Growth hormone
and the heart in children

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To my dearly beloved wife, Daniella, who has
been a constant source of support to me during
all the years we have spent together

&

To my wonderful children Andrea, Amanda and Jonathan,
who fill my heart with profound joy and pride

There is a theory which states that if ever anyone discovers exactly what the
Universe is for and why it is here, it will instantly disappear and be replaced
by something even more bizarre and inexplicable

There is another theory which states that this has already happened

— Douglas Adams (1952–2001), from the book:
The restaurant at the end of the universe

If the Lord Almighty had consulted me before embarking upon creation, I
should have recommended something simpler

— Alfonso X (1221–1284), also called
Alfonso el sabio (the wise)

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ABSTRACT

Background and Aims: The fact that growth hormone (GH) influences cardiovascular structure and function is well established through both human and animal studies. Despite being secreted in a pulsatile fashion, only the impact of peak GH concentrations on cardiac parameters has previously been reported, and the time-dependency of cardiovascular effects during GH treatment has not been detailed. The aims of this pediatric study were (i) to establish the expression of GH-receptor (GH-R) and insulin-like growth factor I (IGF-I) mRNA locally in the heart in children of different ages, (ii) to study in detail the relationship between the heart and endogenous GH secretion pattern, (iii) to study the cardiovascular effects of GH treatment and (iv) to examine organ/tissue-specific responses to GH.

Patients & Methods: Two trials were conducted. In the first, a cardiac biopsy was taken from 18 children undergoing heart surgery. GH-R and IGF-I mRNA were quantified by real-time polymerase chain reaction. In the second trial, 153 short prepubertal children were randomized to receive either a standard or an individualized GH dose. Echocardiography, blood pressure measurements and electrocardiography were performed at study start, and after 3 months, 1 year and 2 years of GH treatment.

Results: GH-R and IGF-I mRNA was found in all children studied. There was a significant relationship between their relative amounts (r=0.75, *p*<0.001), and body mass index was correlated with the relative expression of both genes (r=0.59, *p*=0.01 and r=0.50, *p*=0.04 respectively). Cardiac dimensions were not correlated with peak endogenous GH concentration but were negatively correlated with GH trough levels (r= –0.41, *p*<0.001) and positively correlated with GH secretion rate above baseline level (r=0.44, *p*<0.001). During treatment, a biphasic, time-dependent, cardiac response was seen. Initially, there was an increase in both standard deviation scores (SDS) for left ventricular (LV) diameter in diastole SDS (95% confidence interval (CI) for the increase in SDS from baseline to 3 months (ΔLVDd$_{SDS0-3m}$): 0.05 to 0.36) and LV wall thickness, exemplified by septal thickness (ΔIVDd$_{SDS0-3m}$: 95% CI 0.08 to 0.54). At 2 years, wall thickness returned to baseline values (ΔIVDd$_{SDS0-24m}$: 95% CI -0.41 to 0.06) but LV diameter remained increased (ΔLVDd$_{SDS0-24m}$: 95% CI 0.19 to 0.47). The heart was also found to be more responsive than both skeletal muscle and bone tissue to GH treatment. The dose resulting in a 50% response (ED50%) was as low as 33 µg/kg/d (90% confidence bounds: 24–38 µg/kg/d)) for LVDd compared with an ED50% of 51 (47–56) µg/kg/d for longitudinal growth and 57 (52–65) µg/kg/d for IGF-I.

Conclusion: With the presence of local GH-R and high sensitivity of the heart to GH, cardiac tissue is a primary target for GH. The GH trough levels seem to be of greater importance for cardiac dimensions than the peak GH concentrations, and the response to GH treatment is time-dependent and differs between LV wall thickness and LV diameter. This demonstrates that GH regulation of cardiovascular variables is more complex than previously demonstrated.

Keywords: Growth hormone secretion pattern, cardiovascular dimensions, Growth hormone treatment

SAMMANFATTNING

Bakgrund: Både patienter med över- och underproduktion av tillväxthormon (GH) har förändringar i hjärtats struktur och funktion. Avhandlingens syfte var att närmare undersöka hur GH påverkar hjärtat hos växande barn.

Metod: Två projekt genomfördes. I det ena togs biopsier/bitar från hjärtat i samband med planerad hjärtkirurgi från arton barn. I dessa bitar undersöktes hur mycket mRNA som producerades för GH receptorn (GH-R) och IGF-I. mRNA är ett mellansteg från genen i cellkärnan till färdig produkt. I det andra projektet undersöktes 153 korta barn före puberteten. De genomgick dels omfattande utredningar om hur GH insöndras och dels undersöktes hur deras hjärta reagerade på GH behandling i olika doser.

Resultat: mRNA för GH-R och IGF-I kunde påvisas i hjärtvävnad från samtliga undersökta barn. Ju mer GH-R mRNA vävnaden innehöll desto mer IGF-I mRNA fanns det i den. Smalare barn (lägre BMI) hade mindre GH-R och IGF-I i hjärtat. Insöndring av GH sker pulsatilt med flera toppar per dygn och mellanliggande dalar. GH nivåerna mellan topparna visade sig vara viktigare för hur stort hjärtat var än höjden på topparna. Ingen skillnad i hjärtundersökningarna mellan barn med allvarlig GH brist och mindre uttalad GH brist kunde påvisas. Vid behandling med GH sågs först en snabb ökning av hjärtats diameter och hjärtväggens tjocklek. Efter två års behandling hade väggtjockleken återgått till de ursprungliga förväntade värdena, medan kammarväggens diameter fortfarande var ökad. Genom att studera den dos som behövde ges för att olika vävnader i kroppen skulle reagera påvisades att hjärtats tillväxt var mer känslig än tex längdtillväxt och ökning av IGF-I värdena.

Sammanfattning: Det finns receptorer för GH i hjärtat vilket gör direkta effekter möjliga. Hos korta barn, före puberteten, påverkas hjärtat mer av nivåerna av GH mellan topparna än höjden på topparna, som man sedan tidigare vet optimerar längdtillväxten. Hjärtat är ett av de känsligaste organen för GH behandling och reagerar snabbt med ökad storlek och tjocklek. Efter två års behandling är endast hjärtats storlek ökad.

Betydelse: Avhandlingen visar hur viktigt det är att framtida forskning av GHs effekter på hjärtat tar hänsyn till det pulsatila insöndringsmönstret. Den visar också att olika delar av hjärtat reagerar olika beroende på hur länge hjärtat utsätts för behandling med GH. Detta ger en förståelse för vilka grupper med dålig hjärtfunktion som skulle kunna ha glädje av GH behandling i framtiden.

LIST OF PAPERS

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

I.   **Nygren A**, Sunnegårdh J, Albertsson-Wikland K, Berggren H, Isgaard J. **Relative Expression of growth hormone receptor and insulin-like growth factor-I mRNA in congenital heart disease.** J.Endocrinol Invest **2008**;31:196-200

II.   **Nygren A**, Andersson B, Decker R, Nierop AF, Sunnegårdh J, Kriström B, Albertsson-Wikland K. **Cardiac structure and function in short prepubertal children. Association with spontaneous GH secretion pattern and metabolic factors.** Submitted to Clinical Endocrinology (Oxf) **2012**.

III.   **Nygren A**, Sunnegårdh J, Teien D, Jonzon A, Björkhem G, Lindell S, Albertsson-Wikland K, Kriström B. **Rapid Cardiovascular Effects of Growth Hormone treatment in short prepubertal children. Impact of treatment duration.** Clinical Endocrinology (Oxf) **2012**, In Press. DOI: 10.1111/j.1365-2265.2012.04456.x

IV.   Decker R*, **Nygren A***, Kriström B, Nierop A, Gustafsson J, Albertsson-Wikland K, Dahlgren J. **Different thresholds of tissue-specific dose-responses to growth hormone in short prepubertal children.** Accepted for publication in BMC Endocrine Disorders the 11-Oct-**2012**.

* indicates that these authors contributed equally to this publication.

CONTENT

ABBREVIATIONS

Δ	Change
AITT	Arginine–insulin tolerance test
AL	Area–length
ALP	Alkaline phosphatase
ALS	Acid labile subunit
Ao	Aortic diameter
$Area_{In}$	Inner left ventricular area
$Area_{Out}$	Outer left ventricular area
ASD	Atrial septal defect
AUCb	Area under the curve above baseline level
AUCt	Total area under the curve
avPeak	Average peak value above the zero-line
BMC	Bone mineral content
BMI	Body mass index
BP	Blood pressure
BSA	Body surface area
Ca^{2+}	Calcium
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
CO	Cardiac output
CoA	Coarctation of the aorta
CV	Coefficient of variation
Da	Dalton
DELFIA	Dissociation-enhanced lanthanide fluorescence immunoassay
DXA	Dual-energy X-ray absorptiometry
DILV	Double inlet left ventricle
ECD	Extra cellular domain
ECG	Electrocardiography
ECW	Extracellular water
ED50%	Effective GH dose predicted to result in 50% change effect
EF	Ejection fraction
ERK	Extracellular signal-regulated kinase
ESS	End systolic strain
ET	Ejection time
F	female
FS	Fractional shortening
GH	Growth hormone
GHb	Growth hormone secretion rate above baseline level
GHBP	Growth hormone-binding protein
GHD	Growth hormone deficiency
GHI	Growth hormone insensitivity
GH_{max}	Maximum GH peak
GH-R	Growth hormone receptor
GHRH	Growth hormone-releasing hormone
GHt	Total growth hormone secretion rate
HOMA	Homeostasis model assessment
IGFBP	Insulin-like growth factor-binding protein
IGF-I	Insulin-like growth factor-I

IGF1R	Insulin like growth factor-I receptor
inv	Inverted
IRP	International reference preparation
ISS	Idiopathic short stature
IVSd	Interventricular septal thickness in diastole
JAK2	Janus (tyrosine) kinase 2
Lasso	Least absolute shrinkage and selection operator
ln	Natural logarithm
LST	Lean soft tissue
LV	Left ventricular
LVDd	Left ventricular diameter in diastole
LVDs	Left ventricular diameter in systole
LVPWd	Left ventricular posterior wall in diastole
LVVd	Left ventricular volume in diastole
LVVs	Left ventricular volume in systole
M	male
MAPK	Mitogen-activated protein kinases
MM	M-mode
MPH	Mid-parental height
mRNA	Messenger ribonucleotide acid
mVCFc	Corrected mean velocity of circumferential shortening
n	Number
ns	Not significant
PAPVD	Partial anomalous pulmonary venous drainage
PCA	Principal component analysis
PEP	Pre-ejection period
PHT	Pulmonary hypertension
RA	Right atrium
Ras	Rat sarcoma
rtPCR	Real-time polymerase chain reaction
RV	Right ventricle
SD	Standard deviation
SDS	Standard deviation score
SGA	Small for gestational age
sq	Square root
Src	Sarcoma
SMR	Standardized mortality ratio
SS	Somatostatin
SSI	Systolic strain index
STAT5	Signal transduction and activators of transcription
SV	Stroke volume
TA	Tricuspid atresia
TGA	Transposition of the great arteries
TMD	Trans membrane domain
ToF	Tetralogy of Fallot
TrS	Tracheal stenosis
Truncus	Truncus arteriosus communis type II
UVH	Univentricular heart
VO_2max	Maximal oxygen consumption
VSD	Ventricular septal defect
VTI	Velocity time integral

1 INTRODUCTION AND AIMS

The first association between growth hormone (GH) and the heart was observed in studies of patients with GH hypersecretion. Before the discovery of pituitary GH, the term acromegaly was suggested to describe this condition in 1886 by Pierre Marie[1]. Huchard was the first to describe cardiovascular changes in these patients in 1895[2] and, as early as 1912, Cushing postulated that a "Hormone of growth" was present in the pituitary gland. He was also one of the first to describe pituitary enlargement in acromegalic patients[3]. It was not until 1944 that Li and Evans isolated and described pituitary GH[4]. In 1952 and 1954, Beznak published studies on rats with experimental aortic constriction that showed GH to be of importance in maintaining normal hypertrophic response[5-7]. Following these discoveries, there were a few decades during which there was little research activity in this area. Interest in the role of GH in the regulation of cardiovascular structure and function then increased substantially during the last 25 years, most likely due to the ready availability of GH produced by recombinant techniques from 1985. The cardiovascular effects of GH hypersecretion, GH deficiency (GHD) and GH treatment have now been studied in both animal models and humans[8]. In the pediatric age group, studies have mainly focused on the cardiovascular effects during GH treatment of short stature, with the first study being reported as late as 1991[9]. In 1999, when the current project was planned, there were only a few studies available, and these had yielded varying results varying results[9-17]. Until now, there has been no published information on the importance of endogenous GH secretion pattern in the regulation of cardiovascular structure and function in humans.

1.1 Aims

The overall aim of this thesis was to increase knowledge of the effects of GH on the heart in children, both in terms of the effects of endogenous secretion and of administered GH. More specifically:

- to study whether GH receptor (GH-R) mRNA and insulin-like growth factor (IGF-I) mRNA are expressed in the heart in children
- to study the association between endogenous GH secretion pattern and the heart in children
- to study the cardiovascular effects of GH treatment in children
- to study whether the heart is more or less sensitive to GH treatment than other organs.

2   BACKGROUND

The purpose of this section is to give the relevant background information necessary to enable critical evaluation of the thesis. Current knowledge of GH and the heart in children will be reviewed in the *Discussion* section.

2.1   Growth hormone and insulin-like growth factor-I axis

2.1.1   Growth hormone

GH is a protein hormone that consists of several isoforms. On chromosome 17q there are two *GH* genes. *GH1* or *GH-N* is expressed in the pituitary gland and *GH2* or *GH-V* is expressed in the placenta. Originating from *GH-N*, the most abundant form of GH is a 191 amino acid single-chain protein with a molecular weight of 22 129 Dalton (Da), also called 22K-GH. By alternative mRNA splicing, the second most common GH isoform is a 20 274 Da, 176 amino acid-long protein (20K-GH). In addition to a third isoform, 17.5K-GH, GH is also found in deaminated forms, *N*-acylated forms, glycosylated forms, dimeric forms and oligomeric forms (up to pentameric)[18]. In the pituitary gland, the majority of GH is the monomeric 22K-GH form (55%), with only a small proportion being the monomeric 20K-GH form (6%). Following a secretory pulse from the somatotrophic cells in the anterior pituitary, about 50% of 22K-GH and 25% of 20K-GH becomes bound to a high-affinity GH-binding protein (GHBP), and 5–8% and 50%, respectively, to a low-affinity GHBP, an α2-macroglobulin[19]. The half-life of 22K-GH is significantly shorter than the half-life of 20K-GH; as a result, 20K-GH is relatively more abundant during GH troughs[18]. Although further research is needed on the different properties of the GH isoforms, it has been suggested that an increased proportion of GH forms other than 22K-GH might impair growth in children[20]. GH secretion from the anterior pituitary is stimulated mainly by GH releasing hormone (GHRH) and inhibited by somatostatin. Both of these hormones are secreted from the hypothalamus in a pulsatile manner[21]. GH release is also stimulated by ghrelin, a GH secretagogue, that is mainly produced in the stomach, and to a lesser degree in other organs[22]. Receptors for ghrelin are found in the heart in addition to the pituitary gland.

