

Growth hormone secretion in Turner's syndrome and influence of oxandrolone and ethinyl oestradiol

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SUMMARY We investigated 24 hour growth hormone secretion by intermittent 20 minute blood sampling in 34 prepubertal patients with Turner's syndrome, aged 4.3–12.4 years. Growth hormone profiles were analysed by the PULSAR programme and results expressed as the sum of growth hormone pulse amplitudes. Six patients had abnormal growth hormone pulse frequencies. In the remaining 28, growth hormone pulse amplitudes declined significantly with increasing age, but there was no correlation between growth hormone pulse amplitudes and growth rates. Concentrations of insulin like growth factor-1 (IGF-1) rose with age but did not correlate with either growth rates or growth hormone secretion. Fifteen patients were given oxandrolone and 11 low dose ethinyl oestradiol. Both agents increased height velocity without increasing growth hormone secretion.

We conclude that the relation between growth hormone secretion and growth in Turner's syndrome is less certain than in normal children. End organ resistance is probably due to a skeletal dysplasia. Both oxandrolone and low dose ethinyl oestradiol improve the growth of girls with Turner's syndrome, but their mechanism of action remains uncertain.

Patients with Turner's syndrome are born short, grow slowly, and achieve a reduced adult stature.^{1 2} Although individual cases of growth hormone deficiency have been reported in Turner's syndrome,³ growth hormone responses to pharmacological stimuli are similar to those seen in normal short children.^{4 5}

Endogenous growth hormone secretion is known to be pulsatile and may be assessed in children using intermittent blood sampling over 24 hours.⁶ In some children, considerable discrepancies have been noted between the growth hormone concentrations reached on such assessments and the results of pharmacological provocation tests.⁷ Tall children produce more growth hormone over 24 hours than short children,⁸ and it appears to be growth hormone pulse amplitude that correlates with growth rate in otherwise normal children.⁹ The periodicity of growth hormone secretion is also relevant, abnormal patterns being associated with inadequate growth.⁸

We sought to investigate the qualitative and quantitative aspects of growth hormone secretion in Turner's syndrome and to determine the effect of

growth promoting agents. The anabolic steroid oxandrolone is known to increase height velocity in Turner's syndrome.^{10 11} Low dose ethinyl oestradiol increases height velocity in both the short¹² and long term.¹³ The effects of these treatments on growth hormone secretion in Turner's syndrome is unknown.

Patients and methods

Thirty four patients aged 4.3 to 12.4 years (median 9.1) were studied. Karyotypes, performed by culture of peripheral lymphocytes, are shown in table 1. All had phenotypic features of Turner's syndrome. None had evidence of an emotional or eating disorder. Three patients had previously received either growth hormone, oxandrolone, or ethinyl oestradiol; these drugs had been stopped at least three months previously. One was receiving thyroxine replacement treatment. All the children were prepubertal.

Subjects were admitted the evening before the commencement of sampling when a cannula was

Table 1 *Karyotypes of 34 patients with Turner's syndrome*

No of patients	Karyotype
22	45,X
3	46,X,Xi(Xq)
4	45,X/46,Xi(Xq)
2	45,X/46,X,r(X)
2	45,X/46,XX
1	46,XXp ⁻

inserted and kept patent with a heparin in saline solution. They were freely mobile and took meals at regular times. Blood samples (1–2 ml) were withdrawn every 20 minutes, centrifuged, separated, and the serum stored at -20°C before analysis. Specimens for assay of insulin like growth factor-1 (IGF-1) were withdrawn at 06.00.

Of the 34 patients, 26 then received growth promoting treatment. Fifteen patients, aged 4.3–12.6 years (median 10.2) received oxandrolone 1.25 mg daily, or 0.625 mg if <7 years. Eleven patients, aged 5.1–11.7 years (median 9.7) received ethinyl oestradiol 1 μg daily, or 0.5 μg if <7 years; the two eldest received 2 μg . The treatments were continued for 0.5–1.0 years. Growth hormone sampling was repeated in all patients after six to 10 weeks of treatment.

Growth hormone concentrations were measured by a solid phase immunoradiometric assay (Tandem R, Hybritech¹⁴), all specimens from one subject being analysed in a single batch. The lower limit of sensitivity of the assay was 0.5 mU/l. The intra-assay coefficients of variation were 10.6, 7.8, and 4.8% at 1.4, 7.1, and 26.4 mU/l respectively.

Computerised pulse analysis was performed using the PULSAR programme¹⁵ modified for growth hormone.⁹ This generates a smoothed but variable baseline and defines pulses above that baseline, by taking into account the amplitude and duration of rises and the standard deviation of the assay. We have used simple arithmetic addition of the maximum amplitude of the defined pulses to generate the statistic 'sum of peak amplitudes'.

