Growth hormone responsiveness in children

Results from Swedish multicenter clinical trials of growth hormone treatment

Department of Clinical Sciences
Unit of Pediatrics

Umeå 2017
И вечный бой! Покой нам только снится...

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Fri översättning: I motvind stiger draken
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Abstract

Growth hormone (GH) is a highly dynamic hormone which stimulates linear growth and influences metabolism, body composition and neuropsychological performance. GH has direct effects by targeting the GH receptor and indirect effects through insulin-like growth factor I (IGF-I) produced in the liver (endocrine effect) and/or locally (paracrine effects). In healthy children, endogenous GH secretion varies with height and pubertal maturation, but GH treatment regimens have not adapted the dosing accordingly. Administration as a daily subcutaneous GH injection at bedtime is standard practice worldwide, but no studies on GH uptake in a clinical setting have been published.

The general aims of the thesis were to study GH responsiveness by estimation of:

1. pharmacokinetics and bioavailability of injected recombinant human GH (rhGH),
2. GH treatment growth response as gain in height$^{SDS}$ during childhood and puberty,
3. GH treatment IGF-I response as change in circulating IGF-I$^{SDS}$ and IGFBP3$^{SDS}$.

Methods: Study populations: Short children were recruited during 1988–1999 into two national randomized multicentre clinical trials on growth until adult height. Those diagnosed with GHD were included in the GHD trial, while those above a GH response cut-off were included in the non-GHD trial.

A group of 117 GHD patients who had been treated from prepuberty with a single GH dose of 33µg/kg/day for at least 1 year were randomized at onset of puberty either to remain on this dose regimen or to an increased dose, GH$^{67}$µg/kg/day, administered once daily or divided into two doses, GH$^{33x2}$µg/kg/day. Data on IGF-I$^{SDS}$ and IGF binding protein 3 (IGFBP3)$^{SDS}$ were available from 111 patients and analysed as stated below. The 151 short prepubertal non-GHD patients were randomized into three groups: untreated controls, GH$^{33}$ µg/kg/day or GH$^{67}$µg/kg/day.

A subpopulation from both trials, 128 patients examined annually in Gothenburg, formed the study sample on GH uptake. They received sc GH injections to obtain 16–24 hour GH curves and the GH pharmacokinetics and bioavailability was calculated.

Results: In the study on pharmacokinetics and bioavailability of subcutaneously injected rhGH, a dose-dependent effect on $C_{max}$ was found (p<0.0001) with a coefficient of variation (CV) for $C_{max}$ of 39% and 44% for intra- and inter-individual variability, respectively. Of the $C_{max}$ variability, 43% was explained by the rhGH dose and proxies for injection depth. Median
bioavailability of the injected dose was 71%, with CV 32% and 35% for intra- and inter-individual variability, respectively, mainly dependent on injection depth. The quantified amount of bioavailable injected rhGH with the dose 33 or 67 µg/kg/day corresponded to the low range of endogenous GH secretion rate found in healthy prepubertal or pubertal children (Paper I).

In the patients with IGHD: GH responsiveness was estimated by the on-treatment gain in height\textsubscript{SDS}. A dose-dependent difference in pubertal gain in height\textsubscript{SDS} was found, with mean gain in height\textsubscript{SDS} of 0.8 for the GH\textsuperscript{67} group and 0.4 for GH\textsuperscript{33}, p<0.01. The mean total gain in height\textsubscript{SDS} during treatment was 1.9 for GH\textsuperscript{67} and 1.4 for GH\textsuperscript{33}, p<0.01 (Paper II). GH responsiveness was estimated by the on-treatment increase in circulating IGF-I\textsubscript{SDS}. Pubertal ΔIGF-I\textsubscript{SDS} was 0.5 in the GH\textsuperscript{67} group vs −0.1 in the GH\textsuperscript{33} group, p=0.007, correlating to pubertal gain in height\textsubscript{SDS}, r=0.32, p=0.003. ΔIGF-I\textsubscript{SDS} was the most important variable to explain the variation in pubertal gain in height\textsubscript{SDS} (Paper III).

In the short non-GHD patients: GH responsiveness was estimated by the on-treatment increase in circulating IGF-I\textsubscript{SDS}. The ΔIGF-I\textsubscript{SDS} from baseline to mean study level was 2.07 for GH\textsuperscript{67} vs 1.20 for GH\textsuperscript{33}, p=0.001. The ΔIGF-I\textsubscript{SDS} correlated negatively with baseline values of IGF-I\textsubscript{SDS}, rho=−0.56 for GH\textsuperscript{67}, p=0.001, vs rho=−0.82 for GH\textsuperscript{33}, p=0.0001, and positive correlated with gain in height\textsubscript{SDS} in both GH-treated groups, rho=0.42, p<0.001. In multivariable regression analyses, ΔIGF-I\textsubscript{SDS} was always an important explanatory variable for long-term growth response from the prepubertal period until adult height, while the IGF-I\textsubscript{SDS} study level per se was not (Paper IV).

Conclusions: Growth response to GH treatment was dose dependent with great variability between patients. More pubertal growth could be attained by an increased daily rhGH dose, mimicking the physiology of healthy children, in whom GH secretion rate increases during puberty. This resulted in a gain in IGF-I\textsubscript{SDS} closely correlating to pubertal gain in height\textsubscript{SDS} in both GHD and non-GHD patients followed until adult height. A broad range in GH responsiveness was found for both growth response and IGF response in both diagnostic groups, but lower in the non-GHD group. The uptake of injected rhGH showed great intra- and inter-individual variability. Higher uptake of a given GH dose was observed after a deep injection and a higher GH concentration.

These results are clinically applicable for individuals who remain short close to onset of puberty; by identifying and deeply injecting a rhGH dose that accounts for individual responsiveness, we can stimulate an increment in IGF-I\textsubscript{SDS} that correlates to gain in height\textsubscript{SDS} during puberty.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AITT</td>
<td>Arginine-insulin tolerance test</td>
</tr>
<tr>
<td>AH</td>
<td>Adult height</td>
</tr>
<tr>
<td>ALS</td>
<td>Acid labile subunits</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>AUC24</td>
<td>Area under the curve for 24 hours</td>
</tr>
<tr>
<td>AUCadj</td>
<td>Area under the curve adjusted to baseline</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>Cmax</td>
<td>Maximum serum concentration of GH</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>diffH-MPH&lt;sub&gt;SDS&lt;/sub&gt;</td>
<td>The difference between the child’s Height&lt;sub&gt;SDS&lt;/sub&gt; and MPH&lt;sub&gt;SDS&lt;/sub&gt;</td>
</tr>
<tr>
<td>GH</td>
<td>Growth hormone</td>
</tr>
<tr>
<td>GH&lt;sup&gt;33&lt;/sup&gt;</td>
<td>Growth hormone dose 33 µg/kg/day</td>
</tr>
<tr>
<td>GH&lt;sup&gt;33x1&lt;/sup&gt;</td>
<td>GH, Standard dose 33 µg/kg, once daily injection</td>
</tr>
<tr>
<td>GH&lt;sup&gt;33x2&lt;/sup&gt;</td>
<td>GH, Standard dose 33 µg/kg, twice daily injection</td>
</tr>
<tr>
<td>GH&lt;sup&gt;67&lt;/sup&gt;</td>
<td>Growth hormone dose 67 µg/kg/day</td>
</tr>
<tr>
<td>GH&lt;sup&gt;67x1&lt;/sup&gt;</td>
<td>Growth hormone dose 67 µg/kg/day, once-daily injection</td>
</tr>
<tr>
<td>GHBP</td>
<td>Growth hormone binding protein</td>
</tr>
<tr>
<td>GHD</td>
<td>Growth hormone deficiency</td>
</tr>
<tr>
<td>GH&lt;sub&gt;max&lt;/sub&gt;24h</td>
<td>Maximum GH level during a spontaneous 24h secretion/GH profile</td>
</tr>
<tr>
<td>GH&lt;sub&gt;max&lt;/sub&gt;AITT</td>
<td>Maximum GH level during an AITT</td>
</tr>
<tr>
<td>GH&lt;sub&gt;peak&lt;/sub&gt;</td>
<td>Sign of spontaneous GH secretion peak in the GH curve</td>
</tr>
<tr>
<td>GH&lt;sub&gt;nopeak&lt;/sub&gt;</td>
<td>No sign of spontaneous GH secretion peak in the GH curve</td>
</tr>
<tr>
<td>GH&lt;sub&gt;peak&lt;/sub&gt;width</td>
<td>FWHM, i.e. at half C&lt;sub&gt;max&lt;/sub&gt; of each GH curve</td>
</tr>
<tr>
<td>GHRH</td>
<td>GH-releasing hormone</td>
</tr>
<tr>
<td>GP-GRC</td>
<td>Gothenburg Pediatric Growth Research Center</td>
</tr>
<tr>
<td>hGH</td>
<td>Human growth hormone</td>
</tr>
<tr>
<td>ICP</td>
<td>Infancy-childhood-puberty growth model</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>ICT</td>
<td>Infancy-childhood transition (in months)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td>IGF-II</td>
<td>Insulin-like growth factor II</td>
</tr>
<tr>
<td>IGFBP3</td>
<td>Insulin-like growth factor-binding protein 3</td>
</tr>
<tr>
<td>IIIGHD</td>
<td>Isolated idiopathic growth hormone deficiency</td>
</tr>
<tr>
<td>ISS</td>
<td>Idiopathic short stature</td>
</tr>
<tr>
<td>IRP</td>
<td>International reference preparation</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-to-treat</td>
</tr>
<tr>
<td>JAK-2</td>
<td>Janus-type tyrosine kinase</td>
</tr>
<tr>
<td>$K_{ov}$</td>
<td>An algorithm constant for elimination rate, for calculation of GH bioavailability</td>
</tr>
<tr>
<td>kDa</td>
<td>kiloDalton</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MPH</td>
<td>Midparental height</td>
</tr>
<tr>
<td>MPHDI</td>
<td>Multiple pituitary hormone deficiency</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>PP</td>
<td>Per-protocol</td>
</tr>
<tr>
<td>QEPS</td>
<td>Quadratic Exponential Puberty Stop growth model</td>
</tr>
<tr>
<td>rhGH</td>
<td>Recombinant human growth hormone</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
</tr>
<tr>
<td>STAT5</td>
<td>Signal transducer and activator of transcription 5</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>Time in hours to reach maximum GH concentration</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid-stimulating hormone</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>$V_1$</td>
<td>An algorithm constant, in percent of body mass, for calculation of GH bioavailability</td>
</tr>
</tbody>
</table>
List of papers

This thesis is based on the following studies, referred in the text by their Roman numerals.

I. Elena Lundberg, Björn Andersson, Berit Kriström, Sten Rosberg and Kerstin Albertsson-Wikland
   Broad variability in pharmacokinetics and bioavailability of GH following rhGH injections in children
   Manuscript, submitted to Hormone Research in Paediatrics

II. Kerstin Albertsson-Wikland, Berit Kriström, Elena Lundberg, Stefan Aronson, Jan Gustafsson, Lars Hagenäs, Sten-A. Ivarsson, Björn Jonsson, Martin Ritzén, Torsten Tuvemo, Ulf Westgren, Otto Westphal and Jan Åman
   Growth Hormone Dose-Dependent Pubertal Growth: A Randomized Trial in Short Children with Low Growth Hormone Secretion.
   Horm Res Paediatr 2014; 82:158–170

III. Elena Lundberg, Berit Kriström, Björn Jonsson, Kerstin Albertsson-Wikland and on behalf of the study group
   Growth hormone (GH) dose-dependent IGF-I response relates to pubertal height gain
   BMC Endocrine Disorders 2015, 15:84

IV. Berit Kriström, Elena Lundberg, Björn Jonsson, and Kerstin Albertsson-Wikland on behalf of the study group
   IGF-1 and Growth Response to Adult Height in a Randomized GH Treatment Trial in Short Non-GH Deficient Children
   J Clin Endocrinol Metab, August 2014, 99(8):2917–2924

Hormonet tillförs genom injektion i eller under underhudsfettet varje kväll, vilket barn eller förälder gör själva hemma. Dos baseras på barnets vikt, och har varit lika till alla. Det har observerats att tillväxteffekten med lika dos varierar mycket mellan olika barn, vilket uppfattas bero på olika känslighet. Det är även känt att GH nivåerna stiger under puberteten hos båda könen, men mer hos flickor än hos pojkar.


Studierna visade att de barn som fått den högre GH dosen växte mer än de som fått den lägre dosen, och deras IGF-I nivåer ökade också mer och det gällde båda diagnosgrupperna. Högre tillväxtsvar korrelerade med högre IGF-I svar. Dock, det var stor variation i effekt av GH på både tillväxt och IGF-I vilket speglar varierande känslighet/responsiveness, där effekt (response) beror av känslighet per given dos (response= responsiveness x GH-dos). Högre responsiveness sågs
hos barn med GH brist. IGF-I ökningen visade sig vara den viktigaste variabeln att förklara variationer i tillväxtvar både före och under pubertet, men tillväxteffekten av IGF-I ökning varierade också mycket. Studien av hormonupptag visade att en högre blodkonzentration av GH korrelerade med bättre tillväxt och för detta har både dos och injektionsteknik betydelse. 

Dessa studier genomfördes med konventionell viktbaserad dosering av GH, med resultatet stor variation i effekt mellan barnen oavsett given dos eller diagnos. Detta visar hormon-mottaglighetens stora betydelse och att dosen borde anpassas efter individen också under pubertetsväxandet. Vidare var effekterna överlappande mellan diagnosgrupperna, vilket talar för att mottaglighet för behandling vore bättre grund för behandlingsbeslut än hur mycket hormon som hypofysen maximalt kan frisätta vid kortvuxenhetsutredningen.
1. Introduction

1.1 Childhood growth

Childhood growth, here identified as longitudinal bone growth, is a complex biological process. Factors influencing growth include genetics, psychosocial environment, nutritional and disease status and hormones.

1.1.1 Genetic influence on growth

Height is largely hereditary and this is reflected in the fact that some families are tall, some are short while most have average height. An individual's adult height has a high correlation with mid-parental height (1), but no single gene controls the outcome; the polygenic inheritance of height was long suspected (2) and has since been identified through whole genome mapping (3, 4).

The tempo of growth, timing of puberty and subsequent adult height are all influenced by genetic factors (5). It has been estimated that up to 80% of the variability in adult height is controlled by genes (1, 6), while the remaining 20% varies due to environmental and hormonal factors. The contribution of heredity to adult height is dependent on environmental circumstances, and these interact throughout the entire period of growth (7). Children with similar genotypes, who would reach the same adult height under optimal conditions, may be differently affected by adverse circumstances, as shown in twin studies (2, 3).

1.1.2 Environmental influences on growth

The effect of psychosocial factors on growth, such as short stature resulting from abuse or neglect, was described more than 50 years ago, but this problem is still a reality for millions of children worldwide (8, 9).

Socio-economic factors, i.e. quality of the environment and living conditions as well as availability and quality of clean water and nutritional supply, greatly affect growth. In those countries where these conditions have been improved over generations, changes in adult height and an earlier onset of puberty have been observed (10-12). This change in height in a population over generations is called a 'secular trend'. A positive secular trend regarding adult height was seen in Sweden even as late as in the 20th century, when the growth reference based on data from children born in 1974 was compared to data from children born in 1956 (13); recently it was shown that the trend has continued, when the 1974 cohort was compared with a 1990 birth cohort (14).
Nutrition is a major determinant of growth and has a significant effect on the timing of sexual development (15). There is a relationship to growth over the entire BMI range in childhood, such that higher childhood BMI was associated with more growth during childhood, less growth during puberty and unaffected adult height (16). The nutritional extremes, obesity and anorexia nervosa, illustrate the different effects on growth and puberty. The child with obesity has a normal or accelerated growth velocity with increased tempo in biological maturation demonstrated by the onset of puberty at an early age, but still ending up with an adult height within the child’s genetic potential. The energy-malnourished child with anorexia nervosa has a reduced height velocity, with low levels of insulin and thyroxin and reduced tempo of maturation, with no or late onset of puberty, due to the deficient leptin signal to the hypothalamus (17).

The degree of physical activity affects growth. Moderate activity has a positive effect while excessive physical activity has a negative effect (18). At the other extreme, inactivity of a limb, e.g. due to cerebral palsy or a post-polio syndrome, often results in severely impaired longitudinal growth of that limb, while the rest of the body grows normally (19).

Light: At northern latitudes there is seasonal variation in growth velocity, with greater growth velocity during light months and lower growth velocity during the dark season (20). In Sweden, this has the implication that measurements over a full year are needed to evaluate one-year growth velocity.

Any burden of an acute or chronic disease influences growth, more so if the disease is severe and longstanding and especially if the child is young, when growth velocity would normally be high. Even if successful treatment is introduced, if the height deficit by that time is already substantial, it is difficult to regain the height the child would have had if no disease had occurred. This regained height is called ‘catch-up growth’ and is commonly used as a measure of successful intervention (21). Even acute illness immediately affects growth, as shown by daily measurements of lower leg length by knemometer (22).

The effects of hormones on growth will be discussed later in this thesis but, in short, the hormones that stimulate growth are thyroxine and cortisone, which act permissively, i.e. too low or too high levels inhibit growth, whereas growth hormone (GH) has a dose-dependent effect on growth; the sex hormones estradiol and testosterone, which convert to estrogen, are special in that low serum levels stimulate growth while high estradiol serum levels trigger the end of the growth process by fusing the growth plate.
1.1.3 Growth periods

Normal human postnatal growth can be divided into three main periods: infancy, childhood and puberty from the late intrauterine period to adult height. All growth periods have a special growth pattern that is regulated differently. Modern auxology uses a mathematical description of child and adolescent growth to describe growth patterns. A growth model serves as an essential tool when elucidating the regulation of growth. The first growth model to describe growth from birth to adult height was the infancy-childhood-puberty (ICP) model, which mathematically divides postnatal longitudinal growth into three components corresponding to the growth periods infancy, childhood and puberty (23, 24). A juvenile growth period has been proposed, but has so far not been identified in any growth models. It refers to the period just before puberty, when growth rate decelerates until the onset of puberty, with substantial increase in height velocity (7). Figure 1, Figure 2.

![Figure 1. ICP model of endocrine regulation during infancy, childhood and puberty. The transition phases between different growths periods are marked with circles. Fetal growth is influenced by IGF-I from the placenta and thyroid hormone. Thyroid hormone is a major regulator during infancy and together with cortisol influences the whole growth period afterwards. GH regulates growth from 6–12 months and the GH/IGF-I axis is important during childhood. At the infancy-childhood transition (ICT), the main role is played by the GH-IGF-I axis, while at the childhood-juvenility transition (CJT) adrenal hormones take the lead. The juvenility-puberty transition (JPT) is influenced by the gonadal hormone. Modified with the permission of Albertsson-Wikland from Hochberg, Albertsson-Wikland, Ped res 64; 2008.

The only other growth model that can mathematically describe growth from fetal life to adult height is the recently developed shape-invariant QEPS (Quadratic Exponential Puberty Stop) growth model, with the quadratic, exponential
functions starting in early foetal life, and with specific pubertal and stop functions (25).

\[ \text{Fetal growth} \]

Growth before birth is divided into three trimesters during pregnancy and is the fastest growth period in the lifespan. The formation of most organs takes place during the first 8 weeks. The maximum longitudinal growth rate occurs during the second trimester (26): from 2.5 cm to 35 cm by the end of the second trimester. Fetal growth and brain development (27) depend mainly on maternal nutrition and placental function (28). Fetal growth is mainly regulated by growth factors such as insulin-like growth factor-I (IGF-I), IGF-II and insulin (29-31). At term birth, boys are often heavier and longer, due to androgen effects (32-34). Genetic factors affect birth length less than maternal and placental factors. Birth length and adult height were found to have a correlation coefficient of only 0.25, compared to 0.80 when comparing 2 years of age and adult height, which indicated that not all genes are expressed at birth (35).

\[ \text{Infancy growth} \]

Growth during infancy is a continuation of fetal growth and starts during early fetal life, as described by the QEPS model (25). Using the ICP model for description, it was assumed to start around mid-gestation with a decelerating influence on total growth until 3–4 years of age (24). Infants continue to grow rapidly during the first 3 months of life, with decreasing height velocity after that. On average, children grow 25 cm in the 1st year (13, 36). Infancy growth is mainly dependent on nutrition, and regulated by insulin, thyroxine and insulin-like growth factors (IGFs). The timing of infancy-childhood transition (ICT) adjusts an individual's height in response to environmental cues (37). Age at ICT determines adult height and therefore a delayed ICT will limit the adult height; 40% of children with idiopathic short stature (ISS) have a delayed ICT (38).

\[ \text{Childhood growth} \]

The onset of childhood growth, i.e. at ICT, normally starts before 1 year of age, at 9–12 months. During this growth period, growth velocity is rather stable until puberty and any healthy child will maintain its individual growth trajectory. In this period there is relatively little difference in height between boys and girls. There might be a mild spurt around mid-childhood, possibly related to adrenarche, occurring at age 6–7 years (39, 40), but this has so far not been observed in any other longitudinal study (13, 41). Nutrition and psychosocial factors continue to be of great importance (24, 42).
**Figure 2.** Height velocity chart with markers for the different growth phases and concomitant important growth factors (F=fetal, I=infancy, C=childhood, J=juvenility, P=puberty. IGF=insulin-like growth factor, GH=growth hormone). With permission of Anna-Karin Albin.