2.1.2   Growth hormone receptors

GH-Rs are present to various extents in different organs, being most abundant in the liver followed by the heart (in the rat)[23]. The GH-R is a, so called, 620-residue glycosylated class 1 cytokine receptor with two β-sandwich modules, a

transmembrane domain (TMD) and a cytoplasmatic box 1 sequence interacting with a box 2 sequence, enabling binding to Janus (tyrosine) kinase 2 (JAK2)[24]. The extracellular domain (ECD) of the GH-R is similar to the high affinity GHBP[25]. Two GH-R units are dimerized at the TMD and subsequent binding of GH to the ECD results in rotation and shift of the subunits leading to the activation of JAK2[24]. JAK2 phosphorylates signal transduction and activators of transcription 5 (STAT5) which forms a dimer that regulates gene expression. Other signaling pathways are also activated in parallel. For example, a sarcoma (Src) family kinase activates the Ras (rat sarcoma) signaling pathway, probably through activation of phospholipase Cγ which increases cytoplasmatic calcium (Ca^{2+}). Activation of this pathway subsequently activates mitogen-activated protein kinases (MAPK) and the extracellular signal-regulated kinase (ERK), that participate in the regulation of gene expression[24, 26] (Figure 1).
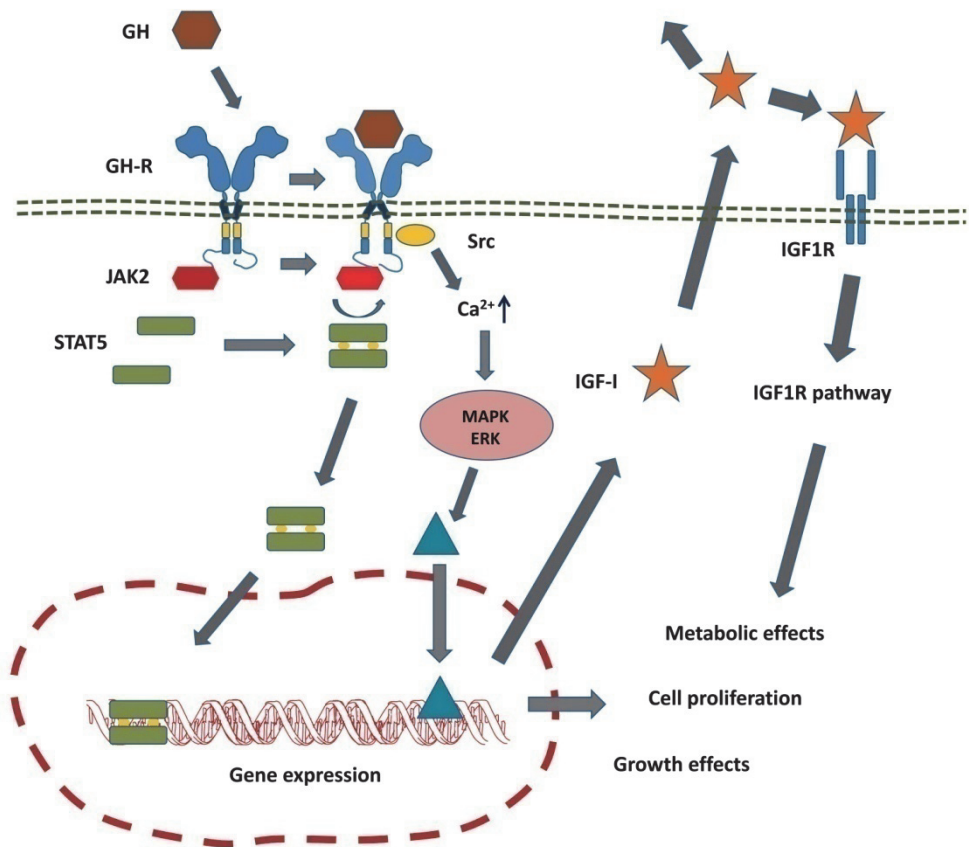

Figure 1. GH-R and IGF1R signaling pathway at a glance. See Subchapter 2.1 for abbreviations and a brief review.

2.1.3 Insulin-like growth factor and associated binding proteins

IGFs are expressed after activation of the GH-R. The signaling pathway involved is of equal complexity to the one for GH. In brief, the system includes two ligands, IGF-I (the major form in postnatal life) and IGF-II (important for fetal growth), at least four receptors (IGF1R having the highest affinity for IGF-I)[27]. In addition there are six IGF-binding proteins (IGFBPs), the most abundant being IGFBP3[28], and the acid-labile subunit (ALS). IGFs have mitogenic and anti-apoptotic effects through post-receptor effects utilizing several signaling cascades (some of which are also utilized by the GH-R)[28]. IGF-I increases the contractility of the cardiomyocyte[29]. The mechanism responsible for this is not entirely clear, but calcium handling seems to be of importance, and there is evidence of increased calcium handling in the short term[30] and also a change in myosin isoforms[31]. IGF-I also acts as a vasodilator, most likely through direct nitric oxide-releasing effects[32].

2.1.4 Growth hormone secretion pattern

GH is secreted from the pituitary gland in a highly pulsatile fashion[33] (Figure 2). This pulsatility is maintained mainly as a result of regulation by the stimulating GHRH and the inhibiting somatostatin secreted from the hypothalamus. GH secretion is modified by negative feedback both from IGF-I and GH[34, 35] (Figure 3).
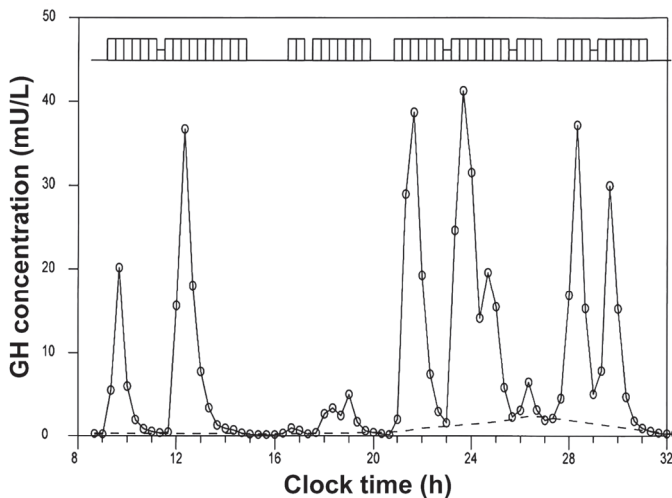
Figure 2. Example of 24-hour GH secretion measurements analyzed by the PULSAR program. Details are given in the Patients and methods section.

The GH secretion pattern in rats is sexually dimorphic. Male rats have high GH peaks every 3 hours with low trough levels in-between[33], whereas female rats have a higher baseline GH level and lower, more irregular, peaks. Injecting Somatostatin into female rats every 3 hours results in a more male-like GH secretion pattern[36]. Moreover, the sexually dimorphic GH pattern corresponds to differences in expression of the hepatic enzyme carbonic anhydras-III[36]. Other evidence for the importance of the pulsatility of GH from studies in rats is that injection of GH seems to acutely down-regulate the GH-R[37], that intermittent administration of GH increases IGF-I more than higher daily doses given as a continuous infusion, and that continuous infusion upregulates GH-Rs in the liver[38].

Figure 3. Possible sites of action for the GH/IGF-I system and GH secretagogues (green lines). Red lines indicate direct or indirect inhibition of GH secretion and blue lines indicate direct or indirect stimulation. See chapter 2.1 for abbreviations and details.

In humans it has been shown that daily injection of GH in children with GHD results in better growth than if the same weekly dose were given in three injections during a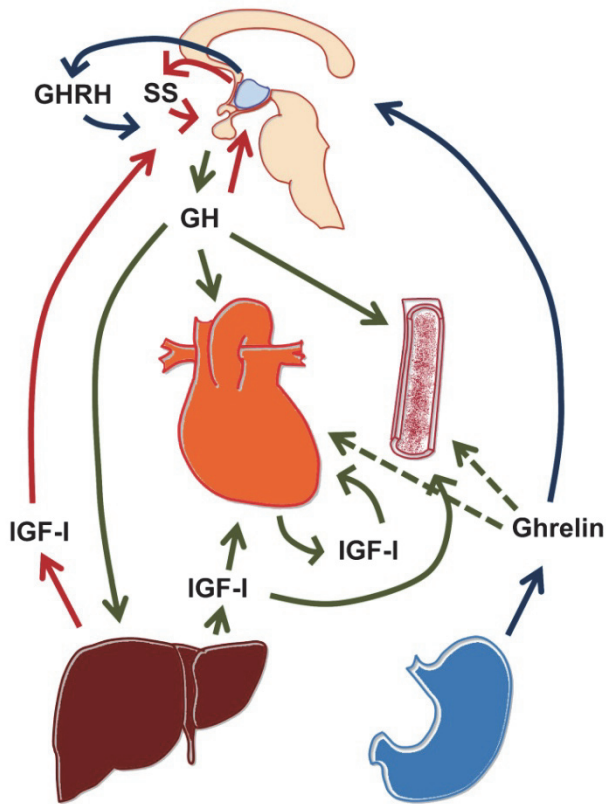 week[39], that high GH peaks are related to a higher growth velocity, and that children growing at the slowest rate had low GH peaks and high trough GH levels[40]. During puberty there are several GH peaks of greater amplitude both day and night[41, 42]. Spontaneous GH secretion can be evaluated using the PULSAR program in both research and clinical settings[43, 44]. This program defines the different variables that are studied in this thesis consistently and in a semi objective manner (Figure 2).

2.1.5   Growth hormone sensitivity

The effects of GH depend not only on the actual level of GH secretion, but also on peripheral tissue sensitivity to GH, as well as the complicated interactions discussed above. In Laron syndrome there is a defect in the GH-R that arises due to a variety of different gene mutations. As a result, children with Laron syndrome have short stature despite high serum levels of GH[45]. This condition is a typical form of GH insensitivity (GHI). Less extreme forms of GHI can be observed in children with short stature who have low growth velocity but may have varying levels of GH secretion, ranging from normal to severely impaired[46].
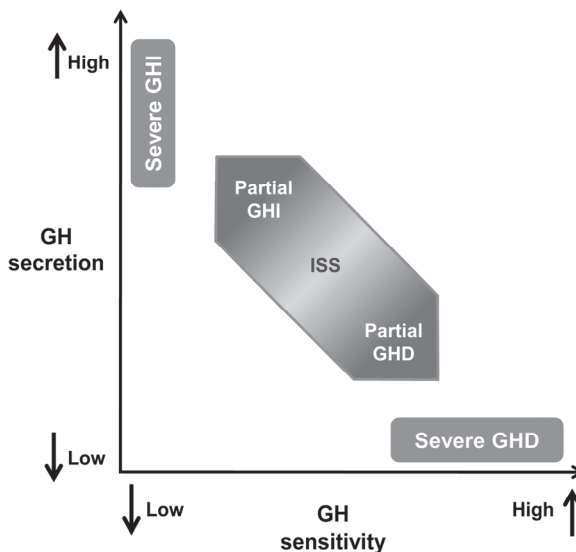

Figure 4. Principal sketch of the relationship between GH secretion and GH sensitivity for different conditions. Children with Laron syndrome (GHI), ISS and GHD are short compared with peers of equal age.

By convention, the adequacy of GH secretion is determined based on peak GH concentrations following two stimulation tests[47]. GHD is diagnosed based on a predetermined cut-off point; however, it is well recognized that the value used is largely arbitrary and that the precise cut-off value has changed with increased availability of exogenous GH[48, 49]. Children with short stature and maximum GH secretion above the agreed cut-off are traditionally considered as having idiopathic short stature [50]. This group is starting to be better characterized, with a growing number showing mutation in the GH-R or defects at the post-receptor level[51, 52]. Figure 4 shows the relation between GH secretion and sensitivity in GHD, ISS and Laron syndrome (referred to as GHI).

2.1.6 Growth hormone and statural growth

GH is important for the maintenance of somatic growth during the childhood period and well into adult life. During the childhood period, GH acts dose dependently and seems to be the most important hormone in this context. During juvenility, adrenergic hormones modulate the effects of GH and during puberty and adolescence, gonadal hormones have an additional impact on growth[53]. During postnatal life and during all growth periods, thyroid hormone and cortisol have permissive effects.

GH/IGF-I axis:
GH is secreted from the pituitary gland in a pulsatile fashion. GH acts both directly on target tissues and through IGF-I produced locally and in the liver. The balance between secretion and peripheral sensitivity determines GH effects. During the childhood period, GH is the main regulator of somatic growth.

2.2 Age-dependent changes in the cardiovascular system

The fact that cardiovascular dimensions and function change with age is obvious. A larger body requires a larger heart. Although several cross-sectional studies of cardiovascular structure and function have been published, our knowledge of the regulation of cardiovascular changes is limited. To put the changes seen during GH treatment into context, I will briefly review the normal development of the heart, focusing on the childhood period where longitudinal growth is most dependent on the GH/IGF-I axis[53, 54].

2.2.1   Blood pressure

During childhood and throughout puberty there is a slow but steady increase in both systolic and diastolic blood pressure (BP)[55]. Average systolic/diastolic BP is about 91/46 mmHg at 1 year of age and 102/63 mmHg at 10 years of age, with no major differences reported between boys and girls. By 17 years of age, BP is slightly higher in boys than in girls (118/64 vs. 110/62 mmHg, respectively) and racial differences have also been reported[56, 57].

2.2.2   Time intervals in the electrocardiogram

Heart rate declines with increasing age. Between 1 and 3 years of age, heart rate is slightly higher in girls than in boys (median 128 bpm vs. 119 bpm, respectively). By 5 to 8 years of age, heart rate is the same in both genders (median 88 bpm) and no big differences are reported during puberty (76 bpm in girls, 73 bpm in boys)[58]. The PR interval increases with age and does not differ between genders. QRS duration also increases with age, but the median duration is slightly longer in boys than in girls. The QT interval increases as heart rate decreases and, thus, QT intervals increase with age during childhood. When corrected using the Bazett formula, QT interval remains stable with age[58].

2.2.3   Left ventricular dimensions

Although LV mass is not commonly assessed in the clinical setting of pediatric cardiology, several reports on LV mass in normal children have been published. In the majority of studies, echocardiography was used to estimate LV mass and a couple of methods have been evaluated[59, 60]. Up to 12 years of age there were no significant differences in LV mass between girls and boys, probably reflecting similarities in height and weight[61]. After 12 years of age, LV mass is greater in boys than in girls, even when indexed to body surface area (BSA)[61]. Different methods have been used to normalize LV mass for body size. As adjusting for BSA introduces an artificial relationship with body size, indexing by dividing with height to the allometric power of 2.7 has been suggested[62]. However, at heights less than 140 cm, this index has a significant negative relationship with height and LV-mass-for-height centile curves have been shown to be superior to LV mass/height$^{2.7}$ in normalizing for body size in shorter children[63] (Figure 5). LV diameter and wall thickness have the same relationship to body size as LV mass. This is not surprising because these variables are usually used for the calculation of LV mass. They are often normalized by the use of standard deviation scores (SDS)[64].
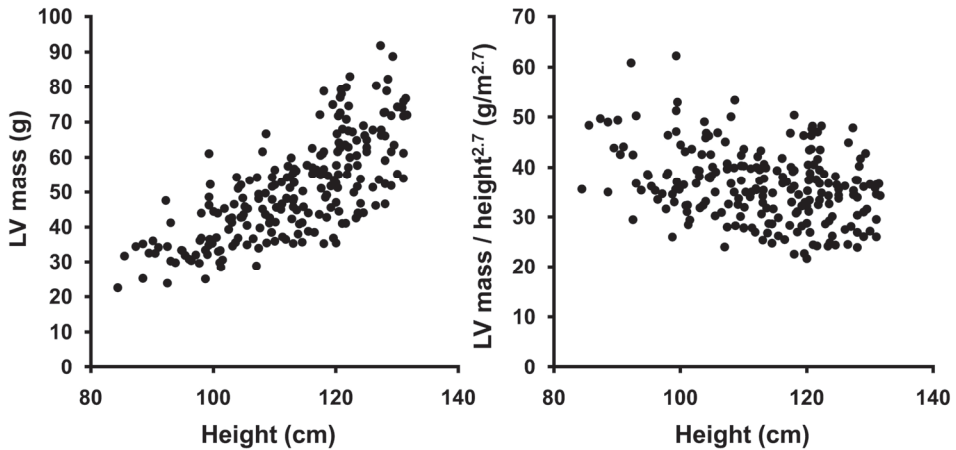
Figure 5. Example of the relationship between LV mass (g) and height (cm) and the overcorrection that occurs when dividing LV mass by height to the allometric power of 2.7.

2.2.4   Cardiac systolic function

The fraction of blood ejected from the LV with each beat, the ejection fraction (EF), varies little over time. Stroke volume (SV) and the volume ejected during one minute, the cardiac output (CO) however varies with body size and age, increasing in a non-linear fashion[65].

2.2.5   Cardiac diastolic function

The general notion is that the diastolic function deteriorates with age. This is not evident in children. Measurements of LV filling pattern, as an estimate of diastolic function, change during childhood in a way that in adult life would constitute an improvement[66]. As an example, the mitral E/A ratio increases from a median of ~1.2 at a BSA of 0.5 m^2 to ~1.7 at a BSA of 0.8 m^2, thereafter, remaining stable during childhood[66].

> *Age-dependent changes in the cardiovascular system:*
> *LV dimensions increase with age. The resulting increase in SV is followed by a reduction in heart rate. LV contractility seems to be independent of longitudinal growth and during the childhood and juvenile period, parameters of diastolic function improve. Although the relationship between body size and cardiac mass has been well established in cross sectional studies, there is no published information on cardiac growth velocity during the different phases of longitudinal growth.*

3 PATIENTS AND METHODS

3.1 Study design

This thesis is based on two projects. The first is called the "Heart biopsy" study and the second the "GH-dose catch-up" study.

