Abnormal growth hormone secretion : clinical aspects

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Abnormal Growth Hormone Secretion
Clinical aspects

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Voor mijn ouders

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Chapter 1

Introduction

Clinical aspects of abnormal GH secretion

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1. Introduction

This thesis describes several studies, which illustrate the physiology and pathophysiology of growth hormone. This chapter provides an overview of the concepts underlaying the studies.

1.1 Regulation of growth hormone secretion

Growth hormone (GH) is synthesized in the anterior pituitary gland by somatotropic cells. This gland is situated within the bony sella turcica and is overlain by the dural diaphragma sella, through which the stalk connects to the hypothalamus. Most of the functioning anterior pituitary cells consist of somatotroph cells (35-45 %)(1). In recent years, much knowledge has emerged on the embryogenesis of the pituitary. It has been discovered that the differentiation of pituitary stem cells into somatotroph cells is regulated by two transcription factors, PROP-1 and Pit-1 (2;3), which has clinical relevance for the understanding of congenital GH deficiency syndromes.

The two most important regulators of GH secretion are the hypothalamic peptides Growth Hormone Releasing Hormone (GHRH) and Somatostatin. Both hypothalamic peptides are secreted in independent waves and interact together to generate, and control, GH release. GHRH stimulates GH release, whereas somatostatin has an inhibitory effect. After GHRH is secreted by the hypothalamus, it is transported through the portal system to the somatotroph cells, where it binds to the GHRH receptor (1;4). Stimulation of the GHRH receptor results in the release of presynthesized GH, that is stored within the cells. In contrast to the GHRH receptor, of which no receptor subtypes are known, 5 different somatostatin receptor subtypes can be distinghuished. In the pituitary gland, the 2 most important somatostatin receptor subtypes are the subtypes 2 and 5 (5). After binding of somatostatin to its receptor, somatostatin inhibits GH secretion and/or cell proliferation (6).

In addition to the effects on the pituitary with respect to the release of GH, somatostatin and GHRH influence each other's release. GHRH stimulates somatostatin secretion, whereas somatostatin inhibits GHRH secretion (4). The integrated effect of GHRH and somatostatin on the pituitary gland ultimately leads to GH secretion. This is characterized by a pulsatile pattern with high amplitude pulses, especially at night, and low amplitude pulses predominantly during daytime.

GH secretion is regulated in a feedback system. The most important peptide in this system is Insulin-like-Growth factor I (IGF-I). Plasma IGF-I is predominantly produced by the liver, whereas IGF-I synthesis occurs in virtually all tissues and acts in a paracrine fashion. Most circulating IGF-I is bound to IGF-binding protein 3 (IGF-BP3) and acid labile sub unit (ALS). IGF-I is transported to the brain, where it has numerous effects. The most important action of IGF-1 in the brain is the stimulation of somatostatin production, which will eventually reduce GH production (7).

In addition to these important regulators of GH secretion, recently a novel potent GH secretagogue has been discovered, Ghrelin (1). Ghrelin appears to be the natural ligand of the growth hormone secretagogue receptor (GHS-R). Activation of this receptor by Ghrelin leads to potent GH secretion.

Ghrelin is predominantly produced by the stomach and released into the bloodstream. In addition to the GH releasing activity, it also stimulates the release of other pituitary hormones like cortisol and prolactin and plays a role in stimulating appetite, controling energy balance and gastric motility. Although Ghrelin is a potent GH releasing peptide, recent insight indicates that Ghrelin contributes more to the regulation of diverse functions of the gut-brain axis than to GH secretion *per se* (8).

1.2 GH and IGF-I

GH binds membrane-anchored GH receptors(9). The extra-cellular domain of this receptor can be released into the circulation and is referred to as growth hormone binding protein (GHBP), which may serve as a stabilizer of GH availability in the circulation (10). GH receptors are found in many peripheral tissues, especially in the liver. After binding of GH to the GH receptor, the receptor initiates a phosphorylation cascade involving a JAK/STAT pathway, which is ultimately leads to the biological actions of GH (11;12).

In general, GH acts on peripheral tissues by two mechanisms: 1) a direct effect, and 2) an indirect effect via IGF-I, produced, and secreted into the blood, by the liver or produced locally within a certain tissue. Of the circulating IGF-I, approximately 75 % is produced in the liver, the remainder being produced locally (13). IGF-I binds to the IGF-I receptor, which results in tyrosine phosphorylation to initiate its effect (14).

1.3 Physiological effects of GH

The physiological actions of GH involve many organs and physiological systems. Although a complete overview of GH action falls beyond the scope of this chapter, important effects include longitudional bone growth, metabolism and - relevant for the scope of this thesis - the heart and general well-being.

GH plays an important role, through IGF-I dependent processes, in postnatal longitudinal bone growth and remodelling by inducing proliferation or/and differentiation of chondrocytes, osteoblasts, osteoclasts and collagen type I synthesis (15-18). In addition to bone

remodelling, IGF-I increases the fiber content and strength of skeletal muscles (19).

GH exerts many metabolic effects that persist throughout life. GH has a lipolytic effect in fat and muscle. After acute administration of GH, a rise in circulating free fatty acids (FFA) and glycerol is observed (20;21). Moreover, a reduction of LDL and elevations of HDL levels is observed with GH administration (22). Acute administration of GH causes a temporary effect on glucose uptake similar to insulin, whereas chronic GH administration leads to insulin resistance with hyperinsulinemia, due to a post receptor defect in insulin signaling (23). These effects may be partially explained by GH induced lipolysis and elevated plasma FFA, that inhibit insulin activity at its target tissues. GH therapy also increases lean body mass by enhancing protein synthesis, with a small inhibiting effect of protein degradation (24).

In addition to these metabolic effects, GH plays a role in immunomodulation, like B and T- cell proliferation, macrophage activity, immunoglobulin production etc. Therefore, GH exerts pleiotropic effects in many physiological systems. Remarkably, however, these effects are in general very subtle in adult patients and cannot be easily quantified by clinicometric approaches. This is probably the reason, that, in general, there is a long delay between the start of the disease acromegaly and the time of diagnosis (see below). In addition, the effects of GH substitution on GH deficient patients exemplify these subtle effects.

1.4 GH and the heart

The GH - IGF-I axis plays an important role in cardiac development and function. In cardiomyocytes both GH and IGF-I receptors are expressed (25). Interestingly, the GH receptor gene is expressed to a greater extent in the myocardium than in many other tissues (26). In hypophysectomized rats, GH induces cardiac IGF-I mRNA expression and increases cardiac IGF-I content. In turn, IGF-I increases the size of cultured cardiomyocytes and enhances myofibril development (27). Concomitantly, IGF-I down-regulates sms-actin, a protein that forms stress fiber-like structures (28). GH and IGF-I have a direct effect on myocardial contractility. By increasing the intracellular calcium content

and enhancing the calcium sensitivity of myofilaments in cardiomyocytes, GH and IGF-I promote the contractility of the myocardium (29;30). Thus, it appears that GH and IGF-I have positive inotropic effects on the heart by increasing cardiac growth and by increasing the sensitivity of the myofilament apparatus to Ca^{2+}.

1.5 GH and quality of life

As discused above, GH has effects in almost every organ system. These effects are translated in a subtle way into quality of life. Many studies on GH deficient subjects have shown that GH deficiency leads to impaired quality of life (31-33). In most studies, restoration of GH levels improves quality of life in the majority of the patients (34). GH may act directly or indirectly on neural sites. In the human hippocampus, putamen, thalamus, hypothalamus and pituitary, GH receptors are found, suggesting a direct role of GH in the brain (35;36). The hippocampus may be important with respect to neural effects of GH as this region plays an important role in memory, motivation and attention (37). The mechanism whereby GH exerts direct effects on psychological functions, is largely unresolved (38;39).

GH may enhance cognition by stimulating brain growth and development. Studies in GH deficient mice have shown impaired brain growth, glial and neuronal proliferation, and myelinisation. Conversely, brain size is increased in GH transgenic mice (37). Accordingly, GH plays an important role in neural function during brain injury. Studies in rats have shown that GH can prevent cell loss in the hippocampus (40), following hypoxic/ischemic injury. GH enhances cerebral blood flow and intracellular communication and, which improves neural function (41;42).

GH may be involved in normal sleep generation. In GH deficient patients decreased deep sleep, increased total sleep time and a decreased REM sleep have been observed, which could be reversed by restoration of GH levels (43;44). Nonetheless, the importance of GH in sleep generation has been questioned by other authors (45;46).

1.6 Pathophysiolocal effects of GH: GH excess

Acromegaly is a rare disorder of GH excess, first described by Pierre Marie in 1886. This syndrome is characterized by elevated GH and IGF-I levels and by progressive somatic disfigurement and systemic manifestations. The prevalence is currently approximately 40 cases per million subjects with an estimated annual incidence of three to four patients per million subjects (47-49).

In almost all cases, GH excess is caused by a GH -secreting pituitary adenoma. In very rare cases, the syndrome is caused by extra-pituitary production of GH or GHRH in neuroendocrine tumors, like carcinoid tumors (50).

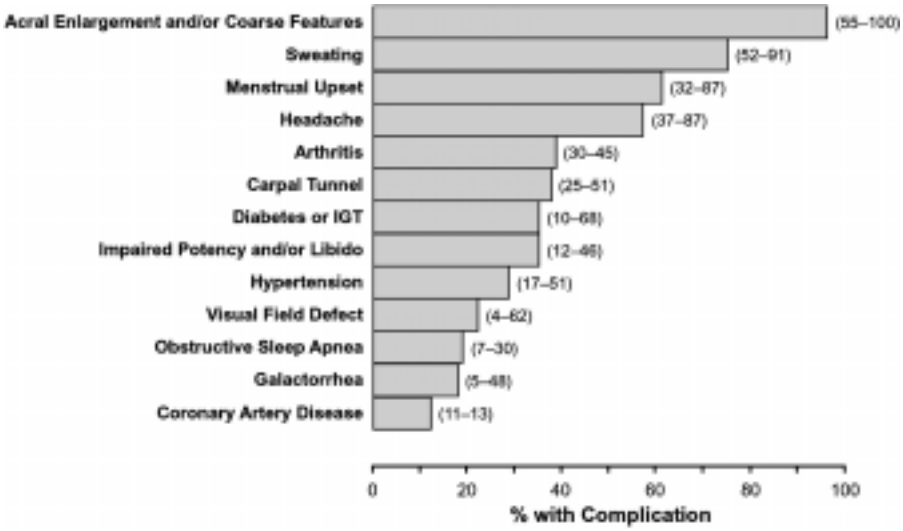

Fig. 1 Clinical features of acromegaly

Acromegaly is a disease that develops slowly, with only little and subtle clinical symptoms in the beginning (see Figure 1). Therefore, there is an average delay of 8 years in the diagnosis of acromegaly. At the time of diagnosis patients have coarsened facial features, soft tissue hypertrophy and exaggerated growth of hands and feet. Other characteristics consist of an increased number of skin tags, sleep apnoe, colonic polyps, insulin resistance, carpal tunnel syndrome and

cardiovascular disease like hypertension and cardiac hypertrophy (50-52).

The treatment of first choice is still trans-sphenoidal surgery, with success percentages ranging 60-70 % on the short term (53-56). The success of surgery depends on the skill and experience of the surgeon. Side effects of the surgical procedure are related to the size of the tumor and on the invasiveness of the tumor into adjacent structures. The most important side effects are hypopituitarism and permanent diabetes insipidus (57). Meningitis is a direct and serious surgical complication. After a follow up of more than 10 years, recurrences occur in 19 % of the patients, resulting in a 10 year cure rate of only 40 % (55). Therefore, adjuvant treatment is necessary in many patients to treat persistent or recurrent disease.

The first choice of adjuvant therapy is medical therapy. Current medical treatment options are mostly based on the fact that somatotroph adenomas express high levels of somatostatin receptors subtypes 2 and 5 (58;59). By stimulating these receptors, GH secretion will be suppressed, leading to decreased IGF-I levels. Two long-acting somatostatin analogues, octreotide and lanreotide, are currently available, which have a high binding affinity to somatostatin receptor subtype 2 and to a lesser extent subtype 5 (5). With the introduction of the octreotide long acting repeatable (LAR) formulation, patients only require an intramuscularly injection once monthly. In approximately 60 % of the patients octreotide treatment decreases GH levels beneath 2.5 μg/L and normalizes IGF-I levels (60;61).

In clinical practice, octreotide LAR appeared to be more effective in suppressing GH secretion than the slow release (SR) preparation of lanreotide (62-65). Lanreotide (SR) had to be injected every 2-3 weeks and effectively controlled GH and IGF-I levels in fewer patients. Recently, a new slow release depot preparation of lanreotide was introduced, Lanreotide Autogel (66). This new depot formula of lanreotide has to be injected subcutaneously only once a month. **Chapter 2** of this thesis describes a prospective study in which the efficacy of octreotide LAR and lanreotide Autogel in suppressing GH and IGF-I levels was compared in patients with active acromegaly.

Another new perspective in somatostatin analogue treatment is the development of SOM 230 (67). This novel drug has a broader binding affinity for somatostatin receptor subtypes. Compared with octreotide it has higher binding affinity to somatostatin receptor subtype 5, and to a lesser extent also to somatostatin receptor subtypes 1 and 4 (5;68). Preliminary data are promising, but further investigations are needed to establish the additional value of this new drug in the treatment of acromegaly (69).

In the development of drugs aimed at decreasing GH levels in acromegalic patients, recently a new approach was introduced. Pegvisomant is a competitive inhibitor of the GH receptor. Pegvisomant is highly effective and normalizes IGF-I levels in 97% of the patients (70;71). One of the concerns of this new drug is that by blocking the GH receptor the patient may become GH deficient. The clinical value of this drug in acromegaly has therefore to be studied in long-term studies (72). Currently, Pegvisomant is used in patients who do not effectively respond to treatment with somatostatin analogues.

The last therapeutical option in acromegaly is the use of radiotherapy. Although effective in reducing GH secretion, the response is slow and takes many years (73). After 6 years, 70% of the patients have normal IGF-I levels, whereas after 10 years, 10-15 % of the patients still have high levels of GH (73;74). One of the major side effects of radiotherapy is hypopituitarism, which develops in almost 55 % of the patients after 10 years (75). Lethargy, impaired cognitive function and personality changes are also observed in patients who were treated with radiotherapy (76). Therefore radiotherapy is currently used as an adjuvant therapy in acromegaly only when surgical and/or medical therapy have failed.

The goal of therapy in acromegaly is biochemical cure (according to consensus defined as GH levels below 2.5 μg/L (or 5 mU/L) and IGF-I levels in the normal age and sex adjusted range. These criteria are based on historical outcome parameters of therapy in acromegaly: Mortality risk normalizes when these treatment goals ar reached (53;77;78). The morbidity that accompanies acromegaly, like impaired glucose tolerance, an adverse lipid profile, sleeping disorders and acromegalic arthropathy (e.g. osteoartrithitis, cartilage thickness)

improves, when effective treatment is instituted. Also the cardiac abnormalities observed in active acromegaly, like biventricular-concentric hypertrophy, diastolic dysfunction at rest, systolic dysfunction at exercise and diastolic heart failure improve or normalize after curation (26;79-81). The above defined biochemical criteria for cure are supported by a recent study that showed that GH secretion in cured acromegalic patients assessed by with detailed 24-hour GH secretion profile did not differ from normal subjects (82).

Patients, in whom the biochemical treatment goals can only be reached by continuous treatment with medicial therapy, have so called "well-controlled disease". Studies have shown that morbidity in these well controlled patients improves to the same extent as in cured acromegalic patients: after 12 months of somatostatin therapy, a decrease in left ventricular mass, an improvement in diastolic function and - to a lesser extent - systolic function is observed (51), despite the fact that 24-hour GH secretion is not completely restored like in cured acromegalic patients (83). The question, therefore, remains, whether these patients still have persisting subtle effects of GH overproduction.

Moreover, most studies investigating the effect of treatment on cardiac function in acromegaly used heterogeneous groups, including de novo acromegaly patients in combination with uncontrolled treated patients, or well-controlled patients in combination with cured patients for analyses (81;84-89). One study used a homogenous group of patients (90), but did not include all relevant diastolic and systolic parameters. In **Chapter 3** it is investigated whether cardiac function in well-controlled acromegalic patients is really normalized as compared with cured patients, using 2 dimensional echocardiography, as well as Tissue Doppler echography, which allows detailed measurement of diastolic function.

A remarkable lack in the knowledge on cardiac consequences of acromegaly is that despite the many investigations on cardiac function in these patients, (26;51;79;80;91-93) little is known about cardiac valve function. It is known that GH excess changes the cardiac structure and diastolic function in acromegalic patients. It is also known that GH excess affects the composition and structure of collagenous tissue in

general. Heart valves consist mainly of collagen but it is has not been documented whether GH excess has any effect on cardiac valves. **Chapter 4** addresses this issue in different groups of acromegalic patients.

1.7 Pathophysiological effects of GH: GH deficiency

GH deficiency occurs when the pituitary secretes an insufficient amount of GH levels. This occurs in congenital pituitary deficiencies, like a Pit-1 or PROP-1 mutation, or in macroadenoma of the pituitary, (e.g. non-functional adenoma), or through other factors that damage the pituitary and/or hypothalamus (e.g. trans-sphenoidal surgery, or irradiation). Interestingly, disturbances in the GH-IGF-I axis can also occur in patients with a normal hypothalamus/pituitary axis but with a chronic disease, e.g. chronic heart failure or obesity.

Patients with adult-onset GH deficiency have many signs and symptoms (Table 2). GH deficiency is associated with a twofold increased risk of death from cardiovascular disease as compared with healthy controls (94-98). The high cardiovascular mortality risk is associated with an favourable metabolioc profile (abdominal obesity, insulin resistance, an abnormal lipid profile, atherosclerosis, endothelial dysfunction and hypercoagulability) and abnormal cardiac function (decreased cardiac function, with reduced left ventricular mass, left ventricular diameters resulting in an impaired systolic function at exercise). Other abnormalities are reduced pulmonary function, muscle dysfunction and decreased performance capacity (80;93;99-104).

Background
• Need for GH treatment as a child (GHD proven on retesting)
• Known pituitary pathology ± previous treatment
• Full "conventional" pituitary hormone replacement
Symptoms
• Abnormal body composition
Reduced lean body mass
Increased abdominal adiposity
• Reduced strength and exercise capacity
• Impaired psychological well-being
Reduced vitality and energy
Depressed mood
Emotional lability
Impaired self-control
Anxiety
Increased social isolation
Signs
• Overweight, with predominantly central (abdominal) adiposity
• Thin, dry skin; cool peripheries; poor venous access
• Reduced muscle strength
• Reduction exercise performance
• Depressed affect, labile emotions
Investigations
• Stimulated GH level below 3 μg/L
• Low or low-normal serum insulin-like growth factor-I (IGF-I)
• Elevated serum lipids, particularly low-density lipoprotein (LDL) cholesterol
• Reduced lean body mass/increased fat mass
• Reduced bone mineral density

Table 2. GH deficiency: Signs and symptoms (ref.108)

The introduction of recombinant human GH (rhGH) has been an important development for the treatment of GH deficiency. RhGH therapy restores IGF-I levels into the normal range (105;106). However, physiogical GH profiles cannot be restored, as GH levels after rhGH administration follow a pharmacological pattern with a T max of 2-4 hours with a slightly down slope GH level during 24 hour (107). Restoration of normal IGF-I levels, and to a lesser extent GH levels, result in an improvement of many organ functions, including insulin tolerance, lean body mass, lipid metabolism, exercise tolerance, bone mass, and cardiac function (102;108-110). When rhGH was introduced as a treatment for adult GH deficiency, studies suggested that quality of life (QoL) also improved. However, all these studies were limited with respect to the fact, that they only focussed on one dimension of QoL (see the review of Hull et al. (34)). There are only a few studies that studied different aspects of QoL, like depression and general well being

(114-116) . In addition, most studies investigated QoL in GH deficiency patients before and after rhGH administration, but did not compare QoL with healthy subjects (114). **Chapter 5** will address the QoL in GHD patients on long-term treatment with rhGH as compared to healthy subjects.

In patients with panhypopituitarism currently not all deficient hormones are replaced. The adrenal cortex produces more hormones than cortisol, which is the only adrenal hormone that is substituted in patients with ACTH insufficiency. One of the key products of the adrenal gland is the hormone DHEA (di-hydro-epi-androsterone) and its sulfate DHEA-S, from which many other steroid hormones are synthesized through intracrine pathways (117;118). Using DHEA replacement in patients with *primary* adrenal insufficiency, an improvement in quality of life, and sexual functioning was observed (119-121). Interestingly, some studies also showed, that DHEA replacement increased IGF-I levels (120;122-124). It was unclear, however, whether the increase in IGF-I levels was due to altered GH secretion and/or to an effect of DHEA on IGF-I production. We hypothesized that patients with secondary adrenal insufficiency will benefit of DHEA replacement, and that the increase of IGF-I could play a role in this improvement. In **chapter 5** the effect of DHEA replacement on QoL and IGF-I will be described in GH and ACTH deficient patients, treated with a fixed dose of rhGH.

In patients with alterations in the GH-IGF-I axis, like chronic non-endocrine disease, or even elderly subjects, the idea emerged that restoration of GH levels would benefit the patients. In line with this assumption, studies performed in the early nineties suggested that patients with idiopathic dilated cardiomyopathy or ischemic cardiac failure would benefit from the positive effects of GH on cardiac muscle (125;126). The idea was, that by improving the strength of the cardiac muscle cardiac function improves. Studies indeed showed promising results, but most studies were only observational. Randomised (controlled) studies could not confirm the results (127-129). These studies mainly focused on the systolic function, although the majority of patients with cardiac failure have diastolic dysfunction (130). Therefore, in **chapter 6**, the effect of rhGH in ischemic cardiomyopathy

on cardiac function with special focus on diastolic function is described.

1.8 Aims of this thesis

GH plays an important role in the human body, by direct and/or indirect mechanisms. The aim of this thesis is to focus on different clinical aspects of the physiological role of GH in humans, with a special focus on three different models. The first model is the model of GH excess: acromegaly. In this model we compared the effects of two somatostatin analogues on GH secretion, the effect of the treatment of acromegaly on cardiac function and on cardiac valves. In the second model, GH deficiency, the investigations focused on the effects of DHEA replacement on quality of life and IGF-I concentrations. The third and last model is the model of relative GH deficiency in a chronic non-endocrine disease (ischemic cardiomyopathy), where we tested the hypothesis that restoring GH levels could have beneficial effects on cardiac function.

In **chapter 2** data are presented of a comparison between a new depot somatostatin analogue, Lanreotide Autogel, and the only other available depot preparation, Octreotide LAR. Lanreotide Autogel is a new slow-release depot preparation that requires monthly injections. To compare the two medications in effectively suppressing GH levels, seven patients were first analysed during treatment with octreotide LAR before they were analysed on the new drug. The effects of GH suppression was analysed with two different approaches. First using GH profiles (an average of GH levels taken every 30 minutes for 3,5 hour) assessed 2, 4 and 6 weeks after an injection. Secondly, we compared GH secretion characteristics in detail via deconvolution analysis of 24 h plasma GH concentration profiles.

Chapter 3 presents data of cardiac function in patients with so-called well-controlled acromegaly during treatment with somatostatin analogues compared with other therapeutic modalities. One of the remarkable observations presented in chapter 2 is that although patients are well controlled by somatostatin analogues according to strict biochemical criteria, the total 24-hour GH production remains relatively

high, in comparison to acromegalic patients cured by surgery, who exhibit normal 24 GH secretion. It is uncertain, whether this difference in GH levels translates into biological effects. A sensitive organ that reacts to GH excess is the heart. Therefore, *de novo*, active and well-controlled patients, as well as cured acromegalic patients, underwent echocardiography to compare systolic and diastolic function in detail. Although there are many studies that assessed cardiac function in acromegaly patients, this is the first study in which diastolic and systolic functions in the four categories of therapeutic modalities were compared with each other.

Chapter 4 presents data of an observational study on the prevalence of myocardial valve dysfunction in acromegaly. Despite some case reports, the prevalence or incidence of valvular insufficiency has not been documented in acromegalic patients. To investigate the effects of active acromegaly on valvular insufficiency both active (*de novo* and treated active patients) and inactive (cured and well-controlled) patients were investigated.

In **chapter 5** the results of a double-blind, placebo controlled randomised cross-over study are presented. In this study the effects of DHEA on IGF-I and QoL were investigated in patients with GH deficiency and secondary adrenal failure, who were on stable hormone substitution. Any difference in QoL, measured with a broad spectrum of parameters, was assessed. Together with a general test (SF-36), the effects on depression, and anxiety (HADS), fatigue (MFI-20) and sexual functioning were investigated.

In **chapter 6** the data are presented of a randomised study in which rhGH in a fixed dose was given for 6 months to patients with ischemic cardiac failure. At baseline and after 6 months cardiac function was assessed using MR cardiac imaging. The goal was to investigate the change in systolic and diastolic function after rhGH therapy, as was suggested in a few studies with patients with dilated cardiomyopathy.

In **chapter 7** the data of the studies presented in this thesis are summarized and discussed.

