of 6 IU/m^2/day. Twice daily injections were given as one third in the morning and two thirds at bedtime. All girls concurrently received low dose oestradiol (0.05 µg ethinyl oestradiol/kg/day, increased to 0.10 µg/kg/day after 2.25 years).
Patients—Nineteen girls with Turner's syndrome aged ≥ 11 years (mean (SD) 13.6 (1.7) years).
Measurements—To determine final height gain, we assessed the difference between the attained final height and the final height predictions at the start of treatment. These final height predictions were calculated using the Bayley-Pinneau (BP) prediction method, the modified projected adult height (mPAH), the modified index of potential height (mIPH$_{RUS}$), and the Turner's specific prediction method (PTS$_{RUS}$).
Results—The gain in final height (mean (SD)) was not significantly different between the once daily and the twice daily regimens (7.6 (2.3) *v* 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 final height gain > 5.0 cm. Mean (SD) attained final height was 155.5 (5.4) cm. A "younger bone age" at baseline and a higher increase in height standard deviation score for chronological age (Dutch–Swedish–Danish references) in the first year of GH treatment predicted a higher final height gain after GH treatment.
Conclusions—Division of the total daily GH dose (6 IU/m^2/day) into two thirds in the evening and one third in the morning is not advantageous over the once daily GH regimen with respect to final height gain. Treatment with a GH dose of 6 IU/m^2/day in combination with low dose oestrogens can result in a significant increase in adult height in girls with Turner's syndrome, even if they start GH treatment at a relatively late age.
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Keywords: Turner's syndrome; growth hormone treatment; final height; growth

Growth failure and subsequently short adult stature is one of the main features in Turner's syndrome.[1][2] It has been shown that short stature, independent of aetiology, can be associated with psychosocial problems.[3] Growth hormone (GH) treatment improves height velocity and adult height in most girls with Turner's syndrome.[4–12] Studies in patients with Turner's syndrome have shown that the initial growth response to GH treatment is dependent on the dose and frequency of administration.[4–6][13][14] 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 Turner's syndrome is characterised 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] Thus, a more frequent injection regimen might 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/m^2 body surface/day was divided into two thirds in the evening and one third in the morning in one group of patients. The other group received the same total GH dose once daily, in the evening. A total dose of 6 IU/m^2/day was chosen instead of the more commonly used 4 IU/m^2/day because an earlier study has shown that on the latter dose the growth response in a somewhat older subgroup of girls with Turner's syndrome 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 twice daily and once daily GH administration in 19 girls with Turner's syndrome aged 11 years or over concurrently receiving low dose ethinyl oestradiol.

Patients and methods
STUDY GROUP
We studied 19 previously untreated girls with Turner's syndrome, confirmed by lymphocyte

chromosomal analysis. Before treatment, 10 girls were enrolled in a 10 week (crossover design) 24 hour GH profile study, as described earlier.[24] In brief, they started taking ethinyl oestradiol (0.05 µg/kg/day) four weeks before they were divided randomly into the once daily or twice daily GH injection groups. GH was then administered for two weeks. Following a washout interval of two weeks, GH treatment was resumed for another period of two weeks, using the alternative injection frequency. The additional nine girls followed the same schedule without 24 hour GH profile testing. 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. At the start of the crossover study, all girls had Tanner puberty stage B1,[25] were aged 11 years or over, and had a Tanner and Whitehouse RUS (radius, ulna, short bones) bone age (RUS BA)[26] of less than 13.5 years. Exclusion criteria were: associated endocrine and/or metabolic disorders, growth failure caused by other disorders or emotional deprivation, hydrocephalus, and previous use of drugs that might interfere with GH treatment. 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 divided randomly into two GH injection frequency groups. One group (n = 9) received 6 IU/m^2 body surface once daily, in the evening. A second group (n = 10) received the same total GH dose divided into one third in the morning and two thirds at bedtime. GH (r-hGH Norditropin; Novo Nordisk A/S, Bagsvaerd, Denmark) was injected by a pen injection system (Nordiject 24; Novo Nodisk A/S). Compliance was carefully monitored. GH treatment was stopped when height velocity had decreased to < 1 cm/six months. The figure for final height was recorded six months after the end of treatment. From the start of the 10 week crossover study, all girls received 0.05 µg ethinyl oestradiol/kg/day, once daily. After the first 2.25 years of GH treatment, the dose of ethinyl oestradiol was increased to 0.10 µg/kg/day and cyclic progestagen treatment was added.

