Pituitary diseases: long-term clinical consequences

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Long-term clinical consequences

Omslag, lay-out en druk verzorgd door Optima Grafische Communicatie, Rotterdam.

Een deel van de studies beschreven in dit proefschrift werd uitgevoerd als AGIKO, met gedeeltelijke financiele ondersteuning van ZonMW.

De druk van dit proefschrift werd gedeeltelijk financieel ondersteund door ZonMW en het onderzoeksfonds van het Bronovo Ziekenhuis.

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Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden
op gezag van de Rector Magnificus prof.mr. P.F. van der Heijden,
volgens besluit van het College voor Promoties
te verdedigen op donderdag 18 december 2008

klokke 11.15 uur

door

Agatha Apolonia van der Klaauw

geboren te Leiderdorp
in 1980

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I. INTRODUCTION

The pituitary gland is the master regulator of the endocrine system. Pathophysiological conditions alter the functioning of the gland, the endocrine system and ultimately the patient. Diseases per se of the pituitary gland can be managed by surgery, radiotherapy, and drug therapy. In general, these approaches enable adequate control of the pituitary disease. The consequences of partial or complete failure of pituitary secretion, caused by the initial disease and/or its treatment, are treated by appropriate replacement strategies with different hormones. From a superficial perspective these treatments of the pituitary diseases and their respective consequences seem to be rather successful, because they result in stable or cured pituitary disease with appropriate hormonal replacement strategies. However, careful assessment during long-term follow-up of these patients indicates that these approaches are not perfect because they do not result in normal biological functioning of these patients with a normal quality of life.

The studies described in this thesis focus on long-term clinical consequences of pituitary diseases with a special focus on acromegaly, growth hormone deficiency and quality of life.

II. THE PITUITARY GLAND: OVERVIEW OF PHYSIOLOGY AND PATHOPHYSIOLOGY.

The hypothalamus and the pituitary are often referred to as the master glands of the endocrine system. This neuro-endocrine axis in the brain orchestrates many complex regulatory functions of multiple endocrine glands and homeostatic processes.

The pituitary is located within a bony cavity, the sella turcica, and is attached to the hypothalamus by the pituitary stalk. The pituitary has two lobes: the adenohypophysis (or anterior pituitary) and neurohypophysis (or posterior pituitary).

The pituitary stalk delivers hypothalamic releasing or inhibiting factors from the hypothalamus to the anterior part of pituitary gland through the hypothalamic-pituitary portal system. These factors modulate the secretion of the anterior pituitary hormones. In the anterior pituitary various cell types synthesize and secrete different hormones: growth hormone (GH), prolactin, thyroid stimulating hormone (TSH), adrenocorticotroph hormone (ACTH), and luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Pituitary adenomas are the most common pathophysiological processes disrupting normal pituitary functions. These adenomas are neoplasms of the pituitary gland, composed of cells of the anterior pituitary. Pituitary adenomas are classified by their size and functionality. An adenoma smaller than 1 cm is classified as a microadenoma, whereas adenomas more than 1 cm are called macroadenomas. This distinction is clinically relevant, since microadenomas seldomly cause pituitary insufficiency or visual field defects. In addition, these adenomas are

classified as functioning or non-functioning, according to their hormonal secretion profiles. Functioning adenomas produce one, or in some cases, multiple anterior pituitary hormones. The hormonal secretion profile is a reflection of the underlying cell type of the pituitary that expanded to form the adenoma. The clinical presentation of a pituitary adenoma depends on several factors. The clinical manifestations of a pituitary macroadenoma, irrespective of the presence or absence of hormone overproduction, are caused by pressure on local tissue such as the optic chiasm causing visual field defects or the surrounding healthy pituitary tissue causing hypofunction of the other hormone secreting cells. In addition, the clinical manifestations of hormone producing adenomas are caused by the effects of excess secretion of one or more anterior pituitary hormones.

In addition to pituitary adenomas, various other pathophysiological conditions can disrupt normal hypothalamic-pituitary function, including craniopharyngioma, infiltrative diseases such as lymphocytic hypophysitis, infarction (Sheehan's syndrome) and mutations in genes that are involved in pituitary development such as Pit1 and PROP1 mutations.

III. ACROMEGALY

The clinical syndrome of acromegaly is caused by excessive circulating GH concentrations.

In most cases the source of the excessive GH concentrations is a pituitary somatotrope tumor. Growth hormone releasing hormone (GHRH) secreting bronchial or gastrointestinal carcinoid tumors, pheochromocytoma and small cell bronchus carcinoma, causing secondary somatotrope hyperplasia, cause acromegaly in only a minority of cases (~1%). The incidence of acromegaly was estimated to be 3-4 per million inhabitants and the prevalence of 60-70 per one million in the UK, Spain and Sweden (1-4).

Clinical signs and symptoms

Clinical signs and symptoms in patients with acromegaly arise from excessive GH secretion, pituitary hormone deficiencies and local, tumor size related aspects. Most pituitary tumors in acromegaly produce only GH, while mixed GH and prolactin production is present in ~30% of cases.

Clinical signs of excessive GH secretion include prognathism, malocclusion and frontal bossing due to growth of enchondral bone of the nose and ears and periosteal bone formation. In addition, the clinical features in the mouth are diasthemata and macroglossia. Hand and feet are enlarged due to soft tissue swelling and periosteal bone formation leading to the characteristic increased ring and shoe size. Organomegaly of liver, heart, kidneys, colon, spleen and thyroid is often present. Other symptoms associated with GH hypersecretion are increased perspiration, tiredness, a low hoarse voice, paraesthesias, carpal tunnel syndrome, arthropathy, sleep apnea syndrome, hirsutism, snoring, and a thick moist skin. Metabolic diseases such as

hypertension, cardiovascular disease, diabetes mellitus and impaired glucose tolerance are frequent. Concomittent hyperprolactinemia and/or pituitary hormone deficiencies can present with galactorrhea, amenorrhea, hirsutism, impotence, infertility and symptoms related to hypothyroidism and hypocortisolism.

Local mass effects include headache, visual field defects with bilateral hemianopsia, and rarely cerebral nerves dysfunction, especially of the trigeminal, trochlear or abducens nerve.

The heart is frequently affected in acromegaly (5). This seems to be related to the direct effects of GH and/or insulin-like growth factor-I (IGF-I) on the myocardium (6). GH excess leads to myocardial hypertrophy with interstitial fibrosis, followed by impaired diastolic and systolic function (5). Reversal of GH and IGF-I excess by surgical removal of the GH secreting pituitary tumour and/or medication attenuates or even reverses abnormal LV measurements and function in acromegalic cardiomyopathy (7;8). Abnormal extracellular matrix regulation by overproduction of growth hormone (GH) and/or IGF-I in patients with acromegaly has been proposed as the cause of the abovementioned cardiac manifestations.

Recent, cross-sectional studies have also documented an increase in a higher prevalence of regurgitant valvular heart disease compared to healthy controls in both active and inactive acromegaly (9;10). However, at present it is unknown how quickly cardiac valve disease occurs in patients with active acromegaly and whether cardiac valve disease is stable in patients with biochemical remission. Therefore, **Chapter 2** of this thesis describes a prospective study to evaluate regurgitant cardiac valve disease in patients with active and inactive acromegaly.

Patients with acromegaly have an abnormal extracellular matrix regulation that is seen in cardiac valves, with severe regurgitation, in patients with acromegaly who have been operated on to replace these valves (9). This histological picture is also present in connective tissue diseases such as Marfan's syndrome and in this syndrome myxoid degeneration extends to the aortic root (11). Prior to the present thesis, only a single study assessed aortic root diameters in a limited number of patients and aortic root enlargement was found in 1 of 25 patients with active and inactive acromegaly (12). In **Chapter 3** it was investigated whether the aortic root is also affected by abnormal extracellular matrix regulation in acromegaly and whether this results in an increased diameter of the aortic root in patients with active and inactive acromegaly compared to healthy controls using two dimensional echocardiography.

Treatment

The current treatment of acromegaly includes surgery and drug treatment with somatostatin analogs and GH receptor blockade drugs. The aims of treatment in active acromegaly are to relieve the symptoms of GH and IGF-I excess, to decrease mass effects, and to reduce the increased mortality risk associated with active acromegaly (13).