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Chapter 2

Octreotide Long-Acting Repeatable and Lanreotide Autogel are equally effective in controlling growth hormone secretion in acromegalic patients

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Abstract

Objective Recently a new depot preparation of the long-acting somatostatin analogue, lanreotide Autogel was introduced for the treatment of acromegaly. Like octreotide long-acting repeatable (LAR), it has high binding affinity for the somatostatin receptor subtype SSTR 2 and less binding affinity for SSTR 5. We hypothesized that the ability to suppress GH secretion in patients with acromegaly would be similar for these depot preparations.

Patients and study design Seven patients (mean age 48.4 ± 7 yr) on long-term octreotide LAR treatment at a monthly injection interval for a mean of 2.8 yr were enrolled in the study. They underwent a GH secretory profile study with 10 min sampling for 24 hr, 28 days after an injection. At two, four and six weeks after the next injection fasting GH profiles (every 30 min for 3.5 hours) and serum IGF-I measurements were measured. These investigations were repeated 12 months later, when the patients were on an individually titrated stable dose of lanreotide Autogel.

Results Secretory characteristics and total 24 h GH secretion, estimated by deconvolution analysis of the 10 min 24 h plasma GH concentrations, did not show differences between these two long-acting somatostatin analogues. Both drugs were equally effective in GH and IGF-I suppression as measured at 2, 4 and also at 6 weeks following an injection.

Conclusion The efficacy of lanreotide Autogel and octreotide LAR was equal, notwithstanding that these drugs are administered in a different way and have different pharmacokinetics.

Introduction

Acromegaly is a syndrome caused by overproduction of growth hormone (GH) from a GH-secreting pituitary adenoma. The high levels of GH and IGF-I are associated with increased morbidity and mortality, which necessitates adequate control of the disease (1,2). Although transsphenoidal microsurgery is the first choice treatment in eligible patients, somatostatin analogues are the preferred secondary therapy in the 30-40 % of patients not in remission after surgery and in the 10-15 % who develop recurrence of disease during long-term follow-up(1,3,4). Furthermore, primary treatment of acromegaly with somatostatin analogues is increasingly applied (5).

Somatotrope adenomas express high levels of somatostatin receptor subtypes SSTR 2 and 5 (6,7). The somatostatin analogues octreotide (Novartis Pharma AG, Basel Switzerland) and lanreotide (Ipsen Biotech, Paris, France) both have high binding affinity for the SSTR 2 and to a lesser extent for the SSTR 5 (8). Octreotide LAR, the depot preparation of octreotide given by monthly i.m. injections is able to normalize GH concentrations in 56% and IGF-1 in 66% of the patients (9). Lanreotide Slow Release (SR), the more frequently i.m. injected depot preparation of lanreotide, seems less effective in normalizing GH and IGF-I concentration (10-13). Recently, lanreotide Autogel, a new slow-release depot preparation of lanreotide was introduced. This new delivery formulation is available in small-volume, prefilled syringes, and is administrated by monthly deep s.c. injections (14).

Considering the comparable binding affinity of octreotide and lanreotide for SSTR's, we hypothesized that there would be no difference between the two depot preparations in their ability to suppress GH secretion in patients with acromegaly. To test this hypothesis, we applied two different independent approaches: first by measuring GH secretion characteristics via deconvolution analysis of 24 h plasma GH concentrations profiles. We have recently shown that sustained blockade by octreotide can not restore all these parameters of abnormal GH secretion, but we have not established that for lanreotide (15). Secondly, we evaluated the extent and duration to which both depot somatostatin

analogues suppressed GH and IGF-I secretion.

Methods

Patients

For this study we included 7 patients with active acromegaly (of whom two were studied previously), who showed relatively good responsiveness to octreotide(15). The diagnosis was based on the characteristic clinical features and confirmed by insufficient suppression of GH concentration during the glucose tolerance test, the presence of a pituitary adenoma on radiological imaging, and elevated age-adjusted IGF-I concentrations. The clinical characteristics of the patients are described in Table 1. All patients used octreotide LAR (20 or 30 mg, at a monthly scheme) for an average duration of 2.8 years. None of the patients received a dopamine agonist before or during the study.

Although the treatment goals were similar for both analogues, i.e. GH < 5 mU/L and a normal age-related IGF-I, four out of seven patients were using 20 mg of octreotide LAR, whereas six patients required the highest dose (120 mg) of lanreotide. The patients were first titrated on octreotide LAR. When there was a discrepancy between GH and IGF-I concentrations we gave preference to IGF-I, at least when the clinical response was satisfactory. The resultant octreotide LAR dose required was 20 mg in four, and 30 mg in three patients. Three out of four patients already had reached the treatment goals, and the fourth patient did not have better results on 30 mg octreotide LAR.

The local Medical Ethical Committee approved the protocol and all patients gave written informed consent.

Study protocol (Figure 1)

The patients were investigated in a prospective study design according to the following protocol. The patients were first analysed on their regular octreotide LAR treatment. To assess details of GH secretory characteristics during chronic octreotide treatment, a 24 h plasma GH profile (with 10 min intervals) was performed 4 weeks after an octreotide LAR injection. To assess the extent and duration of GH suppression

during chronic treatment fasting morning GH profiles were obtained at 2, 4 and 6 weeks after the last octreotide LAR injection. Subsequently, patients were included in a Phase II International Multicentre Trial for Evaluating the Efficacy and Safety of Lanreotide Autogel (Data on file Ipsen –Beaufort, study E2852030717). Patients were randomised to receive 60, 90, or 120 mg of lanreotide Autogel, the dose of which was subsequently adjusted according to individual fasting GH profiles and IGF-I concentrations during the course of the trial aiming at a serum GH concentration < 5mU/L and a normal IGF-1 for age. Six of the seven patients required the highest lanreotide dose. At the end of the international trial, one year later, the investigations were repeated, i.e. a 24 h plasma GH profile 4 weeks after a lanreotide Autogel injection followed by a GH profile 2, 4 and 6 weeks after an injection. Radiological imaging (MRI or CT scan) of the pituitary was performed before the start on each analogue and twice during follow up.

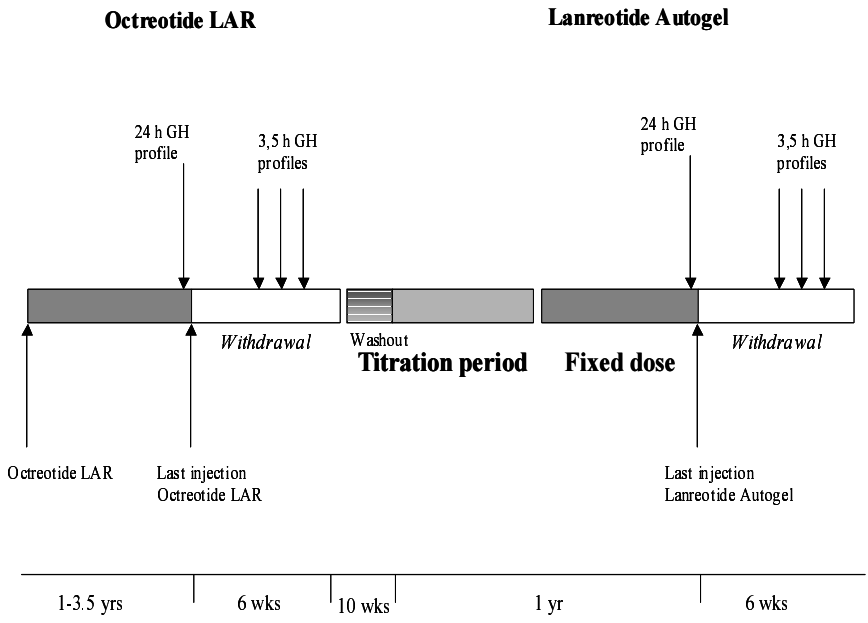

Figure 1. Study protocol.

Study parameters

24 hours GH profile

Patients were hospitalised the evening before the sampling studies, 27 days after the last injection with octreotide LAR or lanreotide Autogel. The following morning, an intravenous cannula was inserted into a large forearm vein, and blood samples were withdrawn at 10-min intervals for the next 24 h, starting at 9.00 h. Standard meals were served at predetermined time points, 7.30 h, 11.30 h and 17.30 h. Lights were turned off between 22.00 h and 07.00 h. All plasma samples were frozen immediately and stored at –20 ^{0} C until analysis.

Short-withdrawal study

After an overnight fast, patients were admitted to the Clinical Research Centre of the Department of Endocrinology between 08.00 and 09.00 h. An intravenous catheter was inserted in a forearm vein for collection of all blood samples. The patients were fasting during the blood sampling procedure. From the first blood sample plasma IGF-I concentration was determined. Subsequently, blood samples were obtained every thirty minutes for 3.5 hours. The mean GH was calculated from 8 samples. All samples from the patients were stored at – 20 ^{0} C until analysis in the same GH and IGF-I assay runs.

Assays

GH concentrations were measured with a sensitive time-resolved fluoro-immunoassay (Wallac, Turku, Finland) specific for the 22-kDa GH. The standard was recombinant human GH (Genotropin, KabiVitrium, Uppsala, Sweden), which was calibrated against the WHO First International Reference Preparation 80/505. To convert mU/L to μg/L, divide by 2.6). The limit of detection (defined as the value 2 SD above the mean value of the zero standard) was 0.03 mU/L. The intra-assay coefficient of variation (CV) ranged from 1.6 to 8.4 % in the assay range between 0.26-47 mU/L, with corresponding inter-assay CV's of 2.0 –9.9 %. The total serum IGF-I concentration was measured by IRMA after dissociation and blocking of the IGF-binding proteins with IGF-

II. (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The references values (95 % CI) per decade ranged form 11-77 nmol/l for 20 - 30 yrs, 10 - 42 nmol/l for 30 - 40 yrs, 8 – 33 nmol/l for 40-50 yrs, 8-24 nmol/l for 50-60 yrs and 8-22 nmol/l for 60-70 yrs.

Analytical techniques

Multiparameter deconvolution analysis was used to quantitate basal GH secretion and the GH half-life (16). This waveform-specific technique estimates the rate of basal release, the number and mass of randomly ordered secretory bursts, and the subject-specific (mono-exponential) half- life (17). The daily secretion rate is the product of secretory burst frequency and mean mass of GH released per event. Total GH secretion is the sum of basal and pulsatile secretion (16).(17)

Statistical Analysis

Data are given as the mean ± SEM, unless otherwise noted. Statistical analysis were carried out using Student's t-test when applicable and with multivariate repeat measures analysis to compare differences between and within groups. Calculations were performed with SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL). $P < 0.05$ was considered significant.

Table 1. Patient characteristics of 7 patients studied during chronic octreotide LAR and lanreotide Autogel therapy.

Number	Sex	Age (Yrs)	Transsphenoidal Surgery	Radiotherapy	Years on Octreotide LAR	IGF-1 at Presentation (nmol/L)	Suppressed GH during OGTT (mU/L)	Octreotide LAR Dose (mg)	Lanreotide Autogel Dose (mg)
1	f	57	1986	No	3.4	97	30	20	120
2	m	32	1997	No	3.1	*	7.15	30	120
3	m	42	1995	1996	2.1	97	10.47	20	60
4	m	53	No	No	3.5	37	196	20	120
5	m	20	No	No	3.5	30	*	30	120
6	m	68	No	No	3.2	69	19.8	30	120
7	m	67	No	No	1.0	27	14.71	20	120

Results

All patients completed the study. Chronic treatment with lanreotide Autogel was well tolerated by all patients, as was octreotide LAR treatment. Three patients experienced bowel cramps and diarrhoea for several days after the first injections of lanreotide Autogel, resolving after the fourth injection. No adverse events were reported during the study.

Of the three patients who had previous surgery, no change in residual tumor volume was noted on both analogues. In one out of four non-operated patients, a 50% decrease in tumor volume was found during octreotide LAR treatment, but no further change in tumor volume could be detected in these patients during subsequent lanreotide treatment.

24 hour plasma GH Profile

Fig.1 describes the 24 h plasma GH concentrations of all seven patients during treatment with both somatostatin analogues, showing a remarkable similar pattern. The secretory characteristics as analysed by deconvolution analysis were similar during both treatments and are detailed in Table 2. The pulsatile and total GH secretion per 24 h were not different between octreotide LAR and lanreotide Autogel.

Short withdrawal study

Table 3 describes the mean GH levels and IGF-I concentrations during the 6 weeks of withdrawal. Mean GH and IGF-I levels obtained 2, 4, and 6 weeks were not different between the two treatment groups. In time, no significant changes in GH levels and IGF-I concentrations could be detected within the two treatments.

The number of patients achieving both control of GH and IGF-1 were three under both treatment modalities. In only one patient of the remaining four, GH was normal on octreotide and slightly elevated on lanreotide, achieving concordance on both criteria in six out of seven patients.

Six weeks after a lanreotide Autogel and octreotide LAR injection only one had a normal mean GH and IGF-I for age.

Table 2. Deconvolution of 24 h secretory GH profiles in acromegalic patients on treatment with octreotide LAR and lanreotide Autogel.

	Octreotide LAR	Lanreotide Autogel	*P*-value
Secretory half duration (min)	27.8 ± 2.9	28.1 ± 3.3	0.95
Half life (min)	16.5 ± 1.5	15.1 ± 1.1	0.43
Number of secretory bursts/ 24 h	36 ± 1.2	37 ± 1.7	0.50
Interburst interval duration (min)	40.3 ± 1.2	38.7 ± 1.9	0.60
Secretory-burst amplitude (mU/L/min)	0.27 ± 0.06	0.29 ± 0.06	0.51
Burst mass (mU/L)	8.59 ± 2.46	9.02 ± 2.43	0.80
Basal secretion (mU/L/24 h)	246 ± 91	269 ± 114	0.49
Pulsatile secretion (mU/L/24 h)	296 ± 78	337 ± 91	0.34
Total secretion (mU/L/24 h)	543 ± 166	606 ± 199	0.24

Data are presented as mean ± SEM. Statistical comparisons were made with the paired Student's t-test

Table 3. **Mean GH and IGF-1 concentrations in acromegalic patients obtained 2, 4, and 6 weeks after injection of octreotide LAR or lanreotide Autogel.**

Weeks after injection	Octreotide LAR		Lanreotide Autogel	
	Mean GH (mU/L)	IGF-1 (nmol/L)	Mean GH (mU/L)	IGF-1 (nmol/L)
2	6.5 ± 1.8	46 ± 9	7.1 ± 1.8	47 ± 7
4	7.7 ± 2.0	40 ± 4	13.7 ± 6.9	55 ± 8
6	10.1 ± 2.3	47 ± 10	10.9 ± 3.1	53 ± 8

Data are shown as mean ± SEM. Statistical calculations were performed with multivariate repeat measures analysis. For mean GH levels no differences were found between lanreotide and octreotide treatment (P=0.53) and GH concentrations did not change in time (P=0.33). The interaction term (time x drug) was non-significant (P=0.53). For IGF-1 comparable results are shown. The P-values were 0.39, 0.43 and 0.34, respectively.

Discussion

This study is the first report that compares lanreotide Autogel, a new slow release formulation, with octreotide LAR. Both drugs are long-acting octapeptide depot somatostatin analogues used for the treatment of acromegaly (14,18). Both analogues showed similarly suppressed GH levels during a detailed 24 h study. Moreover, during the regular injection interval (2 and 4 weeks) and also 6 weeks after the injection, both drugs equally suppressed mean serum GH and IGF-I concentrations. The results from this study on lanreotide Autogel differ from previous studies, comparing lanreotide SR with octreotide LAR. Octreotide LAR, using one monthly injections generally seems to show a higher efficacy in suppressing serum GH and IGF-I levels than lanreotide SR, injected i.m. every 7-21 days (10-13). Lanreotide SR, however, is a different

preparation than lanreotide Autogel. Lanreotide SR consists of lanreotide incorporated into micro particles (like octreotide LAR) and is injected intramuscularly. In contrast, lanreotide Autogel consists of lanreotide-acetate dissolved in water, and is injected deep-subcutaneously. Pharmacokinetic studies of lanreotide Autogel in healthy subjects have shown a release pattern with an almost log-linear decrease of lanreotide serum levels after a single subcutaneous injection, with a terminal half-life of approximately 4 weeks (unpublished data from Ipsen- Beaufour). There is no evidence of accumulation of lanreotide after multiple doses at any dose neither in healthy subjects nor in patients. Steady state serum lanreotide levels are reached after 4 doses in most patients (unpublished data from Ipsen- Beaufour). To date, therapeutic lanreotide levels (> 1000 ng/L) in patients under chronic treatment were documented only 30 days after an injection. On the contrary, octreotide levels following a single octreotide LAR injection show a totally different release pattern: immediately after injection there is a small peak followed by an increase in octreotide levels after 7 days reaching a maximum at 28 days, while therapeutic octreotide levels (>600 ng/L) are maintained up to 42 days (19). These differences in pharmacokinetics between the two depot somatostatin analogues apparently do not result in different efficacy to suppress GH secretion.

In order to obtain the best possible assessment of the GH suppressive effect of both analogues we used detailed 24 hr data analyzed with deconvolution analysis. Both depot preparations are registered for clinical use with an injection interval of 4 weeks, implicating that a safe suppression of GH and IGF-I is guaranteed up to 28 days after an injection in octreotide or lanreotide sensitive patients. Hence, we considered this time point to be the optimal time point for evaluation of GH secretory profiles in patients on chronic octreotide LAR or lanreotide Autogel treatment. The 24 h GH deconvolution analyses illustrate that both long-acting somatostatin analogues, octreotide LAR and lanreotide Autogel induced comparable suppression of GH secretion. Our *in vivo* data are thus in accordance with the *in vitro* data showing equal binding affinity for the SSTR 2 and 5 (8).

Recently we showed that patients who had well-controlled GH and IGF-

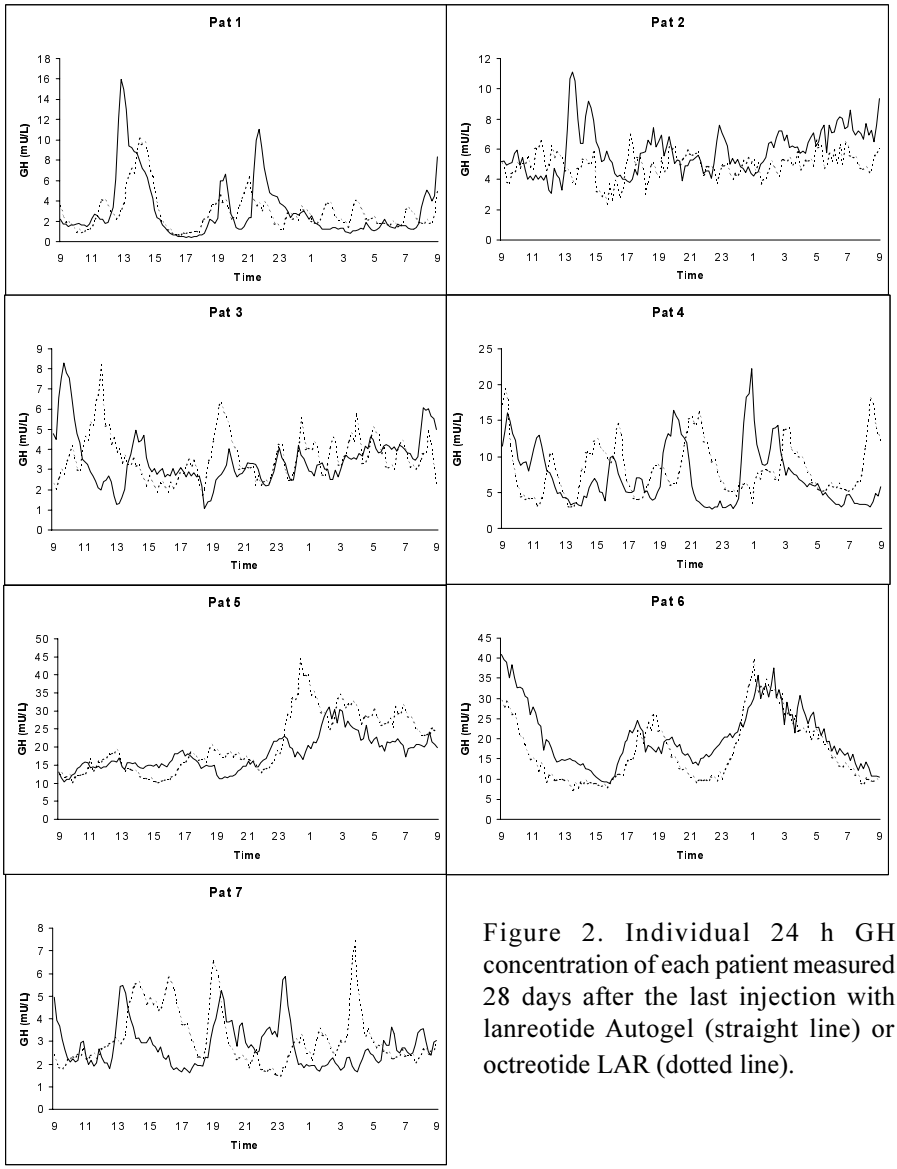

Figure 2. Individual 24 h GH concentration of each patient measured 28 days after the last injection with lanreotide Autogel (straight line) or octreotide LAR (dotted line).

I levels with monthly octreotide LAR injections, remained well-controlled in the long-term when the injection interval was extended to six weeks (20). Octreotide LAR and lanreotide Autogel showed similar GH and IGF-I suppression at 2, 4 and 6 weeks. Although the measurements after four weeks showed a small increase in mean GH and IGF-1 concentrations in lanreotide treated patients, this was not statistically significant. Furthermore, mean simulated GH profiles calculated from 8 comparable time points_during the 24h sampling studies and the IGF-I concentrations were statistically similar to the data obtained at 4 weeks of the withdrawal experiment. Therefore, it might be worthwhile to explore the extension of the injection interval for chronic treatment with lanreotide Autogel.

With regard to this observation, one should be cautious to interpret immediate surgical results in patients pre-treated with lanreotide Autogel. Similar to preoperative octreotide LAR treatment, we suggest postponing the postoperative biochemical evaluation to 3 months after the last injection of lanreotide Autogel (19).

Three patients well controlled on octreotide LAR 20 mg, were individually titrated to the maximum dose of lanreotide Autogel. One patient using octreotide LAR 20 mg was subsequently well- controlled with the lowest dose of lanreotide Autogel, e.g. 60 mg/4 weeks. Thus on an individual basis, different pharmacokinetics, for instance bioavailability, could lead to discrepancies in effective dosing.

In conclusion, lanreotide Autogel and octreotide LAR were equally effective in controlling GH secretion in active acromegaly as measured by suppression of total 24 h GH secretion and by mean GH levels and IGF-I concentrations. Further analysis should be focused on efficacy in the long-term.

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Chapter 3

Persistent Diastolic Dysfunction Despite Successful Long-term Octreotide Treatment in Acromegaly

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Abstract

Introduction: This study was designed to evaluate potential reversibility of left ventricular (LV) dysfunction in patients with acromegaly following long-term control of disease. It is unknown, whether the cardiac changes induced by acromegaly can be completely reversed by long-term strict control of growth hormone (GH) excess by octreotide.
Patients and Methods: We compared LV systolic and diastolic function in inactive patients with acromegaly (n=22) between patients with long-term control by octreotide (n=14) and patients with long-term cure by surgery/radiotherapy (n=8). We also assessed these parameters in patients with active acromegaly (n=17).
Results: In patients with active acromegaly, systolic function at rest was decreased by 18 % ($p<0.01$), LV mass index (LVMI) increased by 40 % ($p<0.04$) and isovolumetric relaxation time (IVRT) increased by 19 % ($p<0.01$), compared to patients with inactive acromegaly. These parameters were not different between well-controlled and cured patients. Using tissue Doppler imaging, the ratio between early- and late diastolic velocity (E'/A' ratio) was decreased in active, compared to inactive acromegaly (0.75 ± 0.07 *vs.* 1.24 ± 0.15, $p < 0.01$). This E'/A' ratio was considerably higher in cured, compared to octreotide treated patients (1.75 ± 0.41 *vs.* 1.05 ± 0.1, $p < 0.01$).
Conclusion: Diastolic function is persistently, and significantly more impaired in acromegalic patients with long-term control by octreotide than in surgically cured patients, which points to biological effects of subtle abnormalities in GH secretion. Criteria for strict biochemical control of acromegaly, should thus be reconsidered.

Introduction

Prolonged Growth Hormone (GH) excess can induce myocardial changes (1-4). These changes include left ventricular hypertrophy, diastolic dysfunction, systolic dysfunction during exercise, arrhythmias, and heart failure (5). Recently, regurgitant valve disease has also been documented in acromegalic patients (6;7). The incidence and severity of these cardiac changes are related to disease activity and disease duration. Left ventricular hypertrophy (LVH) appears to be an early consequence of GH excess (8-11), whereas arrhythmias and valvular disease are associated with longstanding disease (6;12).

Adequate treatment of GH excess can arrest, and even reverse, several of these cardiac changes. A total of 19 studies evaluated the effect of long-term suppression, i.e. 6 months or more, of GH excess on cardiac function, summarized in Table 1**.** From these studies, it is evident, that LV mass decreases, associated with improved systolic and diastolic function in patients, in whom GH excess is well-controlled. Nonetheless, it is not entirely clear to which extent long-term successful biochemical control of GH excess can reverse cardiac function. For instance, although octreotide treatment improved LV ejection fraction (LVEF), measured after one year of treatment, LVEF did not normalize completely (8). Only one study compared cardiac function 5 years after normalization of GH/IGF-I excess and compared patients with controlled disease and patients with cured disease. There were no differences in cardiac function, assessed by radionuclide ventriculography, between patients wellcontrolled by octreotide and those cured by surgery (13). However, all non-invasive techniques have major pitfalls insofar as they cannot measure directly LV pressures.