GROWTH EVALUATION

Height (H) was measured at baseline and three monthly until final height was reached. Heights were determined according to Cameron,[27] using a Harpenden stadiometer; four measurements were made on each visit by two trained observers (AvT and later ThS). Height was expressed as $HSDS_{CA}$ using the Roede en van Wieringen references for healthy Dutch girls ($HSDS_{CA}$ (RvW))[28] and the Dutch–Swedish–Danish Turner's references ($HSDS_{CA}$ (DSD)).[2] At final height, $HSDS_{CA}$ (DSD) was calculated by using the mean height (146.95 cm) and the standard deviation (6.37 cm) of untreated girls

with Turner's syndrome (DSD references) of 21 years of age. Target height (TH) was adapted from Dutch reference data[28] with the addition of 3 cm for secular trend: TH = ½* (H_{mother} + H_{father} − 12 cm) + 3 cm. Pubertal stages were assessed according to Tanner.[25] Bone age was determined by the same two investigators (AvT and later ThS) according to Tanner and Whitehouse RUS BA[26] and to Greulich and Pyle (GP BA).[29] To determine final height gain, we assessed the difference between the attained final height and the final height predictions at start of treatment. These final height 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's specific prediction method (PTS_{RUS}), based on regression coefficients for height, chronological age, and RUS BA.[32] The latter three methods are based on Dutch Turner's references, as described previously.[32]

Before any treatment, all girls underwent a GH provocation test by infusion of arginine (0.5 g/kg body weight in 30 minutes). In addition, blood was taken for the determination of insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), and GH binding protein (GHBP) at the start of our study (pretreatment) and at six and 18 months after it started. The methods and the results of these measurements were described previously.[23 24 34–37]

STATISTICS

Results are expressed as mean (SD) unless indicated otherwise. The Student's t test or the χ^2 test were 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 final height between the once daily and twice daily groups, a multiple linear regression analysis adjusted for baseline variables was performed. For this analysis, RUS BA and the average of the four final height predictions were chosen as baseline variables. In the search for determinants of treatment success (final height gain = attained final height minus predicted final height), multiple linear regression analyses, adjusted for treatment group, were done. For each possible predictive factor, separate analyses were performed. Possible predictive factors for final height gain after GH treatment were at baseline: chronological age, RUS BA, $HSDS_{CA}$ (DSD), target height, concentrations of IGF-I, IGFBP-3, IGF-I to IGFBP-3 ratio, GHBP, and the maximal GH value during a provocation test; after the first six months of GH treatment: the change from baseline of IGF-I, IGFBP-3, IGF-I to 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.

Table 1   Pretreatment clinical data

	Once daily group (n = 9)	Twice daily group (n = 10)
CA (years)	13.3 (1.7)	13.8 (1.8)
RUS BA (years)	12.2 (1.0)	12.7 (0.9)
GP BA (years)	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)
Target height (cm)	166.5 (4.5)	168.3 (6.3)
Karyotype		
45,X	6	8
Other	3	2

Results are mean (SD).
CA, chronological age; BA, bone age, RUS, radius, ulna, short bones; GP Greulich and 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's syndrome.