**Juvenile growth**

The years preceding puberty, the juvenility period, are characterized by declining height velocity (7). The transition from juvenility to puberty is a result of maturation of the hypothalamic-pituitary-gonadal (HPG) axis. Poor quality of life and epigenetic factors during this transition delay fertility and increase longevity (7).

**Pubertal growth**

Pubertal growth, often referred to as the ‘pubertal growth spurt’, takes place during a dynamic period with rapid changes in body size, body composition, sex characteristics and mental development. This is the most rapid period of growth after the neonatal period. It is characterized by rapid acceleration of height velocity, with peak height velocity (PHV) in mid-puberty. Puberty onset in girls starts at age 9–11 years and is characterized by breast stage 2; in boys, puberty onset starts at age 11 years and is characterized by testicular volume of 4 ml, Figure 3. The onset of puberty corresponds to a skeletal age of approximately 11
years in girls and 13 years in boys (43). The timing of puberty shows a wide variation among children of the same gender and ethnic background (44). In girls, increased height velocity occurs about 6 months before breast budding is seen (15, 45). Girls attain PHV at breast stage 3. Total gain in height for girls is on average 20–25 cm.

In boys, the increasing level of testosterone, which aromatizes to estradiol, reaches growth-stimulating serum concentration at testicular volume of at least 6 ml (46, 47). Boys attain PHV at genital stage 4 and their total pubertal gain is up to 25–30 cm (48).

**End of growth and adult height**

The longer duration of prepubertal growth, in combination with a greater PHV, results in the average adult height difference of 13 cm between men and women (28), present in all populations (7).

Recently, using the different components and growth functions of the newly developed QEPS growth model, it became possible to identify different periods of the pubertal growth spurt. Thus, age at onset, middle and end of pubertal growth could be identified with confidence intervals (CI) for the population (14).

### 1.1.2 Pubertal development

**Hormonal regulation**

The HPG axis is active during the fetal period and the first postnatal year. The hypothalamic gonadotropin-releasing hormone (GnRH) pulses stimulate the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which stimulates the gonads to produce testosterone and estrogen, respectively. The sex steroids peak at mid-gestation and decrease until birth, due to negative feedback of the steroids. The interruption of feedback directly after birth results in a rise of LH and FSH and increased sex steroid secretion, reaching peak level at 2–3 months of age; thereafter, the decrease to prepubertal level starts. The prepubertal level is reached at 6–9 months of age in boys (49); in girls, the level of gonadotropins stays higher, until up to 2–3 years of age, before GnRH is downregulated and sex steroids are reduced to prepubertal levels (49, 50). This period with an active HPG axis is called ‘mini-puberty’ (51).

At onset of puberty, GnRH from the hypothalamus is upregulated, becoming active with pulses mainly at night and early morning (52, 53). In response to these pulses, the pituitary starts to secrete LH and FSH, which in turn stimulates the gonadal production of sex steroids (54). In serum, sex steroids are first detected
only during the night, with a circadian rhythm, but following maturation there is also an increase during the day (53).

Gonadal steroids promote the development of secondary sex characteristics and body composition during puberty. In girls, LH stimulates the theca cells in the ovary, which produce androstenedione and testosterone. FSH stimulates granulosa cells to produce estradiol. In boys, LH stimulates Leydig cells to produce testosterone and FSH stimulates Sertoli cells to spermatogenesis (50, 55).

Sex characteristics

Puberty is clinically assessed from secondary sex characteristics, i.e. physical effects of sex hormones. Secondary sex characteristics go through five stages, suggested by Tanner and thereafter used worldwide (45, 56). For boys, it includes genital development and testicular volume and, for girls, breast development, Figure 3.

![Figure 3](image)

**Figure 3.** Left panel: Tanner pubertal stages for boys (A) and girls (B genital, C breast).
Right panel: Orchidometer for testicular volume and volume for boys.

In girls, breast stage 1 corresponds to prepuberty and stages 4–5 to full pubertal female maturation (45). In boys, genital development stage 1 is prepuberty and stage 5 is complete male maturation. Testicular volume is estimated using an orchidometer introduced by Prader (57), Figure 3, and measured in millilitres from the prepubertal volume of 1 ml to approximately 25 ml at complete masculinization (48).

Onset of puberty is well established among paediatric endocrinologists as breast stage 2 in girls and testicular volume 4 ml in boys (15, 45, 56). However, when highly sensitive assays for sex hormones became available to measure serum
levels during pubertal development in healthy children, it was found that the gonads were activated much earlier (53, 58). In girls, estradiol was measurable 2 years ahead of clinical breast development (58). In boys, testosterone could similarly be detected at testicular volume 3 ml (53). However, paediatric endocrinologists worldwide have so far kept the old clinical definitions for onset of puberty.

The following subchapters will describe the secretion and interaction of the GH/IGF-I axis.

1.1.3 Growth hormone

Structure

The GH gene cluster is present in humans on chromosome 17q and contains two GHs: GH-N (normal) is expressed in the pituitary gland whereas GH-V (variant) is expressed only in the placenta (59).

GH in humans is a protein consisting of several isoforms (59, 60). The most abundant form is the 191-amino-acid single-chain protein with a molecular weight of 22 kDa representing more than 90% of the secreted GH. The second most abundant isoform, 20 kDa, comes by alternative mRNA splicing, representing 5–7% of pituitary GH (59). The 20 kDa is generally less biologically active than 22 kDa but has a longer half-life in circulation and is therefore more abundant during GH troughs (60). Figure 4.

More isoforms of GH (27 kDa, 17 kDa, 5 kDa) were identified with different biological activity due to their different binding affinity to the GH receptor. Little is known about the bioactivity or significance of these minor forms, although the 20 kDa has been claimed to have more metabolic effects (60). In addition to the described monomeric forms, GH also exists in oligomeric forms, composed of the various monomeric forms in the pituitary and plasma. The increased proportion of isoforms in the circulation, other than 22 kDa, has been suggested to be a cause of impaired growth in children (61). The existence of many GH isoforms is one reason for the discrepancies in results from different GH assays, since assays using polyclonal antibodies will detect most forms of GH in the circulation, whereas the assays using only monoclonal antibodies (which are still in use) detect only the 22 kDa form (62).
Figure 4. GH/IGF-I. A schematic overview of the GH/IGF-I feedback loop. Secretion of GH from the pituitary gland is regulated by two peptides, GH releasing hormone and somatostatin, in turn influenced by many factors, such as physical activity, nutrition, sleep, stress, sex steroid and thyroxin. Secreted GH consists of several isoforms, with the major 22kD form being the most biologically active and a smaller proportion existing as the 20kD isoform. Almost 50% of the GH in blood is bound to the high-affinity GH-binding protein (GHBP), which is structurally similar to the extracellular domain of the GH receptor. After binding to the receptor, GH induces secretion of insulin-like growth factor I (IGF-I) which can elicit autocrine, paracrine and endocrine effects, as well as feedback effects on GH secretion. The activity of IGF-I is modulated by binding proteins, the most abundant being IGFBP3 and acid-labile subunit (ALS). (SS = somatostatin, GHRH = growth hormone releasing hormone).

Regulation of GH secretion

GH is synthesized in the somatotrophs in the anterior pituitary (63). Regulation of GH secretion from the pituitary is controlled by two hypothalamic peptides: GH-releasing hormone (GHRH) and GH-inhibiting hormone, also named somatostatin (63). GH is secreted in a pulsatile pattern and regulated by the balanced release of GHRH and somatostatin peptides. GHRH is secreted in pulses every 3 hours and is stimulated by hypoglycaemia, nutrition, stress, physical activity, sleep, estrogen, androgen and thyroxine, whereas somatostatin is secreted continuously and regulated by cortisol, IGF-I and free fatty acid (64, 65).

GH in the circulation has a feedback on hypothalamic GHRH secretion and an auto-feedback on its own secretion in the somatotrophs in the pituitary. Negative feedback by endocrine IGF-I also contributes (63, 66, 67).
GH secretion pattern during the lifespan

During the late fetal and neonatal period, GH is present in fetal circulation at a high and unregulated level due to the immaturity of the GHR system. GH is not of great importance for size at birth (28, 42), and infants with congenital GH deficiency and defects in the GH-receptor gene have only a mild reduction in birth size (42,69), whereas defects in the IGF-I gene or any interference with IGF-I action are both associated with severe growth retardation (31).

During infancy, the GH pattern of pulsatile secretion starts to be established (70). Longitudinal growth is still not GH dependent (42, 71). Therefore, growth failure in children with growth hormone deficiency (GHD) commonly does not present until the childhood growth period (42).

During childhood, GH secretion is gradually reduced and starts to establish a diurnal peak-and-trough pattern, with higher peaks during night-time. During this period, GH secretion is similar for both sexes (77). GH is the main regulator of childhood growth (74), with a dose-dependent effect both directly at the growth plate (75) and indirectly through the IGF-I (76). Thyroxine, insulin and cortisol are all necessary and permissive hormones. In addition, nutrition and psychosocial factors continue to be of great importance (42, 73).

During puberty, GH becomes upregulated as a response to estrogen. The pulsatile GH secretion increases, with high peaks during both day and night in a gender-specific manner: about a threefold increase in girls and a doubling in boys (78). The pulse amplitude and amount of GH per secretory burst increase but the pulse frequency does not change (79).

In adulthood, the GH secretory pattern in men is characterized by peaks about every 3 hours (80, 81) and low basal circulating level troughs in between (87). A diurnal rhythm of pulsatile secretion is fairly constant in periodicity but varies in amplitude (83). The highest GH peaks are seen during slow-wave sleep, and smaller pulses occur during the day (84). In women, the estrogen secretion will give rise to a GH pulsatile pattern that is characterized by a sustained basal level and more irregular peaks with different amplitudes (85).

After completed growth, i.e. when adult height is attained, GH secretion progressively declines, falling to very low levels in old age (86). The GH amplitude is reduced while pulsatility remains (87).

In rodents, sex differences were found in the pattern of GH secretion (88-90). The adult male rats had a high-amplitude, three-hourly, pulsatile pattern of GH secretion, with low troughs in between (91), whereas females secreted GH in a
more continuous pattern with a high basal level and irregular peaks (88). The pattern of GH secretion in rodents is an important determinant of the sexually dimorphic pattern of growth (89), liver enzyme function (92), circulating GH-binding protein (GHBP) concentration (93) and IGF-I mRNA expression in skeletal muscle and the liver (94) in these animals.

**The GH receptor**

The human GH receptor gene was cloned in the late 1980s (95). The gene provides instructions for making a protein called the GH receptor (GHR) (96). GH action starts with binding to and activating the GHR. This receptor is embedded in the outer membrane of cells throughout the body and is most abundant in the liver (97). GHR belongs to the cytokine receptor superfamily (98), which also includes a receptor for prolactin and others. The GHR consists of a large extracellular part containing the GH-binding site and occurs separately as the GH-binding protein found in the circulation (99). The intracellular site is involved in the GH signalling process. It was believed earlier that binding of the GH molecule to the GHR started a dimerization (63). Studies have since shown that it is GH that induces the changes in the GHR (96). Intracellular signalling starts by binding to Janus kinase 2 (JAK-2). This initiates the tyrosine phosphorylation cascade, where JAK-2 provides the intracellular transduction followed by activation of substrates for signal transducer including mitogen-activated protein kinase (MAPK) and the activator of transcription 5 (STAT5). The STAT5b pathway mediates the activation of different genes (100), such as a transcription of IGF-I, IGF binding protein 3 (IGFBP3) and the acid-labile subunit (ALS) gene in the liver (101, 102). It has been demonstrated that a deletion in the GHR gene explained the GH insensitivity in some patients with Laron syndrome (103). Interestingly, the GHR gene has now been found in most tissues in humans.

**GH-binding protein**

In the circulation, approximately 40% of GH binds to GHBP. GHBP is identical to the extracellular part of the GHR gene (95) and is generated from GHR by proteolysis. It circulates in plasma as a substantial part of plasma GH. Therefore, the GHBP level reflects the abundance of GHR in the organism and provides the reservoir of GH between secretion pulses (104, 105).

GHBP modulates GH availability to target tissue (106), competes with receptors for GH and influences the response to GH (107). The GHBP level varies considerably among individuals and correlates mostly with BMI or fat mass (108). A modest variation during the day (109) and a significant monthly variation (110) have been observed in children. The GHBP level was found to be
slightly decreased during the 1st month of GH treatment, whereas the GHBP level in the follow-up was equal to the baseline level (111).

**Effects of GH**

GH receptors have been found in most tissue in humans, indicating that GH has an effect in most tissues including protein synthesis, lipolysis and reduced glucose utilization. Our hypothesis on varying responsiveness in different tissues was confirmed in a trial on GH treatment in prepubertal patients with different endogenous secretory capacities (112).

The most responsive to GH is the brain. The GHD patients in this trial showed improved attention, perception and cognitive capacity (113, 114), and earlier studies have shown that GH plays a key role in early brain development, maturation and functionality (115). This was confirmed by a finding of GH immunoreactivity in the first stage of brain development with GH-binding sites in all brain structures (116). In addition, GH has been shown to be important in physiological and reparative neurogenesis, as in brain repair after injury and the development of learning capacity and cognitive function (117).

The second most responsive is lipolysis. GH increases lipase activity in fat tissue, resulting in increased serum free fatty acids (118). Administration of GH has been shown to reduce body fat in both rodents (119) and GHD adults (120, 121). A positive correlation between leptin reduction and 1st year growth response after start of GH treatment was found in a group of short children treated with GH (122).

GH has a positive effect on protein metabolism, i.e. anabolism, and increases muscle tissue both according to mass and strength, as seen in studies where GH was administrated to rodents (94, 119) and to adult GH deficient patients (123), as well as to children (124).

GH acts directly on the growth of myocardium and on inotrope heart function during fetal development (125). Additional support for the GH effect on the heart was shown in both GHD and non-GHD prepubertal patients, in whom increased cardiac size with normalized wall thickness was found (126).

**Bone growth** was the next to least responsive, and the least responsive was the GH effect on *IGF-I serum concentration*, i.e. the GH effect on the liver (112).

In addition, GH induces an apparent *insulin resistance* through increased endogenous gluconeogenesis in the liver and decreased peripheral glucose disposal in muscle (118). Insulin levels are lower than normal in untreated GHD
patients (127) and they increase in a dose-dependent manner with GH treatment, while remaining within the normal range (128) as long as the GH dose is adapted according to individual GH responsiveness (112).

1.1.4 Insulin-like growth factor (IGF-I)

Historical aspects

In 1957, Salmon and Daughaday demonstrated that GH stimulates the incorporation of sulfate into cartilage in vitro by including a secondary growth-promoting substance, which was called the ‘sulfation factor’ (76). Initially it was called somatomedin, as the mediator of somatotropin or GH effects (129), and was the basis of the somatomedin hypothesis. Somatomedin was identified as somatomedin A and somatomedin C (129) and was found to be very similar to insulin, both in structure and metabolism; therefore, these were later renamed as IGF-I and IGF-II (130, 131).

The IGF-I that is measured in serum is produced in the liver and its hepatic expression was highly GH and insulin dependent. Thus, as known today, the liver is the main source of circulating IGF-I (132). However, this peptide is produced in all cells and tissues in an autocrine or paracrine manner (133).

The original somatomedin hypothesis claimed that GH-induced hepatic production of IGF-I promoted growth directly in epiphyseal plate (129). A study on rats showed an independent effect of GH locally on the growth plate by inducing epiphyseal growth (134, 135) and promoting chondrocyte proliferation (136) after GH injection. As a consequence of this finding, the somatomedin hypothesis was modified to the dual effector theory (137, 138), which suggests that the local IGF-I promotes the chondrocyte maturation and bone growth in an autocrine or paracrine manner (133). Figure 5.

Structural similarities between IGF-I and insulin

IGF-I is a 70 amino-acid single-chain polypeptide with molecular size 7.5 kDa and approximately 50% is similar to human proinsulin. IGF-I, like insulin, has an A and B chain connected by disulphide bonds. The greatest difference from insulin is that IGF-I retains the connecting C chain and, while cleaving from proinsulin, it forms C-peptide. The structural similarities give IGF-I an insulin-like effect and similar metabolic and growth-promoting roles (139).
IGF-I is highly GH dependent and is produced in many tissues in response to GH (139). The diurnal level of IGF-I is stable, varying between months (110), Figure 6, in contrast to insulin, which is stored and delivered on demand (140).

**Figure 5.** A schematic overview of IGF-I physiology.

**Figure 6.** Longitudinal serum concentration of IGF-I (left panel) and IGFBP3 (right panel) in healthy prepubertal children. All analysed samples in one child are joined by a line. Reference lines are drawn according to IGF-I reference values (Löfqvist 2001) and show the mean±1SDS and ±2SD, respectively. With permission of Gelander, Pediatr Res 1999.
IGF-I receptor

IGF-I receptor (IGF-IR) and insulin receptor (IR) belong to a family of growth-factor receptors with tyrosine-kinase activity (141-143). These receptors consist of two extracellular α-subunits and two transmembrane β-subunits, which penetrate through plasma and form the tyrosine-kinase part. IGF-IR shares a high similarity of structure and intracellular signalling events with IR (144) but mediates different effects on metabolism, cell proliferation, apoptosis and differentiation. IGF-I has 100 times higher affinity to bind to IGF-IR than to IR, and insulin binds with higher affinity to IR than IGF-I does (142). IGF-I also has a high affinity for hybrids. Therefore, the IGF-I effects are mediated by both IGF-IR and hybrid receptors (145, 146).

IGF-IR was described 30 years ago (140, 147) and is distributed in most tissues including muscles, but not in hepatocytes. IGF-I acts through binding to its own receptor as well as binding to the IR but with a lower affinity and, likewise, insulin binds to IGF-IR (148).

IGF binding protein

In plasma, IGF-I circulates bound to six specific binding proteins (IGFBPs) that act as transport proteins to control the flow of IGF-I from the vascular space, regulate its half-life and allow it to activate surface receptors in targeted cells (149-151).

Serum IGFBP3, as the most abundant form, binds around 90% of IGF-I and IGF-II (152). The half-life of binary complex IGF-I and IGFBP3 was found to be prolonged in the range 16–20 hours, with slightly longer half-life for IGFBP3; together with the liver produced ALS, forming a stable circulating ternary complex of approximately 150 kDa (153, 154). Because of the slow dynamics of IGF-I and IGFBP3, these parameters reflect the GH secretion status over the previous few days (152).

The concentration of IGF-I and IGFBP3 is usually low in patients with GHD, reflecting the reduced GH stimulation of the liver (152). IGFBP3 has a considerably longer half-life than many other peptide hormones, it has no major diurnal variation and it is unaffected by many of the variables that influence GH levels, such as exercise, acute nutrient deprivation or acute fluctuations in blood glucose (155). IGFBP3 is known to independently have an inhibitory effect on cell growth through induction of apoptosis (156).
IGF-I effects

IGF-I is a primary mediator of intrauterine and postnatal growth in mammals. IGF-I-deficient newborn children are short, with low birthweight and with reduced head circumference indicating reduced cerebral development (157). This is not the case in newborns with total GHD, which indicates that the IGF-I prenatal growth-promoting effect is GH independent. After birth, IGF-I becomes largely GH dependent, with some contribution of nutrition and sex steroids through a mechanism that is not known.

In mice with either IGF-I or IGF-II gene knockouts, fetal growth is reduced by 40%. IGF-I deficiency due to knockout of the IGF-I receptor mediating the growth-promoting effect for both IGF-I and IGF-II, results in an approximately 55% reduction in fetal size. These results are supported by findings in humans, where IGF-I receptor mutations on chromosome 15q have been found to be associated with both prenatal and postnatal growth failure (158).