Recent data question, whether the currently accepted definition of biochemical control of GH excess, i.e. GH levels < 2.5mg/L and normal age- and sex-related IGF-1 levels, can be equated with normalization of all aspects of GH secretion in all circumstances. First, we showed (15), that long-term treatment of patients with active acromegaly with somatostatin analogues does not normalize 24-hour GH secretion, even though all these patients fulfilled the abovementioned

criteria for strict biochemical control. In contrast, 24-hour GH secretion in acromegalic patients cured by transsphenoidal surgery was not different from the values in matched controls. Second, discordant results between IGF-1 and GH concentrations have been reported in a significant proportion of newly diagnosed acromegalic patients (16,17). Because somatostatin analogues do not completely normalize GH secretion, it is possible that treatment with these analogues aiming at strict biochemical control of GH excess may not normalize cardiac abnormalities to the extent of the effects of curation by transsphenoidal surgery.

Therefore, we investigated whether there were differences in cardiac parameters between patients with long-term "control" of GH excess by treatment with octreotide and patients cured by surgery, using a group of patients with active acromegaly as a reference group. We used echocardiography including tissue Doppler imaging (TDI), which allows for a detailed and quantitative assessment of cardiac parameters including diastolic and systolic function (14).

Materials and methods

Patients

We studied 39 consecutive patients with acromegaly (19 men) referred from the outpatient clinic (Table 2). The mean age of the patients was 56 years (range 20-83 yrs). The diagnosis of acromegaly was based on the characteristic clinical features and confirmed by insufficient suppression of GH during a glucose tolerance test (GH nadir below 0.5 μg/L), and the presence of a pituitary adenoma on radiological imaging.

We classified patients according to the presence or absence of GH excess as having active or inactive acromegaly, resp. The patients with inactive acromegaly consisted of two groups: 1) well-controlled patients (n=14): mean fasting GH concentration (measured for 3 hours with an interval of 30 minutes) < 2.5mg/L, and normal age- and gender-adjusted IGF-1 concentrations during treatment with depot octreotide acetate, and 2) patients cured after surgery (n=8): no treatment with depot octreotide acetate, GH nadir after a 75 gram oral glucose loading < 0.5mg/L, and normal age- and gender- adjusted IGF-1 concentrations. Pre-therapy disease severity was not different between the two groups. The patients with active acromegaly consisted of two other groups: 3) untreated patients (n=8): no treatment to reduce GH-excess was yet instituted, and 4) uncontrolled patients (n=9): mean fasting GH concentrations (measured every 30 min for 3 hours) > 2.5mg/L, and/or elevated age- and sex adjusted IGF-1 concentrations despite treatment with maximal dosages of depot octreotide acetate (30 mg i.m. every 3 weeks). Cardiac parameters (see below) were analyzed in groups of cured and controlled acromegaly, using untreated and uncontrolled patients as a reference/control groups. None of the patients had hemodynamic instability, previous myocardial infarction, thyrotoxicosis, rheumatic fever, endocarditis, anorexigen use, or connective tissue disease. If hypertension was present (blood pressure > 140/95 mmHg), medication was prescribed to reduce blood pressure to values < 140/90 mmHg. All subjects had a normal blood pressure for at least 1 year prior to the study. Glucose tolerance was assessed according to the 1997 ADA criteria: normal: fasting plasma glucose below 6.1 mmol/L,

impaired fasting glucose (IFG): between 6.1-7.0 mmol/L, and diabetes mellitus: fasting plasma glucose equal to or greater than 7.0 mmol/L. None of the female patients was pregnant during 9 months following echocardiography. The duration of disease was defined by the onset of clinical symptoms related to GH excess (carpal tunnel syndrome, sleep apnoea, and arthralgias), and by careful comparison of old photographs. The end of disease duration was defined as the time of successful (= cure or well-controlled) treatment. The duration of well-controlled disease or cure was defined as the time of successful medical treatment and/or transsphenoidal surgery with/without adjuvant radiotherapy, until the time of echocardiography.

The local institutional ethics committee approved the study, and written informed consent was obtained from all subjects.

Echocardiography, Data Acquisition

Echocardiography was performed with the patients in the left lateral decubitus position using a commercially available system (Vingmed system FiVe/Vivid-7, General Electric – Vingmed, Milwaukee, WI, USA). Standard parasternal (long- and short-axis) and apical views (2-, 4-, and 5-chamber) were obtained. Standard continuous-wave and pulsed-wave Doppler examinations were performed. M-mode images were obtained from the parasternal long-axis views for quantitative assessment of left ventricular (LV) dimensions, fractional shortening (FS) and left ventricular ejection fraction (LVEF) (10;29). LV mass was calculated by the cube formula, and using the correction formula proposed by Devereux, et al.(18;19): 0.8 x {1.04 (LVEDD diameter +PWD + IVSD)^3 + LVEDD^3}+ 0.6. LVM indexation (LVMI) was corrected for body surface area (BSA). LV hypertrophy was defined as a LVMI above 135 g/m^2 for men and 110 g/m^2 for women. Systolic function was evaluated by measurements of FS and LVEF. The following parameters of diastolic function were measured: diastolic transmitral peak velocities (E and A wave), the E/A ratio, the isovolumetric relaxation time (IVRT), and the E-deceleration time. Quantitative diastolic data were derived from tissue Doppler imaging (TDI) analysis. For TDI analysis, the digital cineloops were analyzed using commercial

software (Echopac 6.1, General Electric – Vingmed, Milwaukee, WI, USA). The sample volume (4 mm^3) was placed in the LV basal portion of the septum (using the 4-chamber images). The following parameters (mean values calculated from 3 consecutive beats) were derived: early diastolic velocity (E') and late diastolic velocity (A'), and the E'/A' ratio. All echocardiographic examinations and analyses were performed by a single observer, blinded for treatment modalities.

Hormone Assays

GH concentrations were quantitated in duplicate using a sensitive time-resolved immunofluorescent assay (Wallac, Turku, Finland), specific for 22 kDA GH protein, and calibrated against WHO IRP 80/505. The detection limit was 0.012 ìg/L. Intra-assay coefficients of variation were 1.6-8.4% in the GH-range 0.012-18 ìg/L. The total serum IGF-1 concentration was determined by radioimmunoassay (RIA) after extraction and purification on ODS-silica columns (Incstar corp., Stillwater, MN, USA). The intra- and inter-assay coefficient of variation was less than 11%. The detection limit was 1.5 nmol/l). Age related normal data were determined in the same laboratory. IGF-1 was also expressed as a standard deviation score form age-related normal levels.

Statistical Analysis

Univariate analysis of variance was performed to compare groups, and the Bonferroni multiple comparison as a post hoc test. Linear-by-linear association was performed to investigate a trend for having untreated, uncontrolled, well-controlled or cured disease with E'/A' ratio <1. Data are expressed as mean ± SEM. SPSS software version 11.0 (Inc, Chicago, USA) was used. Differences were considered statistically significant at the $P<0.05$ level.

Table 1. The effect of long-term suppression of GH excess (6 months or more) on cardiac function in acromegaly

study	patients (n=)	definition of cure / well controlled disease	no of patients achieving treatment goals (%)	method of assessment	duration of treatment	effect on ca
Thuesen L, *et al* (1989) (ref 25)	9	decrease of GH by 62%	not defined	2 D echo	1 yr	- systolic functio - ↓ LV mass (but - diastolic functi
Chanson P, *et al* (1990) (ref 35)	3	mean GH 5.2 µg/L normal IGF-1	not defined	right heart cath	2-3 yrs	- cardiac index :r - ↓ filling pressu
Pereira J, *et al* (1991) (ref 28)	5	24 GH profiles: <5 µg/L IGF-1 not measured	4 (80%)	2D echo/Doppler	6 months	- systolic functio - ↓ LV mass - ↑early diastolic
Merola B, *et al* (1993) (ref 24)	11	mean GH 4.6 µg/L mean IGF-1 235 µg/L	11 (100%)	2D echo/Doppler	6 months	- systolic functio - ↓ LV mass - ↑ diastolic fillin
Hradec J, *et al* (1993) (ref 29)	22*	mean GH < 5 µg/L OGTT nadir< µg/L and IGF-1 < 450 µg/L	11 (50%)	2D echo/Doppler	10 yrs	- systolic functio - ↓ LV mass - diastolic functi
Tokgozoglu, *et al* (1994) (ref 26)	6	mean GH 4 µg/L IGF-1 not measured	none	2D echo / max treadmill test	6 months	- systolic functio - ↓ LV mass - diastolic functi
Colao A, *et al* (1999) (ref 23)	30	mean GH ↓ 2.5 1 µg/L nadir GH ↓ 1 µg/L normal IGF-1	13 (43%)	gated blood pool scintigrafy	1 yr	- inactive: ↑ LVE no changes in d - persistent activ
Hradec J, *et al* (1999) (ref 22)	13	not defined (mean GH and IGF-1 not normalized)	not available	2D echo/Doppler	18 months	- systolic functio - ↓ LV mass - diastolic functi
Baldelli, *et al* (1999) (ref 36)	13	GH <2.5 µg/L and/or normal IGF-1	8 (62%)	2D echo/Doppler	1 yr	- systolic functio - ↓ LV mass ↓ IRT and A
Colao A, *et al* (2000) (ref 20)	15	GH <3.5 µg/L / nadir <1.5 µg/L / normal IGF-1	9 (60%)	2D echo/Doppler / gated blood pool scintigrafy	6 months	- systolic functio - ↓ LV mass - diastolic functi

study	patients (n=)	definition of cure / well controlled disease	no of patients achieving treatment goals (%)	method of assessment	duration of treatment	effect on
Colao A, *et al* (2001) (ref 13)	18	fasting GH <2.5 µg/L, or <1 µg/L after OGTT and normal IGF-1	13 (72%) (7 cured 6 well-controlled)	gated blood pool scintigrafy	5 yrs	- contr: LVEF du - diastolic functio
Minniti G, *et al* (2001) (ref 37)	30	OGTT GH nadir <0.75 µg/L; normal IGF-1	15 (50%)	2D echo and Doppler	6 months	- systolic function - ↓ LV mass - diastolic functio
Herrmann B, *et al* (2002) (ref 38)	32	OGGT GH nadir < 1 µg/L and normal IGF-1; WC: normal IGF-1	19 (59%)	2D echo/Doppler + Tissue Doppler Imaging	not documented	- systolic function - diastolic functio cured/well-contr
Vianna C, *et al* (2002) (ref 39)	15 (only OK + RT)	basal GH<5 µg/L OGGT GH nadir <2 µg/L normal IGF-1	15 (100%) (preselected)	2D echo and Doppler	2.7 yr	- ↓ LV mass - diastolic functio
Colao A, *et al* (2002) (ref 9)	25	fasting GH <2.5 µg/L normal IGF-1	13 (53%)	2D echo/Doppler + gated blood pool scintigrafy	6 months	- systolic function LVEF peak ex - ↓ LV mass
Lombardi G, *et al* (2002) (ref 40)	19	fasting GH <2.5 µg/L normal IGF-1	11 (58%)	2D echo and Doppler	6 months	- systolic function - ↓ LV mass - diastolic functio
Gilbert J, *et al* (2003) (ref 20)	8	fasting GH <2.5 µg/L normal IGF-1	1 (13%)	2D echo	6 months	- systolic function - ↓ LV mass - diastolic functio
Colao A, *et al* (2003) (ref 41)	22	mean GH <2.5 µg/L normal IGF-1	22 (100%) preselected	2D echo/Doppler + gated blood pool scintigrafy	1 yr	- systolic function normalized in 8 pts) - ↓ LV mass - diastolic functio
Ciulla M, *et al* (2004) (ref 42)	16	mean GH <2.5 µg/L normal IGF-1	6 (38%)	2D echo/Doppler + Ultrasonic Tissue Characterisation	6-9 months	-systolic/diastolic - ↓ LV mass - normalization o
present series	39	mean GH <2.5 µg/L l, or OGTT GH nadir < 0.5 µg/L normal IGF-1	22 (56%)	2D echo/Doppler + Tissue Doppler Imaging	median 6 yrs (range 1-14)	- systolic function - ↓ LV mass - diastolic functio TDI: ↑, but pers controlled diseas

Results

Clinical Characteristics

The clinical characteristics are provided in Table 2. GH and IGF-1 concentrations were much higher in patients with active acromegaly, compared with the values obtained in patients with inactive acromegaly, reflecting the different inclusion criteria for the different groups. However, there were no differences in GH/IGF-1 levels between untreated and uncontrolled patients nor between well-controlled and cured patients. The duration of controlled GH/IGF-1 levels was not different between well-controlled patients and cured patients (mean 5.8 years, range 1-14 years, *vs.* mean 7.9 years, range 2-16 years, resp., P= NS).

Table 2. Baseline characteristics

	Untreated	Uncontrolled	Well-controlled	Cured
No. of patients	8	9	14	8
Age (yrs)	54.4±4.6	54.1±6.3	56.1±3.2	60.4±6.2
Gender (F/M)	6/2	2/7	6/8	6/2
Estimated disease duration (yrs)	9.9±1.51	18.6±4.4[§]	12.4±1.3	8.8±2.3[#]
controlled GH / normal IGF-I levels (mean + range in yrs)	-	-	5.8 (1-14)	7.9 (2-16)
GH (µg/L)	9.7±3.6	9.8±2.8	1.2±0.2[*‡]	0.9±0.4[*#]
IGF-I (SDS)	+7.63±1.96	+5.56±0.58	+1.22±0.27[*‡]	+1.07±0.42[*#]
Transsphenoidal Surgery	-	5	10	8
Radiotherapy		2	2	2
BMI (kg/m^2)	29.8±1.5	27.6±1.1	27.1±1.3	26.2±0.9
BSA (m^2)	1.96±0.04	2.08±0.6	2.04±0.07	1.95±0.06
Treated Hypertension (No.)	5	2	0	3
Diabetes mellitus / Impaired fasting glucose (No.)	2/2	2/1	2/1	1/1
Duration of octreotide treatment (mean + range in yrs)	-	3.5 (1-8.5)	5.8 (1-14)	-

[§] P<0.05 untreated *vs* uncontrolled

[*]P<0.05 untreated *vs* well-controlled, and [‡] uncontrolled *vs* well-controlled

[#]P<0.05 untreated *vs* cured, and [#] uncontrolled *vs* cured

LV Dimensions and Systolic Function at Rest(Table 3)

LV dimensions were not different between patients with active and inactive acromegaly. However, LVMI was above the normal range and

40 % higher in patients with active acromegaly, compared with patients with inactive acromegaly (140 ± 17.9 *vs.* 99.8 ± 8.8 g/m^2, resp, P<0.04). LVMI was within in the normal range and not different between well-controlled and cured patients.

Systolic function at rest, reflected in FS and LVEF, was decreased by 18-19 % in patients with active acromegaly, compared with patients with inactive acromegaly. FS was 30.3 ± 1.8 *vs.* 37.0 ± 1.2 %, resp (P< 0.01) and LVEF was 58.8 ± 2.3 *vs.* 72.6 ± 1.8 %, resp. (P<0.01). However, FS and LVEF were not different between well-controlled and cured patients.

Table 3. LV dimensions and systolic function at rest

	active acromegaly		inactive acromegaly	
	Untreated	Uncontrolled	Well-controlled	Cured
LVEDD (mm)	49.8±2.5	57.9±3.8	53.6±1.7	50.4±3.5
LVESD (mm)	34.4±2.7	40.2±4.1	34.1±1.9	32.6±2.7
IVSD (mm)	13.1±1.6	13.8±1.1	9.6±0.5*‡	10.6±1.3
PWD (mm)	11.0±0.9	10.3±0.8	9.7±0.5	9.4±0.5
LVM (g)	250±48	314±57	201±20‡	199±43
LVMI(g/m^2)	126.0±22.5	153±27.7	99.6±9.5‡	100.3±19.5
FS (%)	30.9±2.5	29.9±2.6	36.8±1.7‡	37.4±1.4$^{\#}$
LVEF (%)	57.8±3.8	59.7±2.81	72.4±2.6*‡	73±1.8$^{†\#}$

§ P<0.05 untreated *vs* uncontrolled

*P<0.05 untreated *vs* well-controlled, and ‡ uncontrolled *vs* well-controlled

†P<0.05 untreated *vs* cured, and $^{\#}$uncontrolled *vs* cured

$^{\$}$ P<0.05 well-controlled *vs* cured

LVEDD= Left Ventricular End-Diastolic Diameter, LVESD= Left Ventricular End-Systolic Diameter, IVSD= Inter Ventricular Septum Diameter, PWD= Posterior Wall Diameter, LVM= Left Ventricular Mass, LVMI= LVM/BSA, FS= Fractional Shortening, LVEF= Left Ventricular Ejection Fraction

Diastolic Function (Table 4 and Figure 1)

There were no statistically significant differences in diastolic transmural peak velocities (E and A waves) between patients with active and with inactive acromegaly. The isovolumetric relaxation time (IVRT) was increased by 19% in patients with active acromegaly compared with patients with inactive acromegaly (109.7 ± 4.0 *vs.* 88.7 ± 2.5 ms, P<0.01). However, there were no significant differences between untreated and uncontrolled patients, nor between well-controlled and cured patients. Diastolic function, assessed by TDI (Figure 1), showed that the early diastolic velocity (E') was significantly higher in cured patients as compared to the other patient groups (Table 4 and Figure 1). A significant

Table 4. Diastolic function as assessed by echocardiography and tissue Doppler imaging.

	active acromegaly		inactive acromegaly	
	Untreated	Uncontrolled	Well-Controlled	Cured
E (mm/s)	55.5±3.9	56.4±6.8	52.7±3.3	65±5.8
A (mm/s)	59.4±4.9	67.4±5.8	55.7±4.7	55.8±3.7
E/A ratio	1.0±0.2	0.83±0.5	1.01±0.1	1.18±0.1
Edec (ms)	171±10.2	180.3±19.4	180.1±17.2	163.8±12
IVRT(ms)	109.9±3.0	109.6±7.3	90.6±3.0*‡	84.2±4.1$^{†#}$
E' (cm/s)	6.83±0.99	5.23±0.76	7.89±0.34‡	10.06±0.84$^{†#\$}$
A' (cm/s)	9.38±1.21	6.71±0.36§	8.00±0.52	6.6±0.93†
E'/A'	0.72±0.07	0.79±0.19	1.05±0.09	1.75±0.41$^{†#\$}$

§ P<0.05 untreated *vs* uncontrolled

*P<0.05 untreated *vs* well-controlled, and ‡uncontrolled *vs* well-controlled

†P<0.05 untreated *vs* cured, and $^{\#}$uncontrolled *vs* cured

$^{\$}$ P<0.05 well-controlled *vs* cured

E= early transmitral peak velocity, A= late transmitral peak velocity, IVRT= isovolumeteric relaxation time, Edec= E-deceleration time, E'= early diastolic velocity, A'= late diastolic velocity.

difference in E' was also noted between uncontrolled and well-controlled patients: E' was higher in well-controlled patients ($P<0.01$), but was still significantly lower than in those who were cured of disease ($P<0.04$). The E'/A' ratio was considerably decreased in patients with active acromegaly compared with patients with inactive acromegaly (0.75 ± 0.07 *vs* 1.24 ± 0.15, $P<0.01$). In cured patients, the E'/A' ratio was significantly higher when compared to well-controlled patients (1.75 ± 0.41 *vs* 1.05 ± 0.1, resp, $P<0.01$). Remarkably, the E'/A' ratio was <1 in all untreated patients and in 75% of uncontrolled patients. The E'/A' ratio was <1 in 50% of the well-controlled patients *versus* in only 12% of the cured patients ($P=0.003$).

Discussion

The present study demonstrates that surgically cured acromegalic patients had significantly improved cardiac function compared to those in long-term remission by octreotide treatment. During long-term follow-up, diastolic function was significantly more impaired in the patients on medication despite optimal treatment with somatostatin analogues, according to strict criteria of GH/IGF-1 concentrations.

The question arises whether other factors may have affected our observations, other than these related to disease activity of acromegaly. First, the duration of GH excess is a determinant of cardiac abnormalities (6;8). The group characterized as having active disease had a longer duration of disease than the cured patients, which will have affected cardiac function. However, there were no significant differences in disease duration between well-controlled and cured patients. Second, the degree of GH excess is a determinant of cardiac abnormalities. However, there were no differences in GH levels obtained during several hours or IGF-1 levels between well-controlled and cured patients. Third, there were no differences in duration of strict control between both groups. Fourth, there were no differences in BMI or age, which may have affected our conclusions. Fifth, treated hypertension, which may have induced diastolic dysfunction, was present in 38% (3/8) of the

cured patients but in none of the well-controlled group. Moreover, all patients with treated hypertension had a blood pressure <140/90 mmHg during the year prior to the study. Finally, the prevalence of diabetes mellitus and impaired glucose tolerance was not different between cured and well-controlled patients (25 *vs* 21%, respectively). Therefore, our observations are not affected by differences in blood pressure or carbohydrate metabolism. Based on these arguments, we feel that it is unlikely that our interpretation of the data is confounded by other parameters than those related to disease activity of acromegaly. However, we cannot exclude the possibility that the persistent cardiac impairment could be due to still unknown factors other than GH hypersecretion, like asymptomatic ischemia.

Treatment of GH excess favourably affects cardiac function and mass. To our knowledge, a total of 19 studies involving 312 acromegalic patients, have been published, which have assessed the effect of treatment on cardiac function (Table 1). Treatment of GH excess decreases LV mass and improves diastolic function invariably, whereas systolic function at rest remained unchanged in most of the studies. Our data are in accordance with these conclusions of the other publications. Of the 312 patients, 53% (166 patients) achieved treatment goals, defined by normalization of IGF-1 and fasting GH levels below 2.5 ìg/L or glucose-suppressed GH levels below 1 ìg/L. The majority of these patients, 120/166 (72%), were well-controlled by somatostatin analogue treatment. In these 120 patients, LV mass normalized and cardiac function improved. This was mainly reflected by an increase in LVEF during exercise (20-28), a feature which is already observed within a few months of treatment with somatostatin analogues. Prolonged suppression of basal or glucose-suppressed GH levels to values below 2.5 or 1 ìg/L, respectively, in combination with normalization of plasma IGF-I levels for at least 1 year, resulted in significant improvement, but not complete normalization, of LVEF either at rest or at peak exercise without significant changes in diastolic filling (23). These data suggest that prolonged suppression of circulating GH and IGF-I levels normalizes systolic cardiac performance. Forty-six of the 166 patients (27%) with inactive acromegaly were biochemically cured by surgery and/or

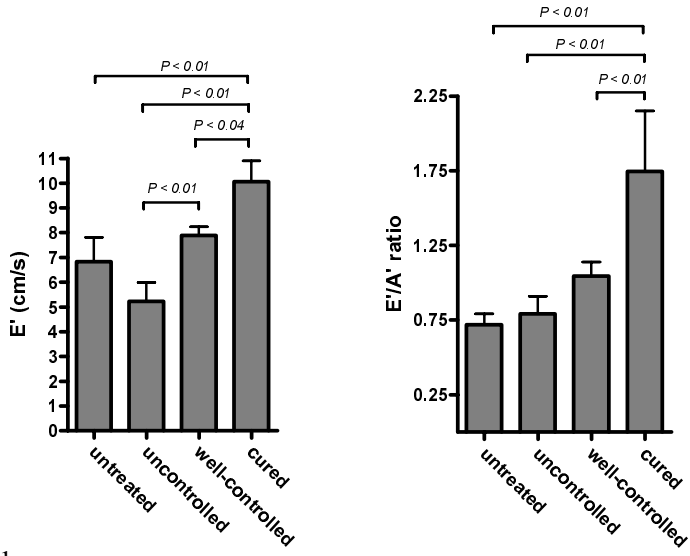

Figure 1

Diastolic function in patients with acromegaly as assessed by Tissue Doppler imaging

radiotherapy and were not treated with somatostatin analogues. Hradec, *et al*. demonstrated a clear beneficial effect of long-term cure on LVMI, but diastolic function was not assessed in that particular study (29). This beneficial effect of cure of GH excess on LVMI was confirmed in other studies, with a concomitant improvement, but not normalization, in diastolic function (27,28). The biochemical criteria used in the studies on cardiac function in acromegaly are based on other studies, which have shown that these criteria are associated with a reversal of the increased risk for malignancies and mortality, associated with GH excess (29,30). However, it is unknown whether biochemical control of GH/IGF-1 excess, according to these criteria, is also sufficient to normalize other GH-related morbidity, like acromegalic cardiomyopathy.