3.1.1 Heart biopsy study

Children scheduled for open heart surgery and their parents were approached regarding participation in the study. If consent was given, a biopsy of cardiac tissue was taken at the time of surgery. Biopsies were analyzed using real-time polymerase chain reaction (rtPCR), as described in detail below. Hormonal evaluations, dual-energy X-ray absorptiometry (DXA) scans and study protocol-specific echocardiograms were not conducted in these children.

Table 1. Inclusion and exclusion criteria in the GH-dose catch-up study

Inclusion criteria
Prepubertal
Girls: 3–10 yrs
Boys 3–11 yrs
Short
$Height_{SDS} < -2$ SDS or
Growth velocity < -1 SDS
Shorter than genetic potential
$MPH_{SDS} < -1$ SDS
Not severely premature
GA > 30 weeks
Additional requirements
Height and weight data
At birth, and at 1 and 2 years of age
1-year pretreatment
Two measurements during the pretreatment year
Exclusion criteria
Chronic illness
Clinical syndrome
Extremely underweight or obese
Catch-up growth during pretreatment year
Tall parents ($MPH_{SDS} > 1.5$ SDS)

3.1.2 GH-dose catch-up study

This thesis concerns the cardiovascular assessment of children included in a large, longitudinal, prospective, multicenter study (study number TRN 98-0198-003). The main objective of this trial was to determine whether catch-up growth in individual children with isolated GHD or ISS could be targeted using individualized GH doses rather than a standard dose. Five Swedish centers participated in the study: Gothenburg, Halmstad, Malmö, Umeå and Uppsala. The children were required to be prepubertal at the start of the study, and free from concomitant disorders. Table 1 summarizes the inclusion and exclusion criteria. A maximum of one-third of the children were allowed to have ISS as determined based on the maximum GH peak (GH_{max}) obtained on the arginine–insulin tolerance test (AITT) which was the gold standard method for diagnosing GHD at the start of the study. The children were randomized in a 1:2 fashion to receive either a standard GH dose (43 µg/kg/d) or an individualized GH dose based on GH responsiveness as estimated by a validated prediction model[67].

Table 2. Schedule for dose selection in the group receiving individualized GH doses.

Predicted $\Delta Height_{SDS}$ after 2 years	Predicted distance from MPH_{SDS} after 2 years				
	< -1.2	-1.2 to -0.8	-0.8 to -0.5	-0.5 to $+0.5$	$> +0.5$
< 1.2	100	66	50	33	17
1.2 to 1.8	66	50	40	33	17
> 1.8	50	33	33	17	17

The selected dose is shown in µg/kg/d. The predicted $\Delta Height_{SDS}$ and predicted distance from MPH_{SDS} after 2 years are based on postulated treatment with a GH dose of 33 µg/kg/d. Reproduced from Kriström et al.[46].

By using pretreatment data, the model accurately predicts the 2-year growth response to a GH dose of 33 µg/kg/d with an error of only 0.28 SDS of the residual. The predicted growth response was used, together with the height$_{SDS}$ difference to mid-parental height (MPH) SDS (diffMPH$_{SDS}$) for dose-selection in the individualized group within the range of 17–100 µg/kg/d. The target was to reach MPH_{SDS} after 2 years of treatment (i.e. the lower the GH responsiveness and the greater the distance to MPH_{SDS}, the higher the GH dose given). The mean GH dose used in this group was 49 µg/kg/d (see Table 2).

3.2 Ethical considerations

3.2.1 Heart biopsy study

There were no potential benefits for the individual child from participating in the heart biopsy study. The researcher approaching the family was not involved in the

clinical management of the patients. The technique for taking biopsies was considered safe based on previous publications and the experience of the thoracic surgeons performing the scheduled cardiac surgery[68, 69]. The protocol was approved by the ethical board of the University of Gothenburg (registration number Ö184-01 with final decision 14-Jun-2001) and conducted in accordance with the declaration of Helsinki (initial statement and amendments found on the homepage of the World Medical Association, www.wma.net). Written informed consent was obtained from all parents and from children where possible.

3.2.2   GH-dose catch-up study

At the start of the trial, the indication for GH treatment in short normal children was GHD, as estimated by two stimulation tests. The potential benefit for children participating in the study was that treatment could be undertaken even if they were categorized as non-GH-deficient. In the standard clinical setting, the medical investigation prior to starting treatment is extensive. Additional tests were conducted in children included in this study which could be considered a disadvantage. GH treatment is considered to have a good safety profile at recommended doses. The dose for the fixed-dose group was slightly higher than the standard dose used in Sweden at the start of the trial. Higher GH doses had, however, been used in other groups of children without significant problems. The dose used was also the standard dose used in the USA at the time. The children randomized to receive an individualized dose could be treated with either lower or significantly higher doses than the standard dose. The individualized dose was, however, considered to be "biologically" similar to the standard dose. Adverse events were monitored carefully throughout the study. The protocol was approved by the ethics boards of the University of Gothenburg (for Gothenburg and Halmstad), Umeå, Uppsala, and Lund (registration number L 553-98 with final decision 2-Jun-1999), and by the Medical Product Agency of Sweden. Written informed consent was obtained from all parents and from children where possible. The trial was performed in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

> ***Study design:***
> *Two trials were performed. In the "Heart biopsy study", included children had cardiac biopsies taken during scheduled open heart surgery. In the "GH-dose catch-up study", short prepubertal children were randomized to receive either a standard GH dose or an individualized dose based on a validated prediction model and taking predicted distance from target height into consideration. The studies were approved by the appropriate ethics boards and the Medical Product Board of Sweden. The Declaration of Helsinki was honored.*
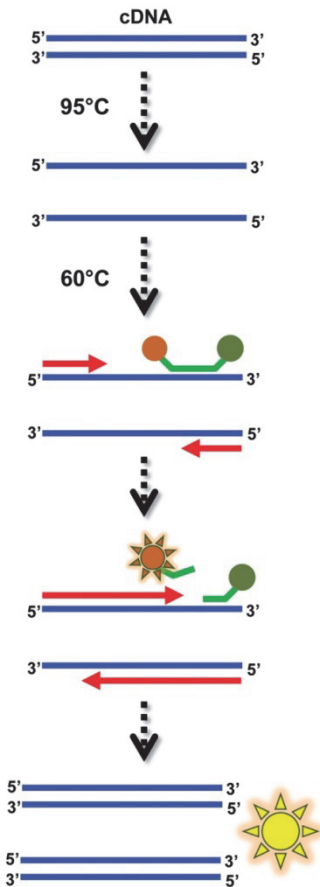
3.3 Methods

3.3.1 Collection and preparation of biopsies

During open heart surgery, catheters are used to divert blood from the heart and return it to the system circulation after oxygenation. A single transmural right auricular biopsy was taken at the time of venous catheterization. Biopsies were promptly frozen in liquid nitrogen and stored at $-70°$ C during the sampling period. All biopsies were analyzed simultaneously. Management of tissues was conducted in a DNA:se free environment.

3.3.2 Real-time polymerase chain reaction

During real-time polymerase chain reaction (rtPCR), cDNA is duplicated in the presence of a signaling probe. When the concentration of the probe is high enough it is detected and the signal intensity increases with increasing amounts of cDNA. The more cDNA there is in the initial sample, the earlier the signal intensity reaches a set threshold (see Figure 6). Primer Express Software (Applied Biosystems, Inc. Foster City, California, USA; currently Life Technologies, Carlsbad, California, USA) was used for the design of pairs of primers and probes from the human mRNA sequence. Sequences used for forward, reverse primer and probe are given in Paper I. Primer and probes were designed for GH-R and IGF-I mRNA. The primers and TaqMan probes were synthesized by Applied Biosystems, Inc. Total mRNA was prepared with TRIZOL® Reagent (Invitrogen, Carlsbad, California, USA, currently Life Technologies, Carlsbad, California, USA), using a standard protocol.

Figure 6. Schematic illustration of one cycle in a real-time polymerase chain reaction. Initially the cDNA is separated by heating to 95°C. Lowering the temperature allows the probe to attach and the primers to initiate replication which will cleave the probe activating a signaling unit. The cycle will result in two cDNA strands instead of one, and the number of cycles needed for the signal to be detected is proportional to the initial quantity of cDNA in the sample.

After cDNA synthesis, TaqMan One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Inc.) was used for rtPCR. As internal standard, 18S rRNA was used. Because of interference, multiplex PCR was not possible and GH-R and IGF-I were analyzed separately. Amplification and detection were performed using the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Inc.). One sample from each patient was analyzed in duplicate. Relative expression of mRNA was calculated with the comparative Ct method, and results were expressed as multiples of the lowest value.

3.3.3 Auxology measurements

Height was measured using a standing Harpenden stadiometer. The mean of three measurements was used. Height was converted into SDS using the prepubertal childhood component[70] of the total reference[71]. Weight was measured using weighing scales with an accuracy of ±0.1 kg. Weight$_{SDS}$ was calculated using the reference population from Albertsson-Wikland et al.[71]. Body mass index (BMI) was calculated using the formula BMI = Weight (kg) / Height^2 (m^2), and converted to SDS[72]. Target height was estimated using MPH in a linear function, as previously described[73], and using reference data from an earlier cohort born in 1956, thus taking the secular trend into account[74]. Body surface area (BSA) was calculated using Mosteller's simple formula BSA = (height(cm) x weight(kg) / 3600)$^{0.5}$.[75, 76]

3.3.4 Cardiovascular assessment

Cardiovascular assessments included serial echocardiograms, electrocardiograms (ECG) and BP measurements. Examinations were performed before study start, after 3 months of treatment, after 1 and 2 years of treatment. Each individual child was examined longitudinally by the same cardiologist/sonographer. The examinations in Umeå, Uppsala, Halmstad and Lund were performed by one pediatric cardiologist at each site. In Gothenburg, the examinations were performed by a single experienced sonographer.

Measured variables

Systolic and diastolic BP was measured in the supine position using a DynaMap system. Heart rate, PR interval, QRS duration and corrected QT (QTc) interval were automatically measured from the ECG. The following M-mode (MM) variables were measured: interventricular septal thickness in diastole (IVSd), LV inner diameter in diastole and systole (LVDd/LVDs) and LV posterior wall thickness in diastole (LVPWd). From two-dimensional pictures, the diameter of the aortic annulus was measured from the parasternal long-axis view. The outer

and inner areas of the left ventricle were measured from the parasternal short-axis view at the level of the papillary muscles, and the length of the left ventricle was measured from the apical four-chamber view. The flow pattern over the mitral valve was recorded using pulsed-wave Doppler.

Table 3. Formulae for the calculation of cardiac variables

LV mass by M-mode method (LV mass MM)		**Ref.**
LV mass MM $=$	$0.8 \times 1.04 \times ([IVSd + LVDd + LVPWd]^3 - LVDd^3) + 0.6$	59
LV volume in diastole (LVVd)		
LVVd $=$	$\dfrac{7LVDd^3}{(2.4 + LVDd)}$	77
LV volume in systole (LVVs)		
LVVs $=$	$\dfrac{7LVDs^3}{(2.4 + LVDs)}$	77
Stroke volume (SV)		
SV $=$	$LVVd - LVVs$	
Fractional shortening (FS)		
FS $=$	$\dfrac{LVDd - LVDs}{LVDd}$	
Cardiac output by M-mode method (CO MM)		
CO MM $=$	$SV \times heart\ rate$	
Corrected mean velocity of circumferential shortening (mVCFc)		
mVCFc $=$	$\dfrac{FS}{ET/\sqrt{R - R\ duration}}$	78
Cardiac output by Doppler and 2D method (CO Doppler)		
CO Doppler $=$	$\dfrac{\pi(Aortic\ diamter)^2}{4} \times VTI \times heart\ rate$	
LV mass by area–length method (LV mass AL)		
LV mass AL $=$	$1.055 \times \dfrac{5}{6} \times (LV\ Outer\ area \times [LV\ length + 1] - LV\ Inner\ area \times LV\ Length)$	60
Mitral E/A ratio		
Mitral E/A ratio $=$	$\dfrac{Mitral\ E\ wave\ velocity}{Mitral\ A\ wave\ velocity}$	

From pulsed wave Doppler in the LV outflow tract, the peak velocity, velocity time integral (VTI), ejection time (ET) and pre-ejection period were measured. Measurements during three consecutive cardiac cycles were averaged to minimize the impact of respiratory variations. Calculation of additional variables was done using formulae given in Table 3.

Normalization of cardiovascular variables for body size

BP and variables associated with cardiac growth are closely related to height and body composition as described in the *Background* section. As GH treatment promotes growth and alters body composition, it is difficult to discern specific effects of GH on the heart in growing children. Most variables were therefore normalized either by indexing them to body size (BSA or height) or by calculating an SDS based on published values. BP was normalized using the formulae provided by Rosner et al.[56], M-mode measurements using the formula by Lester et al.[64], LV mass according to M-mode using the formula by Foster et al.[63] and LV cardiac index using the formula by De Simone et al.[65]. The references used for the SDS of the cardiac variables are summarized in Table 4. QTc was calculated according to the formula by Bazett[79].

Table 4. Measured variables and references used for calculation of SDS

Variable	Ref.	Children (n)	Ages	Predictors
Blood pressure	56	20 225 to 28 664 per subgroup	1 to 17 yrs	Gender
				Height$_{SDS}$
				Age
IVSd	64	202	0 to 23 yrs	Gender
LVDd				Age
LVDs				Height
LVPWd				Weight
				Race
				Heart rate
LV mass MM	63	440	0 to 21 yrs	Height

3.3.5 Laboratory measurements

GH, IGF-I, IGFBP3 and leptin were analysed at the Göteborg Pediatric Growth Research Center (GP-GRC) laboratory (Swedac accredited no.1899)[80-82]. GH was analyzed using a monoclonal assay and international reference preparation (IRP) 80/505. The detection limit for the kit was 0.03 mU/L. At concentrations of 0.4, 5.1 and 21.1 mU/L, the intraassay variations (% coefficient of variation (CV)) were 5.1, 2.7 and 2.2 %, respectively, and the inter-assay variations (% CV) were 2.5, 2.1 and 1.4%, respectively. SDS were calculated for IGF-I, IGFBP3 and for the IGF-I/IGFBP3-ratio[83, 84]. Alkaline phosphatase (ALP), fasting insulin and

fasting glucose levels were assessed at the accredited university hospital laboratories. Insulin resistance was estimated by homoeostasis model assessment (HOMA), using the formula of Matthews et al [(fasting serum insulin × fasting plasma glucose)/22.5].[85]

3.3.6 GH secretion pattern and rate

GH secretion was assessed using both the AITT and a 24-hour GH secretion profile. The AITT was performed by administering arginine, sequentially followed by insulin, as described by Penny et al.[86]. The highest GH measurement obtained during the two provocation tests ($GH_{max}AITT$) was used. The 24-hour GH secretion profile was assessed by taking integrated blood samples every 20 minutes for 24 hours. This method has been described in detail by Albertsson-Wikland et al[43]. GH levels were measured and the results were analyzed by the PULSAR program with settings for GH[43, 44]. The following variables were used: maximum peak amplitude ($GH_{max}24h$), average peak amplitude (avPeak), total area under the curve (AUCt), area under the curve above the baseline level (AUCb), baseline GH level, number of peaks, length of peaks.
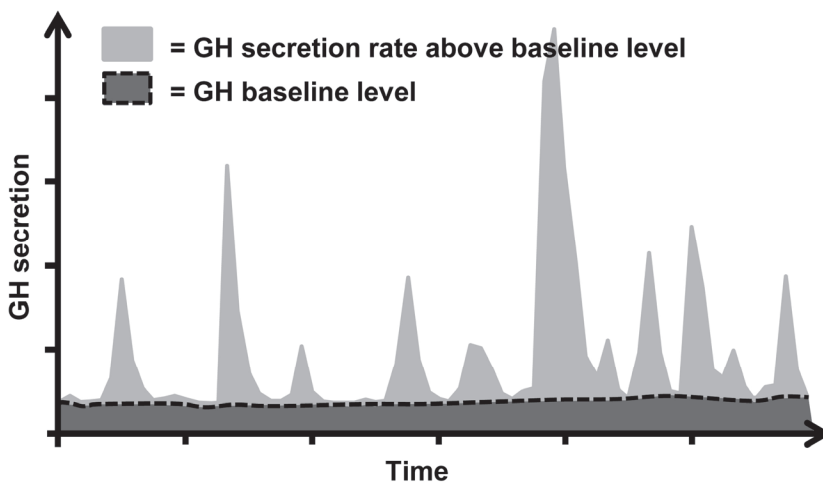
Figure 7. Schematic drawing showing the baseline GH level (the GH trough level) and the GH secretion rate above baseline level (GHb).