Postnatal, circulating IGF-I is GH-dependently produced in the liver, and is, together with locally produced IGF-I, important for maintaining normal structure and function of complex organs such as bone (159). Circulating IGF-I also interacts with GH secretion in a feedback loop system. However, both liver IGF-I-deficient mice and ALS knockout mice showed relatively normal growth and development, even though they had 75% and 65% reductions in serum IGF-I levels, respectively (132). However, when both IGF-I and ALS production were disrupted, the IGF-I serum levels became even more reduced and a significant reduction in linear growth was observed, as well as a negative impact on the growth plate. In addition, bone mineral density was lower than normal, and decreased periosteal circumference and cortical thickness were found in these mice (159). A threshold concentration of circulating IGF-I seems to be necessary for normal bone quality and growth (159).

A minor proportion of total IGF-I circulates in free form. This free IGF-I may reflect bioactivity and be responsible for the negative feedback on GH secretion (150). The concentration of free IGF-I has been reported to be a sensitive indicator of catabolism and GH dysfunction, and it has been suggested that it has greater physiological and clinical relevance than the total IGF-I concentration. As the analysis of free IGF-I is complex (160), one suggestion has been to use the molar ratio between IGF-I and IGFBP3 as an index of bioavailability (161). For clinical use, the methods for evaluation of IGF-I, IGFBP3 and their ratio has been further developed into SD scores (162, 163).

Primary IGF-I deficiency results from GHR mutations, deficient GH signalling and mutations or deletions in the IGF-I gene (31). Treatment with recombinant
IGF-I has been performed in patients with these diagnoses. IGF-I has an immediate insulin-like blood glucose-reducing effect and mediates effects on protein metabolism similar to GH; in addition, it has mitogenic and proliferative action, but cannot alone replace GH for effects on growth.

1.1.5 The growth plate (combined action of GH and IGF-I)

Longitudinal growth takes place in the epiphyseal growth plate, which is a cartilaginous structure between the epiphysis and metaphyseal bone at the distal ends of the long bones. The growth plate contains only one cell type, chondrocytes, which are at different stages of differentiation in three layers: the resting zone, the proliferative zone and the hypertrophic zone, Figure 7. The chondrocytes are surrounded by collagen fibrils and proteoglycans. The proliferation of the chondrocytes, their maturation and production of bone matrix results in bone growth (164). The locally produced factors such as bone proteins, fibroblast growth factors and retinoid regulate the growth plate function by acting locally on growth plate chondrocytes. GH and IGFs are potent stimulators of longitudinal bone growth. GH acts directly on the growth plate by recruiting chondrocytes from the resting zone to the proliferation zone (165). GH also acts by increasing the local paracrine or autocrine production of IGF-I (75), and IGF-I stimulates the hypertrophic chondrocytes, as shown in knockout mice (138). Other systemic factors such as GH, thyroxine, estrogen, androgen, vitamin D and glucocorticoids also act indirectly by modulating other endocrine signals in the network (166). Changes in paracrine factors that control chondrocyte proliferation and differentiation mediate the local effects of hormones (164).

Circulating IGF-I has less influence on bone growth than locally produced IGF-I, which was shown in a study on rats with a selective hepatic IGF-I deletion, in which reduced circulating IGF-I did not impair growth (167). Supporting this, a triple deletion of the ternary complex IGF-I, IGFBP3 and ALS reduced the circulating IGF-I level by more than 90% but gave only a 6% reduction in body length (168). In addition, the severe IGF-I deficiency seen in patients with ALS deficiency, in contrast to their relatively mild growth retardation, supports the hypothesis that preserved autocrine and paracrine action of locally produced IGF-I is the most important factor (169).
Figure 7. Structural organization of the growth plate showing the resting zone, the proliferative zone and the hypertrophic zone.

Thyroxine has an effect on the proliferative and hypertrophic zones. In rats with hypothyroidism, longitudinal bone growth and endochondral ossification slows, while hyperthyroidism accelerates both processes (165, 170).

Glucocorticoids inhibit bone growth through a direct effect on the growth plate by inhibiting chondrocyte proliferation and decreasing hypertrophic cell height, which may be mediated by changes in the local IGF-I system (171), and by stimulating apoptosis in the growth plate (172). Glucocorticoids may also suppress bone growth indirectly by involving other endocrine signals, such as reduced GH secretion (173).

The sex steroid estrogen controls the growth plate acceleration and fusion in both sexes (44, 174). In early puberty, when estrogen serum concentration is low, estrogen stimulates growth by two mechanisms: firstly, by stimulating the hypothalamus and the pituitary to increase GH secretion and, secondly, by the direct effect in all zones on the growth plate. In boys, serum estradiol is dependent on aromatization from testosterone, which means that, at any given pubertal stage, estradiol concentrations are lower in boys; thus, growth stimulation, and later growth cessation, occurs later in the process of puberty in boys than in girls.
When the pubertal process is completed, the growth plate matures and longitudinal growth ceases. Fusion of the growth plate occurs through the estrogen receptors ERα and ERβ, present in the human growth plate in all zones and in both sexes. Activation of ERα signalling decreases the proliferative capacity and advances senescence in the maturity of growth plate chondrocytes, leading to fusion (175). The evidence of the estrogen effect on bones and the growth plate in humans is based on two reports: one described a fully masculinized male patient with tall stature, osteoporosis, open epiphyseal zones and no pubertal spurt in his medical history. He was found to have a homozygous mutation in the ERα receptor (174). The other report described two siblings presenting with a similar phenotype, a male and a female who had aromatase deficiency (P450arom), i.e. inability to convert testosterone to estrogen (176). Estrogen treatment in these patients led to fusion of the growth plate.

1.2 Investigation of low growth velocity and short stature

1.2.1 Definition of short stature

Short stature is defined as individual height more than 2 SD below mean height for the population, sex, and age, when compared to a growth reference (177, 178). Growth of a population is mostly determined from cross-sectional or longitudinal follow-up of all individuals, both healthy and unhealthy. This provides a record of population changes that reflect socioeconomic development of a state or region, but it is not a sensitive instrument for the detection of abnormal growth in individuals. A valid growth reference is a key element in healthcare screening and for monitoring the treatment of diseases (179, 180). To improve utility and reduce variation, only healthy individuals born at term without any factors that might impair their growth pattern should be included. This increases the chance of a narrower SD score (SDS) range and makes the instrument more accurate at detecting abnormal growth. The growth chart presents the reference data for girls and boys separately, in either percentiles or SDS. The SDS is calculated according to the equation:

$$\text{height SDS}_{\text{child}} = \frac{\text{height of the child (cm)} - \text{mean height of the population (cm)}}{\text{SD for the population}}$$

The Swedish growth references used for postnatal growth in this thesis are based on longitudinal data from birth to adult height from children without evidence of disease, chromosome abnormality or syndromes and born at term either during 1955-1958 (181) or during 1973–1975 (13), and are presented in SDScores.
**Height measurement**

Patients included in the trials described in this thesis were measured at the paediatric units at Swedish children’s hospitals according to a standard procedure, using a calibrated Harpenden stadiometer. To reduce variability in the measurements as a result of personnel, locale, time of day or night and season of the year, the measurement was, as far as possible, performed using the same measuring instruments, by the same nurse or physician and at the same time of day; in addition, the mean of three measurements was used, reducing the risk of measurement error. When evaluating earlier growth, the child was measured every three months for at least 1 year before start of GH treatment.

**General investigation of a short child**

Short stature or slow growth velocity is a common reason for referral to paediatric endocrine units.

The primary investigation is focused on possible diseases of genetic or prenatal origin with dysmorphic features, disproportionality or chromosomal abnormalities, as well as defects in bone or connective tissues. In addition, there may be psychosocial problems, malnutrition or undiagnosed somatic disease. Thus, the primary investigation focuses on the possibility of underlying reasons for growth failure (182). Previous measurements should be obtained if possible, not least weight and length at birth, as the child’s growth pattern is of great diagnostic value.

Children born small for gestational age (SGA) are identified at birth as those with birth weight and/or length at least 2 standard deviations (SD) below the mean for gestational age, as proposed by Karlberg & Albertsson-Wikland (183) and later accepted by the professional community (184). These children commonly have a postnatal catch-up growth phase before the age of 2 years (183, 185, 186). However, approximately 10% of these children continue to be short. Children with intrauterine growth restriction diagnosed during pregnancy might have chromosomal disorders or congenital malformations, if not due to restriction of nutrients (187).

For short stature with normal body proportions, no underlying chromosome abnormality, no psychosocial issues or any sign of chronic organic disease, further evaluation of the endocrine system is warranted. If no endocrine problem is found and birth size was normal according to gestational age, the short stature is defined as idiopathic short stature (ISS) (178, 188).

Children with ISS on average are growing channel parallel (when plotted in a growth chart with SDscore-lines) that means with fewer cm/year when growing
along and below -2 SDscore-line compared to a normally growing child along the mean. Usually short children enter puberty 2 years later than those growing along the mean (181). Studies have shown that, at adult height, both boys and girls were 5–6 cm below their target height (189, 190). Children with familial short stature are included in this group, i.e., those who are short compared to the general population but within the normal range when parental height is taken into consideration.

The children who have slow biological maturation with slow growth velocity for many years before their onset of puberty are usually at the later range or even beyond the normal range for puberty onset (191). However, after completed pubertal growth, these children achieve adult height within their target range.

1.2.2 Diagnosis of GHD

Evaluation of endocrine causes of slow growth velocity or short stature includes clinical evaluation of auxology and body proportions, with assessment of puberty and biochemical analysis of thyroid function, cortisol levels and sex hormones, imaging and molecular genetics. Estimation of GH secretion ought to be mandatory and the estimation of the GH/IGF-I axis through evaluation of serum IGF-I and IGFBP3 is often valuable, especially if the results are clearly outside the normal range in repeated samples (192, 193).

In cases of malformations or a family history of short stature, genetic testing can improve the diagnosis. Abnormalities in GH genes or dysfunction in the regulation of GH secretion involving both GHRH and short stature or transcription factors (194-197) can all cause GHD. A number of gene mutations (GH-1, GHRH-1, and PROP-1) (194, 195, 198) resulting in GHD have been determined, allowing for definitive diagnosis of familial GHD in many cases.

GHD is a heterogeneous condition including varying degrees of impaired GH secretion, from complete GHD, an element of multiple hormone deficiency, to isolated GHD (IGHD) with only growth failure; the diagnosis also encompasses GH sensitivity, from severe GH insensitivity to more moderate degrees or normality, taking into account the broad normal continuum (80, 199, 200).

1.2.3 GHD diagnostics and selection for treatment

GH evaluation

Before the development of the GH radioimmunoassay in 1962 by Hunter and Greenwood, the diagnosis of GHD was difficult. Due to the pulsatile nature of GH secretion, a single GH sample is seldom informative. Stimulated GH secretion
tests improved diagnostics when Roth in 1963 published the insulin-induced hypoglycaemia test (201) followed by the arginine test (202). Later, these tests were combined to the standardized arginine-insulin tolerance test (AITT) with the hope of overcoming the reproducibility problems (203). The AITT is still used, despite the hypoglycaemia discomfort and potential danger (205). A variety of GH stimulation tests, both physiological (including physical exercise, fasting, hypoglycaemia) and pharmacological (glucagon, insulin arginine, clonidine and L-dopa), have been introduced to determine an individual capacity for GH release (205). Poor reproducibility of the AITT has been reported from studies in a group of poorly growing children, where no correlation was found in the stimulation test results (206).

The serum GH concentration cut-off peak from a stimulation test has long been the way to identify children with GHD; and has over the years moved from 3 to 5 to 7 to today’s value of <10 µg/L to be diagnosed with a moderate, partial GHD, while most now consider the cut-off for severe GHD to be <5 µg/L.

The response to the stimulus is dependent on the interaction between the two neuropeptides GHRH and the inhibiting hormone somatostatin. If a spontaneous GH pulse has occurred within 3 hours, the response to an exogenous stimulus is reduced or absent (207). This refractoriness contributes to the wide intra-individual variation in response to stimulation tests.

To avoid refractory problems from false interpretation of the data, spontaneous GH secretion capacity can be estimated by repeated blood sampling. In addition, more physiological information on the secretion pattern is gained. Even though 20–30 minute sampling intervals are needed to achieve a reliable result not only about the rate, but also about the pattern of secretion, the procedure is not distressing for the child, who has to follow normal feeding, activity and sleep routines (77, 208). This form of testing considerably decreases the risk of false interpretation, but demands more time, staff and financial resources and the obtained results require professional interpretation. The reproducibility of 24-hour spontaneous GH secretion profiles has been shown to be high, with low intra-individual variation (209), and this method compares favourably with stimulation tests (80, 210). Figure 6.

The results from GH stimulation tests and spontaneous GH secretion are contradictory (206) and stimulation tests have resulted in up to 50% diagnosis of GHD (211) and more over diagnosis (207).
Diagnostics of GH action: IGF-I and IGFBP3

Diagnostic markers for GHD based on measurement of IGF-I and IGFBP3 have become well established, since it was shown in 1990 that a low concentration of IGF-I is associated with GHD (212). Several studies found that an IGF-I level more than 2 SD below the mean is specific to GHD and can therefore be used for diagnostic purposes (205, 213). Nonetheless, it should be noted that it is not unusual for young children to have numerically low levels of IGF-I, which makes it problematic to evaluate a subnormal level in this age group, unless an accurate reference is used. There is an overlap in serum IGF-I between children with normal growth and those with ISS. Up to half of all children with ISS have a subnormal level of IGF-I (213, 214), which is why other markers of GHD are necessary for diagnostic purposes.

IGFBP3 level has been reported as useful for diagnostics (205, 215). The basal level of IGFBP3 is higher than IGF-I and is more useful for diagnosis in young children. In addition, both IGF-I and IGFBP3 show some variation but their fairly constant circadian level make it possible to make an evaluation from a single blood sample.

Patients with GH insensitivity showed low levels of IGF-I and IGFBP3, and testing both is necessary for diagnosis (216, 217).

The IGF-I generation test is a test to assess GH responsiveness (200, 218, 219). Initially, only the ability to generate IGF-I was used for diagnostic evaluation and for estimation of growth rate during GH treatment (220, 221).

IGFBP3 was reported to be a more accurate variable in diagnosis and treatment prognosis (152), and using an IGFBP3 generation test showed higher sensitivity compared to IGF-I, both with high and lower GH doses (222). The serum IGF-I and IGFBP3 measured separately cannot be used for differential diagnosis, while a combined IGF-I and IGFBP3 generation test does give improved diagnostics.

GH responsiveness

The first time the definition of responsiveness was proposed in 1917, in a study of the effects of adrenalin (223). Today, the ability of the GH receptor to act from its binding to molecule or to hormones is defined as sensitivity, while the ability to determine the entire signalling toward a particular GH effect is defined as responsiveness.

Response, defined as change in a chosen variable on GH treatment, is usually dose-dependent. Responsiveness was suggested to explain the observation of a high inter-individual variability in response to a specific dose (224).
A prediction model was developed to suggest the most likely mean growth response to GH treatment for an individual child, rather than just establish the explained variance for the group (211, 225, 226). A valid model for the prediction of growth response will give valuable information about how the individual child will respond to GH treatment on the selected GH dose. In this way, a realistic estimation of the effect of treatment can be obtained. The concept of the prediction models was developed for prognosis of growth in patients with the same diagnosis, based on patient characteristics and modalities of GH treatment (225, 227). The Swedish prediction model was developed initially from a group of prepubertal patients with IGHD and ISS. Later, the model was expanded to include patients with IGHD, ISS, SGA and those born preterm (228). The most important variables were height at GH start, growth deceleration from birth until 2 years of age, midparental height and GH maximum during a 12- or 24-hour spontaneous profile (225, 229). A broad range in growth response when the GH dose was the same per kg for a group of GHD and ISS patients was also found, which reflected the continuum in GH responsiveness (46).

In the past, when no prediction model was available for diagnostics, it was common to start GH treatment immediately and evaluate the 1st year growth response (230). Depending on the health care organisation in a country, this is still the approach applied in many parts of the world. Establishing a sensitive investigation method facilitates the decision about when to initiate GH treatment, and now we know which variables are important in this decision process.

1.3 GH treatment

1.3.1 Using GH as the drug

Pituitary-derived human growth hormone (hGH) has been used for treatment of GHD since 1957 (231, 232). Initially, due to impure hGH preparations, it was injected intramuscularly 2–3 times per week. The supply of hGH was limited and it was only used for treatment of those most severely affected by GHD (233). The production of pituitary hGH slowly increased and more patients could be treated, but in 1984 a link to Creutzfeldt-Jakob disease became apparent and all commercially available growth hormone was withdrawn worldwide (234).

During the 1980s, biosynthetic recombinant hGH (rhGH) preparations were developed, consisting exclusively of 22 kDa (unlike the first preparations, which contained an extra methyl group connected to the hormone) and produced from Escherichia coli through recombinant DNA technology (235).
When rhGH became available in Sweden in 1986, most prepubertal GH deficient patients, together with all Finnish GH deficient patients, were included in the Genotropin® registration study, known as the rhGH study (236).

In 1989, a state of the art workshop was held by the Swedish medical research council to present and discuss the rationale and evidence for optimization of treatment in GH deficient patients, especially during puberty and as adults. Widening the scope of GH treatment to include less severe GHD indications was also discussed (177).

In Sweden, two randomized rhGH treatment trials had been started in 1988. The first involved GH deficient patients (both IGHD and MPHD) randomized at onset of puberty and given rhGH treatment with different doses and administration frequencies; the aim was to study whether mimicking the physiological pubertal GH increment observed in healthy children (78) was of any value for GH substitution during puberty. The second trial involved prepubertal short children (around 10 years of age) with non-GHD (ISS and SGA) just before onset of puberty (258), who were given rhGH treatment at one of two doses and compared to an untreated control group.

1.3.2 Administration: Injection frequency and dose

In the early 1980s, more purified preparations made it possible to change mode of delivery from intramuscular to deep subcutaneous injections (235). This made self-administered injections possible and thereby it became feasible to increase the injection frequency to daily injections (236, 237). This increased frequency resulted in a 30% improvement in growth response despite the same weekly GH dose (236, 237).

In relation to doses used nowadays, doses at the start of GH treatment in the 1960s were relatively low, but with substantial growth response for treated patient compared to untreated patients (233) although only a fraction attained an adult height within the normal range (238). Only one dose-response study on growth was performed during these years, resulting in an exponential dose-response curve with a GH dose range of 30–80 mU/kg/week, corresponding to 33–80 μg/kg/day (239). This group of patients with severe GHD still respond well on a below-average GH dose, even though higher doses are normally used for this group, as the treatment target is an attained adult height within their genetic potential.

After the rhGH trial was completed in 1987, the daily dose of 0.1 mU/kg/day (corresponding to 33 μg/kg/day) became the standard in Sweden. Worldwide, GH dosing still varied between countries and according to diagnosis. It remained
low, not least in Japan and France. In the US, in contrast, the average dose soon became more widely established and 45 µg/kg/day is now often used for prepubertal GH deficient patients, increasing to 100 µg/kg/day during puberty (240). In ISS patients the dose is 50 µg/kg/day (241), while the upper limit of the GH dose used in other paediatric conditions is approximately 70 µg/kg/day, although the use of such doses depends on diagnosis and not least varies according to national health economics (188, 193, 242, 243).

In the two trials presented in this thesis, the outcome of the GH doses of 33 and 67 µg/kg/day had not previously been studied during the prepubertal and pubertal years until adult height. Moreover, the GH dose was the highest acceptable dose among Swedish clinicians.

The GH dose has been based on weight in most countries, whereas the Dutch have used body surface area (BSA) instead, giving doses in the range 0.7–1.4 mg/m²/day (corresponding to 25–50 µg/kg/day). A Dutch–Swedish dosing comparison was performed in girls with Turner syndrome (244), resulting in a higher GH dose for younger patients than older patients when the BSA dose was recalculated into dose per kg of body weight. The BSA dosing resulted in a lower amount of total GH needed to achieve the attained adult height.

Daily nocturnal subcutaneous GH injections are still the worldwide regimen, whereas injection devices have been developed to facilitate the injection procedure and compliance from the first Kabipen that made daily home injections possible (236) to the latest injection devices (245).

1.3.3 Administration: Different routes of GH

To obtain optimal pharmacokinetics and pharmacodynamics, different routes of administration have been tested to mimic the endogenous pulsatile GH pattern, including the circadian variation, pulse frequency, peak amplitude and amount of GH (77, 78, 81, 82).

*Intravenous* administration of exogenous GH resulted in high amplitude peaks with shorter duration than endogenous peaks (81). Elimination with a half-life of 15–30 min and a metabolic clearance rate of 2.2–3.0 mL/kg/min were reported, with a metabolic clearance rate equal to the GH infusion rate when steady state was obtained (246, 247). Serum GH was found to be similar to baseline level 1 hour after intravenous administration, and GH was not detectable before the next bolus when eight bolus doses were administrated over 24 hours (248).