The findings in the current study, in patients with long-term (median of 6 years) control of GH excess, demonstrated that two independent parameters of diastolic function, the E'/A' ratio and the IVRT, improved significantly, indicating ameliorated relaxation and decreased stiffness of the heart muscle (32). However, a significantly higher E'/A' ratio was found in patients cured by surgery when compared

to those well-controlled with long-term octreotide. Furthermore, Sicolo, *et al.* (33) showed that in the presence of diastolic impairment, the incomplete recovery of an adequate preload can affect systolic parameters during physical effort. Since systolic function was only measured at rest, it is possible that systolic function could still be impaired on effort and hence there might be a difference between those cured and those in remission. Therefore, these data suggest that acromegalic patients, well controlled according to stringent criteria, still reveal biological effects of slight GH overproduction. In accordance, there are indications that treatment of active acromegaly with somatostatin analogues resulting in normal IGF-1 and GH levels does not completely normalize GH secretion. Recently, we investigated 24-hour GH profiles in uncontrolled and well-controlled acromegaly patients, treated with long acting somatostatin analogs (34). We applied the same strict biochemical criteria for well-controlled disease (normal IGF-I levels and a GH profile during 24-hour GH sampling < 2.5mg/L) in both groups. However, GH actually was sporadically below 0.5 mg/L, although chronic treatment with somatostatin analogues repressed amplitude-dependent measures of excessive GH secretion in acromegaly. Moreover, tumoral endocrine autonomy was inferred by continued elevations of event frequency, overall pattern disruption (irregularity), and nonsuppressible basal GH secretion. We postulate that these subtle abnormalities in GH secretion relate to the persistently impaired diastolic function despite clinically normal GH and IGF-1 levels.

In conclusion, long-term control of GH/IGF-I excess is associated with normal LV mass and LV dimensions. Nonetheless, diastolic function is more impaired in well-controlled patients than in surgically cured patients, which proves that the current criteria for strict biochemical control of acromegaly may still be associated with subtle effects of excessive GH secretion. Although the clinical relevance of this observation remains to be determined, these patients might benefit from more agressive control of GH production, than obtained by applying the current “strict” criteria of biochemical control of GH excess.

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Chapter 4

Increased Prevalence of Regurgitant Valvular Heart Disease in Acromegaly

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Abstract

Cardiac involvement is common in acromegaly, but the prevalence of valvular abnormalities in patients with acromegaly has not been documented, and was topic of the current study.

In a prospective study design, 40 consecutive patients with acromegaly and 120 control subjects (matched for age, sex, hypertension, and left ventricular systolic function) were studied. All patients and controls were evaluated using conventional 2D and Doppler echocardiography. Significant valve disease was more prevalent in acromegalics compared to controls: 22% *vs* 6.7% (p=0.005). Aortic valve regurgitation (more than or equal to trace severity) was present in 30% of patients *vs* 7% in controls (p<0.001), mitral regurgitation (more than or equal to moderate severity was absent in controls, but present in 5% of acromegalics (p=0.014 *vs* controls). Binary logistic regression analysis showed a significant impact only for disease duration on valvular disease, with an odds ratio of 1.19 (CI 1.028 – 1.376; p=0.019).

Acromegaly is associated with an increased prevalence of regurgitant valvular heart disease. This is dependent on the duration of exposure to increased growth hormone concentrations, with a 19% increase in odds per year. This increased prevalence of occult valvular disease implicates that these patients require appropriate follow-up care and monitoring, especially those with inadequate control of GH overproduction.

Introduction

Acromegaly is associated with increased cardiac morbidity and mortality. Recognized manifestations of cardiac disease in this population include chronic heart failure (CHF) due to either global systolic dysfunction (cardiomyopathy) or to diastolic dysfunction with preserved systolic function. The pathophysiology of these cardiac complications of acromegaly is incompletely understood. It has been hypothesized that abnormal extracellular matrix regulation by overproduction of growth hormone (GH) or IGF-1 in patients with acromegaly may contribute to both systolic and diastolic myocardial dysfunction (1). In addition, GH can increase circulating pro-inflammatory cytokine levels, like IL-1-beta and TNF-alpha (2). These cytokines, in turn, increase gene expression of matrix metalloproteinases (MMPs), that are capable of altering the composition of the extracellular matrix (3). These abnormalities in matrix regulation are associated with cardiac chamber dilation and reduced myocardial tensile strength (4). Abnormalities in matrix regulation have also been implicated in the pathogenesis of aortic and mitral valve disease (5-7) the latter of which is manifest as thickened and redundant valves which are incompetent and have an appearance of myxoid degeneration on pathology.

We hypothesized that patients with acromegaly, in whom GH and IGF-1 are pathologically elevated, have an increased incidence of clinically relevant aortic and mitral valve disease. There are anecdotal reports of aortic or mitral valve operations performed in patients with acromegaly that support this concept (8,9). However, the prevalence of valvular abnormalities in patients with acromegaly has not been documented. Therefore, we prospectively evaluated the prevalence of valvular abnormalities in patients with acromegaly and no prior history of cardiac disease.

Methods

Patients and controls

In a prospective study design, 40 patients (19 male and 21 female) with acromegaly were studied. The median age of the patients was 57 years (range 20-83 yrs). Nine patients were untreated (de novo patients), and 31 patients were treated either by transphenoidal surgery (n=23) or by primary medical treatment (n=8) (Table 1). The diagnosis of acromegaly was based on the characteristic clinical features of acromegaly and confirmed by insufficient suppression of GH concentration during a glucose tolerance test and the presence of a pituitary adenoma on radiological imaging. Disease activity was assessed as follows: Patients were classified as having active disease if they had mean fasting GH concentration (measured every 30 min for 3 hours) >5 mU/L and elevated age- and sex adjusted IGF-1 concentrations. Patients were classified as having inactive disease if mean GH concentration during fasting 3h profile were < 5 mU/L and IGF-1 concentrations were normal. A total of 18 patients were classified as having active disease (nine de novo patients and nine patients treated with depot octreotide acetate), and 22 patients as having inactive disease (eight cured patients and fourteen patients on depot octreotide acetate). Patients with hemodynamic instability, or a prior history of myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, anorexigen use, or other connective tissue disease were excluded from the study. Also, in female patients of childbearing age pregnancy was excluded. None of the patients appeared to be pregnant in the 9 months following echocardiography. The duration of disease was defined by the onset of clinical symptoms related to GH excess (carpal tunnel syndrome, sleep apnea, and arthralgias), and by careful comparison of old photographs. The end of disease duration was defined as the time of successful treatment.

Acromegalic patients ar rare, whereas controls with similar age/sex/comorbidities are not, hence we selected a larger control group. The acromegaly patients were collected first (with history and examinations) and 120 appropriate controls were selected based on age, sex, hypertension, left ventricular systolic function, based on a database with

this info. We controlled for systolic function to eliminate the possibility of getting a lot of patients with mitral regurgitation on the basis of left ventricular enlargement (incomplete closure). Patients were excluded if they were sent for echocardiac evaluation of known valvular disease, murmur, congestive heart failure, or cardiac transplant evaluation. Accordingly, most of the control patients were referred for either atypical chest pain, palpitations, or syncope without murmur. The study was approved by the local institutional ethics committees, and written informed consent was obtained from all subjects.

Echocardiography, Data Acquisition

Patients were imaged in the left lateral decubitus position using a commercially available system (Vingmed system FiVe, General Electric – Vingmed, Milwaukee, WI, USA). B-mode 2-D images were obtained with transmission frequencies of xx-3.5 MHz in the parasternal (standard long- and short-axis) and apical views (2-, 4-, and 5-chamber images). Color Doppler echocardiography was performed in all views after optimizing gain and Nyquist limit. Standard continuous-wave and pulse-wave Doppler examinations were performed. M-mode images were obtained from the parasternal long-axis views for quantitative assessment of LV dimensions, fractional shortening and left ventricular ejection fraction (10). When tricuspid regurgitation was present, pulmonary artery pressure was estimated using the modified Bernoulli equation. The severity of valvular regurgitant was determined by two independent expert readers blinded to the clinical data on a qualitative scale of trace, mild, moderate or severe, using previously described methods (11,12). Significant valvular disease was determined using the FDA case definition: mild or greater aortic regurgitation or mitral regurgitation equal or more than moderate severity (13).

Hormone assays

GH concentrations were quantitated in duplicate using a sensitive time-resolved immunofluorescent assay (Wallac Oy, Turku, Finland), specific for 22 kDA GH protein. The detection limit was 0.03 mU/l (0.01 ìg/L). Intra-assay coefficients of variation were 1.6-8.4% in the GH-range 0.1-

18 ìg/L (0.26-47 mU/L). The total serum IGF-1 concentration was determined by radioimmunoassay (RIA) after extraction and purification on ODS-silica columns (Incstar corp., Stillwater, MN, USA). The inter-assay coefficient of variation was less than 11%. The detection limit was 1.5 nmol/l). Age related normal data were determined in the same laboratory. IGF-1 was also expressed as a standard deviation score form age-related normal levels.

Statistical analysis

Student's *t*-test was used for continuous variables. The Chi-square test and the Cochrane-Mantell test were used to compare continuous and categorical data to detect trends. Binary logistic regression stepwise was performed to explore possible determinants of valvular disease. SPSS software version 10.0 (Inc, Chicago, USA) was used. Differences were considered statistically significant at the $p<0.05$ level.

Table 1. Patients Characteristics

	Patients (n=40)
Age (yrs)	59 ± 13.7
Sex (m/f)	19/21
BMI	28.2 ± 3.3
Cholesterol	5.65 ± 1.1
HDL	1.6 ± 0.39
Triglycerides	1.3 ± 0.52
HbA1c	5.59 ± 0.9
Hypertension	11 (27.5%)
Disease duration (yrs)	12.2 (1-46)
Previous transsphenoidal surgery	23
Active disease	
De novo	9
Active	9
Inactive disease	
Well- controlled	14
Cured	8
Octreotide treatment	23 (57%)
GH at presentation	91.7 ± 126.7 mU/L (range: 5-470)
IGF-1 at presentation	70.6 ± 33.8 nmol/L (range: 28-162)

Data presented as mean ± SD

Results

Left ventricular systolic function and dimensions in acromegalic patients were within the normal range (see Table 2). However, ten patients (25%) had left ventricular hypertrophy, defined by interventricular septum thickness (IVST) above 12 mm (n=9) and/or a posterior wall thickness (PWT) (n=6) above 12 mm. Their corrected Left Ventricular Mass index (LVMi) was above 110 gr/m^2 (for women) and 125 gr/m^2 (for men). Five of these ten patients had significant valve disease. Six patients had slightly increased LV dimensions (left ventricular end diastolic volume below 59 mm and < 32 mm, corrected for height, five of whom had normal left ventricular mass. (four of the six patients had significant valve disease).

Valvular stenosis was neither seen in patients with acromegaly, nor in controls. Any valve disease was seen in 50% of patients and in 50% of controls. Significant valve disease (by FDA criteria) was seen in 22% (9/40) of patients vs 6.7% (8/120) in controls (p=0.005) (or even only 4% (5/120) in control subjects if only mitral and aortic valve were analyzed). Three patients were subsequently operated, two for severe mitral valve regurgitation, and one for severe aortic valve regurgitation. The three patients, that underwent valve surgery, presented with following symptoms prior to echocardiography. The first patient was diagnosed with a systolic and diastolic murmur on preoperative

Table 2. Left ventricular measurements in acromegalic patients

	All patients	Normal values
LVEDD (mm)	53±8	40-59
LVESD (mm)	35±9	26-42
IVST (mm)	11±3	< 13
IVST >12 mm	n=9	
PST (mm)	10±2	< 13
PST>12 mm	n=6	
FS (%)	35±8	26-45
LVEF (%)	70±11	49-79

Data presented as mean ± SD. Normal values derived form : J Am Soc Echocardiography 2001;14:601-11 (ref no 18).
LVEDD:left ventricular enddiastolic diameter;LVESD:left ventricular endsystolic diameter; IVST: inter ventricular septum thickness; PWT: posterior wall thickness; FS: fractional shortening; LVEF: left ventricular ejection fraction

screening for a knee operation. The second patient had palpitations during physical exercise as a presenting symptom. The third patient did not experience any symptoms, although he had hypertension.

Prevalence of regurgitation (Table 3)

Aortic valve regurgitation was present in 30% (12/40) of patients *vs* 7% (8/120) in controls (p<0.001). These differences were significant for all grades of severity of regurgitation detected. Significant aortic valve regurgitation (by FDA criteria) was present in 20% (8/40) of patients *vs* 4% (5/120) in controls (p=0.002).

Mitral valve regurgitation was detectable in 35% (14/40) of the patients *vs* 32% (39/120) of control subjects (NS). However, pathological mitral regurgitation (moderate or severe according to the FDA criteria) was absent in controls, but present in 5% (2/40) of acromegalics (p=0.014, *vs* controls).

Table 3. Valvular regurgitation in acromegalic patients (n=40) compared to controls (n=120).

Valves		None % (n)	Trace % (n)	Mild % (n)	Moderate % (n)	Severe % (n)
Aortic*						
	Patients	70 (28)	10 (4)	17.5 (7)*	0	2.5 (1) *
	Controls	93 (112)	3 (3)	4 (5)	0	0
Mitral						
	Patients	65 (26)	20 (8)	10 (4)	0	5 (2) †
	Controls	68 (81)	18 (22)	14 (17)	0	0
Tricuspid						
	Patients	72 (33)	20 (8)	8 (3)	0	0
	Controls	50 (60)	27 (32)	20 (24)	3 (3)	0

* P=0.002 patients vs controls

†P= 0.014 patients vs controls

Tricuspid valve regurgitation was detectable in 28% (11/40) of the patients *vs* 50% (60/120) of control subjects. However, pathological tricuspid regurgitation (moderate or severe according to the FDA criteria) was absent in all patients and present in only 3% (3/120) of control subjects. This difference was not significantly different.

Valvular regurgitation of one valve was present in ten patients, valvular regurgitation of two valves in two patients, and valvular regurgitation of three valves in two patients.

Prevalence rates of valvular abnormalities were similar between active and inactive patients.

Determinants of valvular disease

Figure 1 describes the impact of disease duration on valvular disease in the acromegalic patients. Binary logistic regression analysis showed a significant impact of disease duration on valvular disease, odds ratio 1.19 (CI 1.028 – 1.376); i.e. every additional year of disease would result in a 19% increase in odds per year.

To explore other possible determinants of valvular disease, binary logistic regression analysis with a stepwise approach was performed. The following parameters were analyzed: IGF-1 and GH concentrations at time of diagnosis, age, active- *vs* inactive disease, (previous) treatment with octreotide, and the presence of hypertension. No significant correlations were found for any of the above mentioned parameters.

Discussion

This study clearly demonstrates that in patients with acromegaly, valvular abnormalities are more prevalent than in control subjects, who were individually matched for left ventricular function, age, sex, and the presence of hypertension. Moreover, we are the first to show that the prevalence of valvular disease in acromegaly proves to be highly significantly correlated to the duration of the disease.

In this study, we compared echocardiographic data in patients with acromegaly with active or inactive disease to a control data base. Matched data base analysis is necessary due to the relatively high prevalence of mild valvular abnormalities in the general population, which tends to increase with age. Studies using 2D color doppler echocardiography with a semiquantitative methods for estimating the severity of regurgitation from trace to severe, have demonstrated that the population-based prevalence of minimal or mild mitral and tricuspid valve regurgitation was quite high (58-77%), whereas aortic valve regurgitation was much less prevalent (14,15). Accordingly, the United States Food and Drug Administration (FDA) has defined pathological regurgitation of the mitral and tricuspid valve as more than or equal to moderate severity, and pathological regurgitation of the aortic valve as more than or equal to mild severity. However, in the normal offspring in the Framingham Heart Study (14), trace severity for mitral and tricuspid valve was present in 75% of the subjects and independent on age, whereas trace aortic regurgitation was only present in about 5% of subjects and strongly dependent on age (from about 2% at the age of 26-39 years to about 10% at the age of 70-83 years). Therefore, it has been postulated, that the FDA's criteria for aortic regurgitation may be too narrow, and the case definition for pathologic regurgitation may need to be modified or made age specific (15). In our study, we therefore individually matched each acromegalic patient for age, sex and the presence of hypertension, to three non acromegalic control subjects. We observed prevalence rates of any and significant valvular regurgitation in these 120 control subjects comparable to those reported in the normal offspring in the Framingham Heart Study. Aortic

regurgitation (present in 13% of men and 8% of women in the Framingham Heart Study) was present in 7% of our control subjects, *vs* in 30% of our patients with acromegaly. However, the abnormalities detected were predominantly mitral regurgitation of trace severity. This is due to the temporal/spatial high resolution of the echo equipment available (GE vivid 7, Vingmed system FiVe).

The pathogenesis of myxomatous heart valve degeneration remains uncertain. Rabkin and co-workers (6), proposed a model in which activation of interstitial cardiac valve cells leads to the release of proteolytic enzymes, phenotypic modulation, and proliferation. Subsequently, degradation of collagen, elastin fragmentation, and glycosaminoglycans accumulation produces extracellular matrix remodeling, and characteristic leaflet myxomatous thickening and redundancy. However, the primary stimulus for activation of these resting fibroblast-like interstitial cells remains to be elucidated, but mechanical stress and genetic abnormalities are proposed to play a key role. In our three patients who underwent valve replacement surgery, similar

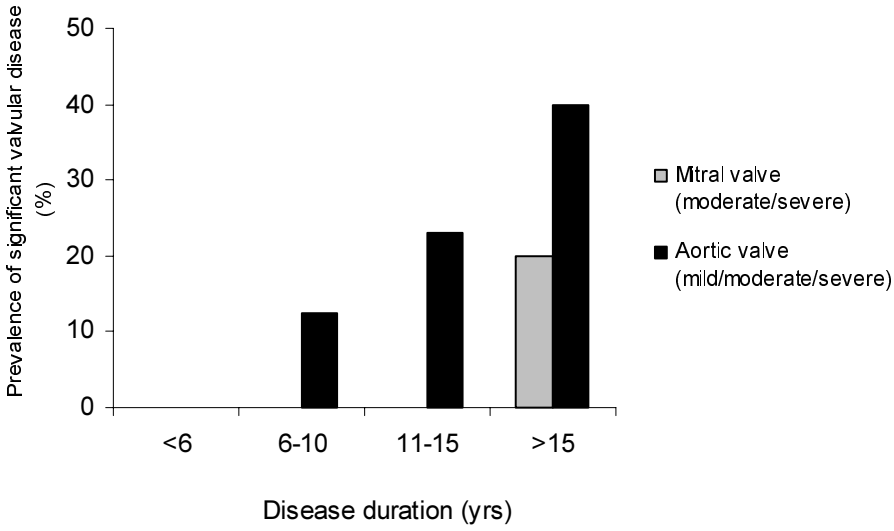

Figure 1: The impact of disease duration on the prevalence of significant valvular disease

pathological changes of myxomatous degeneration were observed (Figure 2). It is likely that direct or indirect effects of overproduction of GH are the causes for the observed valvular incompetence, for the following reasons. First, GH increases gene expression of the matrix metalloproteinases (MMPs)(16), resulting in abnormal matrix regulation. Second, recent data indicate that pro-inflammatory cytokine levels are increased in acromegalic patients with active disease (17). These cytokines, in turn, can also increase gene expression of MMPs, resulting in abnormal matrix regulation. Finally, the prevalence of valvular regurgitation observed in our acromegalic patients proved to be highly significantly correlated to the duration of the disease. Whereas pathological valvular regurgitation was absent in patients with an estimated disease duration of less than 6 years, the prevalence of aortic valve regurgitation (more than or equal to mild severity), increased from 12.5% for patients with a disease duration of 6 to 10 years, to 40% in patients with disease duration of more than 16 years. Similarly, prevalence of mitral valve regurgitation (more than or equal to moderate severity) was absent in controls, but present in 20% of acromegalic patients with disease duration of more than 16 years. Whether the patients had active disease or not at the time of evaluation did not influence these prevalence rates. From a pathophysiological point of view this is very interesting, since the valvular damage is apparently irreversible (in contrast to the already published regression of LVH in succesfully treated patients). This is also in accordance with the recently published study by Colao, et al. who reports an unexpectedly high prevalence of valve abnormalities in patients succesfully cured of acromegaly (19). Hence, chronic exposure to increased GH or IGF-1 production predisposes for myxomatous degeneration, with a calculated increase in odds in our study of 19% for the development of valvular disease for every additional year of exposure to tonically elevated growth hormone concentrations. Because the onset of GH overproduction is gradual, there is in general a long patient delay before the diagnosis of acromegaly is made. Therefore, it can be argued that an acurate assessment of disease duration is cumbersome. Before the onset of clinical symptoms, careful comparison of old photographs reveal (often

subtle, but clear) changes of the face. This retrospective evaluation of the combination of the onset of clinical symptoms and comparison of old photographs proved to be reproducible for estimating disease duration and to find significant associations of this estimated disease duration with mortality (20)**,** as well as with left ventricular hypertrophy and cardiac performance (21). However, this way of assessing disease duration will still lead to inaccuracies. Therefore, we decided to present the impact of disease duration of acromegaly on valvular disease also by stepwise increasing disease duration by very large intervals of 5 years. Highly significant increases in the prevalence of valvular disease were found between every five year interval and the preceding five years (figure 1). Eventhough there remain uncertanties with respect to the exact start of acromegaly, this analysis strongly support our notion that the prevalence of valvular heart disease is dependent on disease duration.

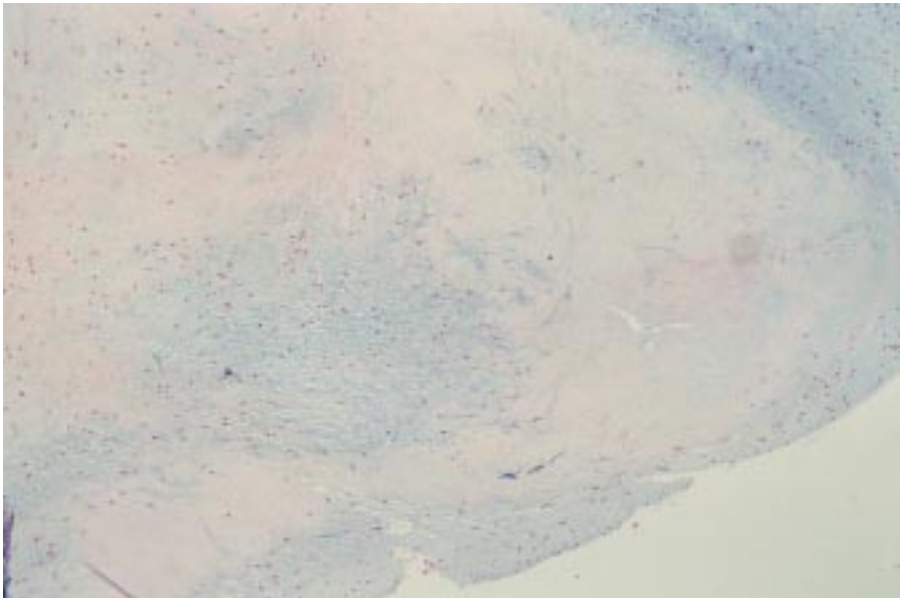

Figure 2: Mitral valve showing degenerative changes such as mucoid alteration of the preexistent collagenous tissue as indicated by the blewish staining (Alcian blue staining, magnification x 100)

It is interesting to note that all patients in whom valvular abnormalities were detected, had normal LV function and that 85% (17/20) of these patients also had normal dimensions. This suggests that valvular disease duration was brief for most patients. The discrepancy between the relative lack of cardiomyopathic responses in most patients in our study compared to the recently published study of Colao et al.(19) is striking, even though we found similar rates of regurgitant valve disease. However, considering the fact that 57% of our patients had been adequately controlled for a long time by octreotide therapy, and that they had similar prevalence rates of regurgitant valve disease as those without octreotide treatment, the following notion emerges. Octreotide is known to reverse the development of LVH (22). However, apparently, this was not reflected in a lower rate of regurgitant valve disease. In line with this notion we found fibrinoid changes in the valves that were removed. These changes are in other diseases associated with irreversible valve disease. Based on the comparison of the data of Colao, et al. (19) and our study, we hypothesize that regurgitant valvular disease in acromegaly may be less amnedable to therapeutic intervention aimed at reducing excessive GH secretion than myocardial complications of acromegaly.

In conclusion, we are the first to report that acromegaly is associated with an increased prevalence of regurgitant valvular heart disease, which is dependent of the duration of exposure to increased growth hormone concentrations, and which is not associated with impaired left ventricular function nor hypertension. This increased likelihood of valve disease may impact the cardiac surveillance of patients with acromegaly and could provide us more insight on the basic pathophysiological interactions of growth hormone with connective tissues. The increased prevalence of occult valve disease implicates that these patients require appropriate follow-up care and monitoring, especially those with inadequate control of GH overproduction. Moreover, antibiotic prophylaxis for any non-sterile procedures may be required.

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Chapter 5

Effects of DHEA, superimposed on growth hormone substitution, on quality of life and IGF-I in patients with secondary adrenal insufficiency: a randomised, placebo controlled, crossover trial

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Abstract

To assess whether DHEA substitution, superimposed on growth hormone (GH) substitution, improves quality of life of patients with secondary adrenal failure, we studied the effects of DHEA (50 mg/day, 16 weeks) *versus* placebo (16 weeks) in GH and ACTH deficient postmenopausal women (n=16, age 61 ± 2 yrs) and men (n=15, age 52 ± 3 yrs), in a double-blind, placebo controlled, cross-over study. All patients were on stable hormone replacement therapy, including a fixed dose of human recombinant GH during the study. The female patients did not receive estrogen substitution. The men received testosterone substitution.