Table 2   Predictions of adult height at start of growth hormone treatment

Prediction method	Once daily group (n = 9)	Twice daily group (n = 10)
Modified projected adult height	146.7 (6.0)	151.5 (6.9)
Modified index of potential height, including RUS bone age	146.9 (3.7)	151.2 (5.6)
Bayley and Pinneau prediction of adult height	145.8 (5.2)	151.5 (6.9)
Turner's specific FH prediction method, including RUS bone age	147.4 (5.7)	151.4 (6.4)
Average of the four predictions	146.7 (4.9)	151.4 (6.3)

Results are mean (SD) centimetres.
FH, Final height; RUS, radius, ulna, short bones.

Results

GROWTH EVALUATION

Table 1 lists the baseline (after the second randomisation) 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. Before treatment, the mean chronological age, bone age, and $HSDS_{CA}$ values of the girls in the twice daily group were slightly (not significantly) higher compared with those in the once daily group.

Table 2 shows the mean predicted adult height according to the four different prediction methods. For both 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 3 shows the clinical data at final height. The difference in final height between the two treatment groups was 2.2 cm in favour of the twice daily group, but this difference was not significant. However, after adjustment for baseline variables (RUS BA and the predicted adult height), the mean final height of the twice daily group minus the mean final height of the once daily group was −1.2 cm (95% confidence interval, −3.8 to 1.4). Thus, the once daily group had a slightly better growth response on GH treatment until final height compared with the twice daily group, but this

difference was not significant. For both treatment groups, final height was significantly higher than the predicted adult height at start ($p \leq 0.001$). Sixteen of the 19 girls have reached a final height > 150 cm, and five of them even had a final height > 160 cm.

The duration of GH treatment (from start of GH treatment until height velocity < 1 cm/six months) was significantly shorter in the twice daily group compared with the once daily group, even after adjustment for RUS BA at baseline ($p < 0.02$). No significant difference in bone maturation from the start of GH treatment until final height was found between the once daily and twice daily groups: bone age to chronological age ratio (year:year) was 0.7 v 0.8, respectively.

At final height, HSDS (RvW) and HSDS (DSD) were significantly increased compared with baseline in the once daily group ($p < 0.001$). In the twice daily group, both HSDS (RvW) and HSDS (DSD) were increased compared with baseline, but only the increase in HSDS (RvW) was significant ($p < 0.001$). The increase in $HSDS_{CA}$ (RvW and DSD) was higher in the once daily group compared with the twice daily group, but this difference was not significant. All girls exceeded their adult height prediction (final height gain range, 1.6–12.3 cm). Thirteen of the 19 girls had a final height gain > 5.0 cm.

RELATIONS WITH GROWTH RESPONSE

Multiple linear regression analyses of the final height gain after adjustment for treatment group revealed a significant negative correlation with baseline RUS BA ($\beta = -1.6$; $p = 0.021$) (fig 1) and a significant positive correlation with increase in $HSDS_{CA}$ (DSD) in the first year of GH treatment ($\beta = 7.8$; $p = < 0.0001$) (fig 2). Final height gain was not significantly related to pretreatment chronological age, $HSDS_{CA}$ (DSD), target height, plasma IGF-I, IGFBP3, IGF-I to IGFBP3 ratio, GHBP, or maximum GH levels after provocation, nor to the change after six months of GH treatment in plasma IGF-I, IGFBP3, IGF-I to IGFBP3 ratio, and GHBP.

Pubertal development

Tanner breast stage development was not significantly different between the once daily and twice daily groups: 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 experienced their menarche.
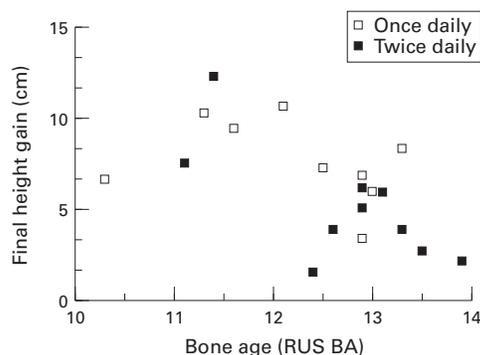
Table 3   Clinical data at final height

Treatment group	n	Final height (cm)	Predicted adult height (cm)	Months of GH treatment	Increase $HSDS_{CA}$ (RvW)	Increase $HSDS_{CA}$ (DSD)	Final height gain (cm)*
Once daily	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)
Twice daily	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)

Results are mean (SD).
*Final height minus predicted adult height.
$HSDS_{CA}$, 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's syndrome.