The measured concentrations of GH during the 24-hour GH secretion profile are dependent on both pituitary secretion and plasma clearance. An algorithm for estimating the actual pituitary secretion rate has previously been developed through single-injection kinetic studies. The formula includes cumulative secretion and body mass (weight) and was used to calculate total GH secretion rate (GHt) over zero-line and GH secretion rate above the baseline level (GHb), see example in Figure 7. [87]

3.3.7 Classification of GH status

Initial diagnostic classification of children as having GHD or ISS was made based on the results of the AITT which was the gold standard method at the start of the study [88]. The cut-off value for the diagnosis of classic GHD was a $GH_{max}AITT$ of <22.6 mU/L (monoclonal dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA)) using IRP 80/505, corresponding to a cut-off value of <32 mU/L for a polyclonal assay and to <20mU/L (<10 µg/L) when using polyclonal antibodies and IRP 66/127[80]. Severe GHD was defined as a $GH_{max}AITT$ of <11.3 mU/L for comparison with data from Capalbo et al. and Salerno et al.[89, 90]. Alternative classification of GHD was done using the GH_{max} from either the 24-hour GH-secretion profile ($GH_{max}24h$) or by combining $GH_{max}24h$ with $GH_{max}AITT$.

3.3.8 Evaluation of GH responsiveness

Individual GH responsiveness was estimated using a validated model for predicting the 1- and 2-year growth response to a standard GH dose (33µg/kg/d)[67]. By using pretreatment data, the residual standard deviation (SD) of the model is 0.19 SDS for the 1-year growth response and 0.28 SDS for the 2-year growth response. The algorithm includes the variables MPH_{SDS}, gender, $weight_{SDS}$ at birth, $height_{SDS}$ at 1 and 2 years of age, $\Delta height_{SDS}$ during the year before GH start, $height_{SDS}$ at GH start, age at GH start and $GH_{max}24h$. The predicted growth response ($\Delta height_{SDS}$ expected after the first and second years on GH treatment) is an indirect estimate of individual GH responsiveness for longitudinal growth.

3.3.9 Measurement of body composition

Body composition was measured by DXA, using either Lunar DPX-L scanner (GE Medical, Madison, WI, USA) or a Lunar Progidy (GE Medical). Each child was measured longitudinally using the same settings. DXA assessment results in a three-compartment model of the body consisting of fat mass, lean soft tissue (LST) mass and bone mineral content (BMC). All analyses were conducted using the extended analysis program for total body analysis with pediatric settings. LST_{SDS} was calculated according to Dutch normative data[91, 92].

3.3.10 Control group

Finding a representative control group with which to compare short prepubertal children is not without problem. The children in the study population are all very short for age and gender (< –2 SDS). Stratifying a normal control group according to age would result in the selected children being significantly taller than the study population, whereas stratifying them for height would make them significantly younger than the study population. As height seems to be the most important factor determining cardiac dimensions, it is usually used for stratification[63, 89]. However, this leaves a question regarding the impact of both age and growth

velocity on cardiac dimensions. The control group in Paper II were drawn from an institutional reference population that was under creation. Echocardiographic examinations, ECGs and BP measurements from children without heart disease were re-analyzed by a single pediatric cardiologist in a retrospective fashion. The sample was strictly stratified by height and gender.

3.4   Statistical considerations

Distribution

The distribution of values was assessed in order to select the most informative presentation method and to identify the appropriate statistical method. The likelihood that a set of data were normally distributed was examined by studying frequency histograms and normal probability plots. The data were also tested using the Kolmogorov–Smirnov test with Lilliefors correction and the Wilk–Shapiro test, and finally by calculation of skewness and kurtosis. If normally distributed, data were presented as mean ± SD, otherwise data were presented as median (25th–75th percentile). In Paper II, median (25th–75th percentile) was used for consistency and non-normality was indicated by a double dagger. Parametric or non-parametric statistical methods were chosen, as appropriate.

Transformations

In the case of multiple linear regressions and principal component analysis (PCA), non–normally distributed data were transformed successfully. Weight, LV mass MM, CO, mVCFc, mitral E/A ratio, insulin, HOMA and GH responsiveness were transformed using the natural logarithm (ln). $GH_{max}AITT$, $GH_{max}24h$, avPeak, baseline GH level, GHt and GHb were transformed using the square root (sq). Leptin was inverted (inv) and presented as a negative inverted value to restore the original order (–inv).

Correction for multiple comparisons

Performing multiple comparisons between groups will inevitably increase the rate of type I error. Correcting for this will on the other hand increase the rate of type II error to the extent that important information is lost or hidden. Consequently, data were presented without correction. If the effect of multiple comparisons was considered important it was pointed out in the results section.

Comparison between groups

Comparisons between groups were conducted using independent sample *t*-tests, paired sample *t*-tests or Mann–Whitney U-tests, as appropriate. A *p*-value less than 0.05 was considered statistically significant.

Bivariate correlations

Pearson's product moment correlation was calculated for normally distributed variables. Otherwise Spearman's rank order correlation was used.

Linear regression

The Lasso (Least absolute shrinkage and selection operator) method was used for the multiple linear regression analysis, and the models were validated with permutation tests. Residuals were required to be normally distributed and data were otherwise transformed.

S-shaped piecewise linear regression and Effective dose at 50% effect

In Paper IV the dose–response relationship was examined for the GH effect on different organ systems. To mimic the S-shaped curve often observed in biological systems, S-shaped piecewise linear regression models were fitted with GH dose as the predictor variable and the Δ-value of the metabolic, cardiac and body composition variables as response variables. The piecewise linear regression consisted of three parts, a horizontal head and tail and a linear piece in the middle. Details are given in Paper IV. The effective GH dose predicted to result in 50% Δ effect (ED50%) was calculated with a 90% confidence interval. A one-way analysis of variance (ANOVA) with GH dose as a bounded continuous predictor was performed to test the piecewise linear GH effect. A non-parametric comparison of group means (robust test of equality of means – Welch test and Brown–Forsythe test) was conducted when variances of dependent variables were not equal across groups. To examine the influence of the 17 µg/kg/d dose group that consisted of only three children, analyses were repeated with these children excluded. Only data that were consistently significant were reported.

Principal component analysis

PCA is a powerful tool, both for reducing a large set of data and for describing complex interactions[93]. In Paper II, cardiac variables were analyzed together with age, height$_{SDS}$, weight$_{SDS}$, GH responsiveness, GH-secretion and metabolic variables. In the PCA, extraction was based on Eigenvalue >1. To simplify the interpretation of data, loadings were rotated using the Varimax method[94]. Missing data were handled by replacing with the mean. For clarity, a few variables have been selected for graphical presentation in this thesis. More detailed results are presented in Paper II. Variables at 90° relative to each other are not correlated with each other; variables pointing in the same direction are strongly and positively correlated with each other, and variables at 180° to each other are strongly and negatively correlated with each other. The length of the line and its projection against the X or Y axis describes how strong the variable is in the component. The percentage of variance explained by the models and different components were taken from the rotation sums of square loadings.

Software

In addition to SPSS 17.0 (SPSS Inc., Chicago, USA), MATLAB version 7.13.0 (R2011b, The Mathworks, Natick, MA, USA) was used for the calculations.

Methods:
Cardiac biopsies were analyzed using rtPCR to estimate the relative expression of GH-R mRNA and IGF-I mRNA. Children in the GH-dose catch-up study were examined using ECG, echocardiography, DXA, and blood pressure, GH secretion and metabolic data were also assessed. Great care was taken to use appropriate statistical methods. Acknowledging the complexity of these data, advanced statistical methods were used in a descriptive manner (referring to PCA, piecewise linear regression and the Lasso method).

4 RESULTS

This section is both a complement to and a summary of the original papers. Detailed information is repeated only when needed for clarity or when it provides additional information

4.1 Heart biopsy study – Paper I

Eighteen children with a variety of cardiac diseases were included in the study. One child were put on cardiac bypass because of resection of a tracheal stenosis, the rest had scheduled open heart surgery. There was a wide range in ages and diagnosis of the children, summarized in Table 5 (for full detail, see Paper I, Table 1).

Table 5. Patient characteristics and results in the heart biopsy study

Group	Age (y)	Gender	Cardiac diagnosis	Length (cm)	Weight (kg)	GH-R mRNA	IGF-I mRNA
Cyanotic infants	0.02	M	TGA	55	3.5	2.49	2.36
	0.02	M	DILV, TGA, CoA	53.5	3.5	1.45	1.00
	0.12	F	TA	51.5	3.44	3.69	5.18
Infants with PHT	0.06	F	Truncus	54	4.04	5.12	6.01
	0.23	M	VSD	55	4.05	2.11	7.97
	0.29	F	VSD	53.5	3.71	1.02	1.99
	0.35	M	VSD	62.5	5.18	2.26	2.03
Children with UVH*	3.3	F	TA	98	16.3	27.36	31.54
	4.1	F	TA	95.5	13.3	1.36	1.34
Volume-loaded RA/RV	3.2	F	PAPVD	99	15.2	11.22	3.24
	3.9	M	ASD	102	15	1.00	1.16
	4.0	M	PAPVD	110	15.7	1.20	1.50
	5.8	M	ASD	123.5	27	5.42	2.95
Mixed group	0.35	F	ToF	69.5	8.56	2.99	4.14
	0.48	M	ToF	63	6.6	2.23	1.82
	1.9	F	CoA	86	12.3	5.04	7.37
	3.8	M	TrS	104	19.3	5.08	5.51
	15.8	M	VSD	185	72	7.31	12.67

GH-R mRNA and IGF-I mRNA are expressed as multiples of the lowest value.
* Biopsies taken at time of Fontan completion. See abbreviation list for details.

Both GH-R mRNA and IGF-I mRNA were detected in biopsies from all children. The relative amounts are displayed in Table 5. Expression of GH-R mRNA and IGF-I mRNA was strongly correlated (r=0.75, $p<0.001$). In Paper I, Figure 1, the correlation is shown for all patients. In Figure 8, below, the outlier has been excluded and the correlation is still preserved (r=0.71, $p<0.001$). The correlations

of GH-R and IGF-I mRNA with age, length, weight, BMI, BP, gender and the occurrence of cyanosis were also studied. GH-R mRNA correlated positively with weight$_{SDS}$ (r=0.65, p=0.004), BMI (r=0.59, p=0.01) and BMI$_{SDS}$ (r=0.59, p=0.01). IGF-I mRNA only correlated with BMI (r=0.50 p=0.04). No correlations were found between GH-R or IGF-I mRNA and age, length, weight, BP, gender or the occurrence of cyanosis.
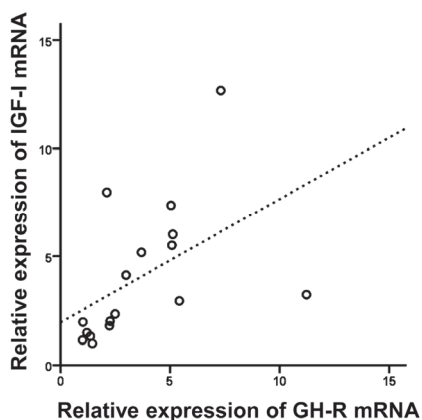

Figure 8. Gene expression is presented as multiples of the lowest value. Excluding the most extreme outlier, the Spearman rank order correlation was still significant (r=0.71, p<0.001).

Results in the heart biopsy study:
GH-R mRNA and IGF-I mRNA were detected in all children studied. The relative amounts were correlated with each other (r=0.75, p<0.001). Expression of GH-R mRNA was correlated with BMI and BMI$_{SDS}$. Expression of IGF-I mRNA was correlated with BMI.

4.2   GH-dose catch-up study. Papers II–IV

A total of 153 children from five pediatric units in Sweden [Gothenburg (n=79), Umeå (n=34), Uppsala (n=17), Malmö (n=13) and Halmstad (n=10)] were included. At 2 years, 128 children (38 girls) had completed the protocol and remained prepubertal, constituting the per-protocol population (Papers III, IV). Sixteen children were excluded because they had entered puberty and four because of poor compliance with GH injections. One was found to have a cranio-pharyngioma and one juvenile rheumatoid arthritis. These two patients were from Gothenburg and were excluded from the analysis in paper II. An additional three children were wrongly included. In addition, data from a child who did not

undergo baseline cardiac evaluation due to a protocol violation was excluded from the analysis in Paper III. The gender of the children who were excluded and the reasons for exclusion were comparable between the randomization groups. In Paper II, only the pretreatment data of the Gothenburg population were studied to avoid inter-observer variability. Inter-observer variability was not considered a problem in the longitudinal analysis of data in Paper III and IV, as only changes in the individual child over time were studied and each child was examined by a single echocardiographer/cardiologist. The study population is summarized in Figure 9.
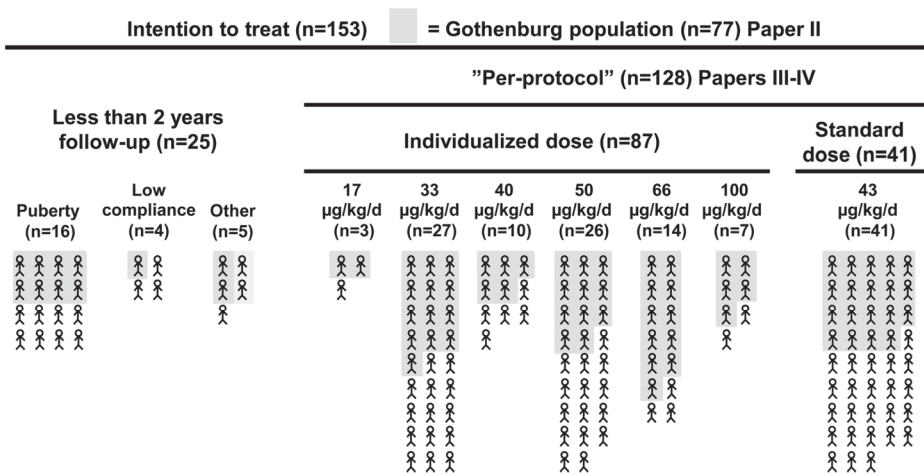
Figure 9. Summary of the GH-dose catch-up study population.

4.2.1 Comparison between stimulation test and spontaneous GH secretion

Maximum GH concentration

In the intention to treat population (n=153), there was a weak correlation between maximum GH concentration during stimulation test ($GH_{max}AITT$) and maximum GH concentration during spontaneous 24-hour GH secretion ($GH_{max}24h$) (r=0.30, $p<0.001$, Figure 10). The number of children defined as being severely GH-deficient, partially GH-deficient or as having ISS will depend on which of the two diagnostic methods is being used. Combining them will result in a different classification (Table 6). The same was found for the Gothenburg population (see Paper II, Figure 1).

Additional information from spontaneous secretion

As discussed in the *Background* section, the secretion of GH has a complex pulsatile pattern. If only the peak GH concentration is taken into account, a great deal of available information will be ignored. This includes the number of peaks, the trough GH level and the GH secretion rate.