At baseline, multiple parameters of quality of life were impaired compared to age- and sex-matched controls, especially in female patients. These parameters were not improved by DHEA treatment. DHEA improved only slightly the depression score (women) and health perception (women and men), although these parameters were not abnormal at baseline. DHEA increased serum IGF-I concentrations in female patients (by ~ 18 %, $p < 0.001$), but not in male patients. In neither group, DHEA affected IGFBP-3 levels.

We conclude, that DHEA, superimposed on GH substitution, does not improve quality of life substantially in patients with secondary adrenal insufficiency, irrespective of gender. In addition, DHEA increases IGF-I levels in estrogen depleted females, but not in testosterone treated males, with secondary adrenal insufficiency.

Introduction

Growth hormone (GH) deficiency is associated with impaired quality of life (1) and substitution with recombinant human GH (rhGH) improves quality of life (1-7). However, despite this beneficial effect of GH substitution and other pituitary hormones, these patients may still have significant impairments in multiple aspects of quality of life (8). It is likely, therefore, that other factors impair quality of life in these patients.

Many patients with GHD will also have secondary adrenal insufficiency, and, therefore, decreased levels of dehydroepiandrostenedione (DHEA) (9;10). DHEA has long been considered as an inactive precursor of sex steroids. However, deficiency of dehydroepiandrostenedione (DHEA) due to adrenal insufficiency is associated with impaired quality of life and treatment with DHEA in subjects with DHEA deficiency significantly improves quality of life (11-13)(See Table 1). In addition, beneficial effects of DHEA substitution are reported on other parameters like insulin resistance and bone mineral density (14-20). These beneficial effects are attributed to the conversion of DHEA into androgens and estrogens. Previously, only one study focussed on the effects of DHEA in female patients with secondary adrenal failure and showed that quality of life parameters improved (12). Remarkably, in that study quality of life parameters were assessed predominantly by the partners of the patients, rather than by the patients themselves.

Therefore, to assess whether DHEA substitution, superimposed on GH substitution, improves quality of life in male and female patients with secondary adrenal failure, we studied the effects of DHEA (50 mg/day, 16 weeks) *versus* placebo (16 weeks) in GH and ACTH deficient postmenopausal women (n=16) and GH and ACTH deficient men (n=15), in a double-blind, placebo controlled, cross-over study. All patients were on stable hormone replacement therapy, including a fixed dose of recombinant human GH (rhGH) during the study. As previous studies had not been controlled for estrogen status (Table 1), we chose to include only postmenopausal women without estrogen replacement therapy. Men were all on stable testosterone replacement.

There are indications that DHEA substitution may increase serum levels of insulin-like growth factor (IGF-I)(Table 1). Because our study was well controlled for growth hormone availability and DHEA might affect IGF-1 independently of GH secretion (21), we also evaluated the effects of DHEA on IGF-1 levels in our study.

Patients and methods

Patients

Patients with pituitary diseases and both ACTH and GH deficiency were recruited from the Outpatients Clinic of the Department Endocrinology and Metabolism from the Leiden University Medical Center. Recruitment of patients took place between October 2001 and April 2002. The Leiden University Medical Center is a large tertiary referral centre for pituitary disorders. Inclusion criteria were GH deficiency, proven by insufficient stimulation of GH secretion (GH < 7 mU/L) during insulin-induced hypoglycemia (minimal glucose concentration after insulin administration 2.2 mmol/L) with stable replacement therapy with rhGH for at least 3 months prior to the start of the study, and ACTH deficiency, proven by insufficient cortisol secretion (cortisol < 0.55 umol/L) during insulin-induced hypoglycemia, with stable hydrocortisone replacement therapy for at least 3 months prior to the start of the study. In all subjects IGF-I levels during treatment with rhGH were in the mean range of sex- and age matched values. Deficiencies of other hormones of the anterior pituitary as well as ADH were allowed, as long as stable substitution with thyroxin and ADH were realized for at least 3 months prior to the study. Thyroxin was dosed to obtain plasma FT4 values in the upper 50 % range of the normal reference values. The dose of thyroxin was stable for at least 3 months prior to starting the study. For all male participants, stable testosterone replacement by transdermal testosterone application (50 mg/day) was required (Testoderm, Ferring Pharmaceuticals, Hoofddorp, The Netherlands). For female participants estrogen replacement therapy was not allowed. Exclusion criteria were liver disease, malignant disease or other severe

Table 1. Overview of studies on the effects of DHEA substitution in patients with primary and/or secondary adrenal failure

Study	N	Sex	Type of Adrenal Failure	Hormone Status [&]	Design [#]	DHEA dose	Effect of DHEA on IGF-I
Arlt *et al* (11)	24	F	14 Primary 10 Secondary (combined analysis)	ER or ED	Double blind RCT, Cross over design DHEA *vs.* placebo Treatment for 4 m Wash-out 4 w Single center	50 mg	Increased only in primary, but not in secondary adrenal failure
Hunt *et al* (12)	39	24 F 15 M	Primary	F: ER or ED M; Testosterone replete	Double blind RCT, Cross over design DHEA *vs.* placebo Treatment for 3 m Wash-out 4 w Single center	50 mg	No effect
Johannsson *et al* (13)	38	F	Secondary	ER or ED Fixed rhGH substitution in 37 pts	Double blind RCT Parallel control group DHEA *vs.* placebo Treatment for 6 m Multicenter	20 (age > 45 yr) or 30 mg (age < 45 yr)	No effect
Lovas *et al* (31)	39	F	32 Primary 6 Secondary 1 unknown (combined analysis)	ER or ED	Double blind RCT Parallel control group DHEA *vs.* placebo Treatment for 9m Multicenter	25 mg	No effect
Present study	31	15 F 16 M	Secondary	F: Estrogen deficient M; Testosterone replete Fixed rhGH substitution	Double blind RCT Crossover design DHEA *vs.* placebo Treatment for 4 m Wash out 8 w Single center	50 mg	F; increase M: no change

[&] ER: estrogen replete (including estrogen replacement therapy (with or without progestagen)), ED: Estroge
or secondary hypogonadism.

[#] duration of trial: w=weeks, m= months, RCT: randomised controlled trial;

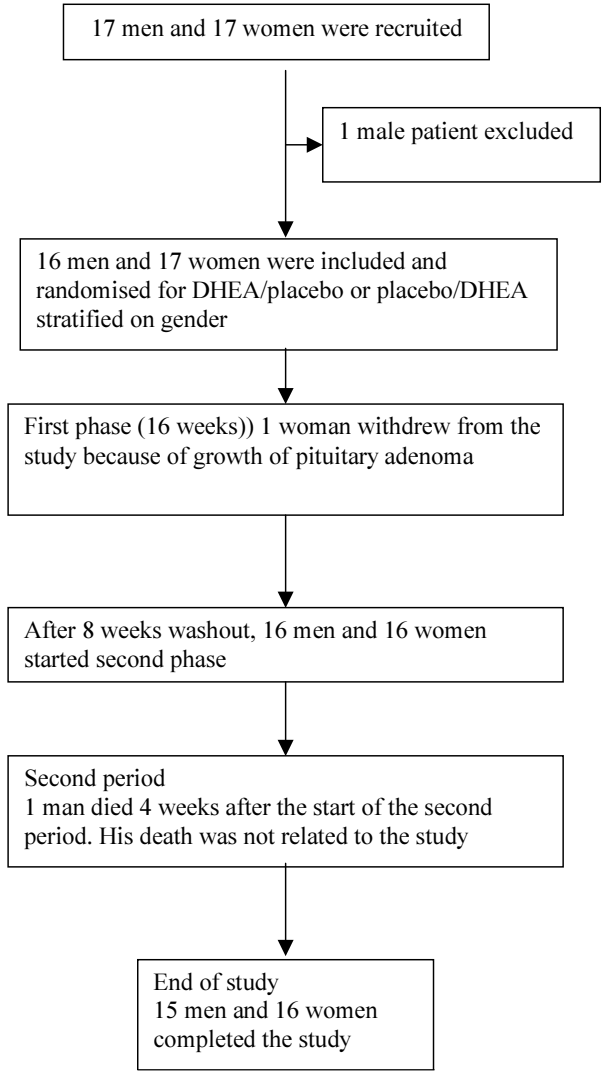
[¶] +: improvement in 1 or more items, =: no effect; Symptom checklist-90: the 90-item Checklist 90 (revised
Multidimensional Mood Questionnaire, VAS: Visual analogue scale, GHQ-30: General Health Questionna
Hospital anxiety and depression scale. MFI-20 : Multidimensional Fatigue Inventory-20, QOL-AGHDA: Q
in adults.

system disease as well as the use of drugs that could potentially interfere with the assessment of study parameters such as psychotropic drugs.

Study protocol

The study was a randomised placebo controlled, double blind crossover

Figure 1 Study Flow-Chart.



study with two treatment periods of 16 weeks separated by an 8-week wash-out period. A block randomisation scheme was used (n=2) with stratification for gender. The randomization schedule was prepared by the Department of Pharmacy. Patients received in random order 50 mg of Dehydroepiandrosterone (Vito Fit Corp., Helmond, The Netherlands) or placebo capsules (containing cellulose). Purity and quantity of DHEA were verified by HPLC analysis at the Department of Pharmacy of Leiden University Medical Center. DHEA or placebo capsules were taken orally each morning. rhGH was injected before bedtime. Compliance for study medication and regular medication was verified at each visit. The treatment allocation was de-blinded after all study data were authorized and introduced in a database, that was closed before deblinding.
The Medical Ethic Committee of The Leiden University Medical Center approved the study protocol, and all patients gave written informed consent.

Measurements
All visits took place at the outpatient clinic between 8.00 and 10.00 a.m.

Quality of life questionnaires
Quality of life investigation was performed with 5 validated questionnaires at baseline and at the end of each treatment period. The questionnaires are described in detail below. Questionnaires were filled-out in a quiet room in the morning. The baseline measurements were compared with an age- and sex-matched control group: for each participant in the DHEA study, 2 age- and sex-matched controls were selected from a group of 114 healthy relatives of GH deficient patients from the Department of Endocrinology and Metabolism of the LUMC (Table 4). The socioeconomic status (level of education, profession, marital state, living area) of controls and participants was comparable.

Short Form-36
The Short Form (SF)-36 comprises 36 items, which record general well being during the previous 30 days (22). The items are formulated as statements or questions and were scored as numbers. Eight parameters

are calculated with a range of 0-100: physical problems, bodily pain, general health, vitality, social functioning, emotional role and mental health. The first three parameters measure physical health, the last three parameters measure mental health, whereas the general health and vitality scales are sensitive to both physical and mental health outcomes. Higher scores represent better quality of life (23).

Quality of life-Assessment of growth hormone deficiency in Adults
The quality of life assessment of GH deficiency in adults (QOL-AGHDA) is developed specifically to assess the impact of GHD and GH replacement in adults (24). The items are formulated as statements and were scored as numbers. Low scores represent better quality of life (24).

Multidimensional Fatigue Inventory-20
The Multidimensional Fatigue Inventory-20 (MFI–20) records fatigue using 20 statements (25). Five parameters are calculated of the statements (general fatigue, physical fatigue, reduced activity, reduced motivation and mental fatigue), with a maximum score of 20 per parameter: A high score indicates a higher level of fatigue or impairment (26).

Hospital Anxiety and Depression Scale
The hospital anxiety and depression scale (HADS) consists of 14 items pertaining to anxiety and depression (27). Each item is scored as a number with a maximal score for each subscale (anxiety or depression) of 21. Higher scores indicate more severe anxiety or depression. A score of ³ 6 on the depression scale or ³ 7 on the anxiety scale, is considered abnormal (28;29).

Eleven Questions on Sexual Function
The Eleven Questions on Sexual Function (ESF) questionnaire is developed by the National Institute for Social Sexual Research (Rutgers Nisso Group, Utrecht, The Netherlands), with the Department of Sexuology of the LUMC. It measures sexual experience during the previous 30 days using 11 questions. For all patients, 3 parameters are

calculated out of 8 questions: Sexual fantasies, libido and general sexual satisfaction. For patients with partners, 3 additional parameters were calculated related to physical sexual functioning: problems with erection or lubrication, problems with orgasm and pain or discomfort during sexual activities. The questions were scored from 1-7, a higher number indicating a higher degree of satisfaction**.**

Endocrine parameters

All blood samples were taken between 08.00 and 10.00 A.M., before regular medication and study drugs were taken, with the exception of cortisol replacement therapy.

Study parameters were serum measurements of IGF-I, IGF-BP3, DHEA, DHEA-S, testosterone, estradiol, estrone, SHBG, serum lipids, HBA1C, and insulin. These measurements were performed at baseline and at the end of each study period. All blood samples were stored immediately at –80^{o}C until measurement. Other study parameters were anthropomorphic measurements (weight, BMI, waist-hip ratio).

Safety parameters

A general health questionnaire was done before the study. Laboratory safety parameters were serum levels of sodium, potassium, ALAT, ASAT, ãGT, AF and creatinin. Weight, heart rate and blood-pressure were recorded at every visit.

Laboratory Assays

All analyses of each subject were analyzed in the same run. Total serum IGF-I concentration was measured by ILMA after dissociation and blocking of the IGF-binding proteins with IGF-II (Nichols Advantage, Nichols Institute Diagnostics, San Clemente, CA, USA). Detection limit was 0.12 ng/ml (0.9 nmol/L). The intra-assay coefficient of variation (CV) was 4.4-5.2% and the inter-assay CV was 5.7-7.4%. Plasma IGF-BP3 concentration was measured by RIA (Nichols Institute Diagnostics, San Clemente, CA, USA). The inter-assay CV was below 6.8 % at the concentrations measured in the present study. The limit of detection was 0.0625 mg/L (2.8 nmol/l). Normal values range form 46 to 122

nmol/l in subjects aged 30-50 years, and 49-112 nmol/l for subjects between 50-70 years. GH concentrations were measured with a sensitive time-resolved fluoro-immunoassay (Wallac, Turku, Finland), specific for the 22-kDa GH. The standard was recombinant human GH (Genotropin, KabiVitrium, Uppsala, Sweden), which was calibrated against the WHO First International Reference Preparation 80/505. To convert mU/L to µg/L, divide by 2.6. The limit of detection (defined as the value 2 SD above the mean value of the zero standard) was 0.03 mU/L. The intra-assay CV ranged from 1.6 to 8.4 % in the assay range between 0.26-47 mU/L, with corresponding inter-assay CV of 2.0–9.9 %. DHEA was measured by RIA after extraction (DHEA-kit, DPC, Bad-Nauheim, Germany). The detection limit was 0.012 ug/L (0.04 nmol/L), the intra-assay CV was 5.2-10.8 %, and the inter-assay CV 5.9-11.7 %. DHEA-sulphate (DHEAS) was measured by ILMA (Immulite DPC, Los Angeles, Ca USA). The detection limit was 148 ug/L (0.4 ìmol/L), the intra-assay CV 7.0-9.5 %, the inter-asay variation 8-15 %. Androstenedione was measured by RIA (SL, Sinsheim, Germany), with a detection limit 0.02 ng/ml (0.07 nmol/L), an intra-assay CV of 2.7-6.3 %, and an inter-assay CV of 9.3-11.7 %. Total testosterone was measured by RIA (DPC, Los Angeles, Ca USA) with a detection limit of 0.08 ng/ml (0.2 nmol/l), and an intra- and interassay CV of 10-19 %. Estrone was measured using RIA (DSL, Veghel, The Netherlands), with a detection limit of 1,1 pg/ml (40 pmol/L), an intra-assay CV of 4.4-9.4%, and an inter-assay CV of 5-17 %. SHBG was measured with ILMA (Immulite, DPC, Bad-Nauheim, Germany) with a detection limit of 0.34 mg/L (4.0 nmol/L), an intra-assay CV of 4.1-7.7 % and an inter-assay CV of 4-20 %. Estradiol was determined with the Elecsys E170 (Roche Diagnostic Systems, Basle, Switzerland), detection limit 1,36 pg/ml (5 pmol/l), with an intra assay CV of 1.6-2.0 % and an inter-assay CV of 1.6-2.7 %. HBA1C was measured with the BioRad Variant method (BioRad Laboratories, Veenendaal, the Netherlands) with a detection limit of 3,6%, an intra-assay CV of 1% and an inter-assay CV of 1-2%. Serum insulin was measured by IRMA (BioSource, Etten-Leur, The Netherlands) with a detection limit of 0.1 mU/L (0.6 pmol/L), an intra-assay CV of 2.1-4.5% and an inter-assay CV of 3.1-4.3%.

A Hitachi 747 autoanalyzer (Roche Diagnostics, Mannheim, Germany) was used to quantify serum concentrations of total-cholesterol and triglycerides with enzymatic tests (all from Roche Diagnostics). High density lipoprotein (HDL) cholesterol was measured with a homogenous enzymatic assay (Hitachi 911, Roche Diagnostics). LDL-cholesterol concentrations were calculated with the Friedewald formula.

Statistics

The sample size was determined by a formal power analysis based on the rise in IGF-I in the study of Arlt et al (11). In this study, a pooled standard deviation of changes in IGF-I of 11% was found. It was calculated that with 2 groups of 15 patients, a rise in IGF-I of 12% could be detected with 80% power and alpha of 0.05. When no carry-over effect would be present, a minimal IGF-I rise of even 8% could be detected.

Data were analysed on a per protocol base. Treatment effects were analysed using univariate analysis of variance (ANOVA). The model associated with the ANOVA had an intercept representing treatment effects. All data were presented separately for men and women. The effects of treatment were also measured by adjusting for carry-over and time effects. The tests for carry-over and time effects followed the procedures described by Hills and Armitage (30). Carry-over and time effects were also tested. If no time or carry over effects were detected, data from both study periods were combined. Categorical data were analysed with the Chi-square test. Data are presented as mean ± SEM. SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL) was used for analysing and a p value of 0.05 was considered to be significant.

Table 2. Baseline characteristics of 31 patients who were treated with 50 mg/day DHEA during 16 weeks or placebo in a randomised cross-over design with 8-weeks washout.

F / M	16 / 15
Age: mean (yr)	57.2±2.0
Men	52.6 ± 3.5
Women	61.5 ± 1.7
Duration of GH therapy	5.2 ± 0.6
	(6 m –17 years)
Dose of GH therapy (mg/day)	
Men	0.41 ± 0.03
Women	0.45 ± 0.04
Cause of pituitary deficiency	
Non-functional pituitary adenoma	13
Cushing's disease	4
Prolactinoma	4
Craniopharyngioma	4
Other	6
Treatment	
Transsphenoidal surgery	14
Transcranial surgery	12
Radiotherapy	14
Surgery and radiotherapy	14
Radiotherapy and Adrenalectomy	3
Other Replacement Therapy	
L-Thyroxin	30
Cortisol	31
Estrogen/testosterone	0/15(only men)

Results

Clinical characteristics

Thirty-four patients were recruited, 17 women, and 17 men (Figure 1). One man was excluded before initiation of treatment, because he developed allergic reactions of the skin to transdermal testosterone replacement therapy. Therefore, 16 men and 17 women started the study. One female patient had progression of a non-endocrine pituitary adenoma, documented by MRI during the first study period, and decided to withdraw from the protocol. One male patient died at home during the second phase of the study probably because of an acute myocardial infarction, but the exact death cause could not be verified as no autopsy was performed. Data from these 2 patients were not included in the analyses of the data (see Figure 1). Thirty-one patients (16 women, 15 men) completed the study. The baseline characteristics of these patients are given in Table 2. No side effects such as acne or greasiness of the skin were observed in any patient

All women were postmenopausal, and did not receive estrogen replacement therapy. All men used transdermal testosterone replacement therapy. All patients had GH deficiency that had been treated for a mean period of 5.2 ± 0.6 years. The causes of pituitary insufficiency are given in Table 2.

Quality of life: baseline values compared with values obtained in controls

Quality of life parameters of patients compared with controls are given in Table 3. Multiple parameters appeared to be worse in patients than in controls despite conventional hormonal replacement therapy. In general, women had more abnormal quality of life parameters than men. Women scored significantly worse than age and sex-matched controls in 7 out of 15 tested parameters, whereas men scored worse in 3 out of 15 tested parameters than age- and sex matched controls. In women, physical functioning (SF-36), role limitations due to physical and emotional problems (SF-36) and general and physical fatigue (MFI-20) were worse than in controls. In men, general health perception was worse

than in controls (MFI-20). Both in men and women, social functioning (SF-36) and activity level (MFI-20) were worse than in controls.

Quality of life: Effects of DHEA versus placebo

The effects of DHEA on the outcome of the quality of life questionnaires in female and male patients are given separately in Table 4. There were

Table 3. Quality of life parameters in 31 patients with substituted ACTH and GH deficiencies obtained at baseline and 62 age and sex matched controls.

	Women			**Men**		
	Controls	*Patients*	*P value vs. Controls*	*Controls*	*Patients*	*P value Vs. Controls*
Number	32	16		30	15	
Age (years)	61.2 ± 1.3	61.1 ± 1.7	P=0.940	53.1 ± 2.5	52.1 ± 3.3	0.800
Questionnaire						
HADS						
Anxiety	4.66 ± 0.62	5.53 ± 0.88	0.424	3.07 ± 0.50	4.88 ± 0.84	0.075
Depression	3.11 ± 0.44	3.76 ± 0.82	0.489	3.60 ± 0.56	4.50 ± 1.05	0.457
Total	7.77 ± 0.96	9.29 ± 1.47	0.392	6.67 ± 0.92	9.38 ± 1.55	0.145
SF-36						
Physical functioning	86.6 ± 2.6	70.9 ± 7.3	0.017	85.2 ± 3.9	87.9 ± 2.5	0.567
Social functioning	93.0 ± 2.6	76.5 ± 6.1	0.003	93.3 ± 2.6	80.5 ± 5.5	0.020
Role limitations due to physical problems	93.4 ± 3.4	66.2 ± 9.3	0.002	86.7 ± 5.8	68.8 ± 9.5	0.121
Role limitations due to emotional problems	93.1 ± 3.4	72.6 ± 10.4	0.022	92.2 ± 4.1	81.3 ± 7.4	0.209
Bodily Pain	86.6 ± 3.2	77.6 ± 6.1	0.201	85.5 ± 3.2	91.2 ± 3.4	0.232
General health perception	71.5 ± 2.9	60.0 ± 6.1	0.062	74.0 ± 3.1	58.8 ± 3.8	0.003
Change in health	55.4 ± 2.5	52.9 ± 4.2	0.766	56.7 ± 3.4	53.1 ± 3.1	0.446
MFI-20						
General fatigue	7.71 ± 0.60	11.35 ± 1.37	0.005	7.72 ± 0.54	9.38 ± 1.22	0.163
Physical fatigue	7.43 ± 0.62	10.12 ± 1.37	0.043	7.83 ± 0.65	9.13 ± 1.09	0.283
Reduced activity	7.17 ± 0.58	9.47 ± 0.91	0.042	6.86 ± 0.50	9.19 ± 0.98	0.023
Reduced motivation	7.17 ± 0.59	8.18 ± 0.98	0.386	7.24 ± 0.60	9.50 ± 1.10	0.083
Mental fatigue	8.43 ± 0.77	9.41 ± 1.29	0.517	7.10 ± 0.69	9.31 ± 1.32	0.151

Data expressed as mean ± SEM
HADS: Hospital anxiety and depression scale
SF-36: Short Form (SF)-36
MFI-20 : Multidimensional Fatigue Inventory-20

Table 4. Quality of life parameters in 31 patients with substituted ACTH and GH deficiencies after 16 weeks treatment with 50 mg/day DHEA or placebo

	Women			**Men**		
Questionnaire	*Placebo*	*DHEA*	*P value vs. placebo*	*Placebo*	*DHEA*	*P value vs. placebo*
HADS						
Anxiety	5.06 ± 0.99	4.69 ± 1.00	0.478	3.00 ± 0.70	3.33 ± 0.76	0.625
Depression	4.00 ± 0.82	2.38 ± 0.52	0.022	4.47 ± 0.99	3.73 ± 0.96	0.661
Total	9.06 ± 1.53	7.06 ± 1.34	0.078	7.47 ± 1.40	7.07 ± 1.62	0.653
SF-36						
Physical functioning	68.1 ± 7.9	71.9 ± 8.1	0.221	93.3 ± 1.6	92.9 ± 1.8	0.792
Social functioning	76.6 ± 7.2	82.8 ± 7.2	0.119	82.5 ± 4.7	85.8 ± 4.0	0.217
Role limitations due to physical problems	60.9 ± 11.2	68.8 ± 10.3	0.370	93.3 ± 3.8	93.3 ± 3.0	1.000
Role limitations due to emotional problems	60.4 ± 11.5	68.8 ± 10.3	0.523	80.0 ± 7.8	82.2 ± 7.9	0.719
Bodily Pain	72.6 ± 6.7	70.0 ± 6.9	0.661	95.1 ± 2.5	95.1 ± 2.9	1.000
General health perception	67.8 ± 5.5	63.8 ± 5.7	0.254	61.3 ± 4.9	63.3 ± 4.2	0.645
Change in health	57.8 ± 5.0	67.2 ± 4.4	0.009	56.3 ± 3.6	65.0 ± 4.5	0.034
MFI-20						
General fatigue	11.1 ± 1.28	10.56 ± 1.24	0.620	9.53 ± 1.23	8.60 ± 1.03	0.178
Physical fatigue	9.88 ± 1.24	10.19 ± 1.37	0.789	9.00 ± 1.01	7.93 ± 0.92	0.064
Reduced activity	10.19 ± 1.11	9.00 ± 1.08	0.284	9.07 ± 0.96	8.60 ± 0.85	0.388
Reduced motivation	8.25 ± 0.83	7.25 ± 0.72	0.198	8.87 ± 1.09	8.80 ± 1.01	0.923
Mental fatigue	9.13 ± 1.35	8.44 ± 1.17	0.491	8.07 ± 1.16	8.20 ± 1.32	0.862
QOL-AGHDA						
Total	7.31 ± 1.46	6.50 ± 1.47	0.422	6.33 ± 1.61	6.60 ± 1.61	0.653
ESF						
All patients						
Fantasies	2.20 ± 0.43	2.13 ± 0.45	0.843	3.57 ± 0.48	3.57 ± 0.52	1.000
Libido	2.27 ± 0.33	2.20 ± 0.37	0.774	3.14 ± 0.28	3.36 ± 0.40	0.533
Satisfaction	3.07 ± 0.25	3.20 ± 0.22	0.582	2.92 ± 0.34	2.71 ± 0.29	0.426
Patients with partners	N=10			N=9		
Problems erection/lubrication	1.60 ± 2.60	2.47 ± 0.36	0.085	1.78 ± 0.28	1.67 ± 0.29	0.347
Problems orgasm	1.70 ± 0.27	2.10 ± 0.31	0.210	1.50 ± 0.28	1.39 ± 0.33	0.347
Pain	1.30 ± 0.21	1.40 ± 0.16	0.591	1.00 ± 0.00	1.11 ± 0.11	0.336

Data expressed as mean ± SEM

QOL-AGHDA: QOL-assessment of GH deficiency in adults
HADS: Hospital anxiety and depression scale
SF-36: Short Form (SF)-36
MFI-20: Multidimensional Fatigue Inventory-20
ESF: Eleven Questions on Sexual Functioning

no carry-over or time effects for any of the study parameters. Remarkably, parameters that were abnormal at baseline compared with controls did not improve significantly upon treatment with DHEA. In women, a significant improvement in the depression score (HADS) was observed. In both women and men, change in health (SF-36) improved significantly. DHEA had no effect on the different dimensions of fatigue, or on parameters of sexual functioning. Patients with partners showed no beneficial effect of DHEA on sexual performance nor did the satisfaction about their sex life change.

