CLINICAL STUDY

Short boys treated with growth hormone show normal progression of testicular size and achieve normal serum testosterone concentrations

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Abstract

Objective: To determine whether there is evidence for impaired testicular function at final height in short boys treated with growth hormone (GH) during their childhood and adolescence.

Study design: The analysis was restricted to males who had isolated GH deficiency or idiopathic short stature, and who were included in the Swedish National Registry and the Swedish GH trials. The subjects had to have been treated with GH for at least 4 years; the treatment had to have been started prepubertally, given for at least one year before the onset of puberty and the subjects had to have reached final height. One hundred and eleven boys fulfilled the criteria.

Methods: Testicular volumes were determined by orchidometer in each boy when GH treatment was started and at final height. Samples for testosterone measurements were collected from 77 boys at final height, and were measured by RIA.

Results: Each subject had normal testicular size (15 ml or more) and for those in whom concentrations were determined, serum testosterone levels and diurnal rhythm were normal.

Conclusions: The results of our survey do not show evidence of testicular impairment following GH therapy.

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Introduction

Treatment with recombinant human growth hormone (GH) has been considered safe, with few short- or long-term adverse effects (1–3). Bertelloni and coworkers, however, reported on four non-growth hormone deficiency (GHD) adult males, treated previously with GH, who had reduced testicular size, impaired spermatogenesis and hypergonadotropic hypogonadism (4). This observation, in conjunction with animal data reporting that short-term treatment of male dogs with high doses of human GH was followed by reduced testicular size and decreased serum testosterone levels (5) have raised concern about the long-term safety of GH treatment. This led us to study all Swedish males with various degrees of GH secretion who had been treated with GH, and who were followed in the Swedish National Registry and the Swedish GH trials for short children. In this analysis, we have used testis volume as a surrogate marker for Sertoli cell function, and serum testosterone concentrations as a surrogate marker for Leydig cell function (6–9).

Subjects and methods

Patient groups

On the 1st January 2000, the Swedish National Registry and the Swedish GH trials for short children contained data on, respectively, 3454 (1383 girls, 2071 boys) and 181 (36 girls, 145 boys) subjects treated with GH. That is nearly 100% of all the children and young adults who have been treated with GH in Sweden. The Swedish National Registry contains data on children treated with GH on registered indications while the register of Swedish GH trials contains information on short children who are not GHD treated with GH. The analysis was restricted to males defined as having isolated growth hormone deficiency (IGHD) or idiopathic short stature (ISS), with no other diseases or syndromes. For inclusion, the subjects had to have received GH for at least 4 years, to have started on treatment at least one year before the onset of puberty (testes ≥ 3 ml) and to have reached final height, defined as height velocity less than 2 cm/year. One hundred
and eleven males fulfilled the inclusion criteria (Fig. 1). These study patients were divided into three groups according to their pretreatment maximal endogenous GH value during an arginine–insulin tolerance test: group A: $\text{GH}_{\text{max}} < 16 \text{ mIU/l}$, $n = 33$; group B: $\text{GH}_{\text{max}} 16–32 \text{ mIU/l}$, $n = 51$; and group C: $\text{GH}_{\text{max}} > 32 \text{ mIU/l}$, $n = 27$. They had been treated with 0.1–0.2 IU GH/kg daily by subcutaneous injections. Table 1 gives heights converted to standard deviation score (SDS) using the Swedish growth reference values for healthy children (10), and chronological age at the start of GH treatment and at final height.

For the boys in the trials, informed consent was obtained from each boy and his parents. The protocol was approved by the Ethical Committee of the Medical Faculty of the Universities in Göteborg, Linköping, Lund, Stockholm, Umeå and Uppsala.

### Study protocol

Testicular volumes were determined by orchidometer (11) in each boy when GH treatment was started and at final height. A testis volume $> 25 \text{ ml}$ was recorded as $25 \text{ ml}$. The larger of the two testes was used in the evaluation.

Samples for sex steroid measurements were collected from 77 young males at final height (39 single samples, 38 24-h profiles), and stored at $-20^\circ\text{C}$ (Fig. 1). Single samples were taken between 0800 and 1800 h, and profile samples were obtained at 1400, 1800, 2200, 0200, 0400, 0600, and 1000 h.