Table 6. Number of children in each category depending on method of diagnosis.

n = 153	Severe GHD <5 µg/L*	Partial GHD 5-10 µg/L*	ISS >10 µg/L*
$GH_{max}AITT$	38	73	42
$GH_{max}24h$	14	50	89
$GH_{max}AITT+24h$	9	43	101

* < 5 µg/L corresponds to < 11.3 mU/L, > 10 µg/L corresponds to > 22.6 mU/L, adjusted for the standard and antibodies used (see paragraph 3.3.7)
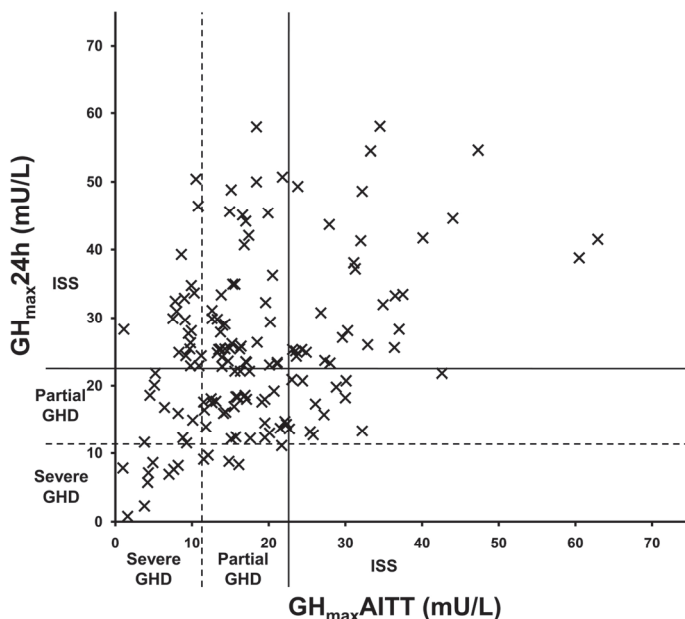

Figure 10. Correlation between $GH_{max}AITT$ and $GH_{max}24h$. Dotted lines mark the cut-off value below which severe GHD is diagnosed. The area between the dotted and solid lines gives the range of values that are considered to warrant a diagnosis of partial GHD.
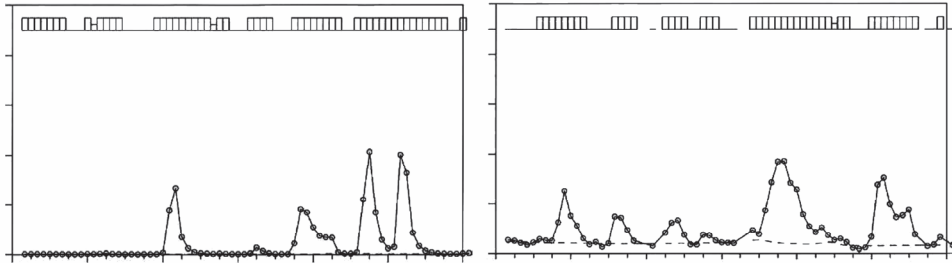
Figure 11. Differences in the GH trough level found during spontaneous secretion for two children with similar peak GH values. The two children had similar Height$_{SDS}$ (–3.0 SDS and –2.9 SDS, respectively) but LV mass$_{SDS}$ differed substantially (–0.2 SDS and –2.4 SDS, respectively), with the higher GH trough level being associated with a lower LV mass.
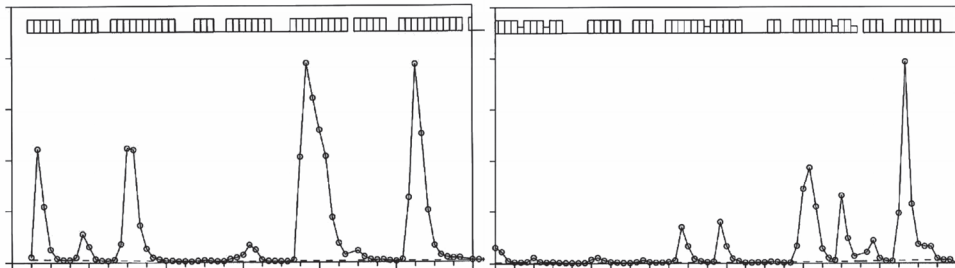
Figure 12. Differences in AUCt found during 24 hours spontaneous profile in two children with similar GH$_{max}$24h. LV mass$_{SDS}$ was similar in these two children (0.1 SDS vs. 0.1 SDS).

Figure 11 shows examples of two patients from the GH-dose catch-up study with similar GH$_{max}$24h and differences in the trough GH levels. Figure 12 shows two other patients from the study with, similar GH$_{max}$24h but the AUCt is almost doubled in the child shown in the left panel (127 mU/(L × 24h) vs. 65 mU/(L × 24h), respectively).

> ***General results from the GH-dose catch-up study:***
> *The correlation between maximal stimulated and spontaneous GH concentration was weak (r=0.30, p<0.001). Classification of severe GHD, partial GHD and ISS is highly dependent on the method being used. Only looking at peak GH concentrations to assess GH levels means that important information from the spontaneous GH secretion pattern is missed.*

4.2.2 Cardiovascular findings according to degree of GHD or ISS – Paper II

Table 7 gives a short summary of the characteristics of the Gothenburg population.

Table 7. Characteristics of the Gothenburg study group. Paper II.

	Mean	SD	Median	Min	Max
Auxology					
Age (y)	7.4	2.0	7.1	3.3	10.9
Height (SDS)	–2.7	0.4	–2.6	–4.1	–1.9
Weight (SDS)	–2.2	0.9	–2.2	–4.1	1.3
Stimulated GH secretion					
$GH_{max}AITT$ (mU/L)	18.2	9.8	16.4	3.8	44.0
Spontaneous GH secretion					
$GH_{max}24h$ (mU/L)	23.8	11.3	23.3	2.3	58.1
Baseline GH level (mU/L)	0.76	0.51	0.63	0.08	2.77
Number of peaks	9	2	9	3	14
AUCb (mU/(L*24h))	74	34	70	3	167
GH secretion rate					
GHb (U/24h)	0.24	0.13	0.22	0.01	0.64
GHt (U/24h)	0.30	0.14	0.27	0.10	0.72
GH growth responsiveness					
Predicted Δheight (SDS)	1.2	0.3	1.2	0.8	2.3
Cardiovascular variables					
Systolic BP	102	9	101	79	127
IVDs (mm)	5.7	0.8	5.6	4.2	7.7
LVPWd (mm)	4.8	0.7	4.8	3.6	7.0
LVDd (mm)	36.5	3.3	36.6	27.5	42.7
LV mass MM (g)	48	13	45	24	80
LV mass MM index (g/m^2)	61	11	59	40	87
EF (%)	69	5	69	55	81
CO MM (l/min)	3.3	0.6	3.2	2.3	4.9
Mitral E/A ratio	2.0	0.5	2.0	1.1	3.3

The control population had a median age of 5.1 years compared with 7.1 years for the study population ($p<0.001$). This was as expected based on the stratification of the groups for height (cm). Heart rate was higher in the control group (median 92 bpm vs. 86 bpm, respectively) and EF was lower in the control group (median 65.0 vs. 68.7, respectively, $p<0.001$) than the study population. No other differences were found. The differences between the subgroups of short children were dependent on whether they were classified according to GH_{max} during a stimulation test, during spontaneous secretion or both. The only consistent finding was a higher EF in the children with GHD and ISS compared with controls (Table 8).

Table 8. Different methods for estimating GH_{max} gives different results when comparing patient groups.

	GHD	ISS	Controls	GHD ISS	Controls vs. GHD	ISS
Height (cm)	Median			P-values		
GH$_{max}$AITT	111.2	112.2	111.3			
GH$_{max}$24h	109.4	111.3				
GH$_{max}$AITT+24h	111.8	111.2				
BMI (kg/m^2)						
GH$_{max}$AITT	15.6‡	14.8	15.3	0.019		0.049
GH$_{max}$24h	15.5‡	15.2				0.049
GH$_{max}$AITT+24h	15.7‡	15.2		0.036		
Systolic BP						
GH$_{max}$AITT	101	101	104			
GH$_{max}$24h	102	99				0.032
GH$_{max}$AITT+24h	104	100				0.037
LV mass MM index (g/m^2)						
GH$_{max}$AITT	56.7	63.8	58.1			0.017
GH$_{max}$24h	58.0	59.1				
GH$_{max}$AITT+24h	56.8	59.3				
EF (%)						
GH$_{max}$AITT	69.8	65.5	65.0	0.004	<0.001	
GH$_{max}$24h	69.2	68.2			<0.001	0.004
GH$_{max}$AITT+24h	70.1	67.6		0.037	<0.001	0.010

Further sub-classification according to GH$_{max}$AITT was done to compare with results from Capalbo et al. and Salerno et al.[89, 90]. They both found a significantly lower LV mass in the subgroup with severe compared with partial GHD classified according to GH$_{max}$AITT. Although including a larger sample size, the difference in the present study was not significant (severe GHD 57.8 g/m^2 vs. partial GHD 60.5 g/m^2, p=0.379).

Classification of GHD or ISS and the heart:
In the population of short prepubertal children, cardiac dimensions were similar in those children classified with severe GHD and ISS. EF (%) was slightly higher in the GH-deficient group compared with the ISS group, but only if classification was based on GH$_{max}$AITT. No differences in cardiovascular structure or function were found between those with severe and partial GHD.

4.2.3 GH secretion, GH growth responsiveness, metabolic data and the heart – Paper II

Bivariate correlation

Several of the GH-related and metabolic variables showed significant correlation with cardiac variables, selected examples are shown in Table 9.

Table 9. Pearson bivariate correlation with selected, GH, metabolic and cardiac variables

	IVSd	LVDd	LV mass MM (ln)
Auxology			
Age	0.46***	0.79***	0.74***
Height$_{SDS}$	ns	ns	ns
Weight$_{SDS}$	0.29*	0.29*	0.38***
Stimulated GH secretion			
GH$_{max}$AITT (sq)	ns	ns	ns
Spontaneous GH secretion			
GH$_{max}$24h (sq)	ns	ns	ns
AUCb (sq)	ns	ns	ns
Baseline GH level (sq)	-0.28*	-0.37**	-0.41***
Number of peaks	ns	-0.23*	-0.24*
Duration of peaks	0.30**	0.29**	0.32**
GH secretion rate			
GHb (sq)	0.30**	0.46***	0.44***
GHt (sq)	0.23*	0.39***	0.35**
GH growth responsiveness			
Pred. Δheight$_{SDS}$ (ln)	-0.23*	-0.36**	-0.34**
Metabolic data			
IGF-I/IGFBP3 ratio$_{SDS}$	0.23*	0.30**	0.30**
Insulin (ln)	0.27*	0.38***	0.40***

*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

After inserting all of the GH-related variables and metabolic variables, together with Weight$_{SDS}$ and Height$_{SDS}$, into a multiple regression analysis (Lasso method) with LV mass MM (ln) as the dependent variable, three variables were found to predict most of the variance. GHb (sq), Baseline GH levels (sq) and Weight$_{SDS}$ together had an r^2 of 0.46, thus, explaining almost 50% of the variance in LV mass MM (ln). There were, however, high degrees of collinearity. Age, for example, was correlated with LV mass MM (ln) (r=0.74, $p<0.001$), baseline GH level (sq) (r= –0.47, $p<0.001$) and GHb (sq) (r=0.43, $p<0.001$).

Principal component analysis

PCA was performed in order to help understand the network of associations between cardiac variables, auxology, GH secretion, GH growth responsiveness and metabolic data. The full rotated component matrix can be found in Paper II, Table 3. In brief, nine components were extracted. Figure 13 shows selected examples from the first two components. LVDd and LV mass were highly correlated with age (first component) but not at all with GH secretion as measured by $GH_{max}24h$ or AUCt (second component). For these two dimensions, the baseline GH level was negatively correlated with the cardiac dimension component (loading of −0.42). This component was also negatively correlated with GH growth responsiveness (loading: −0.38), in contrast to the correlation with GHb (loading: 0.29). The component of LV wall thickness is shown together with the GH secretion component in Paper II, Figure 2, lower section.
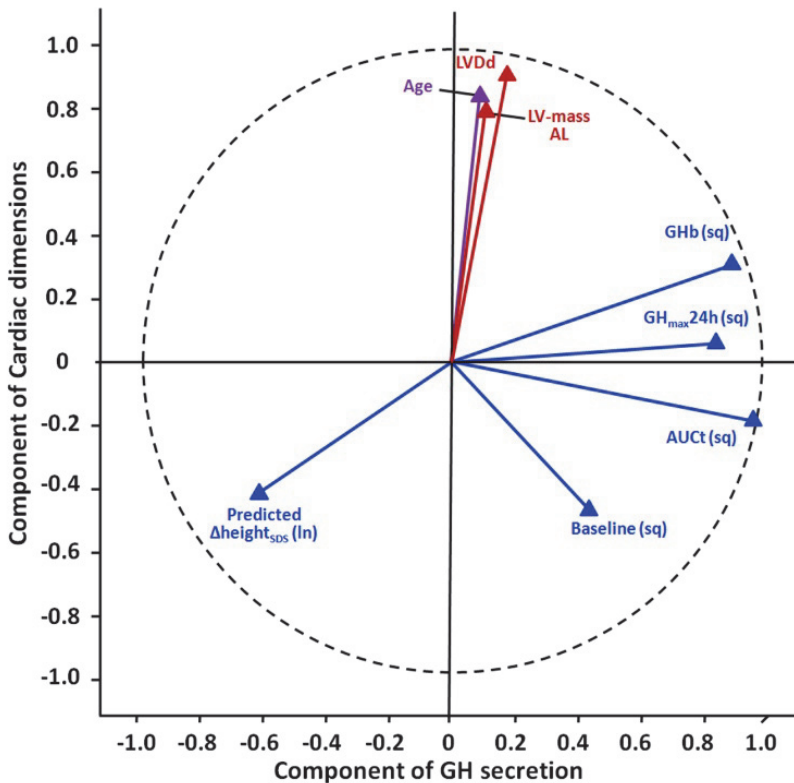

Figure 13. Selected variables from the component of cardiac dimensions and the component of GH secretion. LVDd and LV mass AL is negatively correlated with baseline GH level (GH trough levels) and positively correlated with GH secretion rate above the baseline level (GHb).

There were no correlations between estimates of GH secretion and LV wall thickness. The $GH_{max}AITT$ had a rotated loading of –0.34 in the component of contractility and diastolic function but, otherwise, cardiac function was not correlated with different estimates of spontaneous GH secretion pattern, GH growth responsiveness or metabolic variables (see Paper II, Table 3 for details).

4.2.4   Effect of gender – Paper II

In the study population, the girls were of a similar height (cm) to the boys (median 111.8 vs. 110.2, respectively, corresponding to –2.6 SDS). However, the girls had a lower LVDd (mm) (median 35.9 vs. 37.5, respectively, $p=0.023$) and a lower LV mass MM index (g/m^2) (54.9 vs. 61.1, respectively, $p=0.028$) compared with the boys (Figure 14). In the control group, no differences were found between the genders.
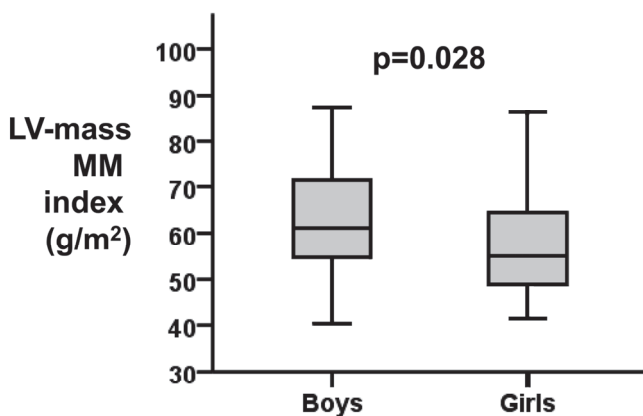
Figure 14. Minor gender differences in LV mass MM index (g/m^2).

Spontaneous GH secretion pattern, GH secretion rate and the heart:
PCA analysis showed that LVDd and LV mass was negatively correlated with GH trough levels and positively correlated with GH secretion rate above the baseline level. This was confirmed by both bivariate correlation and multivariate regression analysis. $GH_{max}24h$ was not correlated with either cardiac dimensions or cardiac function.

4.2.5 Specific effects of treatment – Paper III

In the longitudinal part of the study, all children followed per protocol were described, and changes in cardiovascular variables were analyzed. Eighty-seven children were randomized to receive individualized treatment and 41 to receive a standard GH dose (doses are described in Figure 9). The original publication in the GH-dose trial focused on minimizing the variability in distance of current height from MPH_{SDS} after 2 years of GH treatment by individualizing the dose. The range was reduced by 32%[46]. No such effect could be found for any of the cardiovascular variables that were evaluated (complete data not shown, example is given in Figure 15). As detailed in Paper III, Table 2, most cardiovascular variables changed with treatment.
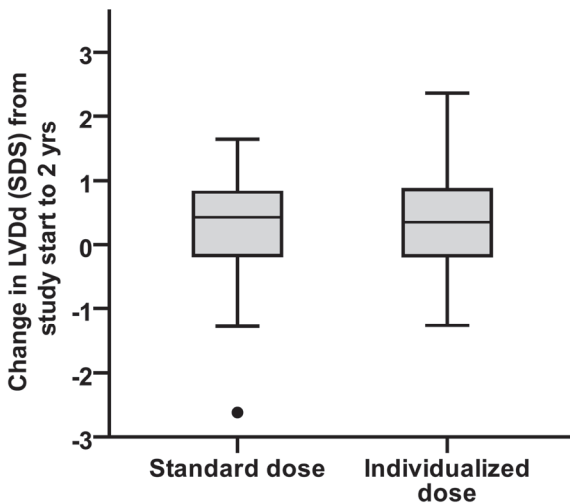

Figure 15. Boxplot of change in LVdd$_{SDS}$ from study start to 2 years of GH treatment, comparing the groups receiving individualized and standard doses. No differences in ΔLVDd$_{SDS}$ or variance were found.