Figure 1 Relation between the final height gain (cm) and the bone age (RUS BA) (years) at start of the GH treatment (β = −1.6; p = 0.021).
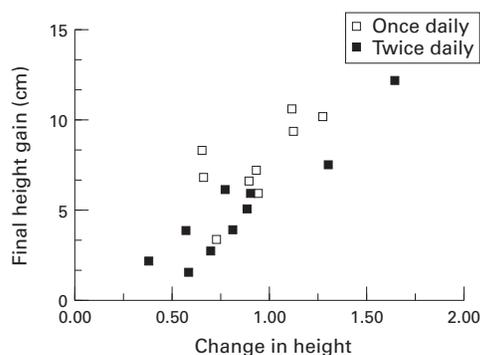

Figure 2 Relation between the final height gain (cm) and the increase in HSDS$_{CA}$ (DSD) in the first year of GH treatment (β = 7.8; p = < 0.0001)

SIDE EFFECTS

No clinically relevant side effects attributable to GH were noted.

Discussion

Division of the total daily GH dose (6 IU/m^2/day) into two thirds in the evening and one third in the morning is not advantageous over the once daily GH regimen with respect to final height gain. Final height tended towards a higher mean value with once daily GH compared with twice daily GH after correction for baseline variables, but a significant difference between the two groups was not found. The mean corrected difference in final height (twice daily minus once daily) was −1.2 cm with a 95% confidence interval between −3.8 and +1.4 cm in favour of the once daily group. The absence of a significant difference in final height between the once daily and twice daily groups might be caused by lack of power as a result of 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 twice daily group can be excluded with 95% confidence.

Our final height data are in line with the two year results.[23] The growth response after two years' GH on a total daily dose of 6 IU/m^2 in combination with 0.05 μg ethinyl oestradiol/kg/day was not significantly different between the two GH injection regimens. However, height velocity 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 once daily group compared with the twice daily group. Thus, a tendency in favour of once daily injections was already seen in the first two years of treatment.

The 24 hour GH profiles in 10 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 twice daily GH treatment, although the GH dose at bedtime was only twice as high as the morning dose. In addition, the mean AUC values for the night time period were 1.9 times higher for the once daily treatment than the twice daily one, while the GH dose at bedtime was only 1.5 times higher in the once daily group.[24] These results suggest a difference in GH bioavailability and are in line with the trend towards a better final height gain in the once daily group.

To our knowledge, our study is the only one in which girls with Turner's syndrome have received twice daily injections of GH until final height. In a study in girls with Turner's syndrome, comparing a once daily regimen with a twice daily regimen in which an equal division of a daily GH dose (25 IU/m^2/week) was made, the change in height velocity in the first year of treatment was somewhat higher in the once daily group (mean (SD), 3.5 (1.3) cm/year) compared with the twice daily group (mean (SD), 2.7 (1.8) cm/year), but also in this study the difference was not significant.[38]

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

The final height gain was, independent of treatment group, negatively related with baseline RUS BA. Therefore, the large interindividual differences in final height 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 the start. Accordingly, this relation with final height gain cannot be explained by regression to the mean. In addition, final height gain was positively related with the increase in HSDS$_{CA}$ (DSD) in the first year of GH treatment. In

