Pulsatile growth hormone release in Turner's syndrome and short normal children

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Abstract. To determine whether the quantitative and qualitative aspects of GH secretion in girls with Turner's syndrome are similar to those of short-normal children we studied the 24-h GH secretion of 10 patients with Turner's syndrome and 9 short-normal children with comparable auxological features. GH profiles, obtained by 30-min sampling, were analysed by the Pulsar programme. The pulsatile GH release over the 24 h in Turner's syndrome was similar to that in normal children. However, when the GH release over the 12 day and night hours were separately analysed, only normal children showed a night-time increase in the sum of peak amplitudes. Moreover, patients with Turner's syndrome had significantly decreased number and frequency of peaks in the night-time compared with short children. In short-normal children but not in Turner's syndrome, height velocity was related to the 24-h integrated concentration of GH, area under the curve over zero-line and over baseline, sum of peak areas, and amplitudes. Night-time GH area over zero-line and over baseline, mean peak amplitude, height, area, sum of peak areas, and amplitudes were positively correlated with height velocity in short children, whereas in Turner's syndrome height velocity was related to daytime parameters only. In conclusion, girls with Turner's syndrome have a discrete pattern of pulsatile GH release. However, the relation of GH secretion to growth, in these patients, is uncertain.

Turner's syndrome is a genetic disorder characterized by several physical abnormalities including short stature. The pathogenesis of the growth disorder is still not understood. Different mechanisms, such as abnormal hormonal regulation, altered end-organ sensitivity or a combination of both, have been considered responsible for the growth failure. Data on pulsatile GH secretion in Turner's syndrome have so far been controversial. In prepubertal patients with Turner's syndrome, GH secretory dynamics have been found to be similar to those of age-matched normal children, with a tendency to become abnormal with advancing of pubertal development (1,2). The presence of a decreased endogenous GH secretion in prepubertal Turner's syndrome girls compared with that of normal short girls, has been described by other authors (3). However, in most of these studies the GH secretory pattern of patients with Turner's syndrome have either been compared with those of regularly growing normal children or analysed by different methods.

Therefore, the current study was undertaken to study the qualitative and quantitative aspects of GH secretion in prepubertal girls with Turner's syndrome and compare them with those in short-normal children with similar auxological features.

Patients and Methods

This investigation was approved by the ethical committee of the Department of Pediatrics of the University of Parma. Informed consent was obtained from all subjects and their parents.

Patients

We evaluated 10 prepubertal patients (Tanner stage I) (4) with Turner's syndrome, aged 6.7 to 11.3 years, and 9
prepubertal short-normal children (4 males and 5 females), aged 5.1 to 12.4 years. Chronological age, bone
age, height standard deviation score, height velocity
based on full year calculations, height velocity standard
deviation score, and weight, expressed as percentage of
the average for height, of all subjects are reported in
Table 1. The diagnosis of Turner’s syndrome was estab-
lished by leucocyte karyotype in all cases. The results of
the karyotype analysis were 9 patients with 45 XO, 1 pa-
tient with 46 X(Xq).

All the children had adequate GH responses (GH peak
>0.46 nmol/l = 10 μg/l) to pharmacological stimuli, such
as insulin-induced hypoglycaemia (0.1 U/kg iv) and/or clo-
nidine (150 μg/m2 po) and normal thyroid function.
Estradiol and testosterone levels were in the normal range
for prepubertal children. None of the patients received
any prior treatment with GH, estrogen or anabolic ste-
roids. All short-normal children had a birth weight ap-
propriate for gestational age and none showed evidence
of systemic diseases, malnutrition, recognizable dysmor-
phic syndrome or psychosocial disturbances.

Methods
At 08.00 h, after an overnight fast, an indwelling non-
thrombogenic catheter was inserted into an antecubital
vein and connected to a portable constant withdrawal
pump, according to the Kowarski method (5). The rate of
withdrawal was of 4 ml/h and the reservoir tubes contain-
ing the blood for subsequent GH measurements were
changed every 30 min for 24 h. During this time children
were encouraged to continue normal activity and ate a
standard hospital diet.

Blood samples for measurement of GH concentrations
were kept at room temperature and centrifuged within 24
h. After centrifugation the serum was stored at −20°C
until assayed. Serum GH concentrations were measured
using the Nichols Institute Diagnostic human GH immu-
noradiometric assay kits (San Juan Capistrano, CA). All
samples from each subject were measured in duplicate in
the same assay. The sensitivity of the assay was 0.09
nmol/l. The mean intra-assay coefficients of variation
were 3.3, 4 and 4.5% at 0.23, 0.69 and 2.3 nmol/l, respec-
tively.