The development of pathological hypertrophy was not reported during the course of the study. LV mass$_{SDS}$ as measured by the MM method was +2.4 SDS after 2 years of treatment in one child assigned to the standard GH dose group (43 µg/kg/d). When examined 2 years later outside the study protocol, this child's LV mass was at 1.7 SDS (Paper III, Figure 1). With the exception of heart rate, changes in cardiovascular variables (described in the *Patients and methods* section) over 2 years of GH treatment did not differ, either between the two treatment groups or between the children with GHD and ISS (as defined by $GH_{max}AITT$). Heart rate decreased on average by 10.0 bpm in the standard dose group compared with 5.4 bpm in the individualized treatment group (p=0.024); however, due to the overall similarity between the treatment groups, data from all children were combined during the detailed analysis.
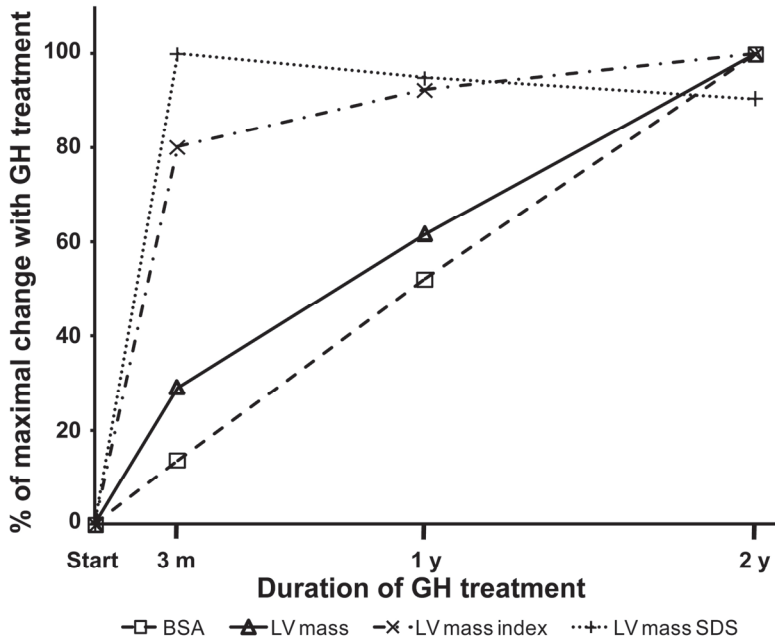
Figure 16. Comparing the effects of GH treatment on BSA, LV mass, LV mass index and LV mass$_{SDS}$ with duration of GH treatment.

Similarly, as no important differences were found in cardiovascular effects between children with GHD and ISS, the two groups were combined for more detailed analysis. In Paper III, Figure 2, LV mass indexed to BSA, and evaluated both by the MM method and the AL method, had already increased after 3 months of treatment. After this point, there was no further increase in LV mass index. In Figure 16, the increases in BSA, LV mass, LV mass index and LV mass$_{SDS}$ are shown as percentages of the maximum increase. The BSA increased in an almost linear manner by 4–5% (of the maximal change) per month (%/month). LV mass doubled during the first 3 months of treatment (10%/month), but later increases were smaller (only 3–4%/month). As a consequence, there was a dramatic increase in LV mass index during the first 3 months (27 %/month), followed by almost no further changes. The same was seen for LV mass$_{SDS}$.

Figure 17 summarizes changes relative to baseline in the most important variables after 3 months, 1 year and 2 years. An initial decrease in diastolic BP$_{SDS}$ was observed, coupled with an increase in LV cardiac index. LVDd$_{SDS}$, IVSd$_{SDS}$ and LVWPd$_{SDS}$ at 3 months in the absence of a change in systolic BP. At 2 years, IVSd$_{SDS}$ and LVPWd$_{SDS}$ had returned to baseline values, whereas LVDd$_{SDS}$ and LV cardiac index were still increased compared with baseline. Diastolic BP$_{SDS}$ remained lower than baseline and systolic BP$_{SDS}$ had decreased.
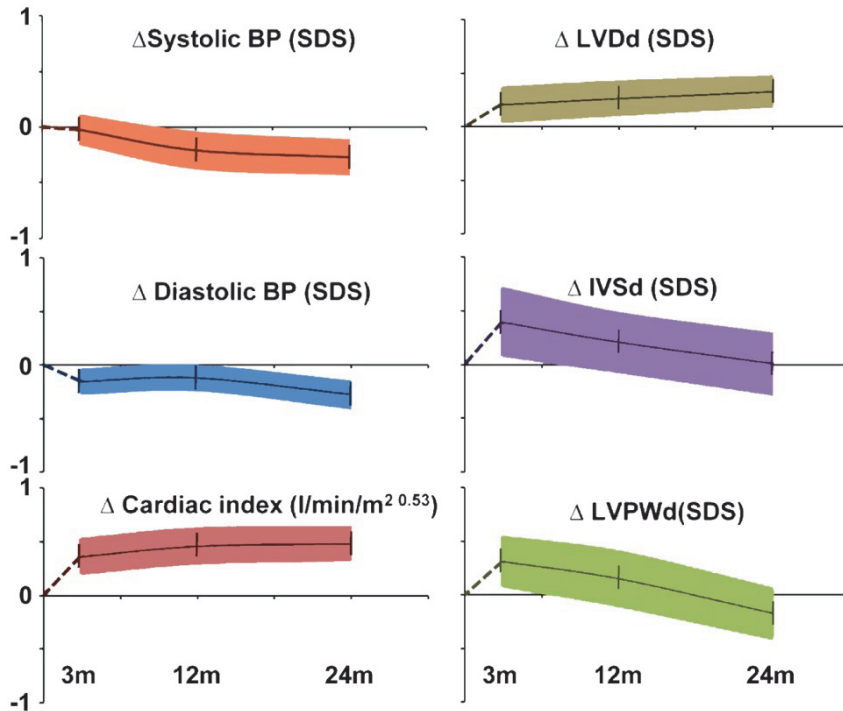
Figure 17. Changes in cardiovascular parameters during GH treatment compared with pretreatment values.

GH treatment and the heart:
During GH treatment there was a rapid change in cardiovascular variables. After 3 months, standardized measurements of cardiac dimensions had increased. At 1 year and 2 years, $LVDd_{SDS}$ remained stable, whereas $IVSd_{SDS}$ and $LVPWd_{SDS}$ had returned to baseline values. Reflecting a decrease in diastolic BP_{SDS}, the LV cardiac index increased rapidly during the first 3 months of treatment, thereafter remaining stable.

4.2.6 Tissue-specific threshold for response to GH – Paper IV

To study the difference in dose-dependency between different tissues, variables representing different organ systems were chosen. Fat mass$_{SDS}$, leptin, bone age, BMD, ALP (µkat/L), LST$_{SDS}$, height$_{SDS}$, insulin (mU/L), IGF-I$_{SDS}$, IVSd (cm), LVPWd (cm), LVDd (cm) and LV mass MM (g) were selected. The variables were analyzed as Δ values between the start and 2 years of GH treatment. For each of the six dose groups, the mean Δ was calculated (Δ dose-group mean), and is shown for the 87 children receiving individualized GH treatment in the per-protocol population. When performing ANOVA and piecewise linear regression, substantial lipolytic effects were seen in all groups as demonstrated by changes in the variables fat mass and leptin from baseline to 2 years of treatment; however, no dose-response differences were observed between the GH-dose groups for Δ fat mass$_{SDS}$, Δ leptin, Δ bone age, Δ BMD, Δ IVSd or Δ LVPWd (data not shown). The Δ LV mass was found to be significant in the initial analysis (ANOVA p-value = 0.013) and had a ED50% of 36 µg/kg/d. After excluding the small 17 µg/kg/d group, significance was lost and these data were not included in Table 10 (complete table shown in Paper IV, Table 1).

Table 10. Increases in variables studied in the different dose groups.

GH dose (µg/kg/d)	ΔLVDd (cm)	ΔLST (SDS)	ΔInsulin (mU/L)	ΔHeight (SDS)	Δ IGF-I (SDS)
17	0.15 (0.10)	0.4 (0.3)	3.7 (0.7)	0.8 (0.3)	1.7 (0.2)
33	0.35 (0.04)	0.6 (0.1)	4.8 (1.2)	0.9 (0.1)	2.0 (0.2)
40	0.38 (0.07)	1.0 (0.1)	5.0 (1.0)	1.5 (0.2)	2.3 (0.3)
50	0.49 (0.04)	1.1 (0.1)	6.2 (1.1)	1.4 (0.1)	2.6 (0.2)
66	0.54 (0.05)	1.4 (0.1)	8.8 (1.4)	1.7 (0.1)	3.5 (0.2)
100	0.50 (0.08)	1.6 (0.2)	8.8 (3.1)	2.0 (0.1)	4.3 (0.6)
ED50%	33	47	48	51	57
90% Conf.bounds	(24–38)	(43–52)	(35–65)	(47–56)	(52–65)
ANOVA (*p*-value)	<0.001	<0.001	0.012	<0.001	<0.001

Δ Dose-group means (calculated between start and 2 years) are given (with their SD). Dose-group means closest to half of the dose effect (50% Δ effect) are marked in bold and with light-grey boxes. The effective GH dose (ED 50%) required to achieve (50% Δ effect) is calculated based on a piecewise linear regression equation of Δ dose. Below the ED 50% is the corresponding 90% confidence bounds. P-values are shown for testing a piecewise linear GH-dose effect, the ANOVA p-value was calculated vs. zero effect.

The variable with the lowest ED50% is the one with the highest responsiveness to GH treatment, indicating higher sensitivity to GH. The highest responsiveness to GH treatment was seen for cardiac dimensions as represented by LVDd and the lowest for IGF-I. Classic dose–response curves for the six different GH doses in the 87 children receiving individualized GH treatment are shown in Figure 18. Absolute dose-group means were set to 0% at study start and compared with values from 1 and 2 years of treatment. IGF-I$_{SDS}$ and LVDd at the start of treatment were also compared with values at 3 months of treatment.
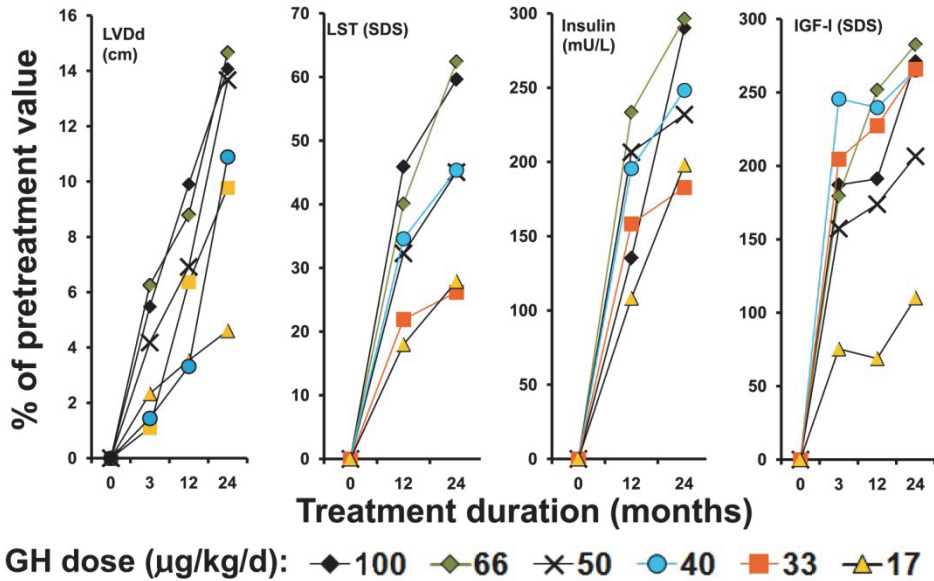

Figure 18. Examples of dose-response relationships. Note the different numerical values on the vertical axis.

Tissue specific dose-response to GH treatment:
LVDd, as a parameter of cardiac dimension, was more sensitive to GH treatment than markers of skeletal muscle (LST), longitudinal growth (height) and IGF-I.

5 MAIN FINDINGS

The relationship between endogenous GH secretion rate, secretion pattern, GH treatment, GH responsiveness and the heart is more complex than previously has been acknowledged.

1. GH-R and IGF-I mRNA are expressed in cardiac tissue in children with congenital heart defects.
2. In short prepubertal children, the GH trough level is negatively correlated with cardiac dimensions and GH secretion rate above baseline level is positively correlated with cardiac dimensions. The maximum GH concentrations do not correlate to cardiac dimensions.
3. During GH treatment there is a rapid change in standardized measurements of cardiac dimensions with an increase in both LV wall thickness and diameter after three months of treatment. In the medium term (2 years of treatment), after fulfilled catch-up growth, LV diameter continues to be increased, relative to pretreatment values, but standardized measurements of wall thickness returns to baseline.
4. LV dimensions are more responsive to GH treatment when compared with insulin, IGF-I and longitudinal growth.

6 DISCUSSION

The most important contribution of this thesis is to highlight the complexity of the actions of GH on the heart in growing children, both in terms of the responses to endogenous GH secretion and to GH treatment. Our current understanding of the influence of the GH/IGF-I axis in the regulation of cardiovascular structure and function during normal conditions is limited. To expand our knowledge, we are confined to studies based on populations already followed within the healthcare system or to experimental data.

In the adult population, the general concept is that GHD is associated with increased cardiovascular risk and mortality[95], reduced LV mass and decreased cardiac output[96, 97]. The validity of these findings has been disputed during the last few years based on studies using cardiac MRI for evaluation of cardiovascular structure and function[98]. On the other side of the spectrum, GH excess, as seen in acromegaly, is also related to increased cardiovascular mortality and structural changes[99]. Normalization of GH levels in both GHD and acromegaly reverses these anomalies to some extent[8].

6.1 GH secretion and the heart

Almost all pediatric studies, analyzing the influence of GH on the heart, have been conducted in children receiving GH treatment. Studies have included short children with and/or without a diagnosis of GHD, children with Turner syndrome, Noonan syndrome or muscular dystrophies, and children who have experienced prior anthracycline therapy, thermal injury or heart transplantation.

Stimulation tests vs. endogenous secretion pattern

The major part of this thesis includes short prepubertal children. In the literature, such children have traditionally been classified as having either GHD or ISS based on their maximum GH secretion levels as assessed during two GH stimulation tests using the arbitrary cut-off point of 10 μg/mL. The cut-off value for the classification of GHD has changed following the increased availability of GH[48]. This thesis has shown that the stimulated GH_{max} often underestimate endogenous GH secretion capacity, as shown in Figure 10. Moreover, in the population of short children, the maximum GH concentration has little relevance for cardiovascular structure or function. GH trough levels and the GH secretion rate above baseline level seem to be of greater importance; however, this information is not available when only using GH stimulation tests.