Fortunately, combinations of surgery, radiotherapy and drug therapy (somatostatin analogs and/or GH receptor blockade drugs) are able to control disease activity in almost all patients (14-17). Surgical treatment alone can reach this target in only 60-80% of the patients. Before the

introduction of somatostatin analogs and GH receptor blockade drugs, the treatment of choice for persistent postoperative disease was radiotherapy. However, since the introduction of effective drug treatment, patients with persistent postoperative disease activity are treated with somatostatin analogs with or without GH receptor blockade drugs or GH receptor blockade drugs alone. Since GH receptor blockade drugs have only been approved for treatment very recently, the long-term treatment effects of GH receptor blockade drugs remain to be studied.

However, despite biochemical control of acromegaly, well-being is not normalized in these patients. Several cross-sectional studies in patients with acromegaly have pointed towards a reduction in quality of life (QoL) even during long term cure or long term biochemical control of the disease (18-22). Some studies revealed a positive influence of adequate control of disease activity on QoL (18-21), whereas in another study no such relationship was found (22). Previous radiotherapy (19;23) and somatostatin treatment (18) were both associated with impaired QoL. However, almost all studies were designed as cross-sectional studies with heterogeneous cohorts, including patients with both cured and active acromegaly. Recently, a longitudinal study documented an overall unchanged QoL in a cohort consisting of patients with cured and active acromegaly, using a disease specific questionnaire and with a median follow up of 21 months (24). Changes in QoL might be due to irreversible effects of previous GH excess and/or treatment which can only be studied in a homogeneous cohort of patients with long-term biochemical remission of acromegaly. Hence, we evaluated whether QoL parameters changed during follow-up in a homogeneous cohort of acromegalic patients with sustained biochemical control of acromegaly in **Chapter 4**. This approach enabled us to identify predictors of QoL during follow-up of acromegaly.

In addition, it is important to note that the various treatment modalities can effectively control disease activity in the majority of patients but on the other hand are associated with side effects. In this thesis we describe some of the side effects of somatostatin treatment (**Chapter 5**) and radiotherapy (**Chapter 6-9**).

Acromegaly is associated with sleep disorders, including sleep disordered breathing, such as snoring and sleep apnoea syndrome. The prevalence of the sleep apnoea syndrome was found to be as high as 80% in patients with active acromegaly (5). Treatment of acromegaly by transsphenoidal surgery or somatostatin analogs has been consistently found to reduce this high prevalence of sleep apnoea syndrome (25-30).

However, somatostatin per se may also adversely influence sleep, because in experimental studies somatostatin and its analog, octreotide, altered sleep (31;32). In healthy elderly subjects, somatostatin impaired sleep especially by decreasing total sleep time and REMS and by increasing the time spent awake in the first sleep cycle (32), although it did not influence sleep in young healthy adults (33). In rats, the long-acting somatostatin analog octreotide suppressed NREMS after repeated injections (31). Moreover, octreotide reduced stage 4 NREMS and REMS during the first half of the night and increased intermittent wakefulness during the second half

of the night in young healthy adults (34). At present, it is unknown whether sleep is affected in patients who are treated with somatostatin analogs to control acromegaly. In **Chapter 5** the effects of acromegaly and somatostatin treatment on perceived daytime sleepiness and sleep patterns in comparison with healthy controls is evaluated.

Radiotherapy for pituitary adenomas frequently leads to the development of anterior pituitary hormone deficiencies (35). GH is most of the times the first to be affected. Therefore, it seems logical to expect GHD in the long-term in acromegaly after such treatment. In accordance, a decreased response of GH to insulin-induced hypoglycemia in 36% of the patients with acromegaly, during long-term follow-up of postoperative radiotherapy (36). Another study has suggested that the prevalence of abnormalities in GH secretion classified as GHD is also high in patients treated by surgery alone (37). It was, however, unclear whether this decreased GH response to insulin-induced hypoglycemia meant that these patients had true GHD. One could hypothesize that the insufficient response results from postoperative radiotherapy, while autonomous activity of the adenoma persists. Therefore, in **Chapter 6** we compared stimulated and spontaneous GH secretion between patients with an insufficient GH response to insulin-induced hypoglycemia after treatment with postoperative radiotherapy for acromegaly and patients with an insufficient GH response to insulin-induced hypoglycemia after treatment with postoperative radiotherapy after similar treatment for other pituitary adenomas.

Cardiac function is dependent on optimal GH and IGF-I regulation. As mentioned above, the heart is affected in acromegaly. On the other hand, a decrease in left ventricular mass and left ventricular ejection fraction in patients with GHD have been observed (38-44), which is correlated to the severity of GHD (40). Additionally, impairment in diastolic function has also been observed in patients with GHD (45). At present it is unknown how the heart adapts to the decreased GH secretion in GHD after previous long-term GH excess in acromegaly. Therefore, cardiac manifestations of GHD after previous acromegaly were assessed in **Chapter 7**.

Radiotherapy for acromegaly is effective in lowering GH concentrations, but it has become apparent that it can induce the converse state: GHD (36;37). In adult patients with GHD due to other reasons without previous radiotherapy, recombinant human GH (rhGH) replacement increases bone mineral density (46), left ventricular mass and stroke volume (47), lean body mass (48), and quality of life (49) and it improves the serum lipid profile (50). These effects are apparent within 6-12 months and are maintained during continued treatment with rhGH in the long-term (47;50-53). However, in almost all studies patients with previous acromegaly were excluded. Only two intervention studies compared the effects of rhGH replacement in patients with GHD after previous treatment for acromegaly (54;55). However, these studies compared these patients with patients with GHD due to other reasons than previous acromegaly and important endpoints of rhGH replacement were not included (cardiac function and QoL with

various general health questionnaires) (54;55). In **Chapter 8** we describe a randomised controlled trial aimed to study the effects of rhGH replacement on body composition, QoL, cardiac function, lipid and glucose concentrations, bone turnover and bone mineral density in patients with GHD after treatment for acromegaly.

Radiotherapy for acromegaly can lead to pituitary deficiencies. In general, the notion is that this is due to side effects of radiotherapy on the pituitary, but the hypothalamus may also be involved. The hypothalamus is considered to be more vulnerable to radiation damage than the pituitary gland (56). Within the hypothalamus, the suprachiasmatic nucleus (SCN) regulates various circadian rhythms, one of its circadian outputs is formed by regulating melatonin secretion of the pineal gland (57). Circadian variations in melatonin secretion are under the control of endogenous clock signals arising from the SCN of the hypothalamus. Circadian variation of melatonin secretion can thus be used as a read-out of SCN functioning. Indeed, adequate sleep quality and quantity is obtained only when aligned with the most favorable circadian timing window for sleep, e.g. during the nocturnal high levels of melatonin (58). In addition, melatonin is suggested to be important for optimal functioning of other human circadian systems (58). At present it is unknown whether SCN function is affected in patients with pituitary tumors treated by radiotherapy and whether altered SCN function contributes to decreased QoL, which is characterized by specifically increased fatigue scores and a negative relationship with radiotherapy (23). Therefore, in **Chapter 9** we compared circadian characteristics of melatonin secretion, as a reflection of SCN function, between acromegalic patients treated with postoperative radiotherapy and patients treated with surgery only for acromegaly and healthy controls.

IV. GROWTH HORMONE DEFICIENCY

Growth hormone deficiency (GHD) in adults has received ample attention since it was recognized to have adverse effects, even when longitudinal growth was completed, and since the availability of growth hormone preparations for treatment of adults (48). GHD in adults can occur as a consequence of various pathological processes in the pituitary and hypothalamic region of which pituitary adenomas and their treatment are the most common (59). In general, the secretion of GH is the first to be affected in pituitary adenomas and their treatment followed by decreased secretion of LH/ FSH, ACTH and TSH (35;60).

Clinical signs and symptoms

Adult GHD is characterized by an adverse cardiovascular metabolic profile: increased serum concentrations of serum total cholesterol (TC), low-density lipoprotein (LDL) and triglycerides (TG), and decreased serum concentrations of high-density lipoprotein cholesterol (HDL), an altered body composition (reduced muscle strength and mass, and visceral obesity) and

decreased bone mass (48). In addition, abnormalities in vascular function and structure have been described in GHD (61-63). Life expectancy is reduced in patients with hypopituitarism during adulthood, despite replacement of anterior pituitary deficiencies (64;65). This has often been ascribed to the negative effects of GHD on cardiovascular risk factors.