The pattern of pulses in the 24-h GH profiles was
analysed using the Pulsar programme (6). The cut-off
parameters Gl-5 were set to 4.0, 2.4, 1.7, 1.2 and 0.9 times
the intra-assay SD as criteria for accepting peaks 1, 2, 3, 4,
and 5 points wide, respectively. The smoothing times
was set to half the total profile time, that is, 12 h (24 points)
for the 24-h profiles. From the Pulsar analyses the fol-
lowing values were extracted: the integrated concentra-
tion, the number of peaks, the mean interpeak interval,
the peak frequency, the maximal peak amplitude, the
mean peak length, the mean peak height, the mean peak
amplitude, and the peak area. The area under the curve
(AUC) was estimated above the zero level (AUC0) as well
as above the calculated baseline (AUCB). In addition, we
used the simple arithmetic addition of amplitude and
area of the defined pulses to generate the statistic sum of
peak amplitudes and areas, respectively.

Bone age was determined by the method of Greulich &
Pyle (7).

Statistical analysis
Values are reported as mean ± SD. Since the data from GH
profiles do not approximate a normal distribution, all
calculations were performed after logarithmic transfor-
mation of the data, which provided a significantly closer
approximation to a normal distribution. Statistical signif-
ance was determined by unpaired or, where appropri-
ate, paired two-tailed Student’s t-test; p<0.05 was consid-
ered significant. Correlations were examined by linear
regression analysis.

Results
Table 1 gives the mean (± SD) age, bone age, height
standard deviation score, height velocity, height ve-
locity standard deviation score, and weight as per-
centage of the average for height for Turner’s syn-
drome patients and short-normal children. No dif-
fferences in any of these parameters were detected
between the two groups.

Fig. 1 illustrates representative 24-h serum GH
profiles from a Turner’s syndrome and a short-
normal child. Visual inspection of such profiles
suggested that the serum GH levels were higher in
short-normal children versus Turner’s syndrome
subjects, perhaps owing in part to the greater fre-
cuency with which nocturnal pulses of GH were
secreted. Objective pulse analysis was performed as
described below.

Integrated GH concentration over 24 h were
comparable in the two groups (Table 2). The same
results were obtained when the integrated GH con-
centrations over the 12 day and night hours were
analysed separately (Table 3).