Impact of being severely GH-deficient, partially GH-deficient or having ISS

Using published data assessing the impact of impaired GH secretion or sensitivity on cardiovascular status, one of the major considerations is finding an appropriate control population. If matched for age or height, normal controls will be taller or younger, respectively, than the short population. Two of the six available studies in the literature included children of varying pubertal status. The control groups either differed from the study population in terms of body size or data on body size were not reported. This unfortunately precluded the drawing of valid conclusions regarding the pretreatment cardiovascular status of the short children in these studies[100, 101]. In a more recent study by Lanes et al., 12 short untreated children with GHD were compared with 14 controls, all of varying pubertal status, but with similar average absolute height[102]. They found no differences in LV thickness between the groups. LV diameters were not reported but both diastolic and systolic volumes were lower in the GH-deficient population than in controls. This was also true for LV mass indexed to BSA. It should be noted that three of the 12 short children had brain tumors as the etiology of their GHD, and four of the children had been previously treated with GH. The three remaining studies are from the Italian group which has published several papers on GH and the heart, both in adults and children[89, 90, 103]. In their reports, all children were prepubertal. In the first study by Salerno et al., 12 short children (one of which had a brain tumor) classified as GH-deficient based on two stimulation tests were compared with 12 controls matched for height and BMI. Neither the age nor height$_{SDS}$ of the control population were reported[103]. They found IVSd to be similar in both groups, but both LVPWd and LVDd were smaller in the GH-deficient population than in controls. As a result, the population with GHD had a smaller LV mass index than controls. Variables of systolic function were similar in both groups. The next study from the Italian group was published in 2006, and most likely included the same 12 patients plus another 18 GH-deficient children (not disclosed in the article)[90]. The GH-deficient population was on average 9.3 years and the control population 9.8 years. Unfortunately, they did not report absolute height, but the control population had an average height$_{SDS}$ of −1.5 SDS with an SD of 1.1. If height were indeed normally distributed as claimed, a significant number of the 30 controls actually had a height$_{SDS}$ <2.0 and most likely could be considered to have ISS. They found no difference in heart rate or BP between groups. IVSd was similar in both groups but LVPWd was reduced in the GH-deficient group relative to controls. As in the previous study, LVDd was smaller in the group with GHD and, as a consequence, LV mass index was reduced compared with controls. As in their previous publication, no differences were found, neither in systolic nor diastolic function compared to controls. More interestingly, when subdividing the patients into those with severe and partial GHD based on the GH$_{max}$ during stimulation tests. They found that LVPWd (but not IVSd), LVDd and LV mass index were reduced in the group with severe GHD relative to those with partial GHD. The

most recent study comparing short children and children of normal stature was published in 2009[89]. This study included 24 GH-deficient children (based on stimulation tests) and compared them with younger children of equal stature. The average height$_{SDS}$ of the controls was –1.2, resulting in a significant number being less than –2 SDS in height. In this study, they were able to demonstrate lower IVSd, LVPWd, LVDd, LVDs and LV mass index in the GH-deficient group compared with controls. They also found subtle reductions in systolic function, as estimated by mVCFc, ESS and SSI. Moreover, they repeated their previous finding, showing a dramatic difference in cardiac parameters between children classified as having severe and partial GHD. These differences between GH-deficient children and controls were not confirmed in the present thesis, either when classified according to the GH$_{max}$ during a stimulation test or according to the endogenous GH secretion. The control group in this thesis was of a similar height to the group investigated in the later study, and had normal height$_{SDS}$, resulting in their age being almost 2 years younger than the controls in the Italian study. The short population in the thesis was also on average 1 year younger then the Italian study-population, which suggests a shorter duration of GHD, which may explain the differences found. No important differences between children classified as having severe or partial GHD were found in the present thesis, something that was clearly demonstrated by both Salerno et al. and Capalbo et al.[89, 90]. This discrepancy is more difficult to explain. Including more children and using the same methodology as the Italian studies, the present study should have had greater power to detect any significant differences between partial and severe GHD. Again, there was a difference in ages. This may indicate that age has an impact of cardiac dimensions that is unrelated to body size. In the present thesis, only children with isolated GHD were reported upon, resulting in few children being severely GHD. This may need to be taken into account when comparing the publications.

Comparison of pretreatment data in short children with normal GH secretion and controls has not been previously reported on. The differences found were minor and dependent on whether a GH stimulation test or a test of spontaneous GH secretion was used. Before drawing any conclusions, larger studies comparing cardiovascular findings in children with ISS and control populations are needed.

Importance of GH secretion pattern
No previous study has investigated the association between estimation of endogenous GH secretion pattern and cardiovascular dimensions or function. Both in the validated multivariate analysis, and in the PCA, trough GH levels, were negatively correlated with LV mass and LV diameter in diastole. This indicates that children with higher baseline GH level have a lower LV mass or smaller LV diameter. Interestingly, some data indicates that children born small for gestational age (SGA) have increased GH trough levels[104], and a recent study has shown that

these children have a lower LV diameter in diastole compared to reference values[105]. Biological importance of elevated GH trough levels are also supported by experimental studies[106]. More human studies are needed to confirm the relationship between GH trough levels and LV dimensions.

Spontaneous GH secretion pattern has distinct effects on different cardiac dimensions

Another interesting finding in this thesis was that the impact of GH secretion pattern on the heart differed for LV diameter and wall thickness. The GH trough levels and the GH secretion rate above baseline values were inversely related to LVDd but showed no relationship to LV wall thickness. This is in line with a study by Andreasen et al. which used cardiac MRI to compare adults with GHD and controls[98]. They found reduced LV diastolic volume but not LV mass in the GH-deficient population. Increased diastolic volume in the absence of an increased mass most likely corresponds to an increased diameter but equal wall thickness.

> ### *GH secretion and heart:*
> *All studies comparing cardiovascular variables in short children with "normal children" have the problem of defining a suitable control population. In a cohort of equally short children, the actual peak GH concentration has little impact on cardiovascular structure and function. Interestingly, other aspects of the GH secretion pattern, like GH trough levels, seem to be of greater importance. Higher GH trough levels are correlated to lower LV mass and LVDd. Measurements of the actual wall thickness seem to be dissociated from GH secretion. This appears to be in line with detailed studies on adult populations.*

6.2   GH treatment and the heart

GH treatment affects the heart in a differentiated and time-dependent manner

A differentiated response to GH treatment was seen in the present study. Initially there was a rapid response to GH, with an increase in standardized measurements of LV wall thickness, diameter and mass after 3 months. However, after 2 years of treatment, LV wall thickness had returned to baseline values, whereas LV diameter and mass remained increased. The mechanism underlying this response is yet to be determined, but possible explanations include both direct effects of GH and IGF-I on sodium retention and cardiac tissue. Johannsson et al. showed that

short-term GH treatment in adults was associated with renal sodium retention and increased extracellular water (ECW)[107]. In their study, renal sodium secretion returned to pretreatment values after 1 year of treatment but ECW remained elevated. In another short-term study by Møller et al., plasma volume was shown to be unchanged[108]. In children, the effect on sodium and fluid homeostasis seems to be of shorter duration. Lampit et al. showed that the increase in sodium and water retention were normalized after 7 and 28 days of GH treatment, respectively[109]. As there is limited information about fluid and sodium homeostasis after 3 months of GH treatment in children it is difficult to know whether sodium and fluid retention alone can explain the increase in LV wall thickness found at this time point. As plasma volume seems to be unchanged, it is unlikely to affect LV diameter. To understand the mechanism responsible for the change in cardiac dimensions, other hemodynamic alterations have to be taken into account. At the 3-month examination, standardized measurements of diastolic BP was reduced in parallel with an increase in cardiac index. This could be explained by reduced peripheral vascular resistance and could be the mechanism responsible for the increased standardized LV diameter in diastole seen at 3 months. However, as the standardized systolic BP was unchanged, this does not explain the increase in myocardial thickness. In animal studies, GH has been shown to be involved in cardiac gene expression and collagen deposition in extracellular matrix[8]. In this thesis, GH-R mRNA and IGF-I mRNA were expressed in cardiac biopsies from children. The early increase in septal and posterior wall thickness could be influenced by direct effects of GH on the heart. Interestingly, at 2 years, the effect on LV diameter, diastolic BP and cardiac index remained, but standardized measurements of myocardial thickness returned to baseline values. This may be a consequence of the reduced systolic BP seen at 2 years, although other mechanisms cannot be excluded.

With individualized dosing, children with GHD and ISS respond similarly to GH treatment

With the dose of GH being individualized to meet the estimated demand, no difference in the response to GH treatment was found when comparing children classified as GH-deficient with those classified as having ISS. This is the first time that data from short children with the complete range of GH secretion have been analyzed together. Previous studies in the literature on the cardiac effects of GH treatment have been confined either to the GH-deficient population or the population with ISS. This is also the first time that the cardiovascular effects of doses as high as 100 µg/kg/d have been examined. Table 11 and 12, summarizes previous studies on short children without Noonan or Turner syndromes. None of the studies report any adverse cardiovascular events due to GH treatment, even though long-term follow-up is lacking.

Cross-sectional studies on GH treatment and the heart in short children

There are four cross sectional studies and nine longitudinal studies regarding GH treatment available in the literature (Table 11).

Table 11. Cross-sectional studies on cardiovascular data in GH-treated children and adolescents compared with untreated controls of normal height.

Year	Author	Ages years	Class	Pts	Cont.	Dose µg/kg/d	Dur. years	BP	LV wall	LVD/ Vd	LV mass index	Sys fx	Dias fx
1991	Rowland[9]	5–17.7	GHD & ISS	16	Ref.	14–30	2.1		↔	↔		↔	
1995	Crepaz[16]	3–17	GHD	22	22	33–56	1.2	↔		↔	↔	↔	↔
2000	Stamoyannou[110]	4.4–16.8	GHD	42	34	33±15	2.9	↔		↔	↔	↔	
2005	Lanes[102]	14.6 ±1.7	GHD	10	14	33	3.8		↔	↓	↓	↔	

↔ indicates no change compared with untreated controls without GHD, ↓ indicates decreased values compared with controls. Values are presented either as mean ± SD or range. Doses were presented differently in the different articles and were recalculated to approximate µg/kg/d for comparison. Abbreviations confined to this table: Dias fx: diastolic function, Dur: duration, Class: classification, Cont: controls, LVD/Vd: left ventricular diameter or volume in diastole, Pts: patients, Ref: reference population, Sys fx: systolic function.

In the four available cross-sectional studies on GH-treated short children, a total of 90 GH-deficient children and 3 children with ISS that had been on treatment for on average 1.2–3.8 years were compared to either controls without GHD or reference data (one study)[9, 16, 102, 110]. For comparison with this thesis, the doses were recalculated into corresponding approximate µg/kg/d and ranged from 14 to 56 µg/kg/d. Where reported upon, blood pressure, wall thickness, systolic and diastolic function was similar in treated children and adolescents compared to controls. Only in one study, the LV diastolic volume and LV mass index was reduced in the treated group compared to controls without GHD. The most valid interpretation of this data is that GH treatment in average does not result in cardiac dimensions or function different from the normal population.

Longitudinal studies on GH treatment and the heart in short children

The first of the longitudinal studies was performed on children classified as having ISS. It was initially published by Barton et al. as a preliminary report in 1992 and subsequently as a full paper in 1995[11, 12]. Compared with pretreatment values, the LV mass index increased only in the group given a higher GH dose

(approximately 67 µg/kg/d). An additional eight longitudinal studies have previously been published (Table 12)[10-12, 15, 17, 89, 90, 103, 111, 112].

Table 12. Longitudinal studies in short GH-treated children and adolescents. Changes in cardiovascular variables during treatment.

Year	Author	Age years	Pts	Class	Dose µg/kg/d	Dur. years	SBP	DBP	LV wall	LVDd	LV mass Index	Sys fx	Dias fx
1995	Barton[12]	5–10	29	ISS	36/72	2	↔		↔		↑	↔	
1995	Daubeney[15]	8 ± 0.5	15	ISS	54	4	↔	↑	↔	↑	↔	↔	
1998	Lampit[10]	5 ± 1	21	ISS	34	3				↔		↔	
1999	Radetti[17]	5–17	14	GHD	34–55	5.5	↔	↓			↑		
2003	Shulman[112]	4–12	10	GHD	43	1	↔				↑	↔	↔
2004	Salerno[103]	6–11	12	GHD	30	1	↔	↓	↑	↑	↑	↔	↔
2006	Salerno[90]	6–12	30	GHD	30	2	↑	↓	↑	↑	↑	↔	↔
2009	Capalbo[89]	4–13	24	GHD	30	2	↔	↔	↑	↑	↑	↑	↔
2011	Ozdemir[111]	11 ± 3	12	GHD	25	0.5			↔	↑	↑	↔	↓
2012	Nygren Paper II	3–11	127	GHD ISS	17–100	2	↓	↓	↔	↑	↑	↔	↔

↔ indicates no change, ↓ indicates decreased values and ↑ indicates increased values at last examination compared with pretreatment measurements. Values are presented either as mean ± SD or range. Doses were presented differently in the different articles and were recalculated to approximate µg/kg/d for comparison.

The most consistent finding was that of increased LV mass index and LVDd. There seems to be a larger discrepancy in earlier studies in populations with ISS[10, 12, 15]. The three most well designed studies from the Italian group all show an increase in LV dimensions following treatment from subnormal values at baseline, and Capalbo et al. show restoration of slightly impaired contractility[89, 90, 103].

Tissue-specific dose-response

This thesis also supports the existence of differential responsiveness to GH treatment among different organ systems. Of the variables studied, LV dimensions appeared to be the most sensitive to GH. Based on the results in Paper IV, a staircase model has been proposed (examples in Figure 19). The hypothesis of the main GH-dose catch-up study was to examine whether the highly variable response usually seen during GH treatment could be reduced by individualizing the dose. This was done using a validated model predicting growth in response to a standard GH dose of 33 µg/kg/d. The dose was then optimized so that the child would end up as close as possible to MPH after 2 years of treatment (i.e. children predicted to end up above MPH$_{SDS}$ had their dose lowered, and children predicted not to reach MPH$_{SDS}$ on the standard dose had their dose increased). This resulted in a 32% reduction in variability in distance to MPH$_{SDS}$ after 2 years of treatment as predicted in this hypothesis generating study[46].
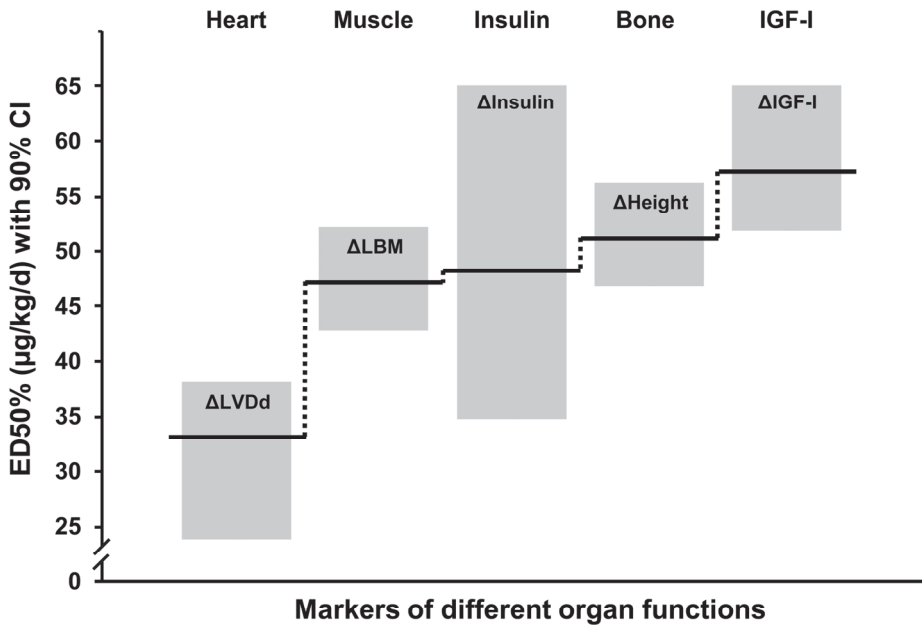
Figure 19. The staircase model of tissue sensitivity. Variables with a low dose predicted to result in 50% increase (ED50%) are more responsive to GH treatment than variables with a high ED50% . The gray area shows the 90% confidence bounds.

Reduced variability of response with individualized treatment has also been found for insulin[113] and behaviour problems[114]. Reduced variability for LV wall thickness, LV diameter or LV mass was not found in this study. This is not surprising considering the higher GH responsiveness of the heart. With an ED50% for change in LV diameter as low as 32 µg/kg/d, the dose selection process was not optimized to reduce the variability in cardiovascular response.

> *GH treatment and the heart in short children:*
> *Cardiac dimensions increased during GH treatment. Initially there was an increase in both standardized measurements of LV diameter and wall thickness, but after 2 years of treatment, only LV diameter stayed increased relative to pretreatment values. Most studies, including ours, indicate that GH does not result in pathological hypertrophy or impaired cardiac function. The longest longitudinal follow up was for 5.5 years and the highest GH dose used was 100 µg/kg/d.*

6.3   GH in pediatric heart disease

6.3.1   GH secretion in congenital heart disease

In late sixties and seventies, several studies examined GH secretion in congenital heart disease[115-118]. In brief, surprisingly high peak GH concentrations were found together with low IGF-I levels. GH levels increased after cardiac catheterization[119] and in response to heart surgery[120]. Interestingly, if cardiac disease was compensated (i.e. without significant hemodynamic compromise), GH levels were normal[121]. These studies indicated that uncompensated heart disease and trauma related to cardiac surgery both are associated with increased peripheral resistance to GH. Moreover Pons Leite et al. showed that prolonged elevation of GH levels was associated with a poorer outcome after heart surgery[122]. Later studies have even shown that the severity of congenital heart defects (shunt size, cyanotic/acyanotic) also influenced GH and IGF-I levels as an indicator of peripheral resistance[123-125]. Although cyanotic heart defects in particular are associated with poor growth, cardiac surgery results in increased IGF-I, increased growth velocity and increased BMI[126]. More recent interest in GH secretagogues has also resulted in a publication showing increased ghrelin levels in both cyanotic and acyanotic heart disease, as well as a relationship between ghrelin and impaired BMI[127]. In conclusion, heart defects in children are most likely associated with peripheral resistance to GH resulting in impaired growth. Fortunately, correction of the heart defect normally results in reversal of GH insensitivity and improved growth. Sasaki et al. studied seven children in whom growth retardation persisted 2 years after correction of their heart defect[128]. GH treatment with approximately 25 μg/kg/d resulted in improved longitudinal growth without any reported adverse events.