*Intramuscular* administration of GH resulted in a higher amplitude and a shorter duration compared to subcutaneous injections (236, 249). The time (T\text{max}) to
reach maximum concentration ($C_{\text{max}}$) was 2–3 hours, and after 12–20 hours GH was no longer detectable in serum (250). A higher bioavailability was obtained after intramuscular compared to subcutaneous administration (248, 249) due to faster uptake of GH through capillaries. Thus, when rhGH is injected intramuscularly, it is rapidly absorbed, resulting in a limited time in the circulation due to the short half-life.

Subcutaneous administration resulted in prolonged absorption and disappearance (251). $T_{\text{max}}$ was 4–6 hours and half-life was calculated as 5.3 hours (248). The GH serum profile following a subcutaneous injection is midway between a pulsatile and continuous GH pattern (252).

The site of subcutaneous GH administration may be of importance. In healthy subjects (253) and in GH deficient patients (248), the absorption of GH after subcutaneous injection was faster from the abdomen than from the thigh, reflected in shorter $T_{\text{max}}$.

When GH is administered by subcutaneous injections it comes into the extracellular space and reaches systemic circulation either by uptake in blood capillaries or in lymph vessels (254), Figure 8.

Molecular size and weight of the proteins affect uptake from the subcutaneous site. Protein with higher molecular weight than 16 kDa enters the systemic circulation through the lymphatic system (255). In a study on sheep, the lymphatic absorption of 22 kDa hGH was 60% of the injected dose (256).

### 1.3.4 Who benefits from GH treatment?

Initially, in the late 1950s, hGH was only used for treatment of the most severe GHD, with the exception of research protocols (231, 232). Only 50% of the boys and 15% of the girls attained a final height above -2 SDS of the population norm or their midparental height (238). A large group of short children were later treated in Finish trial to follow growth outcome in relation to diagnoses and to attempt to establish who would benefit from GH treatment (257). The increased availability of rhGH made it possible for patients with less severe GHD to receive treatment resulting in the extension of indications for treatment to include short patients with endogenous secretion, i.e. those diagnosed with ISS (241, 258), SGA, intrauterine growth restriction and Silver-Russel syndrome (184), among others (259). It also became possible to provide GH treatment not only until patients reached an acceptable height but until they attained adult height in concordance with their individual genetic potential. It also became accepted to continue GH treatment for GHD into adulthood (204, 260).
Age at start of treatment is critical for growth response: the younger the patient, the better the response (46, 225, 261). This is why it is important to monitor growth in a population in order to identify individuals to be able to respond to any deviations. It is thus reassuring that the age at start of GH treatment is decreasing in many countries.

It is observed that more boys than girls are referred to endocrine clinics due to growth disturbances (262, 280). In addition, boys are referred at a younger age. Short stature in girls is often not detected, or it is reported late, because it is considered acceptable for girls to be short. We observe even today that more boys than girls are referred to endocrine clinics for investigation, and that the girls are generally referred too late.

1.3.5 Safety of GH treatment

The Swedish 25-year follow-up of all patients diagnosed with IGHD, ISS and SGA and given GH treatment (GH start 1985–2010 in the national GH-registry and clinical trials) has shown that the increased risk of mortality that was postulated...
from a French study (263) could entirely be linked to specific birth characteristics (birth length, weight, gestational age and malformations); in fact, this accounted for one third of the mortality risk in this GH-treated population (264). However, in a lifespan perspective, this period of follow-up is short.

The past 60 years of GH treatment have shown a good safety profile regarding morbidity (265, 266). Monitoring future morbidity and mortality of adults who were given GH treatment as children will be of great importance for our understanding of the outcomes of treatment.
2. Aim

The overall aim of this thesis was to investigate the balance between GH secretion and GH responsiveness in patients diagnosed with IGHD or non-GHD and the estimated GH uptake during GH treatment.

The aims were to study GH *responsiveness* by estimation of:

1. GH uptake regarding pharmacokinetics and bioavailability of rhGH following a GH injection, as well as intra- and inter-individual variation and factors influencing the uptake of rhGH.

2. Growth response to GH treatment as gain in height$_{SDS}$ during the growth periods childhood and puberty.

3. IGF-I and IGFBP3 response to GH treatment as change in circulating IGF-I$_{SDS}$, IGFBP3$_{SDS}$ and IGF-I/IGFBP3 ratio$_{SDS}$.

3. Patients

Study design and study populations
In 1986, rhGH became available in Sweden for treatment within the registration trial of Genotropin® for GHD, and patients who were diagnosed with GHD started (or restarted) on GH substitution therapy, dose 33 µg/kg/day (267) if they had spent 1 year without GH due to the withdrawal of pituitary GH. The unlimited availability of rhGH for treatment made it possible to initiate trials of the optimization of GH treatment regarding dose and frequency during puberty in patients with the accepted indication GHD (TRN 88-177) (280); it also made it feasible to go beyond this strict indication, to include children with short stature without GHD in a dose-response trial (TRN 88-080) (258). Thus, in 1988 two clinical trials started as national, multicentre, randomized GH dose-response studies in short children diagnosed with GHD or non-GHD. The diagnosis of GHD was based on the GH response cut-off during two GH provocation tests, mainly the AITT, 20mU/L, corresponding to 10 µg/L (62). From 1988, at onset of puberty, isolated idiopathic GHD patients were recruited and randomized in the trial of the optimization of pubertal GH treatment by using different dosages and frequencies, aiming to improve pubertal gain in height and adult height. These patients were included in the growth response study presented in this thesis (Paper II) and in the IGF-I response study (Paper III). Patients assessed as non-GHD were followed in a separate trial during an investigational pre-treatment year and subsequent randomization to different doses of rhGH or as untreated controls to examine growth response, measured as gain in height and adult height (258), and IGF-I response (Paper IV).

During these studies, patients were followed yearly at paediatric endocrine units at university hospitals and every three months at a paediatric clinic at their local hospital (which was the endocrine clinic for over 75% of them) until they attained adult height on treatment and thereafter another 1–2 years. Patients living in the western region of Sweden, who constituted over 50% of both study populations, had their yearly visit at GP-GRC and formed the entire study population for the GH uptake study (Paper I).

3.1 Growth response studies
All patients described here were referred to paediatric clinics in Sweden because of short stature or low growth velocity. Somatic and endocrine investigations were performed. Patients were excluded from the studies if they had chronic disease such as coeliac disease, abnormal karyotypes indicating a syndrome, disproportionate short stature indicating skeletal dysplasia or any hormonal insufficiency in addition to GHD that might influence growth.
3.1.1 Growth response to GH treatment in patients with IGHD (Paper II)

Patients with idiopathic IGHD were included and randomized in the trial of optimization of pubertal GH therapy by using different dosage and frequencies, aiming to improve pubertal gain in height and adult height. Table 1.

Table 1. Inclusion and exclusion criteria for study subjects in the IGHD trial (Papers II and III).

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height below -2SDS or low growth velocity 1</td>
<td>Multiple pituitary hormone deficiency (MPHD)</td>
</tr>
<tr>
<td>GHmaxAITT&lt;10µg/L</td>
<td>Gestational age &lt;32 weeks 2</td>
</tr>
<tr>
<td>Height velocity increase ≥ 50% after 1st year prepubertal GH treatment</td>
<td>Extreme intrauterine growth retardation</td>
</tr>
<tr>
<td>Onset of puberty</td>
<td>Bone age delay more than 3 years 2</td>
</tr>
</tbody>
</table>


Study design

All IGHD patients had received rhGH treatment with a dose of 33 µg/kg/day for at least 1 year prior to inclusion in the trial. After development of clinical signs of puberty, the patients were allocated randomly, without stratification, into three dose groups: 33 µg/kg once daily (GH\textsuperscript{33}), 67 µg/kg once daily (GH\textsuperscript{67}) or divided into two daily doses (GH\textsuperscript{33x2}). The patients were followed until adult height, defined as growth rate <1 cm during the preceding 12 months, and for at least 1 year thereafter. Figure 9.
**Study population**

**Safety population:**
A total of 149 (116 boys) short (< –2 SDS) IGHD patients were enrolled in the study between 1988 and 1999 and formed the safety population. Of the enrolled patients, 38 were excluded from the efficacy analyses due to protocol violation or wrong inclusion: one patient had a bone age delay of 3.6 years at GH start, 24 patients were already pubertal (breast stage >1 or testicular volume >8 ml) at or within 1 year after GH start, one patient was lost from follow-up, six patients were born at a gestational age <32 weeks and six patients were adopted with missing information at birth. At study initiation, there was no accepted distinction between patients born with appropriate size for gestational age (AGA) and those born small for gestational age (SGA), thus SGA patients constituted 30% of the study population.

**Intent-to-treat (ITT) population:**
The remaining 111 patients (90 boys) constituted the ITT population, of whom 98 (85 boys) constituted the per-protocol (PP) population. Of the 12 patients excluded from the ITT population, eight boys stopped GH treatment prematurely (<2.25 years after study start and before adult height was reached) and four patients (three boys) on GH\(^{67}\) decreased their GH dose to GH\(^{33}\). Figure 10.
Figure 10. Flowchart of patients with isolated idiopathic GHD (IGHD) included in the growth response study (framed solid line) and the IGF-I response study (framed broken line). Intention-to-treat (ITT) population and per-protocol (PP) population.

3.1.2 Growth response to GH treatment in non-GHD patients
(Paper IV)

Patients with short stature but not GHD were included in a dose-response growth trial performed during 1988–2005 and published in 2008 (258).

Table 2. Inclusion and Exclusion criteria for study subjects with non-GHD

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Height below -2SDS ¹</td>
</tr>
<tr>
<td>Chronological age 8-13 years for girls, 10-16 years for boys</td>
</tr>
<tr>
<td>Bone age ≤11 years for girls ² Bone age ≤13 years for boys</td>
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<table>
<thead>
<tr>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>GH deficiency</td>
</tr>
<tr>
<td>Gestational age &lt;32 weeks ²</td>
</tr>
<tr>
<td>Extreme intrauterine growth retardation</td>
</tr>
<tr>
<td>Bone age delay more than 3 years ³</td>
</tr>
<tr>
<td>Chronic disease</td>
</tr>
<tr>
<td>Skeletal dysplasia</td>
</tr>
<tr>
<td>Chromosome aberration</td>
</tr>
<tr>
<td>Syndrome</td>
</tr>
</tbody>
</table>

¹Albertsson-Wikland 2002(13), ²Niklasson 1991(268), ³Tanner 1983(271)
**Study design**

All patients were evaluated during a 12-month pre-randomization period and those who were still prepubertal and without a spontaneous catch-up growth were randomized into three treatment groups: standard GH dose, 33 µg/kg/day (GH33), GH dose 67 µg/kg/day (GH67), or no treatment (serving as the control group). Patients who had entered puberty before inclusion into the trial were randomized into the GH67 group or the untreated control group. Figure 11.

**Figure 11.** Study design for trial on patients with non-GHD. Close to onset of puberty, patients who were still prepubertal were randomized into three groups and followed to adult height when GH treatment was stopped: GH-treated with 33 µg/kg/day, GH-treated with 67 µg/kg/day or untreated controls.

**Study population**

In total, 177 patients were enrolled into the study, of whom three did not participate; 174 patients constituted the safety population and, of these, 26 patients were excluded due to protocol violations. The remaining 151 patients (112 boys) constituted the ITT population, of whom 43 were randomized to GH33 and 62 to GH67.

The 108 patients (89 boys) formed the PP population, of whom 32 were randomized to GH33 and 45 to GH67. In the study population, approximately 30% of these 45 patients were born SGA. Figure 12.
3.2 IGF-I response studies

These studies were performed in the two (IGHD and non-GHD) populations in order to evaluate the GH effects on IGF-I and IGFBP3 responses to GH treatment and to assess the relationship to gain in height until adult height.

*Study design:* Serum was sampled for estimation of IGF-I, IGFBP3 and IGF-I/IGFBP3 ratio before the first injection at GH start, then at 10 days, 1 month, 3 months, 1 year and yearly thereafter, and transformed to SDS. The on-treatment changes were expressed as ΔIGF-I, ΔIGFBP3, ΔIGF-I/IGFBP3 ratio and mean on-treatment level, and were expressed in SDS.

3.2.1 IGF-I response study in IGHD patients *(Paper III)*

The study population was based on the same population as the IGHD growth response study (Paper II) except for seven patients (six boys) for whom IGF-I data were missing. The remaining 104 patients (90 boys) formed the ITT population. Further, nine patients (eight boys) were excluded from the ITT population: five boys had stopped GH treatment prematurely (<2.25 years after study start and before adult height was reached) and four patients (three boys) on GH decreased their GH dose to GH. The remaining 95 patients (82 boys) constituted the PP population.

3.2.2 IGF-I response study in non-GHD patients *(Paper IV)*

The study population in Paper IV was based on the same population of non-GHD patients as presented in 3.1.2 and Figure 12.

3.3 GH uptake study in IGHD and non-GHD patients *(Paper I)*

This study included patients from one site, GP-GRC, participating in the two trials: 59 from the IGHD trial and 58 from the non-GHD trial, forming the Clinical setting cohort which was followed annually between 1992 and 1999, up to eight times for some individuals. The GH uptake study includes also a smaller Experimental setting cohort, which was more closely studied and whose data were used for comparison. Figure 13.
Figure 12. Flowchart of patients with non-GHD included in the growth response and IGF-I response study. Intention-to-treat (ITT) population and per-protocol (PP) population.

Figure 13. Flowchart of patients included in the GH uptake study. Patients from a single site, GP-GRC (Gothenburg Pediatric Growth Research Center), 59 participants in the IGHD trial and 58 in the non-GHD trial, formed the Clinical setting group. Four patients were also included in the Experimental setting. Both settings were analysed in the GH uptake study. (GHD = growth hormone deficiency, IGHD = isolated GHD, MPHD = multiple pituitary hormone deficiencies.)
3.3.1 Clinical setting

Study design

Patients came at least 2 hours before receiving the rhGH injection at 18:00. Data were collected about the timing and dose of rhGH given at home the evening before (16–24 hours earlier). The patient or parents injected rhGH at a 90° angle with a 12-mm needle (BD-Microfine®) under the supervision of a nurse, aiming for a deep subcutaneous injection into the thigh of the child. Blood samples were drawn from an intravenous cannula both 2 hours and just before the rhGH injection, and thereafter at 2-hour intervals for 16 hours.

Study population

This cohort included 117 rhGH-treated patients (93 boys), 59 (45 boys) from the IGHD group (six with multiple pituitary hormone deficiencies (MPHD)) and 58 (48 boys) from the non-GHD group. Patients were treated with their daily rhGH injection according to randomization, dose 33 µg/kg or 67µg/kg.

GH curves

In total, 446 GH curves (11 samples per GH curve) were obtained from 117 patients. Seventeen GH curves were excluded from the analysis: eleven due to missing values at either baseline or at 2 hours, three due to an unreliably high starting value and three due to unreliable information on rhGH dose. The remaining 429 GH curves (361 from 93 boys, 68 from 24 girls) constituted the Clinical setting GH curves study.

rhGH-dose subgroups: GH curves were separated into groups according to dose (GH33, GH33x2 or GH67).

GH_{peak} and GH_{nopeak} subgroups: These groups were assigned by visual inspection, based on whether any spontaneous GH-secretion peak was apparent (GH_{peak}) or not apparent (GH_{nopeak}). In some patients, serum GH concentration resembling spontaneous GH peaks were observed at 6 hours after the rhGH injection.

3.3.2 Experimental setting

Study design

The Experimental setting study was conducted as a two-period, crossover design with duplicate injection of two different concentrations, 4 and 16 IU/mL, within each period and rhGH treatment given in-between. Genotropin® 33 µg/kg (0.1 IU/kg) (Pharmacia, Uppsala, Sweden) was administered by deep subcutaneous injection with 12 mm needles (BD-Microfine®) into the thigh by a nurse. Blood
samples were drawn from an intravenous cannula every 15 minutes during the hour before the injection at 09:00, and after the injection every 30 min for 6 hours, and thereafter at 8, 10, 12, 16, 20 and 24 hours (21 samples per GH curve).

**Study population**

This cohort included 15 patients (10 boys) diagnosed with GHD (11 with MPHD), who were assessed twice between 1987 and 1988. Four of the patients also participated later in the Clinical setting.

**GH curves**

Fifty-nine GH curves were obtained for the Experimental setting, as one was excluded due to a sampling error.

### 3.4 Ethics

The prospective trials, TRN 88-177 (IGHD) and TRN 88-080 (non-GHD), were approved by the ethics committees at the Universities of Gothenburg, Lund, Linköping, Uppsala and Umeå, and by the Karolinska Institute, and study TRN 87-164 (GH uptake) was approved by the ethics committee at the University of Gothenburg. Informed consent was obtained from the patients and their parents verbally (considered ethically acceptable at the time of initiation of the studies), with the registration date recorded in the patient’s medical file.

In the 1980s, the patients in the TRN 88-080 trial gave written informed consent to participation and to the use of their data for FDA registration of rhGH for patients with ISS (non-GHD).
4. Methods

4.1 Auxology

Data were collected from obstetric records and from child and health care centres from birth to study inclusion.

*Height* was measured according to a standard procedure using calibrated Harpenden stadiometers at the paediatric units at Swedish children’s hospitals. The mean of three measurements was used and converted into a SD score (SDS) from either the prepubertal childhood component (24) of the total growth reference or at adult height using the total growth reference at age 18 years (13).

*Birth length* and *Birth weight* were converted to SDS for newborns according to sex and gestational age (268).

*Parental height* was measured and converted to SDS at age 18 years (181).

Mid-parental height (MPH) was expressed as SDS (269). Difference between height$_{SDS}$ of the child and the corresponding MPH$_{SDS}$ was calculated and is expressed as diffH-MPH$_{SDS}$.

*Weight* was estimated using calibrated weighing scales, with the child in light underwear, and transformed to SDS (13).

*Body mass index* (BMI) was calculated and expressed as SDS (270).

4.2 Puberty

*Puberty development* was clinically assessed: Tanner breast stage for girls (28) and testicular volume for boys, using an orchidometer (212). Onset of spontaneous pubertal growth acceleration was defined as breast stage ≥B2 for girls or testicular volume ≥6 ml for boys (46).

4.3 Growth outcome

Growth outcome, as gain in height$_{SDS}$ and attained adult height$_{SDS}$ were used for evaluation of growth response. Figure 14.
**Figure 14. Principle for presentation of growth outcome variables**

*Pubertal gain in height*$_{SDS}$ was defined as adult height in cm converted to SDS for age 18 years (AH$_{SDS}$) minus last-recorded prepubertal height$_{SDS}$, the SDS being estimated from the childhood component of the total growth reference (13, 24).

*Prepubertal gain in height*$_{SDS}$ was defined as height$_{SDS}$ at the last prepubertal visit minus height$_{SDS}$ at the visit when GH treatment was started, using the childhood component of the total growth reference (13, 24).

*Total gain in height*$_{SDS}$ was calculated using AH$_{SDS}$ minus height$_{SDS}$ at GH start, using the prepubertal childhood component of the total growth reference (13, 24).

**4.4 Bone age**

Bone age from left carpal and wrist radiographs was assessed yearly during the studies by one radiologist according to the Tanner-Whitehouse II 20-bone method (271).

**4.5 Hormone measurements and evaluation**

Samples for hormone measurement were assayed at the Gothenburg Pediatric Growth Research Center (GP-GRC) laboratory (Swedac accredited no.1899) and at the laboratories in the respective university hospitals.
4.5.1 GH

GH\textsubscript{max} response to provocation tests: Maximal GH response (GH\textsubscript{max}) was assessed during an arginine–insulin tolerance test (AITT), performed by infusion of arginine and sequentially followed by an insulin injection (203). The maximal GH peak (GH\textsubscript{maxAITT}) with cut-off 20mU/L was used for the GHD diagnosis. GH serum concentrations from the AITT were measured in different clinical chemistry laboratories: until 1992 they were measured in all university hospitals with polyclonal antibodies calibrated with the WHO International Reference Preparation (IRP) 66/217 as standard, and for the entire study period at GPGRC with the immunoradiometric assay (IRMA) with polyclonal antibodies (Pharmacia Diagnostics AB, Uppsala, Sweden), also calibrated with IRP 66/217, but from 1992 with the IRP 80/505 as standard. From 1992, the clinical chemistry laboratories in all university hospitals in Sweden changed to the monoclonal antibody-based time-resolved immunofluorometric assay (trIFMA; Wallac, Turku, Finland), calibrated with IRP 80/505 as standard. Figure 15.