IGF-I and IGF-BP3 concentrations: effects of DHEA versus placebo
DHEA treatment significantly increased serum IGF-1 levels by ~18 % in female patients, compared to placebo treatment ($p<0.001$, Table 5 and Figure 2). In contrast, in male patients, there was no significant effect of DHEA, compared to placebo, on IGF-I levels (Table 5, Figure 2). DHEA did not influence IGF–BP3 levels in female or in male patients.

Other plasma concentrations: effects of DHEA versus placebo
DHEA treatment increased serum levels of DHEA, DHEAS, estrone and androstenedione substantially in both men and women (Table 5). DHEA substitution increased estradiol only in women. Interestingly, after DHEA treatment, androstenedione and estrone levels of women reached baseline levels of men.

Other parameters: effects of DHEA versus placebo
BMI, waist and waist-hip ratio were not influenced by DHEA treatment. Fasting serum lipid levels, glucose and insulin levels were not influenced by DHEA (data not shown).

Side effects of DHEA
There were no side effects reported during DHEA or placebo treatment. Some patients experienced an increase in perspiration, but this was not different between both groups. There were no differences observed between DHEA *versus* placebo treatment in systolic or diastolic blood pressures, pulse rate or in safety laboratory parameters.

Table 5. Endocrine parameters of 31 patients with substituted ACTH and GH deficiencies at the end of 16-weeks therapy with 50 mg/day DHEA or placebo

	Women			**Men**		
Hormones	Placebo	DHEA	P value vs. placebo	Placebo	DHEA	*P value vs. placebo*
IGF-I (ng/mL)	169±13.8	200±12.8	<0.001	209±12.4	218±12.3	0.107
IGF-BP3 (mg/L)	2.04±0.08	2.24±0.16	0.116	3.03±0.13	3.04±0.1	0.449
DHEA (nmol/L)	1.0±0.5	8.5±0.8	<0.001	0.5±0.1	5.6±0.5	<0.001
DHEA-S (ng/mL)	8.1±1.1	208.6±27.9	<0.001	10.0±2.0	269.7±30.8	<0.001
Androstenedione (nmol/L)	0.3±0.1	1.7±0.2	<0.001	1.2±0.2	1.9±0.2	0.011
Estradiol (pmol/L)	17.4±4.2	32±2.2	0.006	49.1±8.5	46.8±6.3	0.814
Estrone (pmol/L)	22.0±4.2	94.5±9.4	<0.001	55.5±8.1	108.7±11.8	<0.001
Testosterone (nmol/L)	0.2±0.0	0.7±0.1	0.008	14.3±2.9	12.5±1.5	0.526
SHBG (nmol/L)	60.6±6.6	60.7±8.0	0.990	40.0±5.5	35.7±3.8	0.204

Data expressed as mean ± SEM.
Conversion factors (SI to metric): DHEA 0.288 (ug/L), Androstenedione 0.286 (ng/ml), Estradiol 0.272 (pg/ml), Estrone 0.027 (pg/ml), Testosterone 0.288 (ng/ml), SHBG 0.086 (mg/L).

Discussion

The present study was performed to study if DHEA substitution, superimposed on replacement with rhGH, has effects on quality of life in patients with pituitary diseases, resulting in GH and ACTH deficiencies. At baseline, we found that multiple quality of life parameters were worse in patients than in controls. This observation was more pronounced in women than in men. DHEA treatment showed subtle improvements in a limited number of quality of life parameters in men and women. However, these improvements occurred only in parameters, that were not different from age- and sex matched controls at baseline.

At present 4 randomized trials have been published on the effect of DHEA substitution on quality of life parameters in patients with primary and/or secondary adrenal insufficiency. These studies are summarized in Table 1. Three studies documented beneficial effects on parameters of quality of life (11-13). In contrast, the study of Lovas *et al.* found no significant effects by DHEA on these parameters (31). However, that study was criticized, because of being underpowered (32). They used a parallel group design, which requires a much larger number of patients, compared to the crossover design of the other 3 studies. In addition, Lovas *et al.* used a low dose of DHEA, compared to the other studies and compared to our study.

With respect to the effects of DHEA in secondary adrenal failure, our study can only be compared with the study of Johannsson *et al.* (13). Although 3 other studies also contained patients with secondary adrenal failure, their analyses did not include, or did not permit, separate evaluation of patients with primary *versus* secondary adrenal failure (11;12;31). In contrast with the beneficial effects of DHEA on quality of life predominantly assessed by the partners of the patients reported by Johannsson *et al.*, we observed only subtle beneficial effects of DHEA on quality of life reported by the patients themselves.

In the present study we confirmed the impaired quality of life in female and male patients with pituitary diseases despite conventional hormonal substitution therapy. DHEA substitution had only limited effects on these

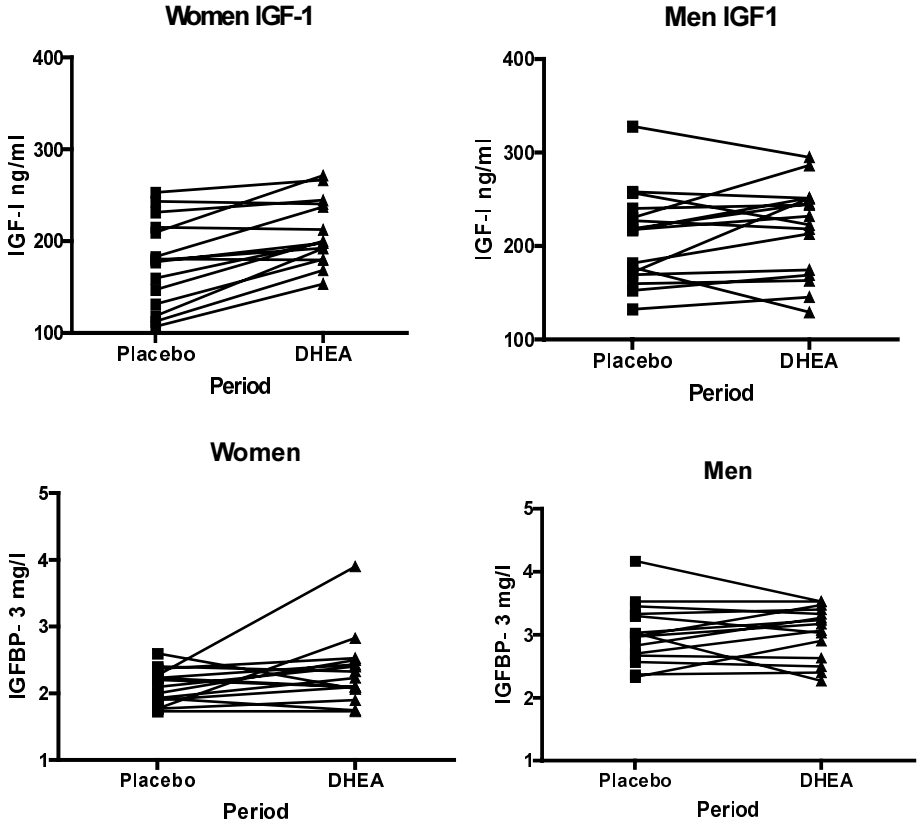
parameters. It can be proposed that the study is underpowered to detect significant changes in quality of life. However, the absolute changes in quality of life scores that were abnormal at baseline were hardly influenced by DHEA. The most severely affected parameter, role limitations due to physical problems (SF-36), changed only from 66.2 (baseline) to 68.8 (DHEA), whereas the control value was 93.4. Therefore, our data argue against a major effect of DHEA on quality of life parameters in such patients.

It is presently unclear by which mechanisms DHEA improves quality of life. The mechanism of action of DHEA is attributed to the conversion of DHEA into estrogens and androgens. Although this is also reflected in the changes in plasma concentrations of the respective hormones in the present study, these hormonal changes were not accompanied by apparent major changes in quality of life.

Another mechanism could be that DHEA increases quality of life by increasing IGF-I levels. Remarkably, in the presence of fixed GH availability, DHEA increased IGF-I levels in estrogen-depleted females, but not in testosterone treated males, with secondary adrenal insufficiency. However, the increase in IGF-I was again not accompanied by an important improvement in quality of life in women.

The study of Arlt *et al.* indicated that treatment with 50 mg of DHEA increased IGF-I slightly only in patients with primary adrenal failure, but not in patients with secondary adrenal failure. These authors suggested, that this differential effect of DHEA on IGF-I in primary *versus* secondary adrenal failure may be due to a GH mediated effect (11;33). In the present study we controlled for an effect of GH, by including only patients with GH deficiency on a fixed dose of rhGH during the whole study. Therefore, an effect of DHEA cannot be caused by any changes in GH availability. In accordance, other studies did not find any effect of DHEA substitution in healthy volunteers on GH secretion (15;21). These observations point to an effect of DHEA, independently of GH, on IGF-I production and/or clearance. Remarkably, this effect of DHEA was only present in estrogen-depleted women. In a study by Span et al (34), it was demonstrated, that estrogen replacement blunts the IGF-I response to rhGH in women. This could explain why

Figure 2 Serum levels of IGF-I and IGF-BP3 in 31 patients at the end of 16-weeks therapy with 50 mg/day DHEA or placebo.



in our study effects of DHEA were found on IGF-I in estrogen deplete women, whereas this effect was not found in the study of Johannsson et al (13). We did not find an effect of DHEA on IGF-I in testosterone-substituted men. It is known that testosterone in healthy subjects and GHD patients enhances IGF-I levels (35-37), which may preclude an additional effect of DHEA. Apparently, the effect of DHEA on IGF-I levels is sex- and/or sex hormone dependent.

The absence of relevant effects of DHEA on quality of life points to a fundamental problem in the concept of conventional hormonal

substitution. Hormonal substitution therapy has been extremely successful in the treatment of the major syndromes of endocrine insufficiency, with respect to reduction of morbidity and mortality. However, in general, many patients treated for endocrine insufficiencies still suffer from more or less vague complaints and a decreased quality of life. It is likely, that these complaints are, at least in part, caused by intrinsic imperfections of hormone replacement strategies in mimicking normal hormone secretion (38). Accordingly, the patients with pituitary diseases evaluated in the present study, showed decreased quality of life for several parameters, compared with age- and sex-matched controls, despite optimal endocrine replacement therapy according to current standards. The fact that DHEA, superimposed on conventional endocrine therapy, causes only subtle improvements points to our limited understanding of the mechanisms by which quality of life in these patients is affected.

DHEA did not affect sexual satisfaction in our study, in contrast to a positive effect of DHEA in other studies. In healthy subjects, and in patients primary and secondary adrenal failure, positive effects of DHEA are described on sexual function (11;13;15;16;31). However, these studies were carried out in younger patients. In our study, the women were postmenopausal, almost half of the patients had no partner, and the men were substituted with testosterone replacement. We cannot exclude the possibility that these factors may have obscured a potential positive effect of DHEA on sexual function.

In conclusion, DHEA substitution, superimposed on replacement with rhGH, has only subtle aspects of quality of life in patients with pituitary diseases with GH and ACTH deficiencies. Remarkably, DHEA increases IGF-I levels only in estrogen depleted females, but not in testosterone treated males, with secondary adrenal insufficiency.

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Chapter 6

Six-months of recombinant human GH therapy in patients with Ischemic Cardiac failure

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Abstract

Growth hormone therapy in patients with idiopathic dilated cardiomyopathy and ischemic cardiac failure has revealed varying effects on systolic function, probably related to the response in serum insulin like growth factor I levels. As diastolic function has not been studied thoroughly, we studied the effects of 6 months of recombinant human growth hormone treatment on systolic *and* diastolic function in patients with ischemic cardiac failure, using cardiovascular Magnetic Resonance imaging.

Nineteenpatients with ischemic cardiac failure (left ventricular ejection fraction < 40 %) were studied in a randomized trial. Ten patients received during 6 months treatment with growth hormone (2 IU/d), 9 served as controls. Systolic and diastolic function were assessed at baseline and after 26 weeks by cardiovascular Magnetic Resonance imaging. No differences were found in systolic and diastolic function between rh GH treated patients and controls. No change was observed in left ventricular mass index, end-diastolic volume, end-systolic-volume and ejection fraction. The treated patients showed no clinical improvement. Six months of treatment with growth hormone therapy in ischemic cardiac failure has no favorable effects on left ventricular mass index, on systolic and diastolic function.

Introduction

Chronic heart failure still has a poor prognosis despite advances in pharmacological therapy.[1] Therefore, new strategies are warranted to improve structural myocardial problems such as tissue growth, repair, and fibrosis, the hallmarks of left ventricular remodeling.[2] In this context, the use of anabolic agents is worthwhile to explore. Experimental data revealed favorable effects of growth hormone and insulin-like growth factor (IGF-I) in vitro and in experimental myocardial infarction in rats.[3-8] However, the experimental treatment with recombinant human growth hormone (rh GH) in patients with cardiac failure of various origins has revealed discrepant results. Although some non-randomized studies revealed favorable effects in idiopathic dilated cardiomyopathy[9] and ischemic cardiac failure,[10] these favorable effects could not be confirmed by randomized controlled studies in idiopathic dilated cardiac failure[11,12] or randomized[13] and non-randomized studies in ischemic cardiac failure.[14]

Although the majority of patients with cardiac failure have diastolic dysfunction,[15] randomized studies on the effects of growth hormone in cardiac failure have only focused on systolic function.[11-13] It is not known, whether growth hormone therapy has a positive effect on diastolic function. Therefore, the aim was to investigate in a randomized study the effects of 6 months therapy with rh GH on systolic and diastolic function in patients with ischemic cardiac failure using cardiovascular Magnetic Resonance (MR) imaging.

Materials and Methods

Patients

Inclusion criteria were the presence of ischemic cardiac disease proven by prior myocardial infarction and/or coronary angiography, a left ventricular ejection fraction less than 40% assessed by gated-SPECT imaging or echocardiography, a stable clinical condition for at least 3 months, and stable medical therapy with ACE-inhibitors, diuretics, digoxin, nitrates or b-blocking agents for at least 3 months. Exclusion criteria were myocardial infarction within 3 months before the study, the presence of a pacemaker or implantable defibrillator, arrhythmias, chronic renal and liver disease, diabetes mellitus, pregnancy and malignant disease. The ethical committee of the Leiden University Medical Center approved the protocol, and all patients gave written informed consent.

Protocol

Baseline measurements included a physical examination, a 12-lead ECG, cardiovascular MR imaging and biochemical tests. After baseline measurements, 22 patients were randomly allocated to treatment with recombinant human growth hormone (Zomacton^{R}, Ferring Pharmaceuticals, Hoofddorp, The Netherlands), or 'non-treatment'. Except for rh GH treatment, the study protocol was identical for the two groups. rh GH treatment started with 0.5 IU/day, self-administered subcutaneously. The dose was increased after two weeks to 1.0 IU/day. Four weeks after entering the study, the final dose of 2.0 IU/day was initiated and continued until the end of the study at 26 weeks. Safety visits were performed at 2, 4, 8 and 16 weeks after initiation. At 26 weeks, cardiovascular MR was performed again. At each visit, weight, waist-hip ratio, blood pressure and heart rate were recorded, and growth hormone vials were collected to assess compliance.

Cardiovascular MR

Cardiovascular MR imaging was performed on a Philips 1.5-T ACS-NT15 MR system with Power Trak 6000 (Philips Medical Systems International, Best, The Netherlands) using ECG triggering. A stack of short-axis images consisting of 2 to 12 slices (depending on heart size), with a thickness of 8 mm and an intersection gap of 1 mm was acquired using breath hold multishot echo planar imaging. Images encompassed the entire left ventricle. The imaging protocol was similar as reported previously. [16]

Phase contrast flow velocity measurements across the mitral valve orifice were acquired using a gradient echo acquisition sequence with retrospective gating. Velocity maps were acquired across the mitral orifice using a flip angle of 20^0 and an echo time of 10-12 ms. The image section had a thickness of 8 mm, a field of view of 350 mm, and consisted of 2 measurements of a 128 ´ 128 acquisition which was interpolated to a display matrix of 256 ´ 256 pixels. Depending on the actual heart rate, between 30 and 45 time frames were evenly distributed over the cardiac cycle, resulting in a temporal resolution of 35 to 39 ms. Total acquisition time was about 3 min. The maximum phase shift of 180^0 was set to occur at a velocity of 100 cm/s.

Cardiovascular MR Analysis

The MR images and velocity maps were analyzed on a remote workstation (Sun Microsystems Computer Corp., Mountain View, California). The left ventricular short axis acquisitions were used to assess LV dimensions, wall mass, ejection fraction. The endocardial, epicardial and papillary muscle borders of the end-diastolic and end-systolic images from each short-axis slice were manually traced using a MR analytical software system developed at our institution.[17] Myocardial borders were detected as previously reported.[18] The left-ventricular mass index (LVMI) and left ventricular ejection fraction (LVEF) were calculated as described before.[16]

Volumetric flow across the mitral valve was calculated by manually tracing the borders of the mitral valve in all time frames of the velocity map series, using flow analytical software package (MEDIS Medical

Imaging Systems, Leiden, The Netherlands)[19]. An experienced observer who was blinded for the treatment modality performed contour tracings. Flow curves were automatically analyzed following a manual indication of the start of early filling, peak early filling, peak atrial contribution to filling, and the end of filling as described previously. [20]

Laboratory tests

At baseline and after completion of the study, fasting blood samples were taken between 0800-0900 h for growth hormone, IGF-I and IGF binding protein-3 (IGFBP-3) measurements. Serum growth hormone was measured with the Delfia human growth hormone assay (Wallac, Turku, Finland). Serum IGF-I was measured by RIA (INCSTAR CORP., Stillwater, MN), intra-assay variability was < 11 %; the detection limit was 1.5 nmol/liter. Normal values are 9-34 nmol/liter for subjects aged 30-50 yr and 8-26 nmol/liter for 50-70 yr. IGFBP-3 was measured by RIA (Nichols Institute Diagnostics, Wychen, The Netherlands) with a detection limit of 0.03 mg/liter and the intra-assay variability of < 8 %. IGF-I and growth hormone determinations of all subjects were measured in one assay. Safety laboratory measurements included serum levels of urea, creatinine, glucose, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase and hematological profile. Other measurements were performed using routine laboratory methods.

Statistics

Continuous variables are expressed as the mean ± SD. Paired or unpaired Student t-tests were used, when appropriate. To analyze treatment effects, the differences in treatment response (post-therapy divided by pre-therapy values) were compared between the groups. A P value of less than 0.5 was considered significant. SPSS 10.0 (SPSS, Inc., Chicago, IL) was used for all statistical calculations.

Results

Baseline measurements

Three patients left the study prematurely for various reasons, not related to the study. Data from these patients were not included in any analysis. The baseline characteristics of the 19 patients who completed the study are shown in Table 1.

Biometric, biochemical aspects at baseline between the two groups are shown in Table 2. No differences in blood pressure and heart rate were found between both groups at the start of the study. Baseline IGF-1 levels were within the normal age-adjusted range in all patients (12-35 nmol/l). The rh GH treatment group showed a significant lower level of IGF-BP3 at baseline compared with the control group (+2.1 ± 0.95 mg/l resp. +2.93 ± 0.45 mg/l; *P*= 0.02).

Cardiac function data measured by cardiovascular MR at baseline are summarized in Table 2. End-diastolic volume, end-systolic volume, stroke volume, LVEF and LVMI were not different between the two groups. In addition, no significant changes were observed in diastolic function between the groups.

Table 1. Baseline Characteristics

	Control	rh GH
	n =10	*n = 9*
Age(yrs)	64	67
Gender (M/F)	9/1	7/2
History of MI [a]	10	9
History of cardiac surgery or PTCA	6	6
NYHA class [b]		
I	1	0
II	4	3
III	5	5
IV	0	1
Treatment		
ACE inhibitors	9	11
Diuretics	6	9
β-Blocking agents	5	3
HMG-CoA reductase inhibitors	9	6
Platelet aggregation inhibitors	5	2
Coumarin derivatives	4	7
Fasting serum GH ± SE (mU/liter)	2.60 ± 1.29	2.61 ± 1.43

[a] Number of subjects
[b] New York heart Association Classification

Effects of rh GH treatment on biochemical and biometric aspects

The effects of rh GH therapy on biochemical and biometric aspects are shown in Table 3. Systolic blood pressure decreased slightly in the treatment group as compared with the control group (+15.8 %; P = 0.05). Diastolic blood pressure and heart rate did not change after therapy.

rh GH therapy induced a significant increase in serum IGF-1 levels in

Table 2. Biochemical and cardiac parameters at baseline between groups

	Control	rh GH treatment	*P-value*
	N=10	N=9	
Biometric aspects			
Systolic blood pressure (mmHg)	141± 26	138± 12	0.75
Diastolic blood pressure (mm Hg)	83 ± 11	80 ± 11	0.51
Heart rate (min^{-1})	63 ± 5.8	63 ± 14	0.97
Biochemical aspects			
IGF-1 (nmol/l)	19.7 ± 5.4	18.4 ± 6.1	0.64
IGFBP-3 (mg/l)	2.93 ± 0.45	2.1 ± 0.95	0.02
LV Dimensions and Systolic function			
EDV (ml)	278 ± 90	327 ± 179	0.46
ESV (ml)	204 ± 79	242 ± 151	0.50
SV (ml)	74 ± 18	85 ± 35	0.41
EF (%)	28 ± 7.4	28± 7.2	0.97
AO acc peak ($(ml/s^2)10^{-3}$)	6.08 ± 1.9	5.03 ± 1.7	0.23
AO PER (ml/s)	381 ± 83	383 ± 114	0.98
AO dec peak ($(ml/s^2)10^{-3}$)	-3.66 ± 1.1	-3.17 ± 0.74	0.28
LVMI (g/m^2)	99.8 ± 22.5	94.2 ± 29.3	0.65
LV Diastolic function			
E acc peak ($(ml/s^2)10^{-3}$)	5.71 ± 3.0	4.54 ± 3.5	0.45
E PFR (ml/s)	358 ± 144	307 ± 190	0.52
E dec peak ($(ml/s^2)10^{-3}$)	-3.31±2.1	-2.98 ± 2.42	0.77
A acc peak ($(ml/s^2)10^{-3}$)	6.88 ± 2.0	4.69 ± 2.76	0.07
A PFR (ml/s)	410 ± 86	349 ± 135	0.28
A dec peak ($(ml/s^2)10^{-3}$)	-6.44 ± 2	-5.24 ± 2.36	0.26
E/A peak flow	0.87 ± 0.52	1.17 ± 1.12	0.48

Abbreviations: rh GH=recombinant human growth hormone, IGF-1: insulin like growth factor 1, IGFBP-3: insulin like growth factor binding protein, EDV=End-diastolic volume, ESV=End-systolic volume, SV=Stroke volume, EF=Ejection fraction, AO acc peak=Aortic acceleration peak, AO PER=Aortic peak ejection rate, AO dec peak=Aortic deceleration peak, LVMI=Left ventricular mass indexed to body-surface area, E acc peak=Early acceleration peak, E PFR=Early peak filling rate, E dec peak=Early deceleration peak, A acc peak=Atrial acceleration peak, A PFR=Atrial peak filling rate, A dec peak=Atrial deceleration peak, E/A peak= E PFR /A PFR

the treatment group, whereas the control group showed a small decrease (+23.6 ± 51 % resp. –14.0 ± 21.2 %; $P = 0.05$). Serum IGF-BP3 showed a significant change in the treated group as compared with the control group (+58.2 ± 68.7 % resp. +4.6 ± 27.8 %; $P = 0.04$).
Serum cholesterol, HDL and triglycerides were not affected by GH therapy (data not shown). Safety parameters were unchanged.