> *GH and congenital heart disease:*
> *Congenital heart disease and surgical trauma are associated with signs of increased peripheral resistance to GH (i.e. high GH levels and low IGF-I levels). This is often seen in parallel with impaired statural growth. Correction of the heart defect usually results in reversal of GH resistance and improved growth.*

6.3.2   GH treatment of heart failure

Dilated cardiomyopathy

Observation of increased cardiomyocyte hypertrophy[129], improved contractility through increased myofilament sensitivity to calcium[29], and regulation of apoptosis[130] has evoked interest in using GH in the treatment of heart failure of various etiologies. McElhinney et al treated eight children with dilated cardiomyopathy with approximately 25 to 40 µg/kg/d for six months in a cross over study[131]. Although not reaching statistical significance, EF showed a trend towards increase during GH treatment. Treatment was concluded to have a good safety profile, but LV dysfunction progressed in two children and they were transplanted during the trial. Rosti et al. described a case of a 6- month-old girl with a complex univentricular heart and dilated cardiomyopathy treated with 0,1 IU/kg/d (33 µg/kg/d) of GH for 3 months[132]. Parents reported an increased exercise tolerance but no echocardiographic improvement could be seen. Ikemoto reported beneficial effects of GH treatment in an infant with dilated cardiomyopathy receiving a dose of 0.2 IU/kg/w every other day (corresponding to approximately 9 µg/kg/d)[133].

Muscular dystrophies

Cittadini et al. reported on six children with Duchenne muscular dystrophy and 10 adults with Becker muscular dystrophy with signs of cardiac involvement[134]. The six children were randomized to receive either 33 µg/kg/d of GH or placebo for 3 months. NT-proBNP as a biochemical marker of heart failure decreased in the GH-treated group (including the adult patients). The small number of children (three in each group) precluded any valid statistical analysis, but the authors noted a 29% increase in LV mass and 33% decrease in end-systolic stress in children receiving GH treatment.

Post anthracycline therapy

Anthracycline treated cancer survivors have a high prevalence of decreased cardiac function and reduced LV dimensions. Lipshultz et al compared 34 anthracycline treated children with 86 controls in a retrospective fashion[135]. GH given two to seven times a week at a dose of 29 to 57 µg/kg/d for a median time of 3.8 years. LV wall thickness was reduced in the anthracycline-treated children and improved during GH treatment. Shortly after stopping treatment, this effect was lost and GH treatment was concluded not to affect progressive LV dysfunction in this population. Studying survival in young adults after acute lymphatic leukemia in childhood, Follin et al. found no difference in LV systolic function between 16 patients treated with GH for 5 years and 13 patients who did not have GH treatment[136]. In a previous report on 2-year data from the same population, there was a significant improvement in LV mass index and EF in those who were GH-treated[137].

GH treatment of heart failure in adults

Le Corvosier et al. published a meta analysis in 2007 of studies of GH treatment in adults with heart failure, highlighting variability in results between papers[138]. Merging the data, they found several cardiovascular benefits of GH doses (approximately 5 to 20 µg/kg/d). LV wall thickness increased, diameter decreased, EF improved, systemic vascular resistance decreased, New York Heart Association (NYHA) classification increased, exercise duration and VO_2max increased. Despite this compelling evidence of the efficiency of GH treatment in heart failure, no large-scale randomized, placebo-controlled studies have been performed with hard end-points like cardiovascular-related mortality.

> ***GH treatment in heart failure:***
> *Data on the effectiveness and safety of GH treatment in pediatric heart failure are limited. There are some evidence of increased LV mass and improved contractility, but these results need to be studied further. In adult heart failure, the evidence in favor of using GH is increasing, but large scale studies are so far missing.*

6.4   GH treatment in other conditions

Turner syndrome

Turner syndrome is caused by the absence of genes from the short arm of one the X chromosomes in girls, ranging from monosomy X to different mosaic karyotypes. Turner syndrome is associated with ovarian failure and short stature. The short stature is partly caused by changed proportions of different isoforms of GH[20, 139]. Girls with Turner syndrome also have a high prevalence of heart defects. Heart defects were found in about one-third of a series of sixty girls with Turner syndrome from our institution (unpublished data). The most common defect was bicuspid aortic valves, which are known to predispose for later aortic stenosis, insufficiency and aortic dilation[140]. There was also a high prevalence of aortic stenosis and coarctation of the aorta. LV mass and LV diameter were not altered in GH-treated compared with non-GH-treated girls. In a larger study by Sas et al., 62 girls treated with randomly assigned GH doses, ranging from 45 to 90 µg/kg/d, were followed for 7 years[13]. Pretreatment diastolic BP was elevated but decreased with duration of treatment. LV wall thickness and LV diameter also decreased with time. Compared with reference values, there were no differences between the different dose groups. Radetti et al. published a cross-sectional study in 2001 comparing 26 girls with Turner syndrome treated for an average of 5 years (dose:

1 U/kg/w corresponding to approximately 47 µg/kg/d) with 37 controls matched for age and body size[141]. They found a lower LV volume in systole, lower systemic vascular resistance, lower E/A ratio, increased mVCFc, higher systolic BP and lower diastolic BP in the GH-treated group. They interpreted these results as reflecting an increased contractile state and signs of impairment in diastolic function, secondary to a GH-induced decrease in peripheral resistance and increase in heart rate in the GH-treated girls with Turner syndrome. Matura retrospectively reviewed 67 GH-treated and 19 non-GH-treated girls with Turner syndrome and found no differences in cardiac dimensions or systolic function[142]. Bondy et al. focused on the impact of GH treatment on aortic dimensions[143]. They compared 53 GH-treated girls (average duration 5 years) with 48 non-GH-treated girls. The GH-treated group was taller and had larger aortic dimensions than the non-GH-treated group, but in a multivariate analysis neither history of GH treatment nor duration of treatment were related to larger descending aortic diameter. Although not a pediatric study, van den Berg et al. found no cases of myocardial hypertrophy, well preserved systolic function, higher heart rate and smaller ventricular volumes in young adult women with Turner syndrome compared with controls[144]. The patients had discontinued GH treatment for at least 6 months when examined by cardiac MRI.

Noonan syndrome

Noonan syndrome is characterized by facial dysmorphism, short stature and cardiac defects, most commonly pulmonary valve stenosis and hypertrophic cardiomyopathy. Final height is improved by GH treatment and so this therapy is currently being used more frequently in this population[145]. Cardiovascular effects of GH in Noonan syndrome have been addressed in five papers reporting on two trials. Cotterill et al. studied 27 children treated with 4 IU/m²/d (approximately 50 µg/kg/d of GH for 3 years. These children were compared with untreated patients with Noonan syndrome. The study included children with heart defects, but required septal thickness to be no more than 1 cm[146]. The first paper, reporting 1-year data, showed increased LV diameter in diastole in GH-treated children but otherwise no differences relative to non-GH-treated patients. The second study reported on 3-year data and no cases of ventricular hypertrophy were found in GH-treated patients[147]. The following year, the subset of patients with heart defects, from the same trial, were analysed, still without showing any development of ventricular hypertrophy[148]. Noordam et al. reported on 27 children followed for 3 years, and 19 children with heart defects followed for 5 years (one child was found to have mild hypertrophic cardiomyopathy at pretreatment examination)[149]. They found no differences in echocardiographic parameters compared with controls, and LV wall thickness did not change in the group followed until the end of treatment[150]. These data are reassuring in terms of safety. However, not believed to be related to GH treatment, there have been some reports of cardiac adverse events from the Kabi International Growth Study (KIGS) database. Of 429 GH-treated

children with Noonan syndrome, three had cardiac arrhythmias, one had severe LV hypertrophy and one had non-defined cardiomyopathy leading to heart transplant[151].

Post heart transplantation
Mital et al. monitored longitudinal GH treatment in ten children following heart transplant (dose: 43 µg/kg/d for an average of 2.5 years)[152]. During treatment, FS, LV mass index, LV volume in diastole and LV cardiac index increased. Discontinuing treatment resulted in echocardiographic variables returning to pretreatment values, with the exception of LV volume which remained increased. No increase in rate of transplant rejection was seen, and GH treatment was considered to have a good safety profile.

Post thermal injury
Severe burn injury is associated with muscle protein catabolism for at least 9 months after the accident and growth delay for up to 2 years[153, 154]. Mlcak et al. have published a randomized, placebo-controlled study on GH treatment in children with severe burns[155]. They randomized 76 severely burned children to receive either daily injection of either saline or GH (dose 50 µg/kg/d) for 1 year. They found an increase in EF from 63 to 69% in the GH-treated group. There were no other differences in cardiovascular variables. GH treatment in severely burnt children was concluded to have a good safety profile and to be beneficial for the heart.

Growth hormone and arrhythmias
In adult GH overproduction, concerns have been raised about an increased risk of ventricular arrhythmias because of the increased incidence of late potentials on signal averaged ECG[156]. There is, however, upcoming evidence that short-term GH treatment might protect against arrhythmias secondary to experimental myocardial infarction in rats[157, 158]. This has also been found for ghrelin[159].

GH treatment in other conditions:
GH has been used to treat short stature in different subpopulations of children including those with Turner syndrome and Noonan syndrome, as well as in children after heart transplant. GH has also been used in the catabolic state after burn injury and in experimental studies on ventricular arrhythmias. Although results are not consistent, there is evidence of improved contractility and reduced ventricular arrhythmias, without any major safety concerns. Children with Noonan syndrome receiving GH treatment should, however, be monitored closely because the natural history of the condition includes an increased prevalence of hypertrophic cardiomyopathy.

6.5   Potential risks with GH treatment

In January 2012 Carel et al. published the data that one year earlier had concerned regulatory authorities and endocrinologists[160]. From the French population in the Safety and Appropriateness of Growth hormone treatments in Europe (SAGhE) study, they showed that mortality was higher in young adults treated with recombinant human GH as children compared to the general population, especially so in those treated with higher doses (> 50 μg/kg/d, mainly children born SGA). For example, the 95% CI of the standardized mortality ratio (SMR) was 1.01–14.63 for bone tumors, 1.40–5.83 for cardiovascular causes and 1.79–17.05 for subarachnoid or intracerebral hemorrhage. In the same issue of The Journal of Clinical Endocrinology and Metabolism, a detailed study of causes of death in the Belgian, Dutch and Swedish cohorts from the SAGhE study was published[161]. Of the 21 deaths identified, 12 were accidents, four were suicides, and one each was due to pneumonia, endocrine dysfunction, primary cardiomyopathy, deficiency of humoral immunity and coagulation defect. None died of bone tumors or intracerebral hemorrhage. The patient who died of primary cardiomyopathy was 19.1 years old and had been on GH for 1.9 years with an average dose of 33 μg/kg/d. He had a GH-treated sibling who also died of cardiomyopathy (the sibling lived in the UK and was therefore not included in the article, personal communication Dr Sävendahl). Albertsson-Wikland et al. have developed a novel mortality model for the general population Swedish population. This model was applied to 3 854 children with isolated GHD, ISS or SGA treated with GH for a total of 44 894 patient-years[162]. In addition to using only age, gender and calendar year, as in SMR, the model also include birth variables (birth weight, birth height and gestational age), concomitant cardiovascular disorders, other malformations and interactions with age. Twenty one deaths were observed in the studied population and the model predicted 22.01, giving a hazard ration not different to the general population. This indicates that the increased mortality seen in the GH treated population most likely is not caused by the GH treatment in itself.

Takala et al. observed increased mortality in critically ill adult patients treated with high GH doses (70–130 μg/kg/d)[163]. In two prospective randomized double-blind, placebo controlled studies, they found the 95% CI of the relative risk of death for those receiving GH to be 1.3–2.9, and 1.6–3.5, respectively. The reason for the increased mortality in these studies is not entirely clear[164].

A few case reports on possible cardiovascular adverse events associated with GH treatment have been published. Kobayashi et al. reported a possible connection between the worsening of hypertrophic cardiomyopathy and GH treatment[165]. Grattan and McCrindle reported a child with a univentricular heart and Fontan circulation who had recurrent exacerbations of protein losing enteropathy during GH treatment. This was thought to be caused by the aldosterone antagonistic

effects of GH[166]. In addition, there has been a case report of a child undergoing GH treatment for short stature who developed complete heart block. The authors discuss the possibility that the heart block was caused by GH treatment[167].

Safety of GH treatment:
Available data so far indicate that GH treatment in children has a good safety profile. This is true both for the heart and for other organ systems. Care should, however, be taken when treating special subgroups of patients: children with Noonan syndrome, children with severe hemodynamic compromise and children with complex cardiac lesions.

6.6 Conclusion

Regulation of the cardiovascular system by GH is complex. This is especially the case in growing children. Previous studies have focused on the importance of associations between maximum GH levels obtained following GH stimulation tests and different cardiovascular variables of interest. By analyzing the spontaneous GH secretion pattern as well as rate, inverse effects of the GH trough levels between peaks and the GH secretion rate above baseline level was demonstrated. There was also a difference in the actions of GH on LV diameter and LV wall thickness. This translated into an effect during GH treatment, where LV diameter and wall thickness behaved differently in response to treatment in a time-dependent manner. Initially, there was an increase in both standardized measurements of LV diameter and wall thickness, but with time LV wall thickness returned to baseline values and LV diameter remained increased. This highlights the importance of not placing too much focus on LV mass, which is generally derived based on a calculation from LV diameter and wall thickness. Based on the present studies and the literature, GH treatment in children appears to have a good safety profile, despite recent concerns about increased mortality in adults previously treated with GH. The development of hypertrophic cardiomyopathy as a result of GH treatment seems unlikely, but subpopulations with a predisposition for the disease should be followed closely.

Currently available information should be interpreted with caution. Studies in the literature include relatively few patients and employ different inclusion criteria and a wide range of GH doses, making it difficult to draw firm conclusions. The presence of GH-R and IGF-I mRNA in cardiac tissue, however, indicates that the heart is a primary target organ for GH.

7 FUTURE PERSPECTIVES

This thesis is only scraping the surface of the complex hormonal regulation of cardiovascular structure and function. Basic knowledge about how the heart grows during different periods in life is limited. What are the growth velocities of cardiac dimensions during infancy, childhood, juvenility, puberty and adulthood? Can future problems in hereditary disorders be predicted by studying this pattern in an individual patient? What parts of the endogenous GH secretion pattern are of importance during the different phases of growth, and how does GH interact with sex steroids and other hormones in the regulation of cardiac dimensions and function?

In the light of emerging evidence that GH doses need to be individualized based on predicted longitudinal growth responses, it is likely that average GH doses given to children will increase in future. It will, therefore, be important to continue to monitor the cardiovascular safety of GH treatment in patients with different conditions who receive this therapy.

Studies on the impact of GH treatment for short stature in congenital heart disease are few and need to be expanded. Is there a way of predicting which children will not experience catch-up growth after cardiac surgery, and what is the best way of managing them?

The most interesting perspective for a cardiologist is the possibility of using GH in the treatment of myocardial dysfunction. The preparation of these protocols will most likely be complex, and the results of clinical trials will be difficult to predict. This is particularly the case because of the apparent discrepancy between the effects of GH on the heart in patients with poor ventricular function and in short children with normal ventricular function. LV diameter increases in GH-treated short children and decreases in adults with heart failure. LV wall thickness is virtually unchanged in short children during GH treatment and increased when treating patients with heart failure. There is also a concern regarding the population with univentricular heart defects and a, so called, Fontan circulation, where caution must be taken to avoid side effects because of fluid retention. Optimal dosing is to be determined.

One emerging field that may reach the hospital floor in 5 to 10 years, is the use of GH in the treatment of ventricular dysrhythmias. With today's anti-arrhythmic drugs having significant side effects and potential adverse impact on systolic function, GH might become an important therapeutic addition, especially for short-term treatment.

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