Diagnosis of GHD

Because the manifestations of GHD are subtle and nonspecific, the evaluation of GHD should be performed only in adults with a high a priori chance to have GHD such as known pituitary disease or GHD during childhood. Prior studies have demonstrated that patients with multiple pituitary hormone deficiencies, including two or more pituitary hormone deficiencies other than GHD, had a likelihood of approximately 95% of having GHDS (66-68). The diagnosis of GHD in adults is established by provocative testing, because IGF-I concentrations and mean 24h GH concentrations overlap in adults considered GH deficient (i.e. due to extensive pituitary disease) and healthy subjects (69). The diagnosis should thus be based on the combination of documented pituitary or hypothalamic disease and a decreased GH response to insulin-induced hypoglycemia or the combined GH releasing hormone (GHRH) and arginine test when insulin-induced hypoglycemia is contraindicated.

Recombinant human GH replacement

Short-term (up to 24 months) replacement therapy with rhGH decreases the plasma concentrations of LDL cholesterol, total cholesterol, as well as fat mass and diastolic blood pressure, and increases lean body mass, fasting glucose and insulin concentrations (50). In addition, rhGH replacement with a maximum duration of 18 months increases left ventricular mass and interventricular septum thickness without changing diastolic function (47). Several studies have reported improved QoL and well-being (49;70-72), suggesting that in selected patients rhGH replacement may have a beneficial effect on QoL (73). Nonetheless, these effect of rhGH replacement on various QoL subscales seems to be limited (49) probably due to the complex pathology in these patients with possible direct treatment effects (radiotherapy) and multiple anterior pituitary hormone deficiencies.

One of the major aims of rhGH replacement is to decrease cardiovascular risk. Since a decade, bone marrow-derived endothelial progenitor cells have been proposed to play an important role in maintenance and repair of the vasculature. These cells carry the cell-surface marker CD34+. Both re-endothelialization and angiogenic capacity have been put forward as mechanisms by which these cells are involved in vascular repair (74). The number of these cells are reduced in patients with type 1 diabetes (75) and in patients with other cardiovascular risk factors or established cardiovascular disease (76;77). RhGH treatment increases the number of circulating endothelial progenitor cells in healthy volunteers (78). In addition, the potential of rhGH to positively influence hematopoiesis has previously been shown in another clinical setting, i.e.

harvesting of CD34+ cells destined for autologous hematopoietic stem cell transplantation in patients with relapsed or refractory hematologic malignancies (79). CD34+ cells express both GH and IGF-I receptors (80), which is also the case for several other cell types that could be involved. Indeed, studies in rodents and on fetal bone marrow demonstrate direct effects of GH and IGF-I on hematopoiesis (80;81). In addition, a recent study reported that IGFBP-3, which is induced by rhGH treatment, promotes migration, tube formation of CD34+ cells and differentiation of these cells into endothelial cells, leading to increased vessel stabilization and quicker blood vessel development (82). At present, it is unknown whether short-term physiological rhGH replacement increases CD34+ cells in patients with GHD, like pharmacological treatment with rhGH in healthy volunteers (78). Therefore, in **Chapter 10** we assessed the effects of 1 year of physiological rhGH replacement in patients with GHD on endothelial function and CD34+ cells.

GHD is in general an irreversible condition, which requires chronic replacement.

Before our study, only 3 single center studies reported the effects of more than 5 years of rhGH replacement (71;83;84). However, in these three studies combined only 33 patients were studied. Only one large multi-center study reported effects of 5 years of rhGH replacement in 118 patients (52). In these studies of 5 years or longer, LDL cholesterol concentrations consistently decreased (52;53;71;83;85), but total cholesterol only decreased in three studies (52;53;83). Several studies found an increase in HDL cholesterol during long-term treatment (52;53;71;85). However, it was unknown whether these changes were sustained when follow-up is prolonged to 7 years. Moreover, initial treatment strategies of rhGH replacement in GHD were based on weight-based regimes adapted from treatment of children with GHD. However, this often resulted in supraphysiological substitution and this treatment regime was subsequently abandoned during long-term studies (46;52). The Growth Hormone Research Society recommended titrating rhGH replacement dose to normalize individual IGF-I concentrations (60). Therefore, in **Chapter 11** we described the effects of prolonged treatment with rhGH (7 years) on biochemical and anthropometric parameters in a large cohort of patients with GHD from a single center treated from the start with an individualized physiological dose of rhGH replacement.

Several studies have focused on the short-term (50) and long-term effects of rhGH replacement on cardiovascular risk factors in adults with GHD (52;53;71;83;85). Most studies reported decreases in LDL cholesterol, body fat and blood pressure. However, at present it is unknown to what extent these changes translate to an overall reduction in cardiovascular risk. The metabolic syndrome is a cluster of metabolic abnormalities, that identifies persons at high risk for cardiovascular disease (86-88). The frequent concomitant occurrence of metabolic risk factors for cardiovascular disease such as abdominal obesity, insulin resistance, dyslipidemia, and hypertension suggested the existence of a "metabolic syndrome" (88). The National Cholesterol

Education Program (NCEP)/ATP III proposed a definition of the metabolic syndrome in 2001, defining cut-off values for fasting plasma glucose, HDL cholesterol, triglycerides, blood pressure and waist circumference (89). However, the prevalence of the metabolic syndrome in patients with GHD was unknown as well as the effects of rhGH replacement on these clustered cardiovascular risk factors. Therefore, in **Chapter 12** we evaluated the prevalence of the metabolic syndrome using the NCEP/ATP III definition in patients with GHD compared to the healthy Dutch population and we evaluated the effects of 5 years of rhGH replacement in these patients. This approach enabled us to translate changes during rhGH replacement into changes in actual cardiovascular risk factors.

It is important to note that several factors influence the efficacy of rhGH replacement in GHD (**Chapter 13 and 14**). These factors are important in the long-term clinical care of these patients since they influence individual treatment response.

Women on estrogen replacement require higher doses of rhGH replacement to achieve similar IGF-I concentrations than eugonadal women and men (90-92). Discontinuation of oral estrogen substitution also leads to an increase in IGF-I levels during continued substitution with rhGH (93). In addition, the route of estrogen administration also affects IGF-I levels. A switch from oral to transdermal estrogen therapy increases IGF-I levels and amplifies the IGF-I response during incremental doses of rhGH (94;95). Several animal studies have shown a relationship between estrogen treatment and hepatic IGF-I RNA expression. In ovariectomized rats, replacement with estradiol dose-dependently suppressed hepatic IGF-I liver mRNA expression and plasma IGF-I concentrations (96;97). Recently, the molecular mechanism underlying the hepatic effect of estrogen on IGF-I synthesis was discovered. Estrogen suppressed GH induced JAK2 phosphorylation through stimulation of SOCS-2 (98).

Although it has been suggested that these differential effects of transdermal and oral estradiol on the GH/ IGF-I axis are due to a first-pass effect of oral estradiol, prior studies in GH-deficient women on stable rhGH replacement were never aimed at identical serum estradiol concentrations. Indeed, in one study this switch from oral to transdermal estrogen administration was paralleled by a decrease in serum levels of estradiol (94). Therefore, in the study described in **Chapter 13** our aim was to study the effects of similar serum estradiol concentrations with different routes of estrogen administration on IGF-I levels in women with secondary hypogonadism and GHD.

Recently, a polymorphism in the growth hormone receptor, a genomic deletion of exon 3 (d3GHR), has been described to increase growth velocity during rhGH replacement in children with GHD (99) and idiopathic short stature or children who were born small for gestational age (100). Due to this polymorphism GH signal transduction is enhanced without any alterations in GH receptor binding (100). The allele-prevalence is estimated to be 25-32% with a frequency of homozygosity of 9-14% (100;101). The read-out for rhGH replacement in children is growth

velocity, which is very straightforward. In contrast, treatment efficacy parameters of rhGH replacement in adults are diverse (QoL, BMD, blood lipids and body composition). At present, it is unknown whether this polymorphism might also contribute to inter-individual variability of the clinical response to rhGH replacement in adults with GHD. Therefore, in **Chapter 14** we evaluated the pharmacogenetic interaction of the effects of rhGH replacement and this GH receptor isoform in patients with GHD.