For comparison of results, the values were transformed to those measured with IRMA polyclonal assay with IRP 80/505 as standard (62).

![Figure 15](image)

**Figure 15.** Influence of the GH assays used for the estimated serum concentration of GH (red circle). The result from one sample estimating serum concentration of GH using two GH assays and two WHO IRPs: 66/127 and 80/505. The assays were an immunoradiometric assay (IRMA), using polyclonal (p) or monoclonal (m) antibodies and a time-resolved monoclonal immunofluorometric assay (trIFMA). Adapted with permission of C Jansson, Clin Chem 1997.

The spontaneous 24-hour GH secretion profile was estimated from integrated blood samples every 20 minutes, measured with IRMA using polyclonal antibodies calibrated against the WHO IRP 66/217, with detection limit 0.4 mU/L. Intra-assay coefficients of variation (CVs) were 7.1, 1.9 and 2.3% at
concentrations of 1, 5–20 and 30 mU/L, respectively. The interassay CVs were 14, 4, 4 and 7% at concentrations of 1, 21, 30 and 46 mU/L, respectively (62). For the data analyses presented here, the results were converted to IRP 80/505.

The Pulsar program (Merriam & Wachter), with algorithms to semi-quantify the GH pulse properties of the 24-hour GH profile (272), was used to identify the GH secretory peaks, amplitude and duration from a baseline using the assay SD as scaling factor. This gives the calculated baseline, number of peaks, amplitude and area under the curve (AUC) above the zero line and above the calculated baseline.

**GH uptake**: GH was analysed using a commercial IRMA with polyclonal antibodies (Pharmacia, Sweden) and IRP 66/217 in the *Experimental setting*. Interassay variation was 3.2 and 3.5% at GH levels of 2 and 100 mU/L, respectively, and intra-assay variation was 5.0 and 2.7% at GH levels of 10 and 40 mU/L, respectively. For comparison, results were converted to the IRP 80/505 standard (62), which was used in the *Clinical setting*. For both studies, a combined CV of 10% was used in further analyses.

### 4.5.2 IGF-I and IGFBP3

*Serum IGF-I* and IGFBP3 were measured using an IGFBP-blocked radioimmunoassay (RIA) with an excess of IGF-II and IGFBP3 by a specific RIA (Mediagnost GmbH, Tübingen, Germany).

For the *IGF-I* assay, the intra-assay CVs were 11.1, 7.2 and 7.4% at concentrations of 36, 204 and 545 µg/L, respectively; the interassay CVs for the same concentrations were 13.5, 8.8 and 9.9%, respectively.

For the *IGFBP3* assay, the intra-assay CVs were 7.1, 7.3 and 7.9% at concentrations of 1800, 3790 and 5776 µg/L, respectively; the interassay CVs for the same concentrations were 13.4, 10.5 and 14.1%, respectively.

Results were converted into SDS according to sex, pubertal stage and age, and the *ratioSDS* of IGF-I to IGFBP3 was calculated (162, 163). Figure 16.

The *on-treatment level of IGF-I*SDS, IGFBP3SDS and of the *IGF-I/IGFBP3 ratioSDS* were based on individual mean levels of the variables: the *total on-treatment level* will be the combination of the *prepubertal on-treatment level*, from one year after GH start until the last prepubertal observation, and the *pubertal on-treatment level*, from onset of puberty to last observation before GH stop.
The change (Δ) in these variables was defined as mean on-treatment level minus level either at GH start or at randomization (at or during puberty); a prepubertal Δ or a pubertal Δ, and the combination of these, a total Δ, was obtained. In addition, the 1st year pubertal ΔIGF-I, defined as mean value at 1st year after randomization minus level at randomization, was calculated. Figure 17.
4.5.3 GHBP

The growth hormone binding protein (GHBP) assay was a ligand-mediated immunofunctional assay (LIFA). The detection range was 15.6–1000 pmol/L. The intra-assay and interassay CVs were 7.3% and 11.3%, respectively (273).

4.6 Pharmacokinetics

Pharmacokinetics of GH in the serum curves were estimated by the time it takes, $T_{\text{max}}$ (h), to reach the maximum serum concentration, $C_{\text{max}}$ (mU/L). The AUC mU/L*h was calculated according to the lineal trapezoidal rule. Figure 18.

**Figure 18.** Pharmacokinetics of serum GH were estimated by the time it takes, $T_{\text{max}}$ (h), to reach the maximum serum concentration, $C_{\text{max}}$ (mU/L). The area under the curve (AUC) mU/L*h was calculated. GH baseline was defined as the individual pre-injection GH level.

*Time for GH level to return to pre-injection baseline:*  
A GH curve was considered to have returned to pre-injection baseline level when the measured GH concentration had fallen to within 10% (the combined intra-assay/interassay CV of the GH assay) of the value observed just prior to the GH injection.

*Time for GH level to decrease to zero level:*  
A GH curve was considered to have a level equal to zero if the measured GH level was within three times the detection limit of the assay (0.4 mU/L, i.e. 1.2 mU/L). $C_{\text{max}}$ was calculated both from the zero level ($C_{\text{max}}$) and from the pre-injection baseline ($C_{\text{maxadj}}$). AUC was also calculated from zero level (AUC) and from the pre-injection baseline until 16 hours after injection (AUC$_{\text{adj}}$), which was the longest time for sampling in the Clinical setting, while 24 hours (AUC$_{24\text{h}}$) was the
longest sampling time in the Experimental setting. A GH injection was given at home 16–24 hours before the visit injection.

4.7 Bioavailability

The cumulative amount of GH was estimated using the previously described algorithm: \( \text{AUC}_0 \times 0.066(k_{01}) \times 0.046(V_1) \times \text{kg} = \text{estimated uptake in mU (converted to Units)} \) (272). The result was compared with the injected dose (Units) (=100%) to give the bioavailability (%), Table 3. As the constants \( k_{01} \), based on elimination rate, and \( V_1 \), based on blood volume, have broad intra-individual variation, the highest (0.132) and the lowest (0.040) values obtained for the algorithm were used in the bioavailability calculations (marked in red in the table) alongside the means (marked in blue in the table) for the constants. In order to avoid underestimation or overestimation of bioavailability, the data from the two youngest individuals (marked with a black box in the table) with body composition and blood volume different from the population in the GH uptake study were excluded. Re-calculation of constants resulted in \( k_{01} 0.069 \) and \( V_1 0.040 \) and were also used in the algorithm.

### Table 3. Bioavailability

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Body Mass, kg</th>
<th>GH</th>
<th>( k_{01} ) min</th>
<th>( k_{10} ) min</th>
<th>( k_{02} ) min</th>
<th>( k_{11} ) min</th>
<th>( C_r )</th>
<th>( V_r )</th>
<th>%body mass</th>
<th>( k_{01} ) V_r</th>
<th>( k_{11} ) V_r</th>
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<td></td>
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<td>197±9</td>
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Means±SD

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<th>( k_{01} ) min</th>
<th>( k_{10} ) min</th>
<th>( k_{02} ) min</th>
<th>( k_{11} ) min</th>
<th>( C_r )</th>
<th>( V_r )</th>
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</thead>
<tbody>
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<td>Mean</td>
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<td>1.00</td>
<td>351±44</td>
<td>1.43</td>
<td>4.3</td>
<td>0.060</td>
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</tbody>
</table>

Values are means±SD. Preparations (prep) of GH: C, Crescormon; S, Somatonorm; G, Genotropin; inj, injection. See text for other abbreviations.


4.8 Statistical analyses

Paper I:
Statistical analyses were conducted by the statistician Björn Andersson PhD, in collaboration with research engineer Sten Rosberg PhD, and were performed
using the R software program (version 3.1.2, The R project, Vienna, Austria). All results are presented as median value, range or CV, if not otherwise stated.

**Analyses between groups:** Differences between groups were analysed with student’s t-test or a non-parametric Mann–Whitney U test where applicable. A $p<0.05$ was considered statistically significant.

The coefficient of variation (CV) was defined as the SD divided by the mean and expressed as a percentage. *Inter-individual CV* was calculated from the individual mean of all GH curves per individual, whereas *intra-individual CV* was calculated as the mean of the CV for each individual for whom two or more GH curves were available.

**GH peakwidth:** In order to describe the duration of the GH peak, an interpolated width at the GH value equal to half of the $C_{\text{max}}$ was estimated (i.e. full width at half maximum (FWHM) of the $GH_{\text{max}}$). Thus, two types of GH curves resulting in the same AUC could be discriminated: curves with a high, narrow peak, associated with intramuscular injections, and curves with a lower, broader peak, associated with subcutaneous injections. Figure 19.

![GH peakwidth diagram](image.png)

**Figure 19.** GH peakwidth: an interpolated width at the GH value equal to half of the $C_{\text{max}}$ was estimated (i.e. full width at half maximum (FWHM) of the $GH_{\text{max}}$). Two types of GH curves resulting in the same AUC could thereby be separated: curves with a high, narrow peak, associated with intramuscular injections, and curves with a lower, broader peak, associated with subcutaneous injections.

**GHBP effect on elimination rate:** The GH elimination rate was calculated as the slope of the GH value at $T_{\text{max}}$ and at the two following time-points (after 2 hours and 4 hours).
Multivariable regression analyses: Tenfold cross-validated stepwise multivariable regressions (both forward and backward) were performed to explain the variance in $C_{\text{max}}$, AUC and bioavailability, using bootstrapping in the R software program. The following variables were included in the models:

- **patient characteristics**: gestational age (weeks), sex, birth-length$_{SDS}$, birth-weight$_{SDS}$, age, height$_{SDS}$, weight$_{SDS}$, $GH_{\text{max}}$AITT and the dichotomous variable puberty (yes/no);
- **information on GH injection**: GH dose ($\mu$g/kg) and GH concentration (IU/ml);
- **proxy estimates of injection depth**: GH level at baseline, $GH_{\text{peak}}$width and BMI$_{SDS}$; and
- **estimates of endogenous GH secretion**: $GH_{\text{max}}$24h and the dichotomous variables $GH$ peak/$GH$ no peak.

For the stepwise multivariable regression, the threshold for inclusion of each variable was set to $p=0.1$ and the models with the best Akaike information criterion were selected in order to avoid overfitting.

Paper II–IV:
Statistical analyses were conducted by statistician Björn Jonsson PhD. Analyses were performed using the standard statistical package SPSS version 20 (IBM Corp., Armonk, NY, USA). Results are expressed as mean ±SD unless otherwise specified. Analyses concerning primary and secondary outcome variables were performed using non-parametric tests of the Wilcoxon type (Wilcoxon signed-rank test for within-group comparisons and Mann–Whitney U test for between-group comparisons). Safety analyses included all patients who received the study drug. Analyses were performed for the entire ITT population and for boys only in the PP population. Results were considered statistically significant at $p<0.05$.

Simple bivariate correlation analyses were performed using Pearson’s r.

Stepwise multivariable regression analysis was used to analyse the influence of IGF-I variables on gain in height$_{SDS}$ and AH$_{SDS}$. Data on birth characteristics and growth until the age of 3 years, as well as characteristics at GH start and during prepubertal growth, were added as predictors in the analysis. Only variables entering the regressions below the significance level $p<0.05$ were used. No correction was performed for multiplicity. Stepwise multivariable forward regression analyses were performed with $p<0.05$ as the entering criterion for predictors and $p<0.10$ for exclusion after inclusion in an earlier step.
5. Results and comments

5.1 Aim 1. Uptake of injected rhGH: pharmacokinetics and bioavailability (Paper I)

Rationale
GH treatment with daily subcutaneous rhGH injections is a regimen used worldwide but there is a lack of longitudinal data on rhGH uptake with this regimen. In Paper I, the uptake of injected rhGH was investigated in two settings regarding pharmacokinetics, bioavailability and factors influencing variability: firstly, as ‘real world data’ in the Clinical setting, in which patients injected GH themselves in the evening as they usually do at home, and secondly in the Experimental setting, in which a professional gave the injections.

5.1.1 Pharmacokinetics
In the Clinical setting, 429 GH curves were obtained from 117 patients with a median C\textsubscript{max} value of 71 mU/L and a median AUC of 534 mU/L*h. Inter-individual variability was expressed as CV% and was found to be broad: 44% for C\textsubscript{max} and 42% for AUC. For those patients who had GH curves measured 2–8 times (n=108), a high degree of intra-individual variability (also expressed as CV%) was found: 39% for C\textsubscript{max} and 36% for AUC. All GH curves are shown in Figure 20, left panel.

In the Experimental setting based on the 59 GH curves from 15 patients, we found a median T\textsubscript{max} of 3 hours, C\textsubscript{max} of 47 mU/L and AUC of 509 mU/L*h, while the AUC\textsubscript{24h} was 685 mU/L*h. Inter-individual variability was 33% for T\textsubscript{max}, 38% for C\textsubscript{max}, 25% for AUC and 24% for AUC\textsubscript{24h}, while intra-individual variability was 22% for T\textsubscript{max}, 36% for C\textsubscript{max}, 28% for AUC and 28% for AUC\textsubscript{24h}. All GH curves are shown in Figure 20, right panel.

Factors influencing the variability in pharmacokinetics

Dosing of rhGH:
The rhGH dose significantly influenced inter- but not intra-individual variability for both C\textsubscript{max} and AUC. There was a dose-dependent positive correlation between GH\textsuperscript{33} and GH\textsuperscript{67} for median C\textsubscript{max}: 63 mU/L (CV 31%) for GH\textsuperscript{33} vs 103 (CV 32%) for GH\textsuperscript{67}, p<0.001, see Figure 21, and the same relationship held for AUC: 464 mU/L*h (CV 29%) for GH\textsuperscript{33} vs 865 (CV 29%) for GH\textsuperscript{67}, p<0.001.
**Figure 20.** Variability in GH curves *Left panel:* Variability in the 429 GH curves obtained in the Clinical setting, using GH doses 33 or 67 µg/kg/day. *Right panel:* Variability in the 59 GH curves obtained in the Experimental setting, using the GH dose 33 µg/kg/day.

**Figure 21.** GH serum concentration from the Clinical setting. The median GH curve from 288 GH curves for 87 patients treated with GH dose 33µg/kg, 63 mU/L (CV 31%) (broken line) and median GH curve of 106 GH curves from 47 patients, GH dose 67µg/kg 103 mU/L (CV 32%) (solid line).

**Concentration of injected rhGH:**
GH-concentration dependency was studied by comparison of rhGH injected with the same dose, GH\textsuperscript{33}, but a concentration of 4 or 16 IU/ml in a double-crossover
design. AUC was positively correlated with GH concentration: AUC was 456 mU/L*h (CV 24%) for 4 IU/ml vs 558 (CV 30%) for 16 IU/ml, p=0.025, and AUC_{adj} was 442 mU/L*h (CV 25%) for 4 IU/ml vs 544 (CV 32%) for 16 IU/ml, p=0.009. Thus, a higher concentration of injected GH using the same dose results in a larger AUC.

**Injection depth:**
In multivariate regression analysis, the variables GH_{peakwidth}, BMI_{SDS} and GH level at injection, which we consider as proxy variables for injection depth, explained 17% of the variability in C_{max} and 7% of the variability in AUC.

For C_{max}, 43% of the overall variability could be explained mainly by the combination of rhGH dose (24%) and proxy for injection depth (17%) and, to a lesser extent, by age and presence/absence of puberty (~1% each). For AUC, 43% of the variability could be explained by the GH dose (35%), proxy for injection depth (7%) and presence/absence of puberty (1%), but still most of the variability could not be explained.

When comparing the GH curve variable GH_{peakwidth} for the group injected by professionals (the *Experimental setting*) with GH_{peakwidth} for the self-injecting group (the *Clinical setting*), a higher proportion of GH curves of the intramuscular type was found in the group injected by professionals. The cut-off for the lowest GH_{peakwidth} tertile was 4.4 hours, compared to 5.7 hours in the self-injecting group, p= 0.021.

**Duration of injected GH level to baseline:**
In the *Experimental setting*, in which all patients were diagnosed with complete GHD, around 70% of GH curves had decreased to the pre-injection baseline level after 16 hours, Figure 22 left panel, with a similar result, 66%, in the *Clinical setting*, including patients who had a broad range of endogenous GH secretion, Figure 22, left panel.

**Duration of GH level in serum to zero line:**
After 24 hours, 85% of the GH curves in the *Experimental setting* no longer had detectable serum GH, Figure 22 right panel, while the corresponding proportion for the *Clinical setting* was 41%, Figure 22, right panel.

**Endogenous GH secretion:**
In the *Clinical setting*, no significant correlations were found between either GH_{max}^{24h} or the dichotomy grouping GH_{peak} / GH_{nopeak} and any pharmacokinetic variable. Spontaneous GH peaks were observed in 115 out of 429 GH curves (37%); the majority of peaks occurred within 6–8 hours of rhGH injection: 41 at
6 hours, 32 at 8 hours, 35 at 10 hours, 24 at 12 hours, and 4 at 14 hours. Repeated peaks were apparent in 18 curves.

Figure 22. Proportion of GH curves with GH levels that returned to baseline (left panel) and to zero level (right panel) after subcutaneous injection. Results for GH\(^{33}\) (open symbols) and GH\(^{67}\) (filled symbols) are shown for the Experimental setting (squares) and the Clinical setting with hospital injection (triangles) and with home injection the day before (circles).

Left panel: In the Experimental setting, GH had returned to baseline levels in 71\% of GH curves (□) by 24 hours after injection; in the Clinical setting, GH had returned to baseline levels in 72\% of GH\(^{33}\) curves (Δ) and in 58\% of GH\(^{67}\) curves (▲) by 16 hours after the injection (p= 0.069).

Right panel: In the Experimental setting, GH had reached zero level in 39\% (23/59) of curves (□) at 16 hours post-injection, and 85\% (50/59) of curves by 24 hours; in the Clinical setting, GH had reached zero level in 25\% (71/288) of GH\(^{33}\) curves (Δ) and 11\% (8/79) of GH\(^{67}\) curves (▲) at 16 hours. Approximately 24 hours after the injection given at home the day before, GH levels in 43\% of GH\(^{33}\) curves (○) and 33\% of GH\(^{67}\) curves (●) were undetectable or had reached zero level.

5.1.2 Comments

The spontaneous GH peak compared to the GH treatment \(C_{\text{max}}\)

The amplitude of the spontaneous GH peak was important for spontaneous growth in children (68, 77), as shown in studies of \(C_{\text{max}}\) in GH-treated hypopituitary rodents (90, 274). During GH treatment, the exogenous \(C_{\text{max}}\) can be regarded as corresponding to the spontaneous GH peak. In our study, \(C_{\text{max}}\) was significantly dose dependent: doubling the rhGH dose gave a doubling of median \(C_{\text{max}}\) while the variability was within the same broad range in both dose groups.
The GH uptake, and thereby the C\(_{\text{max}}\), was dependent on whether the injection was given into the muscle or into the subcutaneous fat, i.e. resorption through capillaries or via the lymphatic system (256). By constructing the variable GH\(_{\text{peakwidth}}\) it was possible to separate two main types of GH curve: the low, broad subcutaneous type and the high and narrow intramuscular type. Thus, a deep injection resulted in a higher C\(_{\text{max}}\) and an improved uptake of the given rhGH dose, and possibly resulted in a greater growth response.

**Inter- and intra-individual variability in pharmacokinetics**

Paper I presents for the first time a finding of a broad inter- as well as intra-individual variability in maximal GH levels (C\(_{\text{max}}\)) of over 100% in such a large group of rhGH-treated patients. The variability was mainly explained by the GH dose and by the proxy for injection depth, and both factors can be influenced by the child, parents and medical team in order to maximize the treatment effect.

**The long GH peak duration and the GH trough**

The proportion of GH curves that had decreased to zero level, with GH no longer detectable in serum at 24 hours, was much higher where a professional gave the injections. The prolonged measurable GH in serum estimated by the GH assay with polyclonal antibodies in our study may reflect endogenously secreted isoforms being retained longer in the circulation. This is supported by the observation of spontaneous peaks after only 6 hours in some GH curves, whereas GH curves from patients with MPHD (i.e. severe GHD) mainly reached the zero line with GH undetectable in serum before the next injection. We interpret the spontaneous GH peaks as early as 6 hours after GH injection as evidence that the negative feedback of the exogenous GH on the endogenous secretion from the pituitary ended after 6 hours in patients who presented with signs of spontaneous endogenous secretion. Therefore we could not support the earlier finding that exogenous injected GH downregulate the endogenous GH secretion for as long as 36 hours (275).