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

Reference	n	Growth hormone		Oxandrolone dose (mg/kg/day)	Ethinyl oestradiol		FH gain over (mean)	
		Age start (years)	Dose (IU/m²/week)		Age start (years)	Dose (ng/kg/day)	BP (cm)	(m)PAH (cm)
Massa *et al* (1995)[10]	45	< 12	24		> 12	100	4.7	2.6
		> 12	2 years GH: 24					
			> 2 years GH: 36					
Van den Broeck *et al* (1995)[7]	78	Mean 11.7–14.6	~ 21–27		Mean 14.5	2–5 µg/day	3.3	3.0
Taback *et al* (1996)[11]	17	Median 12.4	~ 29		Median 13.0	5–20 µg/day	–	−0.2
	14		None		Median 13.3	5–20 µg/day	–	−3.3
Nilsson *et al* (1996)[8]	44	Mean 12.2						
	6		1 year: no GH	0.05	Mean ~15.3	In 4 years from 100 to 400	–	7.4
			> 1 year: ~21					
	7		1 year: no GH	0.05	Mean ~13.3	100	–	4.3
			> 1 year: ~21					
	16		~ 21	0.05	Mean ~15.1	In 4 years from 100 to 400	–	8.5
	15		~ 21	0.05	Mean 12.3	100	–	3.0
					Mean BA 10.7			
Rosenfeld *et al* (1992)[12]	30	9.3	~31.5	0.0625	BA ⩾ 14	–	–	10.4
			~31.5	None			–	5.6
Haeusler *et al* (1996)[9]	20	7.3–16.4	0.9–2.5 years: 12–18, thereafter: 18	0.0625 or 5 mg testosterone/ 2 week intramuscularly	Mean BA 13.3	In 18 months from 50 to 200	–	9.3

BP, Bayley Pinneau prediction method; (m)PAH, (modified) projected adult height.

conclusion, a "younger bone age" at baseline and a higher increase in $HSDS_{CA}$ (DSD) in the first year of GH treatment predict a higher final height gain after GH treatment.

Previous publications on the effect of GH treatment on final height in girls with Turner's syndrome are summarised in table 4. The only other study in which girls with Turner's syndrome used a GH dose comparable with the dose in our study is described by Massa *et al.*[10] The lower final height gain in that study compared with our results can be explained by the lower GH injection frequency and/or total GH dose each week in the first two years of the study. Other studies in which "relatively old" girls were treated with lower GH doses (without oxandrolone) are quite disappointing.[7 11] In the published studies in which girls with Turner's syndrome started GH treatment at a younger age and/or were treated with GH in combination with oxandrolone, 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 final height gain seems to be comparable with the effect of long term lower GH dose treatment or GH combined with oxandrolone 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's syndrome. However, the higher costs of higher GH doses compared with the lower costs of oxandrolone should be taken into consideration when the use of higher GH doses is discussed.

Although oestrogen treatment in girls with Turner's syndrome can result in an acceleration of bone maturation if initiated too early,[41 42] the effect of low dose oestrogen starting within the normal range of age of puberty on final height is not clear. Nilsson *et al* found a significantly lower final height gain in the girls who started at a mean chronological age of 12.3 years with 100 ng ethinyl oestradiol/kg/day compared with the final height gain of the girls who started ethinyl oestradiol at a mean

chronological age of at least 15.1 years. However, the difference in final height gain between these groups seems to be caused by a decreased height velocity in the second and subsequent years of the treatment in the "young starting ethinyl oestradiol 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 oxandrolone) studies in which the induction of puberty was delayed until the epiphysial plates were almost closed,[7 9 12] showed no obvious better final height gain than that of our study, in which low dose oestrogens were combined with a relatively high GH dose. Despite the low oestrogen dose, apart from one girl who was still prepubertal, all girls had breast development at the end of GH treatment. Sixteen 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

Division of the total daily GH dose of 6 IU/m²/day into two thirds in the evening and one third in the morning is not advantageous over the once daily GH regimen with respect to final height gain. In addition, treatment with a GH dose of 6 IU/m²/day in combination with low dose oestrogens can result in a significant increase in adult height in girls with Turner's syndrome, even if they start GH treatment at a relatively late age.

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