IV. QUALITY OF LIFE AND SLEEP

Quality of life (QoL) assessment is becoming an increasingly important tool in medical practice to evaluate well-being and functioning of patients in every day life. In this thesis we used QoL evaluation as a tool to look carefully at residual impact of pituitary diseases on daily functioning during long-term follow-up.

In general, pituitary disease is associated with impaired QoL (102). Several factors have been considered to account for this impairment. Despite optimal endocrine replacement strategies, hypopituitarism is associated with impaired QoL (23;103;104). This may be due to intrinsic imperfections associated with the replacement of hormones to mimic normal biology. In addition specific diseases, such as acromegaly and Cushing's disease, may induce persistent irreversible effects (23;103). Finally, radiotherapy seems to be associated with decreased QoL (23;105). Because the various pituitary adenomas are associated with widely varying clinical presentation, treatment and outcome, there might be disease-specific effects of the different pituitary adenomas on QoL. This is supported by only one study in which QoL was evaluated in patients with different pituitary adenomas by the Short Form-36 QoL questionnaire (SF-36) (102). However, a major limitation of the direct comparison of QoL in patients with different pituitary adenomas is that there are major differences in age and gender distributions between the different pituitary adenomas. Because age and gender per se are major determinants of QoL (106-108), a proper comparison of QoL parameters between patients with different pituitary adenomas can only be performed after adjustment for these differences in age and gender distributions. Therefore, in **Chapter 15** we calculated age- and gender specific standard deviation scores of the various QoL items which enabled us to compare patients previously treated for acromegaly, prolactinoma, Cushing's disease and non-functioning adenomas.

Interestingly, one feature of QoL often encountered in these patients is markedly increased fatigue scores (23;103;104;109). Increased daytime sleepiness has previously been described craniopharyngioma (110), hypothalamic tumors (110), subarachnoid haemorrhage (111), or traumatic brain injury (112), indicative for the relationship between cerebral disease and increased daytime sleepiness. In addition, sleep quality measured with polysomnography is

found to be altered in patients with Cushing's disease (113), acromegaly (114), prolactinoma (115), and patients with craniopharyngioma (116). Conversely, many interactions between nocturnal secretion of different hormones and the sleep electroencephalogram parameters have been described (117). Indeed, altered sleep patterns can induce changes in anterior pituitary hormone secretion (118).

Interestingly, the hypothalamus has been identified as the main regulatory center of sleep: the suprachiasmatic nucleus (SCN) is considered to be the central circadian pacemaker of the body with one of the circadian outputs formed by the regulation of circadian variations in melatonin secretion by the pineal gland (57). Large pituitary tumors and their surgical and/or radiotherapeutical treatment could possibly affect the hypothalamus, which may alter hypothalamic function in the long term. At present, there are hardly any histological studies on hypothalamic tissue obtained from such patients. In addition, there are major limitations in the specificity of clinical or biochemical signs of altered hypothalamic functioning in these patients.

Sleep might thus be a read-out parameter for hypothalamic function in patients treated for large pituitary adenomas and craniopharyngiomas and disturbed sleep characteristics could aid to the understanding of changes in QoL in these patients. Therefore, in **Chapter 16** and **17** we evaluated sleepiness and sleep patterns in patients previously treated for non-functioning macroadenomas and craniopharyngioma.

V. SCOPE OF THE PRESENT THESIS

Careful assessment during long-term follow-up of patient with pituitary disease enables us to study long-term consequences of those diseases and their treatment. These studies provide insight in the complex morbidity present during the long-term follow-up in these patients. The aim of this thesis was to assess the consequences of pituitary disease.

Studies in patients with acromegaly

At present it is unknown how quickly regurgitant cardiac valve disease occurs in patients with active acromegaly and whether cardiac valve disease is stable in patients with biochemical remission. To establish the relation between disease activity and cardiac valve disease, we assessed valvular regurgitation in 37 acromegalic patients (18 patients with active disease, and 19 with controlled disease) by conventional two-dimensional and Doppler echocardiography before and after an interval of almost 2 years in **Chapter 2**.

Abnormal matrix regulation during GH excess might also influence aortic root diameters. In **Chapter 3** we performed a prospective study of aortic root diameters in acromegalic patients with active disease and with controlled disease, comparing the data to healthy controls. Aortic root diameters were prospectively assessed in 37 acromegalic patients (18 patients with active disease, and 19 with controlled disease) by conventional two-dimensional and Doppler

echocardiography before and after an observation period of almost 2 years. Baseline parameters were compared to healthy controls.

Changes in QoL might be due to irreversible effects of previous GH excess and/or treatment which can only be studied in a homogeneous cohort of patients with long-term biochemical remission of acromegaly. In **Chapter 4** we evaluated whether QoL parameters change and to identify predictors that influence changes in QoL during 4 years of follow-up in a homogeneous cohort of acromegalic patients with sustained biochemical control of acromegaly. Quality of life was assessed using four health related quality of life questionnaires ((Hospital Anxiety and Depression Scale (HADS), Multidimensional Fatigue Inventory (MFI-20), Nottingham Healthy Profile (NHP), Short Form-36 (SF-36)) and one disease-specific quality of life questionnaire (Acromegaly-Quality of Life (ACRO-QOL)) in 82 patients (43 men) with strict biochemical control of acromegaly at baseline and after 4 years of follow-up.

Acromegaly has profound effects on sleep characteristics. Somatostatin also affects sleep. It is unknown whether sleep characteristics are altered by somatostatin analogs in the treatment of acromegaly. Therefore, in **Chapter 5** we studied self-reported sleepiness and sleep patterns in patients with biochemical control of acromegaly. We assessed sleepiness and sleep patterns in 62 adult patients controlled by surgery alone or postoperative radiotherapy (69%) and/or somatostatin analogs (31%). We used two validated sleep questionnaires (Epworth sleepiness score and Münchener Chronotype Questionnaire). Patient outcomes were compared to controls.

Postoperative radiotherapy is associated with an increased diminished growth hormone secretion in patients with acromegaly. The value of a decreased GH response to insulin induced hypoglycemia in relation to other GH stimulation tests was not known. Therefore, in **Chapter 6** we studied the diagnostic value of conventional GH stimulation tests to establish the diagnosis of GHD in patients who had been treated with radiotherapy for acromegaly. We compared the characteristics of basal and stimulated GH secretion in 10 patients treated for acromegaly to patients treated similarly for other pituitary adenomas. All patients had a maximal GH concentration by insulin tolerance test (ITT) of 3 µg/l or less, compatible with severe GHD. Stimulated GH release was evaluated by infusion of GH releasing hormone (GHRH), GHRH+arginine and arginine. Spontaneous GH secretion was evaluated by 10 minute blood sampling for 24h and analyzed with Cluster and approximate entropy.

Both acromegaly and GHD have specific effects on cardiac function. However, the effects of GHD in patients with previous acromegaly were not known. Therefore, in **Chapter 7** we assessed cardiac morphology and function in patients with GHD after postoperative radiotherapy for acromegaly in comparison with patients with active acromegaly, patients with biochemical

remission of acromegaly and healthy controls. Cardiac parameters were studied by conventional two-dimensional echocardiography and Tissue Doppler imaging in 53 patients with acromegaly (16 patients with GHD, 20 patients with biochemical remission and 17 patients with active disease). Patients with GHD were also compared to age- and gender-matched controls.

The effects of rhGH replacement for GHD in patients with acromegaly have not been studied in prospective, randomised studies. Therefore, in **Chapter 8** we studied the effects of rhGH replacement for GHD in patients previously treated for acromegaly in a randomised controlled trial. Sixteen patients, treated for acromegaly with surgery and radiotherapy, with an insufficient GH response to insulin-induced hypoglycaemia, were randomized to 1 year of rhGH replacement (n=10), or 1 year of placebo followed by 1 year of rhGH replacement (delayed treatment, n=6). Study parameters were assessed at baseline, after 1 year of placebo (n=6, delayed treatment) and after 1 year of rhGH replacement (n=16). Study parameters were cardiac function, body composition, bone mineral density (BMD), fasting lipids, glucose, bone turnover markers, and QoL.