Plasma concentrations of IGF-1 were measured by radioimmunoassay.¹⁶ The lower limit of sensitivity of the assay was 0.03 U/ml and the coefficients of variation were 11.0, 7.2, and 7.0% at 0.15, 0.47, and 0.94 U/ml respectively.

Height was measured every three months by standard techniques using a Harpenden stadiometer. In order to account for varying height velocities at different ages, height velocity standard deviation scores (SDS) were calculated using normal data for Turner's syndrome² by the formula:

$$\text{SDS} = \frac{x - \text{mean}}{\text{Standard deviation}}$$

Bone ages were estimated by the TW2 method.¹⁷

Approval was obtained from the ethical committees of both the Middlesex Hospital and Hospital for Sick Children, Great Ormond Street, and parental consent was obtained.

Statistical analysis of trends was by Spearman's rank correlation. Effects of treatment were assessed by the Wilcoxon signed rank test. Data are expressed as mean (SD).

Results

Three sample growth hormone profiles are illustrated in fig 1. In fig 1(a) there were eight pulses in 24 hours with nocturnal accentuation of pulse amplitude. Growth hormone concentrations fell to unrecordable concentrations between pulses. Twenty eight patients had a similar pattern, which occurs in normally growing children.⁸

The profile in fig 1(b) was more disorganised, with 11 peaks, a loss of the normal dominant three hour periodicity, and an absence of a return to unrecordable concentrations. Three patients were found to have this more 'rapid' pattern.

In fig 1(c) there was a high amplitude dominant pulse and sparse growth hormone secretion for the remainder of the 24 hours. This pattern was found in three patients. With the exception of one child, pulse frequency was normal below 8.5 years of age, with five to nine identifiable peaks in 24 hours. Above this age both high and low frequencies occurred.

Age related changes in the amplitude of growth hormone secretion are illustrated in fig 2. Analysis of the sum of peak amplitudes in 28 profiles regarded as normal showed a significant decline with age ($p < 0.01$). No decline was apparent in maximum or mean pulse amplitude.

Growth hormone secretion, as expressed by the sum of peak amplitudes, showed no significant correlation with height velocity SDS using standards for either Turner's syndrome (fig 3) or normal children. Attempts to find a correlate using growth hormone pulse frequency, amplitude of maximum growth hormone pulse, or mean growth hormone pulse amplitude were unsuccessful. The growth of those patients judged as having abnormal growth hormone profiles was variable and not obviously different from the other patients (fig 3).

Plasma IGF-1 concentrations fell at the lower end of the range for normal children (fig 4), but increased significantly with age ($p < 0.001$). IGF-1

concentrations did not correlate with the sum of growth hormone pulse amplitudes ($p>0.3$) or with height velocity SDS ($p>0.5$). There was a strong negative correlation with height velocity ($p<0.001$).

EFFECTS OF TREATMENT

Despite producing a consistent and substantial

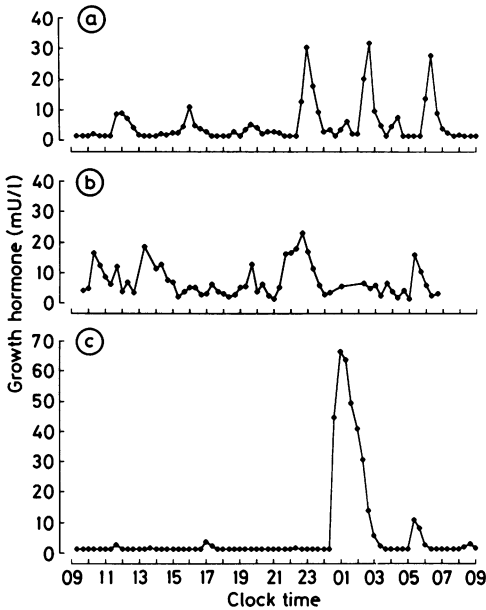


Fig 1 Specimen 24 hour growth hormone profiles from three untreated patients with Turner's syndrome: (a) age 7.4 years; 45,X; (b) age 11.2 years; 45,X/46,X,Xi(Xq); and (c) age 8.6 years; 45,X.

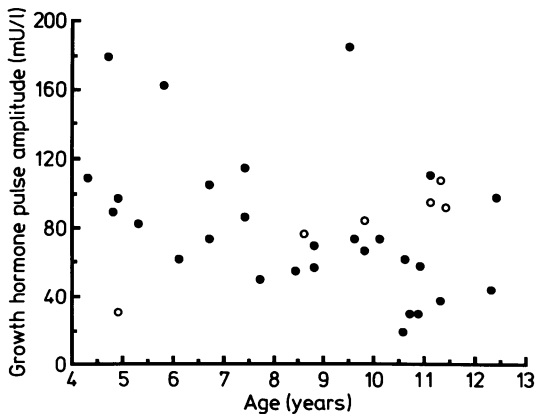


Fig 2 Changes in sum of growth hormone pulse amplitudes with age in 34 patients with Turner's syndrome. Open symbols indicate those patients with growth hormone profiles of abnormal pattern.