The pulsatile pattern of GH secretion promotes growth in humans and rodents (77, 274). In adult male rats, GH is secreted with low troughs in between the high-amplitude peaks occurring every 3 hours. GH secretion in female rats has an almost continuous level, obtained from low irregular peaks and a high baseline. A high baseline was found to be important for modulation of hepatic steroid metabolism (90, 276) and for the sexually dimorphic pattern of growth, with males becoming longer (89). Although results from rodents cannot be directly applied to humans, in adults the trough has been reported to be related to the metabolic effects of GH, reflected by greater increase in IGF-I and IGFBP3 during continuous infusion compared to daily injections (277).
5.1.3 Bioavailability

The median bioavailability of injected rhGH was 71% in the self-injecting group and 85% when a professional technique for deep injection was used; this was not dose dependent. Great inter-individual (CV 32%) and intra-individual (CV 31%) variation was found with deep injection, with no dose effect, p=0.18. However, a positive rhGH-concentration dependency was found when comparing 4 and 16 IU/ml: median bioavailability was 73% for 4 IU/ml and 88% for 16 IU/ml, p=0.035.

Variation in bioavailability

In the multivariate analyses, 22% of the variation in bioavailability could be explained, mainly by the proxy for injection depth (17%) and to a lesser extent by gestational age, puberty and GH dose (~2% each).

Comparison of bioavailable rhGH from the rhGH injection and estimated endogenous GH secretion rate in healthy children

The median bioavailable rhGH was around 70% of the injected dose. The amount was comparable to the endogenous GH secretion rates (U/24h) previously found in healthy children, which has been estimated to be 1–12 U/24h in the prepubertal period, then 1–20 U/24h in boys, and 3–30 U/24h in girls throughout puberty (272). The daily rhGH treatment doses used in Paper I, 33 and 67 µg/kg, gave 5 and 10 U/24h, respectively, and with a bioavailability of 70% this gave 3.25 and 7.5 U/24h, respectively. These amounts correspond to GH secretion rates in the lower range of those found in healthy children (272). Figure 23.

![Figure 23](image)

Figure 23. Bioavailability of injected GH around 70% vs GH secretion rate in healthy children. GH-dosing consequences: GH33 in a prepubertal 25 kg child (shown in green) gives 2.5 U/24h (or, if 70% was used as a median of the normal bioavailability, 70%=1.75 U/24h). GH67 in a pubertal 50 kg child (shown in blue) gives 10 U/24h (70%=7 U/24h). Modified with permission of Albertsson-Wikland, Am J Phys 1989.
5.1.4 Comments

Bioavailability estimation

The mean bioavailability of injected GH of 70% and 85% in our two studies (or 60% and 75% when excluding the two youngest patients from the algorithm) were similar to earlier reports, which estimated bioavailability to be 60–70% in both children (249) and adults (247, 278). Their method for estimation of bioavailability was different from ours, as they compared AUC after an intravenous and a subcutaneous injection.

Moreover, reduced bioavailability found for the subcutaneous injection compared to intravenous injection, which has been suggested to be due to uptake through the lymphatic system (256).

Variation in bioavailability

Our large study included many variables, all with a broad range of values, yet only 22% of the variation in bioavailability could be explained. The main contribution was from the proxy variables for injection depth, which is why injection depth was interpreted as the main reason for this difference in bioavailability. Using the optimal injection technique and repeated training to obtain a deep injection are thus key factors for optimal GH uptake.

Influence of rhGH concentration

A positive rhGH concentration dependency stands in contrast to previous studies in adults (279). Our study population included many growing pubertal individuals with increasing weight, who need to be treated with higher rhGH doses, usually given in higher concentration. The higher concentration resulted in lower volume, which may have given less local tissue damage and thereby an improved uptake of the injected rhGH. Compared to the results from previous reports, our study included twice the number of observations and was performed in a crossover design, which made our results more reliable and accurate than those from previous studies.
Summary
The highest $C_{\text{max}}$, considered to be the signal for growth response, was observed after a deep injection when a higher dose and rhGH concentration was used.

The rhGH doses had a bioavailability of approximately 70% with a wide variation irrespective of the injected dose. As a result, the bioavailable amount of GH with the 33 µg/kg dose corresponded to the lower range of GH secretion in healthy prepubertal children and, with the 67 µg/kg dose, corresponded to the low range in healthy pubertal children.

Thus, the range of rhGH doses used in these trials from the mid 1980s, still used as the treatment doses during both prepuberty and puberty periods, correspond to the lower range of the spontaneous GH secretion rate in healthy children.

5.2 Aim 2. Study on growth response to GH treatment (Paper II)
Rationale
Growth response in the group of patients with IGHD was estimated by the gain in heightSDS from start of GH treatment in prepuberty, through the prepubertal period and during the pubertal growth period until adult height, estimated as total gain in heightSDS during the GH treatment period and attained adult heightSDS (Paper II). The question was whether a GH dose of 33µg/kg was enough or whether an increased GH dose of 67µg/kg, given once or divided into two daily injections, was needed in order to normalize or improve pubertal gain in height and adult height. Preliminary data from the trial population that at that time had reached adult height was published in 1999, including not only IGHD patients but also a subgroup diagnosed with MPHHD (280). Paper II reports an analysis of data exclusively from the IGHD group.

Patients diagnosed as non-GHD were included in another randomized trial investigating whether it was possible to obtain a GH effect, in terms of both gain in height to adult height and improved adult height, by using two different doses of rhGH compared to untreated controls (258). In this thesis, the results from the GH-treated groups in this non-GHD trial were compared with results from the GH-treated IGHD group of the GHD trial. Patients in the non-GHD trial were randomized to GH$^{33}$ (n=43) and to GH$^{67}$ (n=62) and started treatment shortly before puberty onset, which was the reason why it was not possible to distinguish prepubertal from pubertal gain in heightSDS in the entire cohort, and why mainly
the total gain in height_{SDS} will be presented. Nonetheless, 44 patients were treated with GH for at least 1 year before puberty, of whom 17 were randomized to GH^{33} and 27 were randomized to GH^{67}. Sixteen patients, who entered puberty during the pre-study, were randomized only to GH^{67}. The remaining 45 patients were randomized within a year from onset of puberty.

In both trials, approximately 30% of the included patients, both IGHD and non-GHD, were born SGA (183, 268). As the proportion of girls included in the study was only 10%, the results will mainly be presented for boys. The results are presented from the GH-treated ITT population, unless otherwise specified.

5.2.1 Patients with IGHD

Growth response as prepubertal gain in height_{SDS}

At GH start, mean age was 9.9±3.2 years and all patients were pre-pubertal. They all received the same dose, GH^{33}, and 1st year mean gain in height_{SDS} was 1.1±0.63 with no difference between those who later were randomized into different dose groups during the pubertal growth period. Figure 24, left panel.

Growth response as pubertal gain in height_{SDS}

Directly after onset of puberty, patients were randomized to three dose groups: GH^{33} (33 µg/kg once daily), GH^{67x1} (67 µg/kg once daily), or the higher dose divided into two injections per day, GH^{33x2}. Both dose groups gained in height during their pubertal growth. Mean pubertal gain in height_{SDS} for the groups randomized to the higher GH doses, was 0.7±0.87 for GH^{67x1} and 0.7±0.77 for GH^{33x2}, ns; therefore, these groups were combined into one high-dose group, GH^{67}. When comparing dose effect, doubling the GH dose doubled the mean pubertal gain in height_{SDS}; for GH^{33} it was 0.4±0.6 (range -0.6 to 1.5) and for GH^{67} it was 0.7±0.8 (range -0.4 to 2.3), p<0.01. Figure 24, left panel.

Growth response as total gain in height_{SDS}

The mean total gain in height_{SDS} for those who were randomized to continue on GH^{33} throughout puberty was 1.6±0.8 (range 0.2 to 2.8), and it was greater in patients randomized to GH^{67} during puberty, 2.0±1.0 (range 1.2 to 3.5), p<0.01. Figure 24, left panel.

Adult height

Adult height_{SDS} was higher in the dose group GH^{67}, -0.8±0.9 (range -2.5 to 1.1) than in the dose group GH^{33}, -1.2±0.7 (range -2.3 to 0.8), p<0.05. Figure 24, left panel. The difference to mid-parental height_{SDS}, (diffH-MPH_{SDS}) was -0.2 for both
dose groups. Overall, 89% of the patients reached an adult height SDS within the reference population range 0±2.0.

**Factors explaining variability in growth response**

For **pubertal gain in height SDS**, 53% of the variability was explained by (in order of importance) bone age delay at study start, number of pubertal years on GH treatment, a higher age at study start, higher GH dose, a higher birth weight and a taller father.

For **total gain in height SDS**, 67% of the variability was explained by pubertal gain in height SDS, bone age delay at GH start, a higher birth weight, a higher GH dose, a greater difference to parental height at birth and higher age at GH start.

For both these analyses, only the IGF-I variable ‘at GH start’ was available, but it did not contribute to the explanation. At this point no on-treatment IGF-I variables were made available.

### 5.2.2 Non-GHD patients

GH treatment was started near the onset of puberty in non-GHD patients and significantly increased the total gain in height SDS and also the attained adult height SDS in a dose-dependent manner (258).

**Growth response as prepubertal gain in height SDS**

The 1st year prepubertal gain in height SDS was available in 44 GH-treated patients, mean age 11.3±1.4 at GH start. Mean first year gain in height SDS was 0.53±0.20 for the GH33 group (n=17) vs 0.76±0.22 for the GH67 group (n=27), p<0.001.

**Growth response as total gain in height SDS**

The total gain in height SDS was dose-dependent, with a mean of 0.9±0.81 (range -0.7 to 2.8) for GH33 vs 1.3±0.82 (range -0.9 to 2.7) for GH67, p<0.007. Figure 24, right panel.

**Adult height**

Adult height SDS was significantly increased in those patients who were randomized to the higher dose: the mean was -2.0±0.8 for GH33 vs -1.5±0.8 for GH67, p<0.008. Figure 24, right panel. A broad range in attained adult height SDS was found: -2.8 to 0.3 for GH33 and -4.0 to 0.7 for GH67.

At adult height, diffH-MPH SDS was -0.3±0.96 for GH33 vs 0.2±1.05 for GH67, p<0.005.
Figure 24. Growth response for children in the ITT population, included in the studies on IGHD (left panel) and non-GHD (right panel) according to GH dose groups: GH $33\mu$g/kg/day (GH$^{33}$), blue dots, and GH $67\mu$g/kg/day (GH$^{67}$), red dots. All heights are expressed as SDS. For the IGHD group, height SDS is shown at GH start, at study start (randomization) and at adult height. The prepubertal gain in height SDS after the first year of treatment, as well as pubertal and total gain in height SDS are shown as Δ for each period. For the non-GHD group, height SDS is shown at study start (start of GH treatment and randomization), at first treatment year for the 44 children who were still prepubertal and at adult height. The prepubertal and total gain in height SDS are shown as Δ for each period.

Factors explaining the variability in growth response

For total gain in height SDS, 47% of the variability was explained by (in order of importance) bone age delay at randomization or GH start, higher GH dose, diffH-MPH SDS lower at randomization and diffH-MPH SDS greater at birth. Only the IGF-1 SDS variable ‘at GH start’ was available for the analysis; this variable contributed to the explanation in the ITT population but not in the PP population. No on-treatment IGF-I variables were made available for these analyses.

5.2.3 Comparison between growth response in the IGHD and non-GHD groups

Both diagnostic groups gained in height SDS during puberty. The total gain in height SDS was greater in IGHD than non-GHD patients, $p=0.015$, and it was also greater for those randomized to the high dose, GH$^{67}$, than for those receiving the standard dose, GH$^{33}$, in both diagnostic groups, Table 4.
Growth response in the IGHD and the non-GHD groups is shown as prepubertal, pubertal and total gain in heightSDS, diffH-MPHSDS, adult heightsSDS and age at GH start or randomization.

**Table 4. Growth response in the IGHD and the non-GHD groups**

<table>
<thead>
<tr>
<th>GH dose</th>
<th>IGHD</th>
<th></th>
<th></th>
<th>non-GHD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GH&lt;sup&gt;33&lt;/sup&gt;</td>
<td>GH&lt;sup&gt;67&lt;/sup&gt;</td>
<td>p</td>
<td>GH&lt;sup&gt;33&lt;/sup&gt;</td>
<td>GH&lt;sup&gt;67&lt;/sup&gt;</td>
<td>p</td>
</tr>
<tr>
<td>Adult heightSDS</td>
<td>-1.2±0.67</td>
<td>-0.8±0.9</td>
<td>0.05</td>
<td>-2.0±0.85</td>
<td>-1.6±0.89</td>
<td>0.008</td>
</tr>
<tr>
<td>diffH-MPHSDS</td>
<td>0±0.79</td>
<td>0.2±1.02</td>
<td></td>
<td>-0.3±0.96</td>
<td>0.2±1.05</td>
<td>0.005</td>
</tr>
<tr>
<td>Total gain in heightSDS</td>
<td>1.5±0.82</td>
<td>2.0±1.00</td>
<td>0.01</td>
<td>0.9±0.91</td>
<td>1.3±0.82</td>
<td>0.007</td>
</tr>
<tr>
<td>Pubertal gain in heightSDS</td>
<td>0.4±0.59</td>
<td>0.7±0.81</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal gain in heightSDS</td>
<td>1.2±0.82</td>
<td>1.3±1.02</td>
<td></td>
<td>0.5±0.20</td>
<td>0.7±0.22</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Differences in prepubertal characteristics: IGHD patients were younger at GH start than those with non-GHD, mean age 9.9 years and 11.5 years, respectively; they were treated with GH for a longer time before puberty and had their main height gain before puberty. The total growth response was greater in the IGHD patients than the non-GHD patients in both GH dose groups.

Comparing only boys, prepubertal gain in heightSDS was 0.5 for the non-GHD boys in the GH<sup>33</sup> group and 1.3 for IGHD boys on GH<sup>33</sup>, p<0.0001. Pubertal gain in heightSDS for boys was greater for those in the non-GHD group than for those in the IGHD group, p<0.003, Figure 25 (PP population).

Adult heightSDS was greater in patients randomized to the GH<sup>67</sup> dose compared to the standard dose, GH<sup>33</sup>, in both diagnostic groups, Table 4.

A broad range of gain in heightSDS was observed in both diagnostic groups and in both dose groups: the range for the dose GH<sup>67</sup> was 1.2 to 3.5 in the IGHD group and -0.2 to 2.7 in the non-GHD group, while for the dose GH<sup>33</sup> the range was 0.3 to 2.8 in the IGHD group and -0.7 to 2.8 in the non-GHD boys, Figure 25.

The factors found to explain the variation in total gain in heightSDS were the same in both diagnostic groups: GH dose, diffH-MPHSDS, size at birth and bone age; however, for the IGHD group, prepubertal gain and age at GH start were more informative as the range was greater.
Figure 25. Gain in height\textsubscript{SDS} for boys in the PP population in the non-GHD and IGHD groups according to randomization GH dose. Left panel: prepubertal gain in height\textsubscript{SDS}. Centre panel: Pubertal gain in height\textsubscript{SDS}. Right panel: Total gain in height\textsubscript{SDS}. Pubertal gain in height\textsubscript{SDS} was lower in IGHD boys than in non-GHD boys, $p<0.003$, and greater for the GH\textsuperscript{67} dose group than for the GH\textsuperscript{33} group in both IGHD and non-GHD boys, $p<0.010$. However, the IGHD boys had already gained substantially more in height before the onset of puberty compared to the non-GHD boys, $p<0.0001$.

5.2.4 Comments

Paper II presented results from the largest randomized trial on GH treatment in patients with GHD followed until adult height. In addition, this trial was the first dose-response trial during pubertal growth in a group of patients exclusively diagnosed with IGHD. Pubertal gain in height\textsubscript{SDS}, total gain in height\textsubscript{SDS} and attained adult height\textsubscript{SDS} were greater for those patients randomized to a dose of 67 µg/kg/day than to those receiving 33 µg/kg/day during their pubertal growth.

Within the high dose group, GH\textsuperscript{67}, there was no significant difference between growth responses for the injection regimens GH\textsuperscript{67x1} and GH\textsuperscript{33x2}. These results differed from the preliminary report from this trial, where a greater pubertal gain in height\textsubscript{SDS} was found in the GH\textsuperscript{33x2} than in the GH\textsuperscript{67x1} group, 1.3 vs 0.7, respectively (280). This discrepancy may be explained by the difference in study populations, because patients with MPHD were included together with the IGHD patients in the preliminary report.

In the present analysis exclusively on the IGHD group, we found that a higher GH dose during puberty had a positive effect on growth response irrespective of whether one or two doses per day were given. Theoretically, an increased GH
injection frequency could give a pattern with two separated GH peaks, one in the morning and one in the night, with low troughs in-between, i.e. closer to the physiological pubertal GH secretory pattern with high peaks both day and night (78). However, the problem with twice daily injections was that, instead of two distinct pulses, a constantly elevated level could be obtained. We observed the mean value of growth response to be similar for GH\textsuperscript{67x1} and GH\textsuperscript{33x2}, but the variation in growth response was wider for GH\textsuperscript{33x2}, possibly explained by differences in the serum GH-curve profiles in the twice-daily injection group (280).

The GH dose-response effect as pubertal gain in height was underestimated in the IGHD group because many patients stopped GH treatment prematurely or decreased the GH dose from the randomized one long before adult height was reached, when they had attained a height they themselves were satisfied with. In the non-GHD group, the growth response could be either underestimated for the same reason (premature stop of GH treatment) or overestimated because of a catch-up component from the 1\textsuperscript{st} year response included in their total or pubertal gain in height.

An inverse relationship for boys in the IGHD group was observed between prepubertal and pubertal gain in height. The greater the growth response during the prepubertal period, the lower the growth response during puberty. This may partly be explained by the age at GH start in relation to onset of puberty, thereby leading to catch-up growth either during the prepubertal period or during puberty. This is supported by the bone age variable being such an informative variable for growth response.

There are only two previously published randomized studies that followed GHD patients treated with different GH doses during puberty. Our result of a significant GH dose-dependent pubertal gain in height\textsubscript{SDS}, total gain in height\textsubscript{SDS} and increased adult height\textsubscript{SDS}, is the only study exclusively in patients with a diagnosis of IGHD. Mauras et al. reported results from patients with GH and thyroid-stimulating hormone (TSH) deficiency who were randomized at onset of puberty to 43 vs 100 µg/kg/day and followed for at least 3 years (PP population n=48) (240). The pubertal height gain, estimated at adult height, was measured for only a subset. The mean gain was 4.6 cm (range 2.6–6.5), and greater in the high-dose group. Of 75 patients who met the criterion for attaining near-adult height, the date of their last measured height or bone age was extrapolated to near-adult height in 41 patients and measured in 34, giving a less reliable result.

The other previously reported randomized study found a less pronounced GH dose effect on growth response (281). That study included 35 patients diagnosed with IGHD or MPHD who were randomized to 25 or 50 µg/kg/day and followed
from prepuberty to adult height. A 2cm greater gain in height was found in the high-dose group but, possibly due to the small number of participants in each dose group, the result was non-significant.

**Growth response in non-GHD patients**

It has already been established that growth rate increases during first year or two of GH treatment in non-GHD patients (73, 219, 282). However, the majority of studies of GH treatment with non-GHD patients indicated only a modest increment in mean adult height (283-285).

A systematic review to determine the impact of GH therapy on adult height in non-GHD patients with short stature has been published (286). The trials were assessed as having a high standard if designed as randomized controlled trials which allow decision-makers to draw causal inferences linking interventions to outcomes, with protection against bias. Only two randomized clinical trials on non-GHD patients were found to be of a sufficiently high standard for comparison (241, 258). Patients from non-GHD trial (258) achieved a greater mean adult height_{SDS}, -1.6 compared to untreated controls -2.2; and gained more (0.86 SDS) than controls, mean 1.24 vs 0.40, respectively. This was in line with the result of the other randomized, placebo-controlled trial, which treated prepubertal patients with 0.22 mg/kg/week (31 µg/kg/day) or placebo, divided into three doses per week(241) known to result in a 30% reduction in growth response (236, 237). Leshek et al. found an average adult height_{SDS} of -1.7, compared to -2.3 in untreated patients, and with a mean gain in height_{SDS} of 0.5 over the placebo group. A high proportion of patients did not complete the study and thus had missing adult height measurements, 40% of the GH-treated group and 65% of the placebo group. The variable last observed height_{SDS} was used instead of measured adult height, which is less reliable and could give an overestimated result.