A fundamental question is whether macroadenomes and/or their treatment damage the normal function of the hypothalamus in humans. This issue is difficult to assess because we lack sensitive markers for dedicated phenotyping of hypothalamic pathophysiology in humans. Circadian variations in melatonin secretion are under the control of endogenous clock signals arising from the suprachiasmatic nucleus (SCN) of the hypothalamus. We hypothesized that SCN function might be compromised after treatment of pituitary adenomas with radiotherapy. Therefore, the aim of this study described in **Chapter 9** was to assess the effects of postoperative radiotherapy on characteristics of diurnal melatonin secretion in patients cured from acromegaly. We studied 3 groups of 8 subjects matched for age, gender and BMI. The groups consisted of: 1) patients treated with postoperative radiotherapy, 2) patients treated with transsphenoidal surgery and 3) healthy controls. Melatonin concentrations were measured each hour during 24h and circadian rhythmicity was appraised with a skewed baseline cosine curve fit procedure.

Studies in patients with growth hormone deficiency

In **Chapter 10** we report on the effects of rhGH replacement on vasculature and bone marrow derived CD34+ cells, which function as a marker for cardiovascular risk. Vascular function (flow-mediated dilatation (FMD)) and structure (pulse wave velocity (PWV) and analysis) was assessed in 14 adult patients with GHD. In addition, the number of CD34+ cells was evaluated using flow cytometric analysis. Study parameters were analyzed at baseline, and after 6 months and 1 year of rhGH replacement.

GHD requires long-term rhGH replacement. However, studies on the long-term efficacy (> 5 years) have hardly been done. Therefore, in **Chapter 11** we describe the effects of 7 years of rhGH replacement on biochemical parameters and anthropometric parameters in our cohort of GHD adults. Sixty-three adult GHD patients were assessed before and after 2, 5 and 7 years of rhGH replacement.

Many reports demonstrate improvements in single cardiovascular risk factors during rhGH replacement in GHD. However, it remained to be determined to what extent these changes translate into a reduction of increased cardiovascular morbidity and mortality. The aim of this study described in **Chapter 12** was to evaluate the effects of long-term rhGH replacement on the prevalence of the metabolic syndrome. The metabolic syndrome was scored using the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) definition in 50 consecutive patients with adult-onset GHD, before, after 2, and after 5 years of rhGH replacement. The data in untreated patients were compared to the general population using data from a Dutch population based study.

The route of estrogen substitution is a major determinant of the IGF-I response in women on estrogen and rhGH substitution. Nonetheless, studies comparing transdermal and oral estrogen substitution have never been controlled for similar plasma estradiol levels. The aim of the study described in **Chapter 13** was to evaluate the effects of oral versus transdermal estrogen administration at similar plasma estradiol levels on IGF-I, IGF binding protein-3 and sex hormone-binding globulin (SHBG) concentrations. We designed a parallel cross-over study in which two groups of women with fixed and stable rhGH replacement passed through four different estradiol treatment schemes (2 and 4 mg oral and 50 and 100 µg transdermal estradiol) with a duration of 4 cycles each to ensure a new steady state. Group I (18 patients using oral estradiol prior to the study) was treated with oral followed by transdermal estradiol and group II (5 patients with transdermal estradiol prior to inclusion) with transdermal followed by oral estradiol. The dose of rhGH was fixed during the entire study.

There is a common activating polymorphism of the GH receptor. In **Chapter 14** we describe a study aimed at assessing the effects of this exon-3 deletion polymorphism of the GH receptor (d3GHR) on the response to rhGH replacement in adults. We designed a prospective intervention study with rhGH during 1 year (n=99) and in a subset of patients during 5 years (n=53). The presence of the d3GHR variant was established using PCR in GHD patients and linked to short-term and long-term effects of rhGH replacement on IGF-I, lipid metabolism, anthropometric parameters and bone mineral density.

Studies on quality of life in patients with pituitary disease

QoL is impaired in patients treated for pituitary adenomas. However, differences in age and gender distributions hamper a proper comparison of QoL. Therefore, we compared age- and gender-specific standard deviations scores (Z scores) of QoL parameters in patients treated for pituitary adenomas. The results of this study are described in **Chapter 15**. Age- and gender specific Z scores were determined for health-related questionnaires (HADS, MFI-20, NHP, SF-36) in patients during long-term follow-up after treatment for pituitary adenomas. The Z scores were calculated by comparing the data of 403 patients (acromegaly (n=118), Cushing's disease (n=58), prolactinoma (n=128), non-functioning macroadenoma (n=99)) with a control population (n=440) for each subscales of the questionnaires and for total QoL score.

In patients treated for non-functioning pituitary macroadenoma (NFMA) and craniopharyngioma increased fatigue scores on QoL have been reported. Because this may be related to altered sleep patterns, we evaluated daytime sleepiness and sleep patterns in patients successfully treated for NFMA and craniopharyngioma in our center (**Chapter 16 and 17**).

In the case-control study described in **Chapter 16** we assessed sleepiness and sleep patterns in 76 adult patients in remission of NFMA during long-term follow up after surgical (n=76) and additional radiotherapeutical (n=28) treatment. We used two validated questionnaires for sleep parameters (Epworth sleepiness score and Münchener Chronotype Questionnaire) and four validated questionnaires for quality of life (HADS, MFI-20, NHP, SF-36). Patient outcomes were compared to 76 healthy controls.

In **Chapter 17** we describe the results of a study in which we assessed sleepiness and sleep patterns in 27 adult patients with craniopharyngioma after long-term follow up and compared to 50 healthy controls and 38 age-, gender- and BMI matched patients with NFMA. We used two validated questionnaires for sleep parameters (Epworth sleepiness score and Münchener Chronotype Questionnaire).

REFERENCE LIST

1. Alexander L, Appleton D, Hall R, Ross WM, Wilkinson R 1980 Epidemiology of acromegaly in the Newcastle region. Clin Endocrinol (Oxf) 12:71-79
2. Bengtsson BA, Eden S, Ernest I, Oden A, Sjogren B 1988 Epidemiology and long-term survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. Acta Med Scand 223:327-335
3. Etxabe J, Gaztambide S, Latorre P, Vazquez JA 1993 Acromegaly: an epidemiological study. J Endocrinol Invest 16:181-187
4. Ritchie CM, Atkinson AB, Kennedy AL, Lyons AR, Gordon DS, Fannin T, Hadden DR 1990 Ascertainment and natural history of treated acromegaly in Northern Ireland. Ulster Med J 59:55-62
5. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
6. Minniti G, Jaffrain-Rea ML, Moroni C, Baldelli R, Ferretti E, Cassone R, Gulino A, Tamburrano G 1998 Echocardiographic evidence for a direct effect of GH/IGF-I hypersecretion on cardiac mass and function in young acromegalics. Clin Endocrinol (Oxf) 49:101-106
7. Vianna CB, Vieira ML, Mady C, Liberman B, Durazzo AE, Knoepfelmacher M, Salgado LR, Ramires JA 2002 Treatment of acromegaly improves myocardial abnormalities. Am Heart J 143:873-876
8. Colao A, Marzullo P, Ferone D, Spinelli L, Cuocolo A, Bonaduce D, Salvatore M, Boerlin V, Lancranjan I, Lombardi G 2000 Cardiovascular effects of depot long-acting somatostatin analog Sandostatin LAR in acromegaly. J Clin Endocrinol Metab 85:3132-3140
9. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
10. Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, Vitale G, Bonaduce D, Lombardi G 2003 High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 88:3196-3201
11. van Karnebeek CD, Naeff MS, Mulder BJ, Hennekam RC, Offringa M 2001 Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child 84:129-137
12. Savage DD, Henry WL, Eastman RC, Borer JS, Gorden P 1979 Echocardiographic assessment of cardiac anatomy and function in acromegalic patients. Am J Med 67:823-829
13. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
14. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F 2005 Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 6:2393-2405
15. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ 2005 Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365:1644-1646
16. Melmed S 2006 Medical progress: Acromegaly. N Engl J Med 355:2558-2573
17. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO 2001 Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358:1754-1759
18. Hua SC, Yan YH, Chang TC 2006 Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. Eur J Endocrinol 155:831-837
19. Kauppinen-Makelin R, Sane T, Sintonen H, Markkanen H, Valimaki MJ, Loyttyniemi E, Niskanen L, Reunanen A, Stenman UH 2006 Quality of life in treated patients with acromegaly. J Clin Endocrinol Metab 91:3891-3896
20. Trepp R, Everts R, Stettler C, Fischli S, Allemann S, Webb SM, Christ ER 2005 Assessment of quality of life in patients with uncontrolled vs. controlled acromegaly using the Acromegaly Quality of Life Questionnaire (AcroQoL). Clin Endocrinol (Oxf) 63:103-110