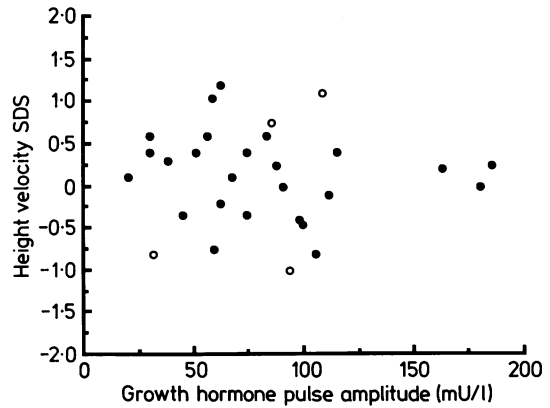


Fig 3 Comparison of height velocity SDS (for Turner's syndrome) with sum of growth hormone pulse amplitudes in 29 patients. Open symbols indicate those patients with abnormal profiles.

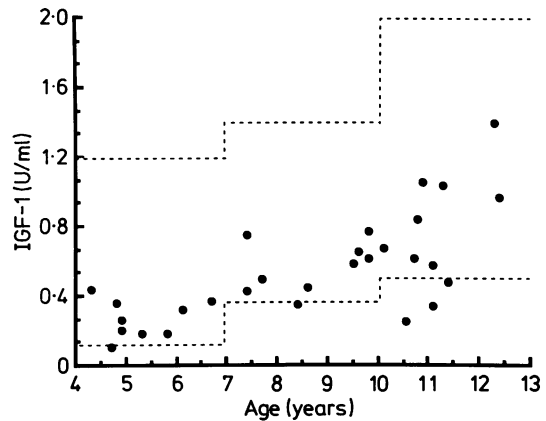


Fig 4 Changes in plasma IGF-1 concentrations with age in 29 patients with Turner's syndrome. Lines indicate the range seen in normal children (2 SD).

improvement in height velocity (table 2(a)), oxandrolone was associated with a decrease in growth hormone pulse amplitude (fig 5) ($p=0.05$). Although there was no overall effect on pulse frequency, abnormal profiles seen in four patients became normal on treatment in three. Plasma IGF-1 concentrations rose significantly.

Ethinyl oestradiol also increased height velocity (table 2(b)). There was a more variable effect on growth hormone secretion and there was no overall significant change in pulse amplitude or frequency (fig 5). There was the suggestion of an association between the change in growth hormone amplitude and the change in height velocity SDS, but this

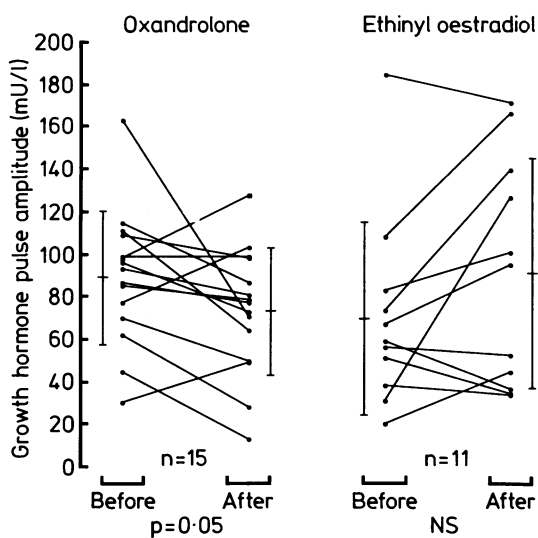


Fig 5 Effects of oxandrolone and ethinyl oestradiol on the sum of growth hormone pulse amplitudes in 26 patients with Turner's syndrome. Bars indicate mean (SD).

failed to reach significance ($p > 0.05$). One of two abnormal pretreatment profiles became normal on ethinyl oestradiol. IGF-1 concentrations were unchanged.

Discussion

Growth in the middle childhood years is largely dependent on growth hormone and independent of sex steroids.¹⁸ Analysis of 24 hour spontaneous growth hormone secretion in short prepubertal children has shown a gradual increase in growth hormone sum of pulse amplitudes over this age range and an asymptotic relation between the

growth hormone sum of pulse amplitudes and height velocity SDS.⁹

In the only previous study of 24 hour growth hormone secretion in Turner's syndrome, Ross *et al* found a decline in both frequency and pulse amplitude after the age of 9 years.¹⁹ Ranke *et al* recently reported a similar decline in amplitude of the maximum nocturnal pulse.²⁰ Our study suggests a steady decline in pulse amplitude over the mid childhood age range but no trend in pulse frequency. Comparison with data on normal short prepubertal subjects²¹ shows similar concentrations of growth hormone sum of pulse amplitudes to 10 years of age with an increasing disparity as growth hormone secretion increases in normal children and decreases in Turner's syndrome.