Effect of rh GH therapy on systolic and diastolic function

Table 4 describes the results of systolic and diastolic function assessed by cardiovascular MR after 26 weeks treatment. rh GH therapy had no effect on systolic function. There was no treatment effect observed in left ventricular dimensions, ejection fraction and left ventricular mass index.
The early peak filling rate showed a positive change in the treatment group (+31.5 ± 37.5 % versus –0.2 ± 17.9 %; $P = 0.03$). No change was observed in atrial peak filling rate, and as a result the E/A ratio increased slightly after 26 weeks in the treatment group (+43.5 ± 59.7 % versus +5.0 ± 22.6 %; $P = 0.09$). Other diastolic function parameters were not affected by rh GH therapy.

Table 3. Biometric and biochemical aspects after 26 weeks between control group and rh GH treatment group

	Control group		% Change	rhGH Treatment group		% Change	*P* value
	T=0	*T=26*		*T=0*	*T=26*		
Biometric aspects							
Systolic blood pressure (mmHg)	141 ± 26	131 ± 17	-5.8 ± 11.2	135 ± 12	116 ± 9	-15.8 ± 4.6	0.05
Diastolic blood pressure (mm Hg)	83 ± 11	82 ± 10	-0.5 ± 14.3	80 ± 10	73 ± 9	-8.6 ± 8.6	0.22
Heart rate (min^{-1})	68 ± 5.7	75 ± 9.9	9.5 ± 14.8	63 ± 14	62 ± 14	8.3 ± 8.7	0.85
Biochemical aspects							
IGF-1 (nmol/l)	19.7 ± 5.4	16.6 ± 5.0	-14.0 ± 21.2	18.4 ± 6.1	21 ± 5.3	23.6 ± 51	0.05
IGFBP-3 (mg/l)	2.93 ± 0.45	3.1 ± 1.07	4.6 ± 27.8	2.1 ± 0.95	2.84 ± 1.05	58.2 ± 68.7	0.04

Abbreviations: IGF-1: insulin like growth factor 1, IGFBP-3: insulin like growth factor binding protein 3

Table 4. Left ventricular dimensions, systolic and diastolic function after 26 weeks between control group and treatment group

	Control group		% Change	
	T−0	*T−26*		*T−0*
LV Dimensions and Systolic function				
EDV (ml)	278 ± 90	298 ± 86	9.1±13.4	327 ± 179
ESV (ml)	204 ± 79	21 ± 78	7.9±10.6	242 ± 151
SV (ml)	74 ± 18	82 ± 18	13.2±25.7	85 ± 35
EF (%)	28 ± 7.4	29 ± 7.5	2.7±11	27 ± 6.5
AO acc peak $((ml/s^2)10^{-3})$	6.08 ± 1.9	6.43 ± 1.6	14.4±37.5	5.03 ± 1.7
AO PER (ml/s)	381 ± 83	378 ± 83	1.9±23.6	383 ± 114
AO dec peak $((ml/s^2)10^{-3})$	-3.66 ± 1.1	-3.74 ± 1.36	6.4±33	-3.17 ± 0.
LVMI (g/m^2)	99.8 ± 22.5	99.4 ± 22.4	-0.4±6.9	94.2 ± 29.
LV Diastolic function				
E acc peak $((ml/s^2)10^{-3})$	5.71 ± 3.0	5.38 ± 2.7	0.1±25.7	4.54 ± 3.5
E PFR (ml/s)	358 ± 144	348 ± 126	-0.2±17.9	307 ± 190
E dec peak $((ml/s^2)10^{-3})$	-3.31 ± 2.1	-3.2 ± 2.37	-5±17.9	-2.98 ± 2.
A acc peak $((ml/s^2)10^{-3})$	6.88 ± 2.0	6.78±1.86	3.6±29.4	4.69 ± 2.7
A PFR (ml/s)	410 ± 86	397 ± 94	-2.8±13.3	349 ± 135
A dec peak $((ml/s^2)10^{-3})$	-6.44 ± 2	-6.82 ± 1.63	11.1±26	-5.24 ± 2.
E/A peak flow	0.87 ± 0.52	0.89 ± 0.52	5.0±22.6	1.17 ± 1.1

Abbreviations: rh GH treatment=recombinant human growth hormone, EDV=End-diastolic volume, ESV=End-systolic volume, SV=Strol
AO acc peak=Aortic acceleration peak, AO PER=Aortic peak ejection rate, AO dec peak=Aortic deceleration peak, LVMI=Left ventricul
area, E acc peak=Early acceleration peak, E PFR=Early peak filling rate, E dec peak=Early deceleration peak, A acc peak=Atrial accelera
rate, A dec peak=Atrial deceleration peak, E/A peak= E PFR /A PFR

Discussion

In the present randomised study, we evaluated the systolic and diastolic function in patients with ischemic cardiac failure after 26 weeks with rh GH therapy, using cardiovascular MR. No beneficial effects of rh Gh therapy were observed on systolic and diastolic function. Left ventricular mass index also remained unchanged.

Chronic heart failure results in increased mortality, despite the developments in pharmacological therapy.[1] Modern drug treatment is based on the prevention of the progression of heart failure, with ACE inhibitors, b-adrenergic blockers, spironolactone, digoxin and diuretics.[1,2] In the near future strategies may be designed to improve the structural myocardial problems like tissue growth, repair and fibrosis (the hallmarks of LV remodeling).[2] In this framework, the use of anabolic agents to improve the remodeling and therefore the cardiac function is a worthwhile option to explore. One of these agents, growth hormone showed promising results in animal studies. Experimental data with adult cardiomyocytes in rats revealed that IGF-1 enhanced myofibril development and, concomitantly, down regulates sms a actin, a protein that forms stressfiberlike structures.[4]

Administration of IGF-1 given to normal rats leads to hypertrophy of the heart.[5] In animals with experimental myocardial infarction, GH therapy leads to an improvement or preservation of cardiac function and induced an increase in myocardial energy reserves.[6,7,8] These observations have led to the assumption that rh GH therapy may result in improvement of cardiac function in patients with cardiac failure of various origins without underlying GH deficiency. Experimental studies with idiopathic dilated cardiomyopathy have shown contradictory results. In an open study, Fazio et al. found an increased myocardial mass, reduced left chamber size and improved hemodynamic parameters in patients with idiopathic dilated cardiomyopathy after 3 months of human Growth hormone administration.[9] In contrast, two randomized studies that focused on dilated cardiomyopathy, demonstrated that administration of rh GH for at least 12 weeks had no effect on cardiac function.[11,12] In addition, Osterziel et al. demonstrated an increase in LV

mass which was not accompanied by a clinical benefit. [11]
Studies in ischemic heart failure revealed similar results. Spallarossa et al. found in a non- randomized study an increase in exercise duration and well-being, but no effect on cardiac function.[14] Genth-Zotz et al. observed in a non–randomised study an improvement in clinical well being, and a decrease in end-diastolic and end-systolic indexes.[10]
Although the treatment group showed a significant change of serum IGF-1, it was observed only in 4 of 9 (44%) patients (Figure 1). Several explanations for the absence of an effect of growth hormone may apply, for instance the advanced stage of cardiac failure [21,22] or the concomitant use of â-blockers.[23] In our opinion, the third and most important explanation may be the existence of growth hormone resistance in chronic cardiac failure, like in many other chronic diseases.[24] Indeed in

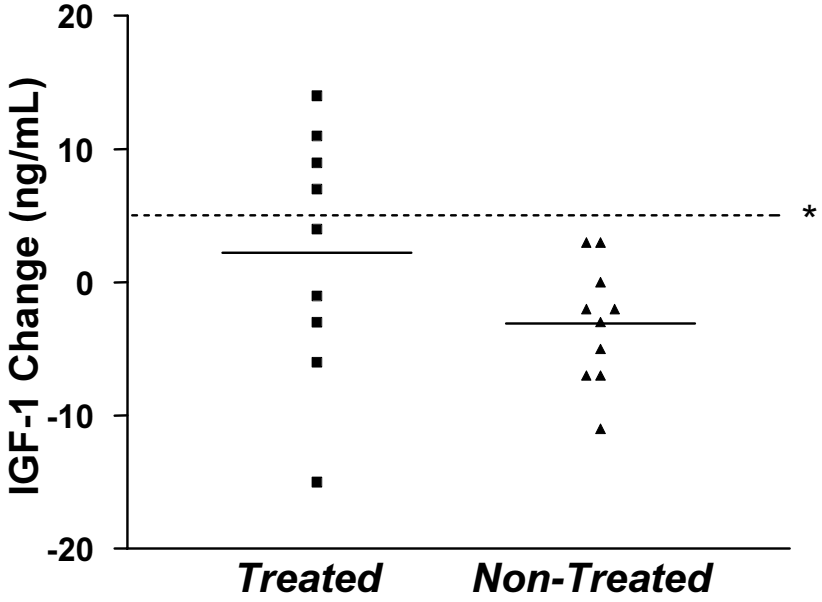

Figure 1. Changes in serum IGF-I in patients with ischemic cardiac failure after 26 weeks of treatment with recombinant human growth hormone (2 IU/d) or non-treatment. The dotted line at 5.8 nmol/l represents the mean IGF-I change of non-treated patients + 2 SD; * P <0.05.

chronic cardiac failure, decreased sensitivity to growth hormone has been observed.[25-27] The moderate increase in serum IGF-I concentration in only 4 of 9 patients in our study as compared with other studies may be in line with this assumption.[10,12,13]

Osterziel *et al*. observed an effect of growth hormone therapy on cardiac output in patients with idiopathic dilated cardiomyopathy who showed an IGF-I response above the median response.[28] In addition, they demonstrated a dose-effect relation between short-term growth hormone application and left ventricular function in ischemic cardiac failure.[29] The IGF-I response to growth hormone was related to the severity of cardiac failure.[28,29] In our study, the patients who responded to rh GH therapy had a comparable LVEF as observed in non-responders; moreover, the responders did not improve their systolic or diastolic function after treatment. The existence of a threshold for growth hormone therapy, could explain why in this study no effect was found of growth hormone on cardiac function. Future studies require a distinct increase in circulating IGF-I by giving each patient an individual dose.

The present study showed, that 26 weeks of 2 IU/day rh GH had no beneficial effect on systolic and diastolic function. Further studies are warranted to evaluate the application of growth hormone in earlier stages of ischemic cardiac disease and regarding individual dose adjustment depending on the IGF-I response.

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Chapter 7

Discussion and summary

Introduction

The clinical approach to new patients in internal medicine proceeds according to a standard diagnostic sequence, before therapy can be instituted. This clinical process starts with a detailed medical history, followed by physical examination. Additional investigations, like laboratory and/or radiological tests, are based on the differential diagnosis made after these first steps. The results of these diagnostic tests enable to confirm the initial diagnosis or to limit the initial differential diagnosis. Sometimes additional tests are required. Finally, based on the principles of evidence-based medicine the patient will receive appropriate treatment. Unfortunately, this clinical approach is not always perfect and doctors need to know the imperfections of the diagnostic and therapeutic approaches. In this thesis some of these imperfections of the diagnosis, treatment and follow up are discussed, with a focus on clinical conditions characterized by growth hormone excess or -deficiency.

Acromegaly

Patients with acromegaly can only be cured by successful trans-sphenoidal surgery. However, this procedure cures only ~60 % of the patients with acromegaly. Moreover, during long term follow up of these initially cured patients, the disease may reoccur, resulting in an overall long term cure rate of only 40-50 %. Patients with persistent recurrent acromegalic disease require additional treatment to prevent increased morbidity and mortality. These additional treatments consist of somatostatin analogues, a growth hormone receptor antagonist, or radiotherapy. Several questions have not been resolved in detail. When is such operation successful? What are the criteria to decide that abnormal growth hormone secretion is well controlled? Which patients require adjuvant therapy? What is the relevance of discrepancies between GH and IGF-I levels? With respect to this last issue, several studies have demonstrated discrepancies between IGF-I and GH levels after surgery. For instance, one study showed that 19 % of the patients had

normal GH levels but high IGF-I, and 8 % of the patients had elevated GH but normal IGF-I. In the group of paients with normal GH levels but high IGF-I another study showed that 50 % had a normal glucose-mediated GH suppression after glucose tolerance test according to the current criteria.

Mortality studies showed that the control of GH hypersecretion leads to a mortality risk, that is not different from the normal population. Based on these studies the biochemical goals of treatment of acromegaly have been defined: random GH levels should be < 2,5 μg/L, the nadir GH levels during OGGT nadir should be < 1 μg/L and/or IGF-I levels should be in the normal range of age- and sex matched controls. The use of the mortality studies to define the criteria for well-control of GH excess has definite limitations. During the long term follow up required in these mortality studies, there is a lot of change in treatment modalities, e.g. better medication, change to more sensitive assays, loss of data, which influence the interpretation of the results. Therefore, the outcome of treatment in mortality studies is a reflection of older treatment modalities confounded by newer developments. Another problem is that these mortality studies used different biochemical parameters, e.g. random or postabsorptive GH measurements, GH levels after OGTT, and IGF-I levels, which make the comparison between the different studies not always straightforward. Also most studies used last known biochemical data what results in selection bias, because the subjects with the longest follow up will have the lowest biochemical parameters.

Somatostatin therapy is currently an accepted medical treatment used as adjunctive treatment after initial transsphenoidal treatment. Several studies have also advocated somatostatin analogues as primary treatment. In both conditions somatostatin analogues are effective in controlling GH excess in ~60 % of the patients, according to the criteria derived from the mortality studies. At present two somatostatin analogues with slow release modalities are available: octreotide LAR and lanreotide Autogel. Instead of the parabolic increase of octreotide levels seen during 4 weeks after subcutaneous injection with octreotide LAR, lanreotide has a more log-linear decrease of its levels during 4 weeks after its

intramuscular injection. In **chapter 2** a comparison was made between these two long acting depot preparations with respect to their ability to suppress GH levels in one group of well-controlled acromegalic patients. Both analogues have almost the similar binding capacities to somatostatin receptor subtype 2 and to a lesser extent to subtype 5. We used two different approaches to compare the efficacy of both methods. First, we measured mean fasting GH levels and IGF-I levels at 2, 4 and 6 weeks after injections of the analogues. Secondly, 24 h GH secretion profiles were compared at 4 weeks after administration of each analogue. There were no major differences between the effects of both analogues on GH levels or IGF-I concentrations. Although the number of subjects included in the study was limited, the detailed analysis of GH dynamics support the emerging notion that there are no major differences in efficacy between both somatostatin analogues, despite the difference in pharmacokinetics.
Although the patients described in chapter 2 were well controlled during treatment with somatostatin analogues according to accepted criteria, the study described in this chapter also showed that patients have persistently high GH levels during 24 h. This indicates that the definitions of biochemical control of GH excess derived from mortality studies should not be equated with normalization of GH secretion. In contrast, normalization of GH secretion occurs after successful transsphenoidal surgery. The question arises to what extent the subtle abnormalities in GH secretion that persist during somatostatin treatment translate into biological effects.

Adequate control of GH excess, employing the criteria derived from the mortality studies, normalizes the reversible manifestations of acromegaly such as sweating, carpal tunnel syndrome, diabetes mellitus, dyslipidemia, and soft tissue swelling. The problem with such parameters is, however, that they cannot easily be quantified by clinicometric or other methods. Therefore, in clinical practice we mostly rely on the effects of treatment on biochemical parameters (GH and IGF-I).

With respect to the heart, control of GH excess has clear effects, reflected

in a decrease of the increased left ventricular mass, improvement of systolic function at exercise and diastolic function. We wondered whether we could use the heart as a biomarker to compare the effects of different treatment modalities of GH excess in patients, who were well controlled for GH excess. Therefore, in **chapter 3**, we compared cardiac function between acromegalic patients cured by transsphenoidal surgery, acromegalic patients well controlled by somatostatin analogues, still active patients despite somatostatin analogues and *de novo* acromegalic patients prior to any treatment. We assessed cardiac parameters, using tissue Doppler assessment. The study showed that diastolic function was improved in patients who were no longer exposed to active disease. However, patients well- controlled by treatment with somatostatin analogues still had impaired diastolic function, indicating persistent effects of GH excess. This observation was present during tissue Doppler imaging, but not during conventional echographic assessment, reflecting the improved sensitivity of tissue Doppler imaging for detecting subtle differences in diastolic function. The clinical relevance of this subtle diastolic impairment is presently unclear. This was one of the first studies that differentiated patients with biochemical control of GH excess by dividing this group in well controlled and cured patients. By using a new technique to assess diastolic function we proved that some acromegalic patients well controlled according to the current biochemicalcriteria, derived from the mortality studies, still have slightly active disease. However, these patients had no other clinical symptoms and the clinical relevance of our observation for patient care is at present uncertain. Nonetheless, the observations in chapters 2 and 3 indicate, that the standard approach of the internist, proceeding from medical history, physical examination and appropriate diagnostic tests, fails to detect subtle, but persistent disease both with respect to biochemical parameters and with respect to the subtle effects on the heart.

During the studies described in the present thesis, we made an intersting obervation. Although acromegaly was known from 1886, and cardiovascular disease plays an important role in the increased morbidity and mortality rate associated with acromegaly, it was astonishing to

conclude that there was hardly any documentation of the effects of GH excess on the function of the cardiac valves. We stumbled upon this question, when several of our patients had documented insufficiencies of the mitral and/or aortic valves. Despite long term follow up studies and many studies involving echocardiography there was no notion of a relation between acromegaly and cardiac valve disease. In **chapter 4** we compared the prevalence of cardiac valve disease in acromegalic patients and matched controls. Therefore, we performed echocardiography in 40 patients with cured, well-controlled disease, or active acromegaly to assess if there was any valvular insufficiency compared with 120 healthy controls, matched for age, sex,blood pressure, and left ventricular systolic function. The prevalence of significant valvular insufficiency was more than threefold increased in the group of the acromegalic patients (22 % vs. 6.7 % in controls). Pathological aortic valve regurgitation was present in 20 % of the patients present compared with 4 % of the controls. Pathological mitral regurgitation was only present in 5 % *vs*. 0 % in controls. Interestingly, none of the patients with valvular insufficiency had symptoms or were previously known to a cardiologist. Nonetheless, the regurgitation found had clinical significance, according to the participating cardiologists. A longer duration of exposition to GH excess was associated with an increased prevalence of valvular insufficiency. We postulate that the damage that occurred to the cardiac valves is irreversible, in contrast to some other manifestations of acromegaly. This is based also on the histological examination of one of the valves obtained from an acromegalic patient after cardiac surgery. In conclusion, there is a high prevalence of valvular regurgitation in acromegalic patients, which most likely depends on the duration of GH excess. Apparently, the standard approach of the internist including medical history and physical examination has failed to detect this important complication of acromegalic disease in the 120 years after this disease was initially described. Therefore, patients with active acromegaly require echocardiographical examination to assess the valvular damage, because this may not be detected by a standard physical examination. This may have consequences for their treatment.

Growth hormone deficieny

Diseases resulting in damage to the somatotrophic cells in the pituitary, cause GH deficiency. The syndrome of adult GH deficiency results in subtle changes in physical and biochemical parameters and in aspecific changes in quality of life. It is currently recommended to treat these patients with recombinant human GH (rhGH) aimed at IGF-I levels in the upper range of normal to improve cardiovascular morbidity. Because the complaints and physical effects of GH deficiency are subtle and aspecific, there are no good clinicometric or biochemical parameters to titrate the treatment with rhGH other than IGF-I concentrations. In addition, there are intrinsic imperfections with respect to physiological GH replacement. GH is secreted in a pulsatile fashion, which cannot be mimicked by a once daily injection of rhGH. Moreover, patients with pituitary insufficiency frequently have additional endocrine insufficiencies, with additional intrinsic limitations in physiological replacement. Despite adequate replacement of these endocrine insufficiencies, the quality of life of patients with pituitary disease may improve, but remains impaired to a certain extent.

In **chapter 5** we evaluated quality of life in patients with long-term replacement with rhGH. A broad spectrum of quality of life parameters was investigated and compared to age- and sex-matched controls. In general, women had a greater impairment in quality of life parameters then men. In GH deficient female patients the domains physical functioning, role limitations due to physical and emotional problems, measured by the SF-36, and general and physical fatigue, measured by MFI-20, were worse than in controls. Moreover, both male and female GH deficient patients had problems in social functioning, together with impaired activation. The general health perception in men was impaired compared with controls. These results were interesting, because although most patients had a long-term follow up, we had limited knowledge of the quality of life of our patients. Apparently, the standard approach of internists is not suited to assess quality of life in patients, even though this is one of the main goals of treatment in patients with GH deficiency. Several years ago positive results of DHEA substitution were reported in subjects with primarily adrenal insufficiency. DHEA and its sulfate,

DHEA-S, are strange steroids. Although their concentrations are several folds higher than that of any other adrenal gland hormone, the function of these hormones is still not clear. In humans, no receptor has been found for DHEA or DHEA-S. It is postulated that they serve as precursors for active steroids intracellularly, for instance in the brain. By restoring DHEA to physiological levels, the quality of life improved. An interesting side effect was noticed in women consisting of a rise in IGF-I levels. Therefore, the question arose whether the improvement of quality of life and the rise of IGF-I were related to each other.

In chapter 5 the data are presented of a double blind crossover study of the effects of DHEA *versus* placebo in GHD patients to address this question. DHEA restored the adrenal gland hormone concentrations to normal levels. In general, the patients perceived a positive change in their health. Nonetheless, we observed that they were not able to describe exactly what aspects improved. The only positive change was observed in women who showed an improvement in the depression score (HADS). The general approach of internists of taking the medical history is inadequate for assessing subtle changes in quality of life, such as assessed by detailed questionnaires. Nonetheless, the use of questionnaires has limitations. Quality of life is something abstract that is difficult to determine and each subject may have slightly different interpretations of his/her quality of life. Another problem is related to the selection of the controls, which may be subject to bias. On the other hand, reference data from the literature may not be applicable to the study population. Therefore, we decided to use each subject as his/her own control.

In women, the small improvement in quality of life was not associated with the increase of IGF-I levels. Remarkably, IGF-BP3 levels, the major GH binding protein in the plasma, did not increase, indicating that the increase in IGF-I levels was not merely the result of increased binding proteins. Because all patients had a fixed dose of rhGH, increased GH availability was another unlikely explanation of the increase in IGF-I levels. We studied only postmenopausal women with hypopituitarism without hormone replacement therapy, whereas we studied men with hypopituitarism who were all on testosterone substitution. We speculate that this difference in hormone substitution between men and women

and/or gender may have been involved in the discrepant results of DHEA substitution on IGF-I levels. Nonetheless, from a practical perspective, at present postmenopausal women do not receive standard estrogen supplementation, whereas men above the age of 50 years still receive testosterone supplementation.

In **chapter 6** the date are presented of a prospective, randomised, controlled study on the effects of GH substitution in patients with ischemic cardiac failure. The basic concept derived from other studies was that cardiomyocytes have receptors for GH and IGF-I and that GH treatment may be of benefit in certain patients with impaired cardiac function. The patients received rhGH in dosages normally given to GH deficient patients. Unfortunately, there was no effect on cardiac function after 6 months of treatment. This randomised study proved that GH substitution in patients with normal endocrine functions is not of benefit for ischemic cardiac dysfunction. The question arises whether the initial ideas of performing such studies in this group of patients without any endocrine dysfunction were solid. In the early nineties observational studies of rhGH treatment were reported in small groups of patients with heart failure, which demonstrated a positive change in left ventricular mass and systolic function. Moreover, there were suggestions that these patients had a disturbed GH-IGF-I axis reflected in decreased IGF-I levels. Subsequently, a large study in intensive care patients proved that GH substitution caused excessive mortality. In this respect, a parallel with the effects of thyroid hormone substitution in patients with non-thyroidal illness can be made. In these patients so far no consistent clinical benefit of thyroid hormone substitution has been demonstrated. The changes in hormone concentrations in response to non-endocrine disease reflect the adaptive capacity of the human body. Before interfering in the human body, one should understand the underlying pathophysiological mechanisms of the changes observed. It is interesting that all the studies performed in cardiac failure with GH were based on one uncontrolled study, and that later this hype was finished by prospective, randomised studies.

Concluding remarks

Internists use a standard approach to evaluate signs and symptoms of patients, supplemented by diagnostic tests, in order to make a diagnosis, to assess the activity of the disease and to evaluate the effects of appropriate treatment. Against this background the clinical syndromes of GH excess and GH deficiency impose several difficulties. With the exception of advanced cases of acromegaly, the signs and symptoms of both syndromes can be subtle and aspecific. Consequently, they cannot easily be quantified by clinicometric methods.