21. Webb SM, Badia X, Surinach NL 2006 Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study. Eur J Endocrinol 155:269-277
22. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ 2005 Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. J Clin Endocrinol Metab 90:3337-3341
23. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F 2004 Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 89:5369-5376
24. Paisley AN, Rowles SV, Roberts ME, Webb SM, Badia X, Prieto L, Shalet SM, Trainer PJ 2007 Treatment of acromegaly improves quality of life, measured by AcroQol. Clin Endocrinol (Oxf) 67:358-362
25. Herrmann BL, Wessendorf TE, Ajaj W, Kahlke S, Teschler H, Mann K 2004 Effects of octreotide on sleep apnoea and tongue volume (magnetic resonance imaging) in patients with acromegaly. Eur J Endocrinol 151:309-315
26. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329
27. Saeki N, Isono S, Tanaka A, Nishino T, Higuchi Y, Uchino Y, Iuchi T, Murai H, Tatsuno I, Yasuda T, Yamaura A 2000 Pre-and post-operative respiratory assessment of acromegalics with sleep apnea--bedside oximetric study for transsphenoidal approach. Endocr J 47 Suppl:S61-S64
28. Buyse B, Michiels E, Bouillon R, Bobbaers H, Demedts M 1997 Relief of sleep apnoea after treatment of acromegaly: report of three cases and review of the literature. Eur Respir J 10:1401-1404
29. Grunstein RR, Ho KK, Sullivan CE 1994 Effect of octreotide, a somatostatin analog, on sleep apnea in patients with acromegaly. Ann Intern Med 121:478-483
30. Leibowitz G, Shapiro MS, Salameh M, Glaser B 1994 Improvement of sleep apnoea due to acromegaly during short-term treatment with octreotide. J Intern Med 236:231-235
31. Beranek L, Obal F, Jr., Taishi P, Bodosi B, Laczi F, Krueger JM 1997 Changes in rat sleep after single and repeated injections of the long-acting somatostatin analog octreotide. Am J Physiol 273:R1484-R1491
32. Frieboes RM, Murck H, Schier T, Holsboer F, Steiger A 1997 Somatostatin impairs sleep in elderly human subjects. Neuropsychopharmacology 16:339-345
33. Steiger A, Guldner J, Hemmeter U, Rothe B, Wiedemann K, Holsboer F 1992 Effects of growth hormone-releasing hormone and somatostatin on sleep EEG and nocturnal hormone secretion in male controls. Neuroendocrinology 56:566-573
34. Ziegenbein M, Held K, Kuenzel HE, Murck H, Antonijevic IA, Steiger A 2004 The somatostatin analogue octreotide impairs sleep and decreases EEG sigma power in young male subjects. Neuropsychopharmacology 29:146-151
35. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
36. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
37. Conceicao FL, Fisker S, Andersen M, Kaal A, Jorgensen JO, Vaisman M, Christiansen JS 2003 Evaluation of growth hormone stimulation tests in cured acromegalic patients. Growth Horm IGF Res 13:347-352
38. Amato G, Carella C, Fazio S, La Montagna G, Cittadini A, Sabatini D, Marciano-Mone C, Sacca L, Bellastella A 1993 Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J Clin Endocrinol Metab 77:1671-1676
39. Colao A, Cuocolo A, Di Somma C, Cerbone G, Della Morte AM, Nicolai E, Lucci R, Salvatore M, Lombardi G 1999 Impaired cardiac performance in elderly patients with growth hormone deficiency. J Clin Endocrinol Metab 84:3950-3955

40. Colao A, Di Somma C, Cuocolo A, Filippella M, Rota F, Acampa W, Savastano S, Salvatore M, Lombardi G 2004 The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. J Clin Endocrinol Metab 89:5998-6004
41. Merola B, Cittadini A, Colao A, Longobardi S, Fazio S, Sabatini D, Sacca L, Lombardi G 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab 77:1658-1661
42. Sartorio A, Ferrero S, Conti A, Bragato R, Malfatto G, Leonetti G, Faglia G 1997 Adults with childhood-onset growth hormone deficiency: effects of growth hormone treatment on cardiac structure. J Intern Med 241:515-520
43. Colao A, Cuocolo A, Di Somma C, Cerbone G, Morte AM, Pivonello R, Nicolai E, Salvatore M, Lombardi G 2000 Does the age of onset of growth hormone deficiency affect cardiac performance? A radionuclide angiography study. Clin Endocrinol (Oxf) 52:447-455
44. Erdogan D, Tukek T, Aral F, Oflaz H, Ozaydin M, Kocaman O, Akkaya V, Goren T, Molvalilar S 2004 Structural, functional and autonomic changes in the cardiovascular system in growth hormone deficient patients. Ann Noninvasive Electrocardiol 9:19-23
45. Yurci A, Oflaz H, Meric M, Ozbey N 2005 Mitral and tricuspid annular velocities determined by Doppler tissue imaging in hypopituitary, growth hormone-deficient patients. Horm Res 64:107-114
46. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 156:55-64
47. Maison P, Chanson P 2003 Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 108:2648-2652
48. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
49. Deijen JB, Arwert LI, Witlox J, Drent ML 2005 Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes 3:63
50. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
51. Davidson P, Milne R, Chase D, Cooper C 2004 Growth hormone replacement in adults and bone mineral density: a systematic review and meta-analysis. Clin Endocrinol (Oxf) 60:92-98
52. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
53. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 92:1442-1445
54. Feldt-Rasmussen U, Abs R, Bengtsson BA, Bennmarker H, Bramnert M, Hernberg-Stahl E, Monson JP, Westberg B, Wilton P, Wuster C 2002 Growth hormone deficiency and replacement in hypopituitary patients previously treated for acromegaly or Cushing's disease. Eur J Endocrinol 146:67-74
55. Norrman LL, Johannsson G, Sunnerhagen KS, Svensson J 2008 Baseline Characteristics and the Effects of Two Years of Growth Hormone (GH) Replacement Therapy in Adults with GH Deficiency Previously Treated for Acromegaly. J Clin Endocrinol Metab 93:2531-2538.
56. Toogood AA 2004 Endocrine consequences of brain irradiation. Growth Horm IGF Res 14 Suppl A:S1-18-S124
57. Mignot E, Taheri S, Nishino S 2002 Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5 Suppl:1071-1075
58. Arendt J 2006 Melatonin and human rhythms. Chronobiol Int 23:21-37

59. Bates AS, Van't Hoff W, Jones PJ, Clayton RN 1996 The effect of hypopituitarism on life expectancy. J Clin Endocrinol Metab 81:1169-1172
60. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
61. Elhadd TA, Abdu TA, Oxtoby J, Kennedy G, McLaren M, Neary R, Belch JJ, Clayton RN 2001 Biochemical and biophysical markers of endothelial dysfunction in adults with hypopituitarism and severe GH deficiency. J Clin Endocrinol Metab 86:4223-4232
62. Evans LM, Davies JS, Goodfellow J, Rees JA, Scanlon MF 1999 Endothelial dysfunction in hypopituitary adults with growth hormone deficiency. Clin Endocrinol (Oxf) 50:457-464
63. Lanes R, Gunczler P, Lopez E, Esaa S, Villaroel O, Revel-Chion R 2001 Cardiac mass and function, carotid artery intima-media thickness, and lipoprotein levels in growth hormone-deficient adolescents. J Clin Endocrinol Metab 86:1061-1065
64. Rosen T, Bengtsson BA 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285-288
65. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM 2001 Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 357:425-431
66. Bates AS, Evans AJ, Jones P, Clayton RN 1995 Assessment of GH status in adults with GH deficiency using serum growth hormone, serum insulin-like growth factor-I and urinary growth hormone excretion. Clin Endocrinol (Oxf) 42:425-430
67. Hartman ML, Crowe BJ, Biller BM, Ho KK, Clemmons DR, Chipman JJ 2002 Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J Clin Endocrinol Metab 87:477-485
68. Toogood AA, Beardwell CG, Shalet SM 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf) 41:511-516
69. Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KK 1994 Diagnosis of growth-hormone deficiency in adults. Lancet 343:1064-1068
70. Blum WF, Machinis K, Shavrikova EP, Keller A, Stobbe H, Pfaeffle RW, Amselem S 2006 The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. J Clin Endocrinol Metab 91:4171-4174
71. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D 1999 The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 84:2596-2602
72. Stouthart PJ, Deijen JB, Roffel M, Delemarre-van de Waal HA 2003 Quality of life of growth hormone (GH) deficient young adults during discontinuation and restart of GH therapy. Psychoneuroendocrinology 28:612-626
73. Sonksen PH, McGauley G 2005 Lies, damn lies and statistics. Growth Horm IGF Res 15:173-176
74. Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ 2004 Endothelial progenitor cells: more than an inflammatory response? Arterioscler Thromb Vasc Biol 24:834-838
75. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ 2004 Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 53:195-199
76. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T 2003 Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348:593-600
77. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G 2005 Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 353:999-1007