The relevance of this decline is uncertain as the growth rates did not correlate with any measure of growth hormone secretion. In addition, those subjects with the lowest growth hormone secretion did not necessarily exhibit particularly poor growth. We have not attempted to use mean growth hormone concentrations; the data from growth hormone profiles do not approximate to a normal distribution and such an artificial smoothing of the data is unlikely to express the dynamics of a pulsatile system meaningfully.

Six of 35 patients (17%) had loss of the normal three hourly periodicity.⁸ This is by way of contrast with our wider experience in which such abnormalities are very few and are associated with particularly poor growth.

IGF-1 concentrations were at the lower end of the normal range, but increased with age confirming previous studies,^{5 11 19} although Cuttler *et al* were unable to find an increase after 10 years of age.²² This increase occurred despite absent gonadal steroids and declining growth hormone secretion. IGF-1 concentrations did not correlate with height velocity SDS. (The strong negative correlation with

Table 2 Effects of treatment with oxandrolone or ethinyl oestradiol

	No of patients	Pretreatment	Treatment	p Value
<i>(a) Oxandrolone</i>				
Height velocity SDS	11	0.0 (0.5)	2.5 (1.0)	<0.005
Growth hormone sum of pulse amplitudes (mU/l)	15	89.3 (31.6)	73.3 (29.7)	0.05
Growth hormone pulses per 24 hours	15	6.9 (2.4)	7.0 (1.9)	NS
IGF-1 (U/ml)	11	0.53 (0.21)	0.84 (0.37)	<0.05
<i>(b) Ethinyl oestradiol</i>				
Height velocity SDS	11	0.1 (0.6)	1.8 (1.4)	<0.01
Growth hormone sum of pulse amplitudes (mU/l)	11	70.2 (45.4)	91.5 (54.0)	NS
Growth hormone pulses per 24 hours	11	6.8 (2.9)	6.7 (2.3)	NS
IGF-1 (U/ml)	6	0.70 (0.31)	0.90 (0.21)	NS

height velocity reflects their opposite age related trends.) The severe limitations of the measurements of circulating concentrations of such a paracrine hormone as IGF-1 have to be born in mind.²³ In this context, however, the data probably do exclude a failure of IGF-1 generation by growth hormone. Although Rosenfeld *et al* showed normal IGF-1 binding and action on fibroblast cultures,²⁴ actions on the growth plate are unknown.

The decline in growth hormone secretion could be ascribed to absence of gonadal steroids. Oestrogens in pharmacological doses have been shown to increase the growth hormone response to pharmacological stimuli in adults²⁵ and children²⁶ and the pubertal growth spurt in girls has been attributed to growth hormone secretion induced by oestrogens, though appropriate gonadotrophin secretion may also be necessary.²⁷

In our study ethinyl oestradiol failed to have a consistent effect on growth hormone secretion, though it should be stressed that oral ethinyl oestradiol does not provide physiological oestrogen replacement. We used somewhat less than the 100 ng/kg dose that Ross *et al* found to induce the maximal short term growth response.¹² In our experience, such a dose commonly induces florid pubertal changes, which would not have been appropriate in the younger patients. A large dose of 100 µg of ethinyl oestradiol per day did increase growth hormone secretion in adult patients with Turner's syndrome.²⁸ It is of note that in castrate baboons, oestrogen treatment only increased growth hormone secretion if used in excessive amounts, 'replacement' doses having no effect.²⁹

Oxandrolone produced a consistent improvement in growth without increasing the amplitude of growth hormone secretion. This has also been observed in boys with constitutional delay of growth and development.³⁰ We noted a qualitative improvement in pulse pattern in three patients. Time series analysis from three other patients showed that oxandrolone induced subtle changes in pulse frequency not apparent in the PULSAR analysis.³¹ Oxandrolone increased IGF-1 as reported by others.¹¹

In conclusion, although growth hormone secretion declined with age in Turner's syndrome, the relation to growth is uncertain, and end organ resistance in the form of a skeletal dysplasia probably plays a major part in the growth failure.¹⁻⁵ The experience that higher doses of growth hormone are required to achieve the same response as normal children supports this view and is against decreased bioactivity of endogenous growth hormone. Long term growth hormone treatment in pharmacological doses may nevertheless improve height prognosis. The onset of

growth failure in early life implies that such treatment should be commenced well within the first decade. Our results suggest that tests of growth hormone secretion may not be helpful in selection of patients for such treatment. Oxandrolone and ethinyl oestradiol remain effective modes of treatment in Turner's syndrome, though their mechanisms of action in growth promotion remain uncertain.

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