The studies described in the current thesis highlight several limitations of the standard approaches of internists. The association between acromegaly and valvular heart disease escaped the attention of the attending physicians for more than 120 years (chapter 4). In the assessment of disease activity and of the effects of treatment we have to rely on biochemical criteria for both GH excess and GH deficiency. For the treatment goals of acromegaly, the current criteria have been derived from mortality studies. However, acromegalic patients still have subtle GH overactivity during treatment with somatostatin analogues despite being "well controlled" according to these criteria. We obtained evidence for biological effects of this subtle GH overactivity at the level of the heart, i.e. diastolic dysfunction was present in these patients, but not in patients cured from active acromegaly by surgery (chapter 3). There appeared to be no difference between two different somatostatin analogues with respect to biochemical control of active acromegaly (chapter 2).

The studies in the patients with GH deficiency indicate that our current clinical approaches are not able to assess the effects of treatment with respect to the effects on quality of life. Using structured questionnaires, we were not able to show improved quality of life in GH deficient patients with secondary adrenal insufficiency by adding DHEA substitution although patients experienced a subjective change (chapter 5). Finally, our treatment of diseases should be based on solid evidence of underlying pathophysiological mechanisms. GH substitution in subjects without endocrine disease but with ischemic cardiomyopathy did not have major effects (chapter 6). However, in retrospect the

evidence for the involvement of GH in the pathophyioslogy of heart disease in patients without GH deficiency or overproduction was only circumstantial.

Hoofdstuk 8

Discussie en samenvatting

Inleiding

De klinische benadering van nieuwe patiënten in de interne geneeskunde verloopt volgens een standaard stramien, voordat therapie wordt gestart. Dit begint met een gedetailleerde anamnese en beschrijving van de medische voorgeschiedenis gevolgd door lichamelijk onderzoek. Aanvullend onderzoek, zoals laboratorium- en/of radiologische tests, zijn gebaseerd op de differentiaal diagnose gemaakt na de eerste stap. De resultaten van deze diagnostische tests bevestigen de initieel al vermoedde diagnose of limiteren het aantal mogelijkheden van de differentiaal diagnose. Soms zijn er extra testen nodig. Uiteindelijk zal de patiënt de juiste behandeling krijgen, gebaseerd op de principes van 'evidence-based medicine'. Helaas is deze klinische benadering niet altijd perfect en de dokter dient dan ook op de hoogte te zijn van de imperfecties en dus beperkingen van de diagnostische en therapeutische mogelijkheden. In dit proefschrift zullen sommige van deze imperfecties bij de diagnose, behandeling en de follow-up worden besproken, met de nadruk op de klinische gevolgen van groeihormoon overschot en -tekort.

Acromegalie

Patiënten met acromegalie kunnen alleen succesvol worden behandeld door transsphenoidale chirurgie. Echter, hierdoor worden maar 60 % van de acromegale patiënten gecureerd. Sterker nog, bij langdurige follow-up blijkt dat er bij een aantal een recidief optreedt bij deze in eerste instantie gecureerde patiënten, hetgeen ertoe leidt dat de overall cure rate daalt naar maximaal 50 %. Patiënten met persisterende ziekte, of een recidief, dienen additionele therapie te ontvangen, zodat het toegenomen risico op mortaliteit en morbiditeit kan worden voorkomen. Deze additionele therapie bestaat uit somatostatine analogen, groeihormoon receptor antagonisten of radiotherapie. Een aantal vragen zijn echter nog niet beantwoord. Wanneer kan men spreken van een succesvolle operatie? Wat zijn de criteria om te bepalen of de abnormale GH secretie goed onder controle is? Welke patiënt heeft additionele therapie nodig? Wat is de relevantie van de discrepantie tussen GH en

IGF-I concentraties? Meerdere studies hebben laten zien dat er een verschil is tussen IGF-I en GH concentraties na operatie. Bijvoorbeeld, een studie toonde aan dat 19 % van zulke patiënten een normale GH concentratie had maar een hoog IGF-I, en 8 % had verhoogde GH concentraties maar een normaal IGF-I. Een andere studie toonde aan, dat 50 % van de patiënten met een discrepantie tussen GH en IGF-I een normale glucose-geïnduceerde suppressie hadden na de OGTT volgens de huidige criteria.

Mortaliteit studies tonen aan, dat de controle van de GH hypersecretie leidt tot daling van het mortaliteits risico tot een waarde die gelijk is aan die van de normale populatie. Op deze studies zijn de biochemische criteria voor de behandeling van acromegalie gebaseerd: een random GH concentratie beneden 2,5 ug/L, een laagste GH spiegel gedurende een glucose tolerantietest van minder dan <1 ug/L en/of normale IGF-I concentraties voor leeftijd en geslacht. Het gebruik van mortaliteitsstudies voor de definitie van goed gecontroleerde GH overschot heeft duidelijk beperkingen. Door de langdurige follow-up nodig die nodig is in deze studies, treden er veranderingen in de behandelingstrategieën, komen er nieuwere, sensitievere laboratorium tests, is er verlies van data, hetgeen allemaal invloed heeft op de interpretatie van de resultaten. Dus, de uitkomst van de mortaliteit studies is een reflectie van oudere behandelingsstrategieën, vertroebeld door nieuwe ontwikkelingen. Een ander probleem is dat mortaliteit studies gebruik maken van verschillende biochemische parameters, zoals random metingen van het GH in de periode na de operatie, GH waarden na OGTT en IGF-I concentraties, wat de vergelijkingen tussen studies moeilijk maakt. Tenslotte, de meeste studies gebruiken de laatst gemeten biochemische data wat resulteert in een selectie bias, omdat patiënten met de langste follow-up de laagste biochemische parameters hebben. vergeleken 4 weken na injectie. Er werden geen verschillen in de effectiviteit van de twee analogen gevonden. Ondanks dat het aantal patiënten beperkt was in de studie, steunden de resultaten van de gedetailleerde analyse van de GH dynamiek de conclusie, dat er geen verschillen waren in effectiviteit van beide somatostatine analogen, ondanks de verschillen in farmacokinetiek.

Ondanks het feit dat de patiënten beschreven in hoofdstuk 2, "well-controlled" waren voor GH overproductie gedurende de behandeling met beide analogen volgens de huidige criteria, toonde de studie aan dat de patiënten gedurende 24 uur verhoogde GH concentraties hadden. Dit betekent dat de definities van biochemische controle gebaseerd op mortaliteit studies niet gelijk staat aan normalisatie van de GH secretie. Dit staat in contrast met de observaties dat na een succesvolle operatie van acromegalie, de GH concentraties wel normaal zijn. De vraag is dan ook in hoeverre de iets hogere GH concentraties bij "well-controlled" patiënten met somatosatine behandeling leiden tot biologische effecten. Adequate controle van de overmatige GH secretie, volgens de criteria gedestilleerd uit mortaliteit studies, normaliseert de reversibele symptomen van acromegalie, zoals overmatig transpiratie, weke delen zwelling, carpaal tunnel syndroom, diabetes mellitus en dyslipidemie. Het probleem met zulke parameters is, dat ze moeilijk zijn te kwantificeren door klinische of andere methodes.
Daarom vertrouwen we in de dagelijkse praktijk meer op biochemische parameters (GH, IGF-I) om het effect van de behandeling van acromegalie te beoordelen.

Met betrekking tot de effecten van acromegalie op het hart, heeft controle van de overmatige GH secretie duidelijke effecten, zoals vermindering van de toegenomen linker ventrikel massa, en verbetering van de systolische- en diastolische functie. Wij vroegen ons af of het hart kan worden gebruikt als biochemische marker om het effect van de verschillende behandelingen van GH overschot te vergelijken. Daarom hebben we in **hoofdstuk 3** de cardiale functie vergeleken tussen acromegale patiënten gecureerd door transsfenoidale chirurgie, "well controlled" door somatostatine analogen, niet goed gecontroleerde patiënten ondanks somatostatine analogen en nieuwe acromegalie patiënten die nog niet behandeld waren. Voor het meten van de verschillende hartfuncties maakten we ook gebruik van tissue Doppler echocardiografie. De studie toonde aan dat in patiënten de diastolische dysfunctie was verbeterd in patiënten die geen actieve ziekte meer hadden. Echter, bij de patiënten die "well-controlled" waren met

analogen was er nog steeds sprake van enige diastolische Therapie met somatostatine analoga is nu een geaccepteerde medische behandeling, die wordt gebruikt als adjuvante therapie na een initiele transsphenoidale behandeling. Meerdere studies tonen aan, dat somatostatine analogen ook kunnen worden toegepast als primaire therapie voor acromegalie. Bij beide mogelijkheden zijn somatostatine analogen effectief in de suppressie van de overmatige GH productie bij ~60 % van de patiënten, volgens de huidige criteria gebaseerd op mortaliteit studies. Er zijn momenteel twee "slow-release" somatostatine analogen op de markt: octreotide LAR en lanreotide Autogel. In plaats van de parabole stijging van octreotide concentraties gedurende 4 weken na een subcutane injectie, laat lanreotide Autogel een meer log-lineaire daling zien van lanreotide concentraties volgend op de piek na een diep-subcutane injectie. In **hoofdstuk 2** is een vergelijkende studie beschreven tussen deze twee depotpreparaten, die beide in staat zijn om GH te onderdrukken, in een groep van acromegale patiënten, die goed gecontroleerd waren voor hun GH overproductie. Beide analogen hebben nagenoeg dezelfde bindingscapaciteiten voor de somatostatine receptor 2, en in minder mate voor receptor 5. We hebben gebruik gemaakt van twee verschillende methoden om de effectiviteit van de beide te vergelijken. Bij de eerste methode hebben we de gemiddelde nuchtere GH concentraties gemeten 2, 4 en 6 weken na een injectie van een van de analogen. Bij de tweede methode hebben we de 24 uurs GH profielendysfunctie, wat betekent dat er nog steeds sprake is van persisterende effecten van GH overmaat. De waarneming werd alleen gevonden bij de metingen met 'tissue doppler', en niet tijdens de normale conventionele meting, wat aangeeft dat Tissue doppler sensitiever is in de meting van subtiele verschillen van diastolische functie. De klinische relevantie van de diastolische dysfunctie is nog niet helemaal duidelijk. Deze studie was een van de eerste die differentieerde tussen de biochemische controle van GH overmaat door acromegale patiënten te verdelen in "well- controlled" en genezen patiënten. Met behulp van nieuwere technieken om diastolische dysfunctie vast te stellen werd bewezen dat de patiënten die volgens de huidige criteria, met behulp van medicatie geen persisterende ziekte zouden moeten hebben, nog

steeds actieve ziekte hadden. De patiënten hadden verder geen andere klinische symptomen, en de uiteindelijke klinische relevantie is op dit moment nog onduidelijk.
De observaties in hoofdstuk 2 en 3 tonen aan dat de standaard benadering van een internist, gebaseerd op medische voorgeschiedenis en anamnese, lichamelijk onderzoek en de juiste diagnostische test(s), falen bij acromegalie in het detecteren van subtiele maar nog wel steeds aanwezige ziekte met betrekking tot de biochemische parameters en subtiele veranderingen op het hart.

Gedurende de studies beschreven in dit proefschrift, gebeurde iets interessants. Ondanks het feit dat acromegalie al bekend is vanaf 1886, en de wetenschap dat cardiovasculaire ziekten een belangrijke rol spelen in de verhoogde mortaliteit en morbiditeit bij acromegalie, was het verbazend dat er geen duidelijke documentatie was over het effect van GH overschot op de functie van de hartkleppen. We werden geconfronteerd met deze vraag toen enkele van onze patiënten een gedocumenteerde insufficiëntie hadden van de mitraal- en/of aortaklep. Ondanks meerdere studies met echocardiografie bij acromegalie, werd er geen relatie beschreven tussen acromegalie en hartklep insufficiëntie. In **hoofdstuk 4** hebben we de prevalentie vergeleken van hartklepinsufficiëntie tussen acromegale patiënten en gematchte controles. Daarvoor ondergingen 40 acromegalen echocardiografie om te bepalen of dat er sprake van was klep insufficiëntie in vergelijking met 120 gezonde controles, gematcht voor leeftijd, geslacht en bloeddruk. De prevalentie van klepinsufficiëntie was drie maal verhoogd in de groep van acromegale patiënten (22% vs. 7% bij de controles). Aorta klepinsufficiëntie kwam in 20 % van de patiënten voor vergeleken met 4 % van de controles. Mitraal klepinsufficiëntie kwam maar in 5 % van de gevallen voor *vs* 0 % bij de controles. Opvallend was dat geen van de patiënten met kleplijden symptomen had, of bekend was bij een cardioloog. Toch was het kleplijden klinisch relevant, volgens de participerende cardiologen. Een langdurigere blootstelling aan groeihormoon overschot was geassocieerd met een verhoogde prevalentie van kleplijden. We postuleren dat de schade die ontstaat bij

acromegalie aan de hartkleppen irreversibel is, in tegenstelling tot een aantal andere symptomen van acromegalie. Dit is onder andere ook gebaseerd op het histologisch onderzoek op een van de hartkleppen, dat was verricht bij een acromegale patiënt na een klepvervanging, beschreven in hoofdstuk 4.
Concluderend kan er gezegd worden dat er is sprake van een hoge prevalentie van kleplijden bij acromegale patiënten, hetgeen samenhangt met de duur van het overmatige groeihormoon productie. Blijkbaar heeft de standaard benadering van de internist gefaald in het detecteren van deze belangrijke complicatie van acromegalie in de afgelopen 120 jaar. Daarom dienen patiënten met actieve ziekte een echocardiografie te ondergaan om kleplijden vast te stellen aangezien dit bij fysisch onderzoek gemist kan worden. Het kan belangrijke consequenties hebben voor hun behandeling.

Groeihormoon tekort

Ziekten, die de somatotrope cellen van de hypofyse beschadigen, veroorzaken GH deficiëntie. Het syndroom van GH deficiëntie bij volwassenen resulteert in subtiele veranderingen in fysiek welbevinden en in biochemische parameters. Het wordt nu aanbevolen om deze patiënten te behandelen met recombinant groeihormoon met als doel IGF-I concentraties te verkrijgen die liggen in het hoge-normale gebied met het doel de kwaliteit van leven en het cardiovasculaire risico te verbeteren. Omdat de klachten en fysieke effecten van GH deficiëntie subtiel en aspecifiek zijn, zijn er geen goede klinische of biochemische parameters om de behandeling met rhGH te verfijnen behoudens dan IGF-I concentraties. Daarbij moet nog worden opgemerkt, dat er tevens sprake is van intrinsieke imperfectie met betrekking tot fysiologische GH vervanging. GH wordt pulsatiel gesecerneerd, hetgeen door een dagelijkse subcutane injectie met rhGH niet kan worden nagebootst. Sterker nog, patiënten met hypofysaire insufficiënties hebben vaak meerdere endocriene insufficiënties, waarbij de substitutie op zichzelf weer fysiologische beperkingen heeft. Door een zo optimaal mogelijke substitutie van deze endocriene insufficiënties mag de kwaliteit van leven van patiënten met een hypofyse ziekte weliswaar verbeterd zijn,

het blijft tot op zekere hoogte beperkt.
In **hoofdstuk 5** evalueerden wij de kwaliteit van leven van patiënten met langdurige GH substitutie. Een breed spectrum van kwaliteit van leven parameters werd onderzocht en vergeleken met leeftijd- en geslacht gematchte controles. In het algemeen hadden vrouwen een meer gestoorde kwaliteit van leven dan mannen. De GH deficiënte vrouwelijke patiënten hadden problemen met fysiek functioneren en zij voelden bovendien, dat hun functioneren was beperkt door hun fysieke en emotionele problemen vergeleken met de controle populatie (SF-36). Tevens was er sprake van algemene en fysieke vermoeidheid (MVI-20). Zowel mannelijke als vrouwelijke GH deficiënte patiënten hadden beide zowel problemen met het sociale functioneren, als met een verminderde activiteit. Alleen bij de mannen was de gezondheidsbeleving gestoord ten opzicht van controles. De resultaten waren interessant aangezien de meeste patiënten langdurig vervolgd waren. Bovendien was er weinig inzicht bij de behandelende dokters in de gestoorde kwaliteit van leven van onze patiënten. Blijkbaar is de standaard benadering van een internist onvoldoende om de kwaliteit van leven te beoordelen, ondanks dit wel een van de doelen is van behandeling van GH deficiënte patiënten.
Een aantal jaren gelden werden er positieve resultaten beschreven van DHEA substitutie bij patiënten met primaire bijnierinsufficiëntie. DHEA en DHEA-S zijn vreemde steroiden. Ondanks het feit dat de concentraties van DHEA en DHEA-S een aantal maal hoger zijn dan die van andere bijnierhormonen, is hun functie nog verre van opgehelderd. Tot nu toe zijn er nog geen receptoren gevonden bij de mens voor deze bijniersteroïden. Er is gepostuleerd dat zij voorlopers zijn van actieve steroïden die intracellulair worden geproduceerd, bijvoorbeeld de hersenen. Door DHEA concentraties te verhogen naar fysiologische spiegels verbeterde de kwaliteit van leven bij patiënten met een bijnierinsufficiëntie. Daarnaast was er een interessant epifenomeen in de vorm van een stijging van het IGF-I gehalte.
De vraag kwam op, of de verbetering in kwaliteit van leven en de stijging van IGF-I in relatie met elkaar stond. In **hoofdstuk 5** is een dubbel blinde cross-over studie naar de effecten van DHEA versus placebo

beschreven bij GH deficiënte patiënten met een ACTH deficiëntie. De DHEA concentraties stegen hierbij naar fysiologische niveaus. In het algemeen ervoeren de patiënten een duidelijke verbetering van hun kwaliteit van leven. Toch observeerden wij, dat zij niet precies spontaan konden aangeven wat er precies veranderd was in hun functioneren. Daarom schiet de algemene benadering van de internist in de anamnese tekort om subtiele veranderingen in kwaliteit van leven te detecteren, zoals gemeten middels gedetailleerde vragenlijsten. Toch heeft ook het gebruik van vragenlijsten zijn beperkingen. Kwaliteit van leven is iets abstracts wat moeilijk is te bepalen en elke persoon heeft zijn/haar verschillende interpretaties van haar/zijn kwaliteit van leven. Een ander probleem is gerelateerd aan het bepalen van een goede controle populatie, wat kan zorgen voor bias. Referentie gegevens uit de literatuur zijn ook niet toepasbaar op de studie populaties. Daarom was elke patiënt zijn/haar eigen controle in onze studie.
Bij vrouwen was de kleine verbetering van kwaliteit van leven niet geassocieerd met een verhoging van IGF-I concentraties. Opvallend was dat IGF-BP3 concentraties, een belangrijk GH bindingseiwit in plasma, niet stegen, wat betekende dat de verhoging van IGF-I concentraties niet alleen een resultaat was van stijging van de IGF-BP3 concentraties. Omdat alle patiënten een gefixeerde dosis rhGH kregen kon een verhoogde beschikbaarheid van GH ook geen verklaring zijn van de stijging van IGF-I concentraties. We hebben alleen postmenopauzale vrouwen onderzocht met hypopituitarisme zonder oestrogeen substitutie, terwijl de mannen allemaal testosteron substitutie kregen. We speculeren dat het verschil van hormonale substitutie tussen mannen en vrouwen en/of het geslacht mogelijk de oorzaak is van het verschil in resultaat gevonden bij DHEA substitutie op IGF-I. Er dient worden opgemerkt dat mannen boven de 50 jaar nog steeds testosteron substitutie krijgen terwijl bij vrouwen dit niet standaard wordt gegeven.

In **hoofdstuk 6** worden de gegevens gepresenteerd van een prospectieve, gerandomiseerde studie naar de effecten van GH substitutie in patiënten met ischemisch hartfalen. Het basis concept gedistilleerd uit nadere studies was dat cardiomyocyten GH en IGF-I receptoren hebben en dat

GH behandeling mogelijk een positieve verandering zo kunnen geven bij patiënten met een gestoorde hartfunctie. De patiënten kregen rhGH in een dosis gelijk aan die van GH deficiënte patiënten. Helaas werd er geen effect waargenomen op hartfunctie gemeten na 6 maanden van behandeling. Deze gerandomiseerde studie toonde aan dat rhGH substitutie in patiënten met verder normale endocriene functie geen voordeel heeft ten aanzien van hun hartfunctie. De vraag kwam op of het initiële idee om dit soort studies te doen in deze groep van patiënten zonder endocriene dysfunctie wel helemaal solide was. In begin van de jaren negentig toonden observationele studies met rhGH behandeling in kleine groepen van patiënten met hartfalen positieve veranderingen aan op de linker ventrikel massa en de systolische functie. Bovendien werd de suggestie gewekt, dat deze patiënten een gestoorde GH/IGF-I as hadden gezien hun lagere IGF-I concentraties. Echter, een grote studie op een intensive care toonde aan dat GH substitutie, weliswaar in suprafysiologische doseringen, leidde tot een verhoogde mortaliteit. In dit licht kan er een parallel gemaakt worden naar de effecten van schildklierhormoon in patiënten met non-thyroidal illness. In deze patiënten is tot nu toe geen consistent positief klinisch effect beschreven van schildklierhormoon substitutie. De veranderingen in hormonale concentraties op basis van niet-endocriene ziekte reflecteert vaak de adaptieve capaciteit van het menselijk lichaam. Voordat men dan ook intervenieert met het menselijk lichaam, moet men de onderliggende pathofysiologische mechanismen begrijpen. Het is interessant dat alle studies met rhGH bij hartfalen waren gebaseerd op een ongecontroleerde studie en dat uiteindelijk de hype werd afgesloten met prospectieve gerandomiseerde studies.

Concluderende opmerkingen

Internisten gebruiken een standaard benadering om te symptomen van patiënten te evalueren, aangevuld met diagnostische tests, om een diagnose of een activiteit van een ziekte vast te stellen en het effect van een behandeling te evalueren. Tegen deze achtergrond hebben de klinische syndromen van GH overschot en -tekort enkele moeilijkheden. Met uitzondering van de zeer lang onderkende casussen van acromegalie,

zijn de symptomen en afwijkingen van beide syndromen subtiel en aspecifiek. Daardoor zijn ze moeilijk te kwantificeren met klinische parameters. De studies beschreven in dit proefschrift tonen aan, dat er enkele tekortkomingen zijn in de standaard benaderingen van de internist. De associatie tussen acromegalie en kleplijden is voor 120 jaar ontsnapt aan de aandacht van de dokters. Bij het vaststellen van de ziekte activiteit en het effect van de behandeling van overmatige en tekortschietende GH secretie, kunnen we slechts vertrouwen op biochemische criteria. De huidige behandelingscriteria voor acromegalie zijn gebaseerd op mortaliteit studies. Echter acromegalie patiënten die volgens deze criteria "well-controlled" zijn, hebben nog steeds subtiele GH overactiviteit ondanks de behandeling met somatostatine analogen. We toonden aan, dat er sprake was van biologische activiteit van deze subtiele GH overproductie ten aanzien van het hart, aangezien er sprake was van diastolische dysfunctie in deze groep patiënten, wat niet aanwezig was in de gecureerde patiënten na transsfenoidale chirurgie. Ook bleken er geen verschillen te zijn tussen de beide verschillende somatostatine analogen in betrekking tot hun controle van de GH hypersecretie.

De studies bij GH deficiënte patiënten toonden aan, dat onze huidige klinische benaderingen niet in staat zijn om het effect van behandeling ten aanzien van de kwaliteit van leven vast te stellen. Gebruikmakend van gestructureerde vragenlijsten waren we niet in staat aan te tonen dat er een verbetering van kwaliteit van leven plaats vindt bij GH deficiënte patiënten met een secundaire bijnierinsufficiëntie door het toevoegen van DHEA substitutie aan de behandeling. Echter de patienten ondervonden een subjectieve positieve verandering.

Uiteindelijk, dienen we de behandeling van ziekte te baseren op solide bewijzen van onderliggende pathofysiologische mechanismen. GH substitutie bij personen zonder endocriene dysfunctie maar met ischemisch hartfalen heeft geen belangrijke toegevoegde waarde. Uiteindelijk was er geen bewijs dat GH een rol heeft in de pathofysiologie van hartziekten in patiënten zonder GH deficiëntie of - excess.

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Nawoord

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Curriculum Vitae

Sjoerd Willem van Thiel werd geboren op 24 mei 1975 te Helmond. In 1993 heeft hij zijn eindexamen Atheneum gedaan op het Lorentz Lyceum te Eindhoven. Aangezien hij was uitgeloot, startte hij in 1993 met de studie Rechten aan de Rijksuniversiteit Leiden. Een jaar later werd hij alsnog ingeloot voor de studie Geneeskunde. In 2000 begon hij aan zijn afstudeer onderzoek op de afdelingen Endocrinologie en Radiologie (supervisors: Dr. J.W.A. Smit en dr. H.J. Lamb). Dit onderzoek naar het effect van GH op systolische en diastolische functie gemeten met Cardiac Imaging resulteerde uiteindelijk in één van de hoofdstukken van dit proefschrift. In 2001 is hij als arts onderzoeker aan dit proefschrift gaan werken op de afdeling Endocrinologie en Stofwisselingziekten van het LUMC onder begeleiding van professor dr. J.A. Romijn, dr. J.W.A. Smit, en dr. A.M. Pereira Arias. Sedert mei 2004 is hij werkzaam in het Bronovo Ziekenhuis te Den Haag (opleider: dr. J.W. van ‘t Wout) als arts-assistent Interne Geneeskunde.