78. Thum T, Fleissner F, Klink I, Tsikas D, Jakob M, Bauersachs J, Stichtenoth DO 2007 Growth hormone treatment improves markers of systemic nitric oxide bioavailability via insulin-like growth factor-1. J Clin Endocrinol Metab 92: 4172-4179
79. Carlo-Stella C, Di Nicola M, Milani R, Guidetti A, Magni M, Milanesi M, Longoni P, Matteucci P, Formelli F, Ravagnani F, Corradini P, Gianni AM 2004 Use of recombinant human growth hormone (rhGH) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the mobilization and collection of CD34+ cells in poor mobilizers. Blood 103:3287-3295
80. Hanley MB, Napolitano LA, McCune JM 2005 Growth hormone-induced stimulation of multilineage human hematopoiesis. Stem Cells 23:1170-1179
81. Merchav S 1998 The haematopoietic effects of growth hormone and insulin-like growth factor-I. J Pediatr Endocrinol Metab 11:677-685
82. Chang KH, Chan-Ling T, McFarland EL, Afzal A, Pan H, Baxter LC, Shaw LC, Caballero S, Sengupta N, Li CS, Sullivan SM, Grant MB 2007 IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor cell function during vascular development. Proc Natl Acad Sci U S A 104:10595-10600
83. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG 2000 Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 85:3762-3769
84. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO 2002 Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 87:2121-2127
85. Svensson J, Stibrant SK, Johannsson G 2003 Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 88:2061-2069
86. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L 2001 Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 24:683-689
87. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT 2002 The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709-2716
88. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607
89. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) 2001 JAMA 285:2486-2497
90. Cook DM, Ludlam WH, Cook MB 1999 Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 84:3956-3960
91. Johansson AG, Engstrom BE, Ljunghall S, Karlsson FA, Burman P 1999 Gender differences in the effects of long term growth hormone (GH) treatment on bone in adults with GH deficiency. J Clin Endocrinol Metab 84:2002-2007
92. Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG 2000 Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab 85:1121-1125
93. Christiansen JJ, Fisker S, Gravholt CH, Bennett P, Svenstrup B, Andersen M, Feldt-Rasmussen U, Christiansen JS, Jorgensen JO 2005 Discontinuation of estrogen replacement therapy in GH-treated hypopituitary women alters androgen status and IGF-I. Eur J Endocrinol 152:719-726
94. Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F 2000 A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. J Clin Endocrinol Metab 85:464-467

95. Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK 2001 Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. Am J Physiol Endocrinol Metab 281:E1191-E1196
96. Borski RJ, Tsai W, DeMott-Friberg R, Barkan AL 1996 Regulation of somatic growth and the somatotropic axis by gonadal steroids: primary effect on insulin-like growth factor I gene expression and secretion. Endocrinology 137:3253-3259
97. Krattenmacher R, Knauthe R, Parczyk K, Walker A, Hilgenfeldt U, Fritzemeier KH 1994 Estrogen action on hepatic synthesis of angiotensinogen and IGF-I: direct and indirect estrogen effects. J Steroid Biochem Mol Biol 48:207-214
98. Leung KC, Doyle N, Ballesteros M, Sjogren K, Watts CK, Low TH, Leong GM, Ross RJ, Ho KK 2003 Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. Proc Natl Acad Sci U S A 100:1016-1021
99. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ 2006 Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91:1076-1080
100. Dos SC, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
101. Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amselem S 2000 Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. J Biol Chem 275:18664-18669
102. Johnson MD, Woodburn CJ, Vance ML 2003 Quality of life in patients with a pituitary adenoma. Pituitary 6:81-87
103. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA 2005 Quality of Life in Patients After Long-term Biochemical Cure of Cushing's Disease. J Clin Endocrinol Metab 90:3279-3286
104. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA 2006 Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:3364-3369
105. Page RC, Hammersley MS, Burke CW, Wass JA 1997 An account of the quality of life of patients after treatment for non-functioning pituitary tumours. Clin Endocrinol (Oxf) 46:401-406
106. Norris CM, Ghali WA, Galbraith PD, Graham MM, Jensen LA, Knudtson ML 2004 Women with coronary artery disease report worse health-related quality of life outcomes compared to men. Health Qual Life Outcomes 2:21
107. Agewall S, Berglund M, Henareh L 2004 Reduced quality of life after myocardial infarction in women compared with men. Clin Cardiol 27:271-274
108. Mainio A, Hakko H, Niemela A, Koivukangas J, Rasanen P 2006 Gender difference in relation to depression and quality of life among patients with a primary brain tumor. Eur Psychiatry 21:194-199
109. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM 2006 Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 154:483-489
110. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589
111. Schuiling WJ, Rinkel GJ, Walchenbach R, de Weerd AW 2005 Disorders of sleep and wake in patients after subarachnoid hemorrhage. Stroke 36:578-582
112. Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR 2006 Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehabil 87:278-285
113. Shipley JE, Schteingart DE, Tandon R, Starkman MN 1992 Sleep architecture and sleep apnea in patients with Cushing's disease. Sleep 15:514-518

114. Astrom C, Christensen L, Gjerris F, Trojaborg W 1991 Sleep in acromegaly before and after treatment with adenomectomy. Neuroendocrinology 53:328-331
115. Frieboes RM, Murck H, Stalla GK, Antonijevic IA, Steiger A 1998 Enhanced slow wave sleep in patients with prolactinoma. J Clin Endocrinol Metab 83:2706-2710
116. Muller HL, Muller-Stover S, Gebhardt U, Kolb R, Sorensen N, Handwerker G 2006 Secondary narcolepsy may be a causative factor of increased daytime sleepiness in obese childhood craniopharyngioma patients. J Pediatr Endocrinol Metab 19 Suppl 1:423-429
117. Van Cauter E, Leproult R, Plat L 2000 Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284:861-868
118. Spiegel K, Leproult R, Van Cauter E 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435-1439

Chapter 2

Uncontrolled Acromegaly is Associated with Progressive Mitral Valvular Regurgitation

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Growth Hormone & IGF Research, 2006

ABSTRACT

Introduction

Recent cross-sectional studies have documented an association between acromegaly and regurgitant valvular heart disease. The aim of this study was to evaluate the change in prevalence of valvular heart disease in relation to the clinical activity, because the natural history of valvular changes in acromegaly is unknown.

Patients and methods

Valvular regurgitation was assessed in 37 acromegalic patients (18 patients with active disease, and 19 with controlled disease) by conventional two-dimensional and Doppler echocardiography before and after an interval of 1.9 yr (range 1.5-3.0 years).

Results

At baseline, valvular regurgitation (mitral and aortic sites combined) was present in 46% of the patients and increased to 67% at follow-up (p=0.008). Mitral regurgitation increased significantly from 32% to 60% (p=0.002), but no change was noted for the aortic valve (27 vs. 31%, NS). In patients with active disease, valvular regurgitation increased significantly from 56% at baseline to 88% at follow-up (p=0.031) due to a significant increase of mitral regurgitation from 39% to 78% at follow-up, p=0.016). In contrast, no increase in valvular regurgitation was found in patients with controlled disease.

Conclusion

The prevalence of mitral, but not aortic, valvular regurgitation increased in patients with active acromegaly during follow-up. Patients with acromegaly require adequate cardiac evaluation and follow-up to establish the extent and progression of valvular involvement.

INTRODUCTION

Active acromegaly alters cardiac structure and function. Cardiac manifestations of acromegaly include left ventricular (LV) hypertrophy, arrhythmias, and heart failure due to diastolic and systolic dysfunction (1-3), but fortunately adequate treatment of growth hormone excess can arrest or even reverse these cardiac changes (4).