The non-GHD trial from Albertsson-Wikland group (258) is still the only randomized, controlled, dose-response study on this diagnostic group followed until adult height; thus, there are no relevant studies available for comparison (286). However, a report on two joint studies on prepubertal non-GHD patients followed to adult height and compared with historical untreated controls is used for comparison (287). A dose-dependent effect on gain in height_{SDS} and adult height_{SDS} was found, as well as an effect of the dose regimen. Most efficacious was a high dose administered right from the start of GH treatment, showing that the major impact of GH on adult height occurred in the first year (287).

In our IGHD group, one of the inclusion criteria for the pubertal trial was that the child should have had a 50% or more increase in growth velocity the first year on GH treatment, representing satisfactory GH responsiveness. Despite this, some
patients had low pubertal gain or none at all, Figure 25, centre panel, resulting in a limited total height gain, if any, at adult height, Figure 25, right panel. However, it can be claimed that we do not know how their pubertal growth would have been without GH treatment. In the non-GHD patients, only five altogether (three in the GH$^{33}$ dose group and two in the GH$^{67}$ group) had a total gain in heights$^{\text{SDS}}$ below zero, i.e. no response, with some additional patients just above zero. The control group showed great variation in $\Delta$ height$^{\text{SDS}}$, indicating that pubertal growth is also highly individual in a non-GHD group, Figure 26.

![Figure 26. Final outcome of the individuals in non-GHD (PP population), expressed as gain in heights$^{\text{SDS}}$ from study start to adult height. Each dot represents one patient. Adapted with permission of Albertsson-Wikland JCEM 2008.](image)

The magnitude of growth response was greater for IGHD patients than for non-GHD patients. A broad range of GH effect as gain in height was found in both GH dose groups in both trials. This highlights the need for individualized GH dosing during puberty, as was previously shown to be possible in prepubertal patients receiving a GH dose according to individually estimated GH responsiveness (211). These results demonstrate the feasibility of improved gain in height even during the pubertal period; however, higher GH dosage will be needed for most individuals.
**Summary**

An increased GH dose was shown to have a clear impact on gain in height during the pubertal growth period. This GH effect was observed both in patients with IGHD and those with non-GHD. The height gain was greater for those randomized to our high GH dose, GH\textsuperscript{67}, than those given the low dose, GH\textsuperscript{33}, and it was greater in the IGHD group than in the non-GHD group.

The range in prepubertal, pubertal and total gain in height\textsubscript{SDS} was great in both diagnostic groups and both GH dose groups, indicating a broad range in GH responsiveness. This treatment regimen may be considered in clinical practice for children who are diagnosed late or remain short in relation to the population or to their parents at onset of puberty.

### 5.3 Aim 3. Study on IGF-I and IGFBP3 response to GH treatment (Paper III & IV)

**Rationale**

The question in this study was whether, in patients receiving a higher and more physiological GH dose during puberty, the IGF-I, IGFBP3 and IGF-I/IGFBP3 ratio, as a proxy for free IGF-I, would follow the concentrations in serum during puberty in healthy children. Response to GH was estimated by changes (Δ) and attained treatment levels of IGF-I\textsubscript{SDS}, IGFBP3\textsubscript{SDS} and IGF-I/IGFBP3 ratio\textsubscript{SDS}, both during the prepubertal period and puberty. IGF-I\textsubscript{SDS} response for IGHD patients (Paper III) was compared to IGF-I\textsubscript{SDS} response in the non-GHD patients (Paper IV). As the proportion of girls included in both studies was only 10%, the results presented here will mainly be based on boys only. The results will also mainly be presented for the GH-treated ITT population, unless otherwise specified.

#### 5.3.1 IGHD patients

**GH response as change in IGF-I\textsubscript{SDS}**

**Prepubertal IGF-I:**

At GH start, the mean serum concentration for IGF-I\textsubscript{SDS} was -1.2±1.6. During the prepubertal period, when all patients received GH\textsuperscript{33}, the observed mean prepubertal ΔIGF-I\textsubscript{SDS} (from GH start in prepuberty until 1 year on GH\textsuperscript{33} treatment) was 2.1±1.48 and the mean prepubertal on-treatment IGF-I\textsubscript{SDS} level was 0.9±1.7, Figure 27, left panel.
**Pubertal IGF-I:**

After randomization, directly after puberty onset, the mean 1st year pubertal ΔIGF-I\(_{\text{SDS}}\) was significantly greater in the high dose group: -0.1±0.96 in the GH\(^{33}\) group vs 0.5±1.1 in the GH\(^{67}\) group, \(p=0.007\).

The mean 1st year pubertal ΔIGF-I\(_{\text{SDS}}\) was significantly higher compared to the mean pubertal on-treatment ΔIGF-I\(_{\text{SDS}}\): ΔIGF-I\(_{\text{SDS}}\) from at onset of puberty to the mean pubertal on-treatment IGF-I\(_{\text{SDS}}\) level was -0.2±0.86 for GH\(^{33}\) and 0.2±0.92 for GH\(^{67}\), \(p=0.028\), Figure 28. There was a broad variation of the individual mean pubertal on-treatment ΔIGF-I\(_{\text{SDS}}\), ranging from -2.2 to 1.7 for GH\(^{33}\) and from -2.8 to 2.3 for GH\(^{67}\).

When comparing the individual mean pubertal on-treatment levels of IGF-I\(_{\text{SDS}}\), there was no significant difference between GH\(^{33}\) and GH\(^{67}\) for the pubertal period, Figure 27, left panel.

![Figure 27. IGF-I\(_{\text{SDS}}\) responses from the study on IGHD (left panel) and non-GHD (right panel) according to GH dose groups: GH 33 μg/kg/day (GH\(^{33}\)), blue dots, and GH 67 μg/kg/day (GH\(^{67}\)), red dots. All IGF-I values are expressed as SDS. For the IGHD group, serum concentration of IGF-I\(_{\text{SDS}}\) is shown at GH start, at study start (randomization) and at adult height. The prepubertal (n=63), pubertal and total IGF-I\(_{\text{SDS}}\) change are shown as Δ for each period.

For the non-GHD group, serum concentration of IGF-I\(_{\text{SDS}}\) is shown at study start (start of GH treatment and randomization), and at adult height. The total IGF-I\(_{\text{SDS}}\) change is shown as Δ for each period.](image-url)
Figure 28. Pubertal $\Delta$IGF-I$(\text{SDS})$ in the IGHD group from randomization (study start) to mean pubertal on-treatment level according to GH randomization dose, 33 µg/kg/day vs 67 µg/kg/day. Box and whisker plots show median and interquartile range within ±1.5.

**GH response as change in IGFBP3$(\text{SDS})$**

**Prepubertal IGFBP3:**
At GH start, the mean prepubertal serum concentration of IGFBP3$(\text{SDS})$ was $0.3±0.8$ for the total group. The observed mean prepubertal $\Delta$IGFBP3$(\text{SDS})$ was $0.6±0.55$ and the mean prepubertal on-treatment level of IGFBP3$(\text{SDS})$ for GH$^{33}$ was $0.3±0.5$, Figure 29.

**Pubertal IGFBP3:**
During the pubertal period, the mean pubertal $\Delta$IGFBP3$(\text{SDS})$ was 0. The mean on-treatment level of IGFBP3$(\text{SDS})$ was very stable with no difference between the dose groups.

Figure 29. Development of IGF-I, IGFBP3 and IGF-I/IGFBP3 ratio in the IGHD group during the prepubertal and pubertal growth period according to GH dose groups: GH 33 µg/kg/day, blue dots, and GH 67 µg/kg/day, red dots. All values are expressed as SDS. IGF-I, IGFBP3 and IGF-I/IGFBP3 ratio are shown at GH start, at randomization, at 1st treatment year on the randomized GH dose, at individual mean pubertal on-treatment level, at GH stop at adult height, and at 1 year after GH treatment.
GH response as change in IGF-I/IGFBP3 ratio\textsubscript{SDS}

**Prepubertal IGF-I/IGFBP3 ratio:**
At GH start, the mean IGF-I/IGFBP3 ratio\textsubscript{SDS} was -1±1.1. The observed mean prepubertal ΔIGF-I/IGFBP3 ratio\textsubscript{SDS} was 1.5±1.24 and the mean prepubertal on-treatment level was 0.4±0.95, Figure 29.

**Pubertal IGF-I/IGFBP3 ratio:**
The mean pubertal on-treatment ΔIGF-I/IGFBP3 ratio\textsubscript{SDS} from the randomization to the individual mean pubertal on-treatment level was 0±1.0 for GH\textsuperscript{33} vs 0.6±0.9 for GH\textsuperscript{67}, p=0.008.

**Factors explaining the variation in pubertal ΔIGF-I\textsubscript{SDS}, ΔIGFBP3\textsubscript{SDS} and ΔIGF-I/IGFBP3 ratio\textsubscript{SDS}**
With only IGF variables allowed, 39% of the variation in pubertal ΔIGF-I\textsubscript{SDS} was explained by IGF-I\textsubscript{SDS} at randomization (the lower the value, the greater the increase during puberty); 28% of the variation in pubertal ΔIGFBP3\textsubscript{SDS} was explained by IGFBP3\textsubscript{SDS} and age at randomization; and 40% of the variation in pubertal ΔIGF-I/IGFBP3 ratio\textsubscript{SDS} was explained by IGF-I/IGFBP3 ratio\textsubscript{SDS} at randomization.

**5.3.2 Non-GHD patients**

GH response as change in IGF-I\textsubscript{SDS}

The study population used in Paper IV was described in detail in section 5.2. Only the total change in IGF-I will be presented here because it was only possible in one subgroup to distinguish between pubertal and total IGF-I response.

*At GH start/randomization, the mean IGF-I\textsubscript{SDS} was -0.76±1.2.*

*On-treatment:* A significant dose-dependent ΔIGF-I\textsubscript{SDS} from pre-treatment baseline to individual mean on-treatment IGF\textsubscript{SDS} level was found: the mean ΔIGF-I\textsubscript{SDS} was 1.3±1.35 for GH\textsuperscript{33} and 1.9±1.27 for GH\textsuperscript{67}, p<0.009, Figure 27 right. There was a broad range in ΔIGF-I\textsubscript{SDS}: -1.2 to 2.3 for GH\textsuperscript{33} and range -1.3 to 3.3 for GH\textsuperscript{67}, (PP). The total ΔIGF-I\textsubscript{SDS} correlated to the baseline value for IGF-I\textsubscript{SDS}, rho= -0.56, p<0.001 (PP).

The attained mean individual pubertal on-treatment level of IGF-I\textsubscript{SDS} was 0.9±1.1 for the total study group (including both dose groups). The mean level was 0.6±1.16 for the GH\textsuperscript{33} group and 1.1±1.05 for the GH\textsuperscript{67} group, p<0.019, Figure 30.
Figure 30. Development of IGF-I and IGFBP3 in the non-GHD ITT group during the prepubertal and pubertal growth period according to GH dose groups: GH 33 µg/kg/day (GH\textsuperscript{33}), blue dots, and GH 67 µg/kg/day (GH\textsuperscript{67}), red dots. All values are expressed as SDS. IGF-I and IGFBP3 levels are shown at GH start (randomization) and at the mean pubertal on-treatment level, calculated on values from mean on-treatment level.

GH response as change in IGFBP3SDS

The mean pre-treatment serum concentration of IGFBP3SDS was -0.1±1.04. The increase to the individual mean on-treatment level, ΔIGFBP3SDS, was 0.8±0.77 for GH\textsuperscript{33} and 1.0 ±0.99 for GH\textsuperscript{67}. The difference between the dose groups was not significant, Figure 30.

GH response as change in IGF-I/IGFBP3 ratioSDS

The ΔIGF-I/IGFBP3 ratioSDS from pre-treatment baseline to on-treatment mean level was 0.7±1.60 for GH\textsuperscript{33} and 1.4±1.07 for GH\textsuperscript{67}, p< 0.005.

Factors explaining the variation in ΔIGF-I\textsubscript{SDS}

With only IGF variables allowed, 43% of the variation in ΔIGF-I\textsubscript{SDS} was explained by the pre-treatment level of IGF-I\textsubscript{SDS} (the lower the baseline IGF-I\textsubscript{SDS}, the greater the increase in IGF-I\textsubscript{SDS} to on-treatment level), increasing to 48% if the GH dose was also allowed into the analyses.

5.3.3 Comparison of IGF-I response between the IGHD and non-GHD groups (Papers III and IV)

There was an IGF-I response to GH treatment in most IGHD and non-GHD patients, observed as ΔIGF-I\textsubscript{SDS}, but with a broad variability in IGF-I response.
The baseline mean IGF-ISDS at GH start was -1.2 in the IGHD group and -0.8 in the non-GHD group. For both diagnostic groups a significant dose-dependent increase in total ΔIGF-ISDS was found for those randomized to GH<sup>67</sup> compared to those on GH<sup>33</sup>. For IGHD, the mean total ΔIGF-ISDS was 2.3 for GH<sup>67</sup> vs 1.9 for GH<sup>33</sup>, p=0.007; for non-GHD, the mean total ΔIGF-ISDS was 1.9 for GH<sup>67</sup> vs 1.3 for GH<sup>33</sup>, p=0.009, Figure 27.

When comparing boys in both diagnostic groups during the pre-pubertal period, a greater mean prepubertal ΔIGF-ISDS was observed within GH<sup>33</sup> group in those with IGHD compared to non-GHD, p<0.012 (PP), Figure 32, left panel. In those in the non-GHD group, the GH<sup>67</sup> dose resulted in a higher ΔIGF-ISDS compared to the GH<sup>33</sup> dose, p< 0.02 (PP), Figure 31, left panel. However, the attained individual mean on-treatment IGF-ISDS levels during puberty were similar in both diagnostic groups, Figure 27, figure 31 right panel. Among boys with non-GHD, the pubertal on-treatment IGF-ISDS level was higher in the GH<sup>67</sup> dose group than in the GH<sup>33</sup> group, p=0.008 (PP), Figure 31, right panel.

**Figure 31. ΔIGF-ISDS for the pre-pubertal period (left panel) and IGF-ISDS mean on-treatment level during the pubertal period (right panel) for boys only, according to GH dose in the non-GHD and IGHD groups.**

When comparing the dose effect within and between the diagnoses IGHD and non-GHD, there were widely ranging on-treatment mean IGF-ISDS levels: in the IGHD group the level ranged from -2.2 to 1.7 for GH<sup>33</sup> and from -2.8 to 2.3 for GH<sup>67</sup>, and in the non-GHD group the level ranged from -1.1 to 2.2 for GH<sup>33</sup> and -1.3 to 3.2 for GH<sup>67</sup>. Moreover, the on-treatment mean pubertal IGF-ISDS level showed substantial overlap between dose groups and diagnostic groups.
The mean IGFBP3_{SDS} was similar at GH start/randomization for IGHD and non-GHD patients, -0.3 versus -0.12, respectively. The mean pubertal ΔIGFBP3_{SDS} was observed to be similar in the IGHD group and the non-GHD group 0.60 vs 0.91.

### 5.3.4 Comments

The current studies on IGHD and non-GHD patients treated with different GH dosage during puberty showed significant GH dose-dependent IGF-I_{SDS} response in both diagnostic groups. This indicates that both patient groups were able to generate IGF-I in response to the GH randomization dose. However, a higher mean ΔIGF-I was observed in IGHD patients, indicating them to be more responsive to GH treatment, compared to non-GHD patients. The non-GHD mean ΔIGF-I_{SDS} with dose GH was similar to the mean ΔIGF-I_{SDS} in IGHD with dose GH, indicating that the non-GHD patients do have somewhat lower GH responsiveness.

The prepubertal ΔIGF-I_{SDS} response in boys who received GH was significantly higher for those with IGHD than for those with non-GHD. To achieve a similar prepubertal ΔIGF-I, the non-GHD group required a higher GH dose. A greater total ΔIGF-I was found for IGHD patients compared to non-GHD patients.

The broad ΔIGF-I ranges indicated a wide range in individual GH responsiveness. Even though the magnitude of IGF-I response was greater in the IGHD group, a broad range in response and an overlap of the ranges obtained in the two diagnostic groups was observed.

Only two randomized studies in IGHD patients treated with different GH doses during the pubertal growth period until adult height were found (240, 281). Mauras, et al. randomized pubertal patients with GHD and TSH deficiency to GH doses of 43 and 100 µg/kg/day during pubertal growth for 3 years. Serum IGF-I levels were assayed at baseline and every 3 months, and expressed with log values or standardized scores. The high GH dose group presented an approximately 30% higher numerical value in IGF-I than those randomized on the low dose. A direct comparison between our studies was not possible due to differences in IGF-I methods and IGF-I references used. Our published Swedish references used the IGF-I_{SDS} considering sex, age and pubertal stage (162) making our study possible with a reliable result.

The other randomized study was small-scale and included both GHD and MPH patients, who were given GH doses 25 or 50 µg/kg/day until adult height. IGF-I estimations were performed by RIA and IGF-I values were transformed to SDS using reference values for healthy children with correction for age and sex (214).
Sas et al. found the mean increase in IGF-I_{SDS} to be twice as high in the high GH dose group than in the low dose group; however, this result was non-significant due to the small number of patients in each dose group (281).

The importance of ΔIGF-I_{SDS} as an indicator of response to GH treatment has previously been reported for prepubertal patients with varying endogenous GH secretion capacity, treated with GH 33 µg/kg/day during two prepubertal years (288). IGF-I was increased for most patients, but with a broad range, as seen in our pubertal study groups. Prepubertal ΔIGF-I was correlated with prepubertal growth response.

The finding from the current studies confirmed the result from IGF-I titration studies (289, 290), where both GHD and ISS (non-GHD) patients, classified on the GH max peak during a stimulation test, were included. Prepubertal short children (< -2SDS) with IGF-I value ≤ -1SDS were randomized to a study target IGF-I level of mean 0SDS or IGF-I +2SDS (without GH dose restriction), or to conventional weight-based GH dosing (40 µg/kg/day) for the control group. A positive ΔIGF-I was found in both groups and the highest Δ was in the group randomized to IGF-I +2SDS, with a broad variation of ΔIGF-I observed in all groups (289). Later, patients were separated according to the diagnosis GHD or ISS (290). To attain the IGF-I_{SDS} target level, GHD patients required a lower GH dose, mean 37 and 91 µg/kg/day to reach IGF-I 0SDS or IGF-I +2SDS, respectively. The patients in the ISS group required a corresponding mean dose of 32 and 114 µg/kg/day for IGF-I 0SDS and IGF-I +2SDS, respectively. This indicated the higher responsiveness to GH in the GHD group compared to ISS. The wide range in GH doses (20 to 346 µg/kg/day) to obtain the IGF-I +2SDS target level was also a sign of the GH responsiveness in both diagnostic groups (289, 290).

In the present study (Paper IV), GH treatment led to substantial changes in IGF-I_{SDS}, in both diagnostic groups and with both GH doses. Compared to changes in IGF-I_{SDS}, the relative rise in IGFBP3_{SDS} was less pronounced, resulting in an increased availability of free IGF-I (160). The mean ΔIGFBP3_{SDS} was similar in the IGHD and non-GHD groups. The on-treatment level of IGFBP3_{SDS} was very stable in both our studies, which indicates that IGFBP3 may prove to be a useful marker of satisfactory compliance (222).
Summary
In both diagnostic groups, i.e. in children with IGHD and non-GHD, a dose-dependent IGF-I_{SDS} response to GH treatment was observed using doses of 33 or 67 µg/kg/day. The ΔIGF-I_{SDS} range was broad in all groups, both for GH dose and diagnosis.

The observed mean IGF-I response was approximately 0.5 SDS greater in the IGHD group, indicating them to be more responsive to GH treatment than the non-GHD children.

5.4 Aim 4. Growth response and IGF-I response to GH treatment (Paper II, III, IV)

Rationale
Since the two randomized clinical trials described in this thesis used the same dose-response design, using GH dose 33 or 67 µg/kg/day, for groups of patients with short stature who differed only in their endogenous GH secretion capacity, the relationship between gain in ΔIGF-I_{SDS} and gain in height_{SDS} from start of GH treatment until adult height could be compared between IGHD and non-GHD patients. Table 5.