The results of the identification and character-
ization of pulsatile GH release using the Pulsar pro-
gramme are shown separately for day and night
hours in Table 3. In both the patients and the short-
normal children AUC0, AUCB and sum of peak
areas in the night-time exceeded the mean daytime
concentrations. Moreover, short-normal children
had a significant nocturnal elevation of the sum of
peak amplitudes, whereas patients with Turner’s
syndrome had no significant day/night difference.
In neither group did the daytime mean number of
peaks, mean interpeak interval, peak frequency,
mean peak area, mean peak amplitude, maximal
Table 1.
Main auxological features of patients with Turner's syndrome and short-normal children.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Chronological age (years)</th>
<th>Bone age (years)</th>
<th>Height standard deviation score</th>
<th>Height velocity (cm/year)</th>
<th>Height velocity standard deviation score for bone age</th>
<th>Weight as % of the average for height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turner's syndrome (N=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>10</td>
<td>10</td>
<td>-3.19</td>
<td>2.02</td>
<td>-4</td>
<td>125</td>
</tr>
<tr>
<td>F</td>
<td>10.6</td>
<td>9.5</td>
<td>-3.12</td>
<td>2.3</td>
<td>-3.4</td>
<td>119</td>
</tr>
<tr>
<td>F</td>
<td>9.5</td>
<td>8.25</td>
<td>-1.6</td>
<td>4.1</td>
<td>-1.5</td>
<td>129</td>
</tr>
<tr>
<td>F</td>
<td>6.7</td>
<td>4</td>
<td>-2.4</td>
<td>4.4</td>
<td>-2.17</td>
<td>93</td>
</tr>
<tr>
<td>F</td>
<td>10.6</td>
<td>8</td>
<td>-4.4</td>
<td>2.7</td>
<td>-3</td>
<td>120</td>
</tr>
<tr>
<td>F</td>
<td>11.33</td>
<td>11.5</td>
<td>-2.17</td>
<td>4.5</td>
<td>-2</td>
<td>127</td>
</tr>
<tr>
<td>F</td>
<td>9.5</td>
<td>7</td>
<td>-2.72</td>
<td>3.7</td>
<td>-2.26</td>
<td>121</td>
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<tr>
<td>F</td>
<td>9.8</td>
<td>8.5</td>
<td>-3.1</td>
<td>3.8</td>
<td>-1.76</td>
<td>106</td>
</tr>
<tr>
<td>F</td>
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<td>8.5</td>
<td>-3.2</td>
<td>3.1</td>
<td>-2.5</td>
<td>108</td>
</tr>
<tr>
<td>F</td>
<td>9.7</td>
<td>7.3</td>
<td>-2.2</td>
<td>2.8</td>
<td>-3.2</td>
<td>96</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>9.79±1.23</td>
<td>8.25±1.99</td>
<td>-2.8±0.77</td>
<td>3.34±0.88</td>
<td>-2.57±0.79</td>
<td>114.4±12.8</td>
</tr>
<tr>
<td>Short-normal children (N=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>10.58</td>
<td>11.4</td>
<td>-4.2</td>
<td>3.8</td>
<td>-1.48</td>
<td>97</td>
</tr>
<tr>
<td>F</td>
<td>8.3</td>
<td>5</td>
<td>-2.5</td>
<td>4.2</td>
<td>-2.26</td>
<td>83</td>
</tr>
<tr>
<td>M</td>
<td>10.9</td>
<td>7.4</td>
<td>-3</td>
<td>2.7</td>
<td>-3.57</td>
<td>123</td>
</tr>
<tr>
<td>F</td>
<td>12.4</td>
<td>10.2</td>
<td>-4</td>
<td>3.16</td>
<td>-2.8</td>
<td>122</td>
</tr>
<tr>
<td>M</td>
<td>7.16</td>
<td>5</td>
<td>-2.1</td>
<td>5.4</td>
<td>-1.13</td>
<td>118</td>
</tr>
<tr>
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<td>5.08</td>
<td>4.77</td>
<td>-1.05</td>
<td>5.8</td>
<td>-0.75</td>
<td>102</td>
</tr>
<tr>
<td>F</td>
<td>11.66</td>
<td>7.69</td>
<td>-2.8</td>
<td>4.34</td>
<td>-1.4</td>
<td>118</td>
</tr>
<tr>
<td>F</td>
<td>6.33</td>
<td>4.5</td>
<td>-2.4</td>
<td>5.1</td>
<td>-1.55</td>
<td>83</td>
</tr>
<tr>
<td>F</td>
<td>9.5</td>
<td>8.25</td>
<td>-2.1</td>
<td>5</td>
<td>-0.5</td>
<td>110</td>
</tr>
<tr>
<td>Mean ± sd</td>
<td>9.10±2.53</td>
<td>7.13±2.52</td>
<td>-2.68±0.97</td>
<td>4.39±1.03</td>
<td>-1.71±0.99</td>
<td>106.2±15.8</td>
</tr>
</tbody>
</table>

peak amplitude, mean peak length, and mean peak height differ from the values obtained at night.

Analysis of the pulsatile GH release over the 24 h showed no differences between children with Turner’s syndrome and short-normal children (Table 2). Nocturnal peak frequency and number of peaks were significantly higher in short-normal children compared with patients with Turner’s syndrome. This was accompanied by a decreased mean interpeak interval in the former group (Table 3). Night AUC<sub>0</sub>, AUC<sub>b</sub>, mean peak amplitude, mean peak height, mean peak length, mean peak area, maximal peak amplitude, sum of peak areas, sum of peak amplitudes were similar in the two groups (Table 3). No differences in the daytime GH secretory pattern were detected when measures from short-normal children were compared with those of Turner’s syndrome patients (Table 3).

Linear regression analysis showed that height velocity of short-normal children correlates positively
24-hour GH integrated concentrations and pulse characteristics in short-normal children and patients with Turner's syndrome (Mean ± sd).

<table>
<thead>
<tr>
<th>Integrated GH concentration (nmol/l)</th>
<th>Short-normal children</th>
<th>Turner's syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the curve over baseline (AUCb) nmol·l⁻¹·(24 h)⁻¹</td>
<td>11.91±3.14</td>
<td>8.81±2.66</td>
</tr>
<tr>
<td>Area under the curve over zero-line (AUC0) nmol·l⁻¹·(24 h)⁻¹</td>
<td>14.32±3.81</td>
<td>11.61±2.99</td>
</tr>
<tr>
<td>Number of peaks</td>
<td>9.55±3.2</td>
<td>11.61±2.99</td>
</tr>
<tr>
<td>Mean interpeak interval (h)</td>
<td>2.41±0.6</td>
<td>2.86±0.69</td>
</tr>
<tr>
<td>Peak frequency (h⁻¹)</td>
<td>0.42±0.14</td>
<td>0.37±0.09</td>
</tr>
<tr>
<td>Mean peak length (h)</td>
<td>1.55±0.34</td>
<td>1.87±0.51</td>
</tr>
<tr>
<td>Mean peak amplitude (nmol/l)</td>
<td>0.60±0.24</td>
<td>0.51±0.16</td>
</tr>
<tr>
<td>Mean peak area (nmol/l)</td>
<td>1.12±0.51</td>
<td>1.07±0.57</td>
</tr>
<tr>
<td>Maximal peak amplitude (nmol/l)</td>
<td>3.83±1.62</td>
<td>3.97±2.5</td>
</tr>
<tr>
<td>Sum peak amplitudes (nmol/l)</td>
<td>5.25±1.46</td>
<td>4.09±0.71</td>
</tr>
<tr>
<td>Sum of peak areas (nmol/l)</td>
<td>9.48±2.46</td>
<td>8.23±2.99</td>
</tr>
</tbody>
</table>

with the 24-h integrated concentrations (r=0.82, p<0.005), total AUC0 (r=0.85, p<0.005), AUCb (r=0.73, p<0.025), and sum of peak areas (r=0.78, p<0.02) and amplitudes (r=0.68, p<0.05). These relationships were not found in patients with Turner's syndrome. Attempts to find a correlation using number of peaks, mean interpeak intervals, pulse frequency, mean pulse amplitude, mean pulse area, maximal pulse amplitude, mean peak height, and mean length over the 24 h were unsuccessful in either group. Short-normal children did not have a significant correlation of height velocity with any of

Table 3
Mean (± sd) daytime and night-time GH integrated concentrations and pulse characteristics in short-normal children and patients with Turner's syndrome.

<table>
<thead>
<tr>
<th>Integrated GH concentration (nmol/l)</th>
<th>Short-normal Children</th>
<th>Turner's Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area under the curve over baseline (AUCb) nmol·l⁻¹·(24 h)⁻¹</td>
<td>4.13±1.21</td>
<td>4.29±1.69</td>
</tr>
<tr>
<td>Area under the curve over zero-line (AUC0) nmol·l⁻¹·(24 h)⁻¹</td>
<td>10.52±3.42a</td>
<td>7.89±1.52a</td>
</tr>
<tr>
<td>Number of peaks</td>
<td>4.77±2.33</td>
<td>4.0±0.89</td>
</tr>
<tr>
<td>Mean interpeak interval (h)</td>
<td>2.52±1.53</td>
<td>2.67±0.46</td>
</tr>
<tr>
<td>Peak frequency (h⁻¹)</td>
<td>0.41±0.2</td>
<td>0.34±0.07</td>
</tr>
<tr>
<td>Mean peak length (h)</td>
<td>1.46±0.54</td>
<td>1.39±0.3</td>
</tr>
<tr>
<td>Mean peak amplitude (nmol/l)</td>
<td>0.47±0.43</td>
<td>0.34±0.15</td>
</tr>
<tr>
<td>Mean peak area (nmol/l)</td>
<td>0.92±0.97</td>
<td>0.66±0.32</td>
</tr>
<tr>
<td>Maximal peak amplitude (nmol/l)</td>
<td>0.89±0.53</td>
<td>0.79±0.43</td>
</tr>
<tr>
<td>Sum of peak amplitude (nmol/l)</td>
<td>1.54±0.73</td>
<td>1.38±0.69</td>
</tr>
<tr>
<td>Sum of peak area (nmol/l)</td>
<td>2.68±1.09</td>
<td>2.61±1.27</td>
</tr>
</tbody>
</table>

a = p < 0.01 vs daytime value
b = p < 0.05 vs Turner's syndrome patients
the daytime parameters of GH secretion analysed, whereas height velocity in patients with Turner’s syndrome was positively correlated with daytime AUCa (r=0.95; p<0.005), AUCb (r=0.85; p<0.05), sum of peak areas (r=0.81; p<0.05) and amplitudes (r=0.80; p<0.005, Fig. 2). Night-time AUCa (r=0.82; p<0.01), AUCb (r=0.93; p<0.001), mean peak amplitude (r=0.82; p<0.01), mean peak height (r=0.81; p<0.01), mean peak area (r=0.83; p<0.005), sum of peak area (r=0.93, p<0.001), and sum of peak amplitudes (r=0.73, p<0.01, Fig. 3) were positively correlated with height velocity in short-normal children, but not in girls with Turner’s syndrome.

Discussion

The findings of the present study indicate that episodic secretion of GH is present in patients with Turner’s syndrome and in short-normal children. A nocturnal elevation of non-pulsatile GH concentration occurs in both groups. However, only short-normal children showed a night-time increase in the sum of peak amplitudes. Patients with Turner’s syndrome had significantly decreased number and frequency of peaks in the night-time compared with those in short-normal children. Moreover, height velocity of short-normal children was found to be related to several 24-h and night-time param
eters of GH secretion, whereas this was not so for patients with Turner's syndrome. In this latter group, in fact, height velocity appears to be dependent on daytime GH release only.

The observations summarized above demonstrate that the 24-h qualitative and quantitative aspects of GH secretion of prepubertal patients with Turner's syndrome are similar to those of short and slow-going children with comparable auxological features. Distinctive patterns of GH release were observed when the daytime and night-time secretions were separately analysed and differences were only observed at night.

The present findings are in part at variance with previous reports in which spontaneous GH secretion of patients with Turner's syndrome was studied (1-3). It should be pointed out, however, that in most of these studies the patients had previously been treated with anabolic steroids or estrogen. In addition, the data from the patients were compared with those of children of normal height and height velocity. In the study by Ross et al. (1), in fact, the 24-h profile of serum GH in patients with Turner's syndrome was compared with that of age-matched normal girls and no differences in GH levels, peak amplitudes and frequencies were observed between the two groups of subjects aged 2 to 8 years. Other investigators have recently reported decreased mean 24-h and daytime and night-time GH levels in prepubertal girls with Turner's syndrome (3). The subjects of this study, however, were older than our patients and on treatment with anabolic steroids up until one week prior to the study. Moreover, the criteria for pulse detection used in the studies referred to above were different from the ones we used, making a comparison of the data inappropriate. In the former report (1), in fact, a GH secretory peak was defined as an increment from nadir to peak exceeding 20%, whereas in the latter (3) a hormonal level twice as high as the nadir value with an absolute value greater than 2 μg/l (0.092 nmol/l) was considered a pulse.

In a recent investigation (8), the GH profiles of prepubertal girls with Turner's syndrome were analysed by the Pulsar programme and the results were expressed as the sum of GH pulse amplitudes and number of peaks. Only 6 patients (18%) had abnormal GH pulse frequencies and there was no correlation between GH pulse amplitudes in all the subjects and their growth rates. However, day and night spontaneous GH secretion were not separately analysed so that differences in daytime and night-time GH release could not be detected.

The absence of nocturnal increase in the sum of peak amplitudes together with the lower number of pulses occurring at night in Turner's syndrome seems to indicate the presence of a distinctive nocturnal pattern of GH release in these patients. Specifically, the decreased frequency of GH pulses suggests that the periods of somatostatin withdrawal and/or GHRH stimulation at night may be abnormally frequent. Thus, while not disproving any specific abnormality of the pituitary, our data are consistent with altered hypothalamic function. These observations are in agreement with studies in which lower GH responses to insulin-induced hypoglycemia were described in patients with Turner's syndrome compared with normal age-matched children (9). The possibility that an altered somatostatinergic tone modulates the response of somatotropes is also suggested by the GHRH-stimulated GH release in Turner's syndrome which appears to be lower than in normal subjects (10,11).

The relationship of the growth rate to some parameters of GH secretion, in particular to the sum of peak amplitudes, was temporarily reversed in patients with Turner's syndrome. Heigh velocity, in fact, correlated with the daytime values in Turner's syndrome and with night-time values in short-normal children. Moreover, 24-h GH secretion was positively correlated with growth rate in normal children only. These discrepancies are difficult to explain. The absence of gonadal steroids is certainly a factor that has to be taken into account when examining the growth pattern of patients with Turner's syndrome. A role of estrogen on GH release would be consistent with the observation that estrogen increases GH responsiveness to provocative testing (12,13). However, analysis of GH secretory dynamics after estrogen replacement therapy failed to demonstrate a consistent effect on GH secretion (8).

Whatever the reason for the distinctive relation between GH secretion and growth in Turner's syndrome, its biological relevance is uncertain. The growth of the two groups examined in this study was actually similar despite the different GH secretory dynamics. It should be remembered, however, that the children we studied all had short stature and abnormal growth rates. Thus, given that the short stature of normal children can partly be attributed to a low GH secretion rate (14,15), the
growth failure of patients with Turner’s syndrome might be ascribed to factors relatively independent of pulsatile GH release.

In conclusion, prepubertal girls with Turner’s syndrome have a discrete pattern of pulsatile GH release by the anterior pituitary. However, the relation of the GH secretory pattern to growth in these patients is uncertain. The cause of short stature is most likely multifactorial, involving probably both genetic and hormonal factors.

References