Recent cross-sectional studies have documented an association between acromegaly and regurgitant valvular heart disease, irrespective of disease activity (5;6). In these studies it was concluded that valvular change is apparently irreversible in contrast to the observed regression of LV changes in successfully treated patients. Because the natural history of valvular changes in patients with acromegaly is unknown, the aim of the present observational study was to evaluate the changes in prevalence of valvular heart disease in relation to the clinical activity. Therefore, we enrolled patients previously described in our cross-sectional study (5) and reassessed valvular regurgitation after an interval of at least 1.5 years. In order to assess the possible influence of disease activity on valvular disease, we studied patients with (mild) active disease and patients with controlled disease. We hypothesized on the basis of the high prevalence of valvular regurgitation found in our cross-sectional study that valvular regurgitation would increase during follow-up in patients with active disease.

PATIENTS AND METHODS

Patients

Thirty-seven patients were enrolled to this study, of whom 35 had participated in the previous study (5). The initial diagnosis of acromegaly was based on the characteristic clinical features and confirmed by insufficient suppression of GH during a glucose tolerance test (normal response: GH nadir <0.5 µg/L), an elevated age- and gender-adjusted IGF-I, and the presence of a pituitary adenoma on radiological imaging.

Patients were classified at study entry as having active or inactive acromegaly.

Active acromegaly (n=18) was defined as: mean fasting GH concentration (measured every 30 minutes for 3 hours) >2.5 µg/L, and an elevated age- and gender-adjusted IGF-I concentration. This study was performed before the introduction of GH receptor blockade drugs. Nineteen patients were classified as inactive acromegaly which was defined in medically well-controlled acromegaly (n=13) as mean fasting GH concentration (measured for 3 hours with an interval of 30 minutes) <2.5 µg/L, and normal age- and gender-adjusted IGF-I concentrations during treatment with depot octreotide acetate (n=13, Novartis Pharma AG, Basel, Switzerland) and in surgically cured acromegaly (n=6) as glucose-suppressed GH <0.5 µg/L, and normal age- and gender-adjusted IGF-I concentration without medical treatment.

None of the patients had hemodynamic instability, previous myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, or connective tissue disease. None of the female patients became pregnant during the study period.

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

Echocardiography, Data Acquisition

The data were collected prospectively with a minimal duration of 1.5 years on the first available occasion at the outpatient clinic.

Echocardiography was performed in the patients in the left lateral decubitus position using a commercially available system (Vingmed 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 (Inter-Ventricular Septum Thickness (IVST), Posterior Wall Thickness (PWT), LV End-Diastolic Diameter (LVEDD), LV End-Systolic Diameter (LVESD)), Fractional Shortening (FS) and LV Ejection Fraction (LVEF) (7).

LV mass (LVM) was calculated by the cube formula, and using the correction formula proposed by Devereux, et al. (8): $0.8 \times \{1.04\ [(\text{LVEDD} + \text{PWT} + \text{IVST})^3 - \text{LVEDD}^3]\} + 0.6$. LVM indexation (LVMi) was corrected for body height (9). LV hypertrophy (LVH) was defined as a LVMi above 49.2 g/m$^{2.7}$ for men and 46.7 g/m$^{2.7}$ for women (9).

The severity of valvular regurgitation was assessed by two independent expert readers blinded to the clinical data on a qualitative scale of trace, mild, moderate, or severe, using previously described methods (10;11).

Hormone Assays

GH concentrations were quantitated using a sensitive time-resolved immunofluorescent assay (Wallac Oy, Turku, Finland), specific for 22 kDa GH protein. The detection limit was 0.012 µg/L. Inter-assay coefficients of variation were 8.4-1.6% in the GH-range 0.1-18 µg/L. 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 coefficients of variation were less than 11%. The detection limit was 1.5 nmol/l. Age- and gender-adjusted IGF-I data was determined in the same laboratory. IGF-1 was expressed as a standard deviation (SD) score from age- and gender-related normal levels.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 12.0 (SPSS Inc. Chicago, Illinois, USA). Results are expressed as the mean ± standard error of the mean (SEM), unless specified otherwise. Paired samples t-tests were used to assess the difference in left ventricular measurements

at baseline and at follow-up. Independent samples t-tests were used to compare baseline values, follow-up values, and the difference in baseline and follow-up values between the two groups. Non-parametric related samples test of McNemar was used to assess the difference in fractions at baseline and follow-up. Chi-square tests were used to assess the difference in fractions between the study groups. A P-value <0.05 was considered to represent a significant difference.

RESULTS

Clinical Characteristics

The interval between the two study occasions was 1.9 years, range 1.5 to 3.0 years. Mean GH concentration and IGF-I SD scores were significantly higher in active patients, compared to inactive patients, both at baseline and at follow-up (see Table 1 for details). The activity of acromegaly decreased during follow-up in the patients with active acromegaly, reflected by a significant decrease of GH and IGF-1 concentrations at the end of follow-up. Six of the 18 patients, who were characterized as having active acromegaly at baseline, were adequately controlled at the end of follow-up. The mean duration of adequate control of acromegaly prior

Table 2/1: Clinical characteristics at baseline and follow-up.

		Active acromegaly (n=18)	Inactive acromegaly (n=19)	P-values
Age (yrs)		52.8 ± 3.8	54.8 ± 3.1	0.689
Gender (male/ female)		8/ 10	7/ 12	0.638
Follow-up duration (yrs)		2.0 ± 0.1	1.8 ± 0.1	0.247
IGF-I (SD scores)	Baseline	8.3 ± 1.9	0.8 ± 0.4	<0.001
	Follow-up	2.9 ± 0.5*	1.0 ± 0.3	0.006
GH (mU/l)	Baseline	19.0 ± 4.9	2.7 ± 0.6	0.004
	Follow-up	5.8 ± 1.4**	2.0 ± 0.5	0.009
Weight (kg)	Baseline	87.4 ± 4.0	85.9 ± 4.2	0.799
	Follow-up	85.2 ± 3.8	86.1 ± 3.9	0.875
BMI (kg/m^2)	Baseline	27.9 ± 1.0	27.7 ± 1.0	0.898
	Follow-up	28.3 ± 1.2	28.1 ± 1.0	0.879
Systolic blood pressure (mmHg)	Baseline	143.8 ± 6.3	144.7 ± 4.3	0.905
	Follow-up	132.8 ± 3.0	134.2 ± 3.7***	0.765
Diastolic blood pressure (mmHg)	Baseline	83.6 ± 2.2	84.9 ± 1.5	0.616
	Follow-up	86.4 ± 2.2	83.5 ± 1.9	0.326

Values are expressed as mean ± SEM. Within the two groups, parameters are compared between baseline and follow-up with paired samples t-tests. The two groups are compared with independent samples t-tests or chi-square tests when appropriate. BMI Body Mass Index. *IGF-I SD scores significantly decreased within active patients (P<0.001). **GH significantly decreased within active patients (P=0.017). ***Systolic blood pressure significantly decreased within inactive patients during follow-up (P=0.007).

to the first echocardiography in patients with inactive acromegaly at study entry was 7.6 years, range 0.8 to 22.7 years.

Left Ventricular Diameters and Systolic Function

At baseline, systolic function was significantly lower in patients with active acromegaly (Table 2). In addition, IVST was significantly higher in patients with active acromegaly compared to patients with inactive acromegaly. LVH was present in 61% of the active patients and in 32% of the inactive patients. This was not significantly different. Left ventricular diameters and systolic function remained unchanged during follow-up in active as well as inactive patients, except for a minimal decrease in LVEF, which was not clinically relevant. There were no differences in the difference between baseline and follow-up between active and inactive patients.

Table 2/2: Left ventricular measurements and systolic function.

		Active acromegaly (n=18)	Inactive acromegaly (n=19)	P-values	Reference values (9;21)
LVEDD (mm)	Baseline	51.1 ± 1.3	51.1 ± 2.0	0.981	40-59
	Follow-up	51.3 ± 1.1	51.3 ± 1.3	0.982	
LVESD (mm)	Baseline	33.9 ± 1.4	33.4 ± 1.5	0.805	26-40
	Follow-up	31.9 ± 1.4	32.6 ± 1.2	0.707	
FS (%)	Baseline	32.