### Control groups

Reference values for testicular volumes of healthy boys from Zachmann et al. were used for the evaluation of the testicular volume at final height (12). Serum testosterone concentrations at final height were compared with the concentrations in healthy, volunteer boys: median age 17.1 years (range 16.6–18.2), median height SDS 0.8 (range $-0.5$–$2.3$), median testis volume 22.5 ml (range 15–25) (13).

### Measurement of growth hormone and testosterone

Plasma GH concentrations were determined in duplicate using a polyclonal antibody-based immunoradiometric assay (Pharmacia human growth hormone (hGH) RIA; Pharmacia Diagnostics, Uppsala, Sweden). The standards in the kit were calibrated against the first international reference preparation (IRP) of hGH 80/505. The detection limit was 0.4 mU/l; the intra-assay coefficient of variation (CV) was 3% for levels above 5 mU/l and the inter assay CV was less than 14%. If the samples had been quantified against the previously used IRP 66/217 standard, a conversion factor was used (14). In the present study we have used a peak GH concentration below 10 $\mu$g/l during the arginine–insulin tolerance test as the criterion for GH deficiency according to the GH Research Society Consensus (15). Since we have used polyclonal
antibodies and the reference 80/505, 10 μg/l corresponds to 32 mU/l (14). However, since GH secretion shows a continuum and not a clear cut-off, we arbitrarily divided the subjects into the following groups according to their peak GH concentration during the arginine–insulin tolerance test: GH max \(= 16 \text{ mU/l} \) (corresponds to 5 μg/ml), GH max \(= 16–32 \text{ mU/l} \) and GH max \(> 32 \text{ mU/l} \).

Serum testosterone concentrations were determined in duplicate by radioimmunoassay (Spectria testosterone; Orion Diagnostica, Espoo, Finland) as described previously (16). The detection limit was 0.03 nmol/l (8.7 pg/ml); the intra-assay CV was less than 7%, and the interassay CV was less than 10%.

**Statistical procedures**

Values are given as medians and ranges. A value of \( P < 0.05 \) was considered significant. The testosterone levels of the serum profiles were calculated as the mean value of seven samples drawn over an interval of 24 h. The diurnal variation of serum testosterone was calculated as the ratio between afternoon-evening (1400–2200 h) and morning (0200–1000 h) concentrations. Non-parametric statistical methods were used for analyses: the Mann–Whitney test for comparison between groups (17).

**Results**

No differences were observed between the 3 patient groups with different levels of GH secretion, in their age at start of GH treatment, age at final height, duration of treatment, testicular volume at start of GH treatment or volume achieved at final height. The testicular volumes attained at final height ranged between 15 and 25 ml, and the median value was 20 ml in each of the three groups. The testicular volumes were within \( \pm 2 \text{s.d.} \) for the mean value of healthy boys (Fig. 2) (12).

Serum testosterone concentrations were compared with the 95% confidence interval for the median of the healthy boys. None of the GH-treated boys had a testosterone concentration below the healthy controls, although a few had values in excess of controls (Fig. 3). Statistical analyses showed no differences in serum testosterone levels or diurnal variation between healthy non-GH treated boys, and study groups A, B or C at final height.

Testosterone measurements in single samples taken at final height were similarly distributed as in the control group (Fig. 4). One of the 48 boys had a level below the 95% confidence interval for the median and 2 boys had levels at the lower border of the 95% confidence interval for the median. Assuming normal distribution, this is what can be expected. The single samples were taken at different clock times, diminishing the possibility of statistical analysis.

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>GH max (mIU/l)</th>
<th>Diagnosis</th>
<th>Age (years) at GH start</th>
<th>Height SDS at GH start</th>
<th>Age (years) at final height</th>
<th>Height SDS at final height</th>
<th>GH duration (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>33</td>
<td>&lt; 16</td>
<td>Isolated GH deficient</td>
<td>9.5 (5.0–14.7)</td>
<td>−2.6 (−5.7–−1.4)</td>
<td>18.7 (15.8–20.4)</td>
<td>−0.6 (−2.4–1.9)</td>
<td>7.5 (4.9–15.4)</td>
</tr>
<tr>
<td>B</td>
<td>51</td>
<td>16–32</td>
<td>Isolated GH deficient</td>
<td>10.8 (5.7–14.6)</td>
<td>−2.5 (−4.4–−1.6)</td>
<td>18.1 (15.3–20.7)</td>
<td>−1.2 (−2.9–1.2)</td>
<td>6.7 (4.6–11.3)</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>&gt; 32</td>
<td>Idiopathic short stature</td>
<td>11.0 (5.0–14.3)</td>
<td>−2.6 (−3.7–−1.9)</td>
<td>18.6 (17.2–20.3)</td>
<td>−1.1 (−3.9–0.1)</td>
<td>7.0 (4.2–13.1)</td>
</tr>
</tbody>
</table>

**Figure 2** Progression of testicular size (at start ○, at final height ●) in (A) boys with isolated GH deficiency, GH max < 16 mU/l, \( n = 33 \), (B) boys with isolated GH deficiency, GH max 16–32 mU/l, \( n = 51 \) and (C) boys with idiopathic short stature, GH max > 32 mU/l, \( n = 27 \), plotted on a testicular growth chart according to Zachmann et al. (12).
Discussion

We found that testicular growth proceeds normally in boys treated with GH during childhood and adolescence for IGHD or for ISS. No instances of diminished testicular volume were found in the Registries.

Normal testicular volume is a prerequisite for, but not a guarantee of fertility. Testicular size is determined primarily by the mass of Sertoli cells and seminiferous tubules. The critical volume required for normal testicular function is estimated to be approximately 15 ml (6, 7, 9). Indeed, each of the four males that Bertelloni et al. reported to have decreased testicular size, also had decreased sperm concentration and motility, elevated serum gonadotropins, but normal serum testosterone concentrations (4). In the present study none of the young men treated with GH during childhood and adolescence had a testis volume below 15 ml.

Among the subjects who had measurements of serum testosterone concentrations, the values were compared with the 95% confidence interval for the median in a group of healthy boys. The patients had normal diurnal variations and a similar distribution of testosterone concentration as the control group. This is not surprising, as low testosterone concentrations are usually observed when testicular volumes are below those found in the present study (6–9).

GH plays a role in normal pubertal development and fertility. IGHD is associated with delayed puberty and infertility, and this can be restored to normal by administration of GH (18–20). Therefore, the results in the present study showing normal growth of the testes and normal increases in serum testosterone concentrations in boys with IGHD is reassuring and in line with the concept that GH has a role in normal pubertal development.

Our observations do not support the notion that GH treatment has a negative effect on testicular growth and function. They do not, however, exclude such a risk. A recent report by Werber Leschek et al. (21) is in line with our results. They studied 32 boys with non-GHD in a randomized double-blind placebo-controlled trial. GH or placebo treatment was initiated...
prepubertally or in early puberty and continued until final height was reached. Their results showed no difference between treatment groups in final testis volume, testosterone or gonadotropin concentrations. Together with the present study these results are reassuring. Nevertheless studies on fertility in males treated with GH during childhood are still needed to be sure that the treatment does not impair normal Sertoli cell development.

The observations by Bertelloni and coworkers of four GH-treated males (4) with decreased testicular size and impaired function also need to be interpreted with caution, because the prevalence of testicular impairment among young men with short stature is not known. Of special interest in this context is the fact that short stature is associated with other diseases, with chromosomal abbreviations and with hormonal deficits (22). To know whether there is a risk of GH treatment in short boys without GHD we need to know the true prevalence of testicular impairment among males with short stature. Such data are needed before a positive or negative effect of GH treatment can be determined with certainty.

Acknowledgements

This work was supported by grants from the Swedish Medical Research Council (no. 7509), The Sahlgrenska Medical Research Council and Pharmacia. The observations by Bertelloni and coworkers of four GH-treated males (4) with decreased testicular size and impaired function also need to be interpreted with caution, because the prevalence of testicular impairment among young men with short stature is not known. Of special interest in this context is the fact that short stature is associated with other diseases, with chromosomal abbreviations and with hormonal deficits (22). To know whether there is a risk of GH treatment in short boys without GHD we need to know the true prevalence of testicular impairment among males with short stature. Such data are needed before a positive or negative effect of GH treatment can be determined with certainty.

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References