Table 5. Growth response and IGF-I response in IGHD and non-GHD groups

<table>
<thead>
<tr>
<th>ITT population</th>
<th>IGHD</th>
<th>Non-GHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>GH33</td>
</tr>
<tr>
<td>IGF-I_{SDS} at start</td>
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<td>-1.0</td>
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<tr>
<td>IGF-I_{SDS} +1yr prepuberty</td>
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<td>0.9</td>
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<tr>
<td>ΔIGF-I_{SDS} +1yr prepuberty</td>
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<td>2.1</td>
</tr>
<tr>
<td>IGFBP3_{SDS} at start</td>
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<td>-0.4</td>
</tr>
<tr>
<td>ΔIGFBP3_{SDS} total</td>
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<td>0.6</td>
</tr>
<tr>
<td>Total Δheight_{SDS} at adult height</td>
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<td>1.6</td>
</tr>
<tr>
<td>Pubertal Δheight_{SDS}</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Prepubertal Δheight_{SDS}</td>
<td>1.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>
5.4.1 IGHD patients

The relationship between \( \Delta IGF-I_{SDS} \) and gain in height_{SDS}

**Prepubertal \( \Delta IGF-I_{SDS} \):** Prepubertal first year \( \Delta IGF-I_{SDS} \) (mean 2.1) correlated with mean prepubertal gain in height_{SDS} (mean 1.3), \( r=0.29, p=0.019 \), Figure 32, left panel (PP boys). Furthermore, the prepubertal first year \( \Delta IGF-I_{SDS} \) also correlated with total gain in height_{SDS}, \( r=0.42, p=0.001 \), in other words, including pubertal height gain in response to the randomized dose GH^{33} or GH^{67} during pubertal growth.

**Pubertal \( \Delta IGF-I_{SDS} \):** Pubertal \( \Delta IGF-I_{SDS} \) (mean 0.1 for the total group, -0.2 for GH^{33} and 0.2 for GH^{67}) correlated with the pubertal gain in height_{SDS} (mean 0.6 for the total group), \( r=0.35, p=0.001 \). Height gain (mean \( \Delta \)height_{SDS}) was 0.4 for the dose group GH^{33}, \( r=0.32, p=0.003 \), and 0.7 for dose group GH^{67}, \( r=0.24, p=0.026 \), Figure 32, centre panel. Pubertal \( \Delta IGF-I_{SDS} \) did not correlate with total gain in height_{SDS}.

**Total \( \Delta IGF-I_{SDS} \):** Total \( \Delta IGF-I_{SDS} \) (prepubertal and pubertal \( \Delta IGF-I_{SDS} \)) correlated with total gain in height_{SDS} for both the GH^{33} (mean \( \Delta IGF-I_{SDS} \) 1.9) and GH^{67} (mean \( \Delta IGF-I_{SDS} \) 2.3) dose groups, \( p<0.01 \) for both, and for the total IGHD group \( r=0.46, p<0.001 \), Figure 32, right panel.

*Figure 32. Results from the IGHD group, comparing \( \Delta IGF-I_{SDS} \) and gain in height_{SDS}. GH dose GH^{33} (unfilled circles, broken line), GH^{67} (filled circles, solid line). Left panel: Correlation of prepubertal \( \Delta IGF-I_{SDS} \) (from GH start to on-treatment mean IGF-I_{SDS}) with prepubertal gain in height_{SDS}, \( r=0.29, p=0.019 \). All participants were receiving GH dose GH^{33}. Centre panel: Correlation of pubertal \( \Delta IGF-I_{SDS} \) (from randomization directly after onset of puberty to on-treatment mean IGF-I_{SDS}) with pubertal gain in height_{SDS}. GH dose GH^{33} (broken line) \( r=0.32, p<0.003 \), GH dose GH^{67} (solid line) \( r=0.24, p=0.026 \). Right panel: Correlation of total \( \Delta IGF-I_{SDS} \) (from GH start to mean pubertal on-treatment IGF-I_{SDS}) with total gain in height_{SDS}. GH dose GH^{33} (unfilled circles broken line) and GH dose GH^{67} (filled circles solid line), both \( p<0.01 \) from zero. For the total IGHD group, \( r=0.46, p<0.001 \).*
The relationship between $\Delta$IGFBP3SDS, and gain in heightSDS

Prepubertal 1st year $\Delta$IGFBP3SDS: Prepubertal 1st year $\Delta$IGFBP3SDS (mean 0.6) did not correlate with prepubertal gain in heightSDS, $r=0.24$, $p=0.057$, nor with pubertal gain in heightSDS (ns), but did correlate with total gain in heightSDS, $r=0.29$, $p=0.022$.

Pubertal $\Delta$IGFBP3SDS: Pubertal $\Delta$IGFBP3SDS (mean -0.1) from randomization to on-treatment level correlated with pubertal gain in heightSDS, $r=0.30$, $p=0.002$, but not with total gain in heightSDS.

The relationship between $\Delta$IGF-I/IGFBP3 ratioSDS and gain in heightSDS

Prepubertal 1st year $\Delta$IGF-I/IGFBP3 ratioSDS: Prepubertal 1st year $\Delta$IGF-I/IGFBP3 ratioSDS (mean 1.5) did not correlate with prepubertal gain in heightSDS, but did correlate with pubertal gain in heightSDS, $r=0.28$, $p=0.026$, and with total gain in heightSDS. $r=0.42$, $p=0.001$.

Pubertal $\Delta$IGF-I/IGFBP3 ratioSDS: Pubertal $\Delta$IGF-I/IGFBP3 ratioSDS (mean 0.3) correlated with pubertal gain in heightSDS, $r=0.22$, $p=0.022$, but not to total gain in heightSDS.

5.4.2 Non-GHD patients

The relationship between $\Delta$IGF-I SDS and total gain in heightSDS

For the GH-treated PP population, the $\Delta$IGF-I SDS from GH start to individual mean on-treatment level (mean 1.7) correlated with total gain in heightSDS (mean 1.3), $\rho=0.42$, $p<0.01$, Figure 33. Separated according to dose group, the corresponding means in $\Delta$IGF-I SDS were 1.20 for GH33 and 2.07 for GH67 (GH33 vs GH67, $p<0.004$) and means for total gain in heightSDS were 0.9 and 1.3, respectively. The correlation between $\Delta$IGF-I SDS for GH-treated group and total gain in heightSDS was $\rho=0.42$, $p<0.0001$.

The relationship between $\Delta$IGFBP3 SDS and total gain in heightSDS

The $\Delta$IGFBP3 SDS (mean 1.03 for the GH-treated PP population, 0.78 for GH33 and 1.20 for GH67) correlated with total gain in heightSDS, $\rho=0.35$, $p<0.01$ (or $\rho=0.49$, $p<0.0001$, when controls were included).
Figure 33. Results from the non-GHD group, comparing ∆IGF-I_{SDS} (from GH start to mean on-treatment pubertal IGF-I_{SDS} level) and total gain in height_{SDS}. GH^{33} (unfilled circles), GH^{67} (filled circles), untreated controls (+). For the total non-GHD group, r=0.58, p<0.001.

The relationship between ∆IGF-I/IGFBP3 ratio_{SDS} and total gain in height_{SDS}

The ∆IGF-I/IGFBP3 ratio_{SDS} (mean 1.2 for the GH-treated PP population, 0.6 for GH^{33} and 1.6 for GH^{67}) correlated with gain in height_{SDS}, rho=0.49, p<0.0001.

5.4.3 Factors explaining the variation in growth response in the IGHD and non-GHD groups

Multivariable analyses with gain in height_{SDS} as the outcome variable revealed great similarities in growth response.

With only IGF variables available, in both diagnostic groups 28% of total gain in height_{SDS} could be explained by the ∆IGF-I_{SDS} variable from baseline to on-treatment level, making this the most important indicator of individual GH responsiveness, Table 6.

When all variables were available, 62–63% of the total gain in height_{SDS} could be explained; for the IGHD group, the variable prepubertal 1st year ∆IGF-I/IGFBP3 ratio_{SDS} was informative and, for both groups, bone age delay and GH dose contributed significantly to the explanation, Table 6.
**Table 6. Multivariable analyses, explained variation in gain in heightSDS in IGHD and non-GHD groups**

<table>
<thead>
<tr>
<th>IGHD, gain in heightSDS</th>
<th>Non-GHD, total gain in heightSDS</th>
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<tr>
<td>26% Pubertal gain in heightSDS</td>
<td>28% Total gain in heightSDS</td>
</tr>
<tr>
<td>Pubertal ΔIGF-I&lt;sub&gt;SDS&lt;/sub&gt;</td>
<td>Pubertal ΔIGF-I&lt;sub&gt;SDS&lt;/sub&gt;</td>
</tr>
<tr>
<td>ΔIGF-I/IGFBP&lt;sub&gt;3&lt;/sub&gt; ratio&lt;sub&gt;SDS&lt;/sub&gt; prepubertal</td>
<td>Prepubertal IGF-I&lt;sub&gt;SDS&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

**5.4.4 ΔIGF-I<sub>SDS</sub> and gain in height<sub>SDS</sub> in the IGHD and non-GHD groups**

*Dose response*: In both diagnostic groups, there was a dose-response association to growth response, estimated as gain in height<sub>SDS</sub> as well as in IGF-I, estimated as ΔIGF-I<sub>SDS</sub>.

*The relationship between IGF-I<sub>SDS</sub> and gain in height<sub>SDS</sub>*

The IGHD group had the lowest IGF-I<sub>SDS</sub> at start and the highest prepubertal ΔIGF-I<sub>SDS</sub> (all on dose GH<sup>33</sup>) compared with the non-GHD group (randomized to dose GH<sup>33</sup> or GH<sup>67</sup>). The IGHD group pubertal ΔIGF-I<sub>SDS</sub> from the time of randomization to GH<sup>33</sup> or GH<sup>67</sup> was modest, equally so when considering only the dose group GH<sup>67</sup>, although they showed a greater range. Those patients with substantial prepubertal ΔIGF-I had the greatest prepubertal height gain, and more patients who in addition increased their IGF-I when changing GH dose from the onset of puberty (see range in Figure 32, centre panel, ended up with more pubertal height gain.

The non-GHD patients randomized to GH<sup>33</sup> showed only a modest increase in their prepubertal IGF-I<sub>SDS</sub> (mean 1.1) during GH treatment, while those on GH<sup>67</sup> had a ΔIGF-I<sub>SDS</sub> (mean 2.3) corresponding to the 1<sup>st</sup> prepubertal year ΔIGF-I<sub>SDS</sub> seen in the IGHD GH<sup>33</sup> group (mean 2.1), indicating lower GH responsiveness in the non-GHD patients.

Total ΔIGF-I<sub>SDS</sub> (prepubertal and pubertal) was greater for the IGHD group (mean 2.2) and for those receiving GH<sup>33</sup> (mean 2.1). The non-GHD group on dose GH<sup>67</sup> achieved a total ΔIGF-I<sub>SDS</sub> (mean 1.9), which was on a level comparable with
the IGHD group; this was not the case for the non-GHD group on dose GH$^{33}$ (mean 1.3).

When comparing effects on height gain, total gain in height$_{SDS}$ for the non-GHD group was 0.9 for dose GH$^{33}$ group and 1.3 for dose GH$^{67}$, while the IGHD group gained 1.6 and 2.0, respectively. Thus, the non-GHD group needed the GH$^{67}$ dose in order to gain in IGF-I almost as much as the IGHD GH$^{33}$ group, but still this resulted in lower mean height gain, 1.3 vs 1.6 SDS. These signals some degree of reduced IGF-I responsiveness in the non-GHD patients.

**IGFBP3 and IGF-I/IGFBP3 ratio$_{SDS}$ in relation to diagnosis**

For IGFBP3$_{SDS}$, again IGHD mean values were lower at GH start, -0.3 vs -0.1 in the non-GHD group. However, mean total ΔIGFBP3$_{SDS}$ was 0.9 for the non-GHD group vs 0.6 for the IGHD group, resulting in on-treatment mean levels 0.9 for the non-GHD group vs 0.3 for the IGHD group. This implies that the total ΔIGF-I/IGFBP3 ratio$_{SDS}$ become lower in the non-GHD group (mean 1.16 vs 1.8). Thereby the IGF-I free fraction may be lower in the non-GHD group, possibly contributing to the reduced growth response.

**Summary**

Comparing the effect of GH dose 33 and 67 µg/kg/day on change in IGF-I$_{SDS}$ there was a lower response in the non-GHD group compared to the IGHD group. In addition, comparing the IGF-I$_{SDS}$ in relation to gain in height$_{SDS}$ also revealed a reduced response in relation to IGF-I$_{SDS}$ in the non-GHD group.
6. General discussion

This thesis is based on trials which started in the late 1980s, studying two diagnostic groups, based on GH secretion capacity, IGHD and non-GHD patients, both randomized to two weight-based GH doses and followed until adult height. From these trials it is possible to make comparisons according to the different GH effects estimated as growth response and IGF-I response. For each diagnostic and dose group, there were broad and overlapping ranges of variation in response, challenging the present opinion that these diagnoses should be handled as different entities. These broad ranges in response are considered to primarily be signs of variable individual GH responsiveness, (i.e. response = responsiveness x dose), although more variables (discussed later) may affect the individual response. Rather than discussing diagnosis, the investigation should seek to answer the question of whether there is a potential for growth response to GH and, if so, what dose would be needed. For a predefined growth outcome, available and future models for prediction of growth response by estimating individual GH responsiveness are valuable tools making individual dosing possible.

From the two trials, which were randomized controlled weight-based dosing studies, a dose-dependent effect was found on gain in heightSDS and ΔIGF-I SDS according to both diagnosis and GH doses, respectively. In addition, there was strong correlation between ΔIGF-I SDS and gain in heightSDS.

When evaluating the degree of response, the non-GHD group randomized to dose GH33 had the lowest response, while the non-GHD GH67 dose group approached responses at the level of the IGHD GH33 group. The interpretation is that the non-GHD group had a reduced responsiveness compared to those in the IGHD group, both for GH and IGF-I.

Our team has reported from a trial on individual GH dosing for a prepubertal two-year period, starting in 2000 (211), when the dose was determined by individually estimated GH growth response using the Swedish prediction model (225, 229). The non-GHD group needed a higher mean GH dose to attain the same mean growth response as the IGHD group, signalling reduced GH responsiveness in the non-GHD group (211). Thus, in the studies reported in this thesis, the previously reported difference in GH responsiveness between IGHD and non-GHD patients during prepuberty has been confirmed to exist also during the pubertal growth period until adult height.

Our study with reduced GH responsiveness in the non-GHD group showed that IGF-I increment to be the most informative variable for growth response in both
dose groups; this also confirms the two-year IGF-titration study by Cohen et al. (289, 290), even though their approach was different and undertaken only in prepubertal patients. Their IGF-I_{HIGH} group had the greatest gain in height. However, the multivariable analysis on the outcome height gain revealed the ΔIGF-I_{SDS} to be a more informative variable than the attained IGF-I level (289). In addition, when the study group was separated according to endogenous GH secretion capacity, GHD or ISS (i.e. non-GHD), the GHD group had greater prepubertal GH responsiveness (290), as was found in our trial, where the patients were observed until adult height. A problem with an IGF-I titration approach is that the IGF-I increase, i.e. measurable IGF in the circulation, may not per se be the same as the GH-stimulated locally produced IGF in the growth plate; in other words, the on-target level that is needed to induce a satisfying growth response on an individual basis is presently not known.

However, GH responsiveness according growth response on a specific GH dose can be individually estimated by models for prediction of growth response for the prepubertal period and with the diagnosis GHD or ISS (225, 227-229), and for the pubertal period with the diagnosis GHD (291). Informative variables for prepubertal responsiveness using information from exclusively before start of treatment were age at start of GH treatment, parental height, size at birth, height_{SDS} at GH start and a GH or IGF-I variable. The most informative GH variable was the maximal nocturnal peak, followed by the total amount secreted, whereas the GH secretion response to the provocation test (AITT) was less informative, and actually in the range of IGF-I concentration in blood (225, 229). After start of treatment, the gain in IGF-I_{SDS} could replace many pre-treatment variables; furthermore, the observed first year gain in height predicts the growth response during the following prepubertal years (292).

For prediction of pubertal growth response, the variables at puberty onset such as sex, age, distance of height_{SDS} to MPH_{SDS} and GH dose were the important ones (291). Contrary to results reported in this thesis, they found the GH dose to be of little importance. This may be due to differences in both the numerical dose and the dose range, 18–39 µg/kg/day, in comparison to 33–67 µg/kg/day in our trials. This interpretation of dose effect on impact of trial dose range is supported in studies using 25 or 50 µg/kg/day (low dose effect) (281), 43 or 100 µg/kg/day (a clear dose effect) (240), or the positive dose effect found in the observational stepwise GH dose increase study (293).

Low age per se is related to high GH responsiveness and results in greater growth response compared to an older child treated with the same dose/kg. This is a reason why treatment should preferably start at as low an age as possible (46, 225, 261, 291). However, starting at an early age demands early detection of the
growth disturbance, which in turn needs well organised growth monitoring in the community (294-296).

Distance in height_{SDS} of the child to MPH_{SDS} is an additional important variable positively related to responsiveness (46, 225, 227). Variability in adult height is to 80% genetically influenced (1), mirrored by the importance of the MPH_{SDS} variable. This means that a great height distance to MPH_{SDS} is an important sign of responsiveness. Due to the importance of this variable, parental height should preferably be measured, as reported height has less accuracy (297).

In the present studies, there were not enough girls to make a gender comparison possible. During prepubertal years, growth is rather stable in both sexes without principal differences. However, at onset of puberty, GH secretion doubles in boys and increases to threefold in girls due to influence of estrogen (272). More participating girls would have been of interest, as in the present trials the doses were the same for both boys and girls.

Responsiveness to GH most often refers to growth, i.e. longitudinal bone responsiveness. However, the responsiveness varies in each individual between tissues and has been ordered according to responsiveness as a ‘staircase’ in prepubertal children (112). Even though the ‘starting level’ varies individually in prepubertal children, the brain and fat tissue had the greatest responsiveness, followed by muscle and insulin sensitivity; lowest responsiveness was found for bone and for IGF-I (112). However, whether this individually set ‘staircase’ in responsiveness is influenced by onset of puberty remains to be studied.

When evaluating the response to GH treatment in a child in terms of individual responsiveness, there are factors that can interact with what we falsely may define as responsiveness. The primary diagnosis may not be correct. In a young child, judgement of body proportions are both hard to measure and to evaluate, and x-rays are not very informative until many bones have become calcified. With increasing height and bone maturation, a previously undiagnosed disproportionate short stature due to skeletal dysplasia may become obvious. Other factors, such as fibroblast growth factor receptor-3 (298), SHOX (299, 300) have an effect on the growth plate. The responsiveness related to the diagnosis non-GHD/ISS varies greatly due to heterogeneity in pathophysiology. However, the growth response, as shown in the present studies, is for many patients with non-GHD short stature comparable with that in the GHD-group (225, 301).

The variation in gain in height may in addition be influenced by the injection technique and thereby the uptake of the injected dose. The degree of influence of GH uptake on what we estimate as responsiveness was not directly investigated in this project.
However, indirectly, information can be gained by the $C_{\text{max}}$. As extrapolating from the physiological relation between $GH_{\text{max}}$ and spontaneous growth, a higher $C_{\text{max}}$ would result in a better growth-promoting effect (68, 77, 79). The injection device should optimally support the injection technique to allow a deep injection required for a high GH uptake. In addition, low compliance should preferably be prevented, as the child may falsely be judged to have low responsiveness (302, 303).

Thus, when considering GH treatment, a thorough investigation is of great value, making it possible to evaluate not only the GH secretion capacity, but also the endogenous balance between spontaneous GH secretion and GH responsiveness. A prediction model estimation of individual responsiveness supports selection of optimal dose right from the start of treatment and will thereby realize the first year growth potential.

The present studies highlight the great impact of individual responsiveness and the necessity of individual dose adaptation during puberty. In addition, the differences between the diagnostic groups in terms of treatment effects in growth and IGF-I were minor, which supports the theory that responsiveness provides a constructive rationale for treatment decisions.
7. Conclusions

1. The highest GH uptake, giving the optimal growth signal, was observed after a deep injection with higher GH dose and concentration; however, there was substantial intra- and inter-individual variation regarding both pharmacokinetics and bioavailability. Around 70% of the injected rhGH reached the circulation.

2. It is possible to improve pubertal gain in height with a higher GH dose in both GHD and non-GHD patients. Prepubertal and pubertal growth response as gain in height_{SDS} and attained adult height_{SDS} was dose dependent, although greater response was found in the GHD group, indicating higher responsiveness. The range for gain in height was broad in all dose and diagnostic groups.

3. GH treatment induced a dose-dependent IGF-I_{SDS} response in both diagnostic groups, but it was higher for the GHD group, indicating that they have higher responsiveness. The range for ΔIGF-I_{SDS} was broad in all dose and diagnostic groups.

4. The increased GH dose during puberty resulted in both higher ΔIGF-I_{SDS} and a greater gain in height_{SDS}. ΔIGF-I_{SDS} correlated with gain in height until adult height_{SDS}. In both diagnostic groups there was a range in GH responsiveness. When gain in height_{SDS} during puberty is the treatment target, a GH dose increase large enough to result in a ΔIGF-I_{SDS} is required from onset of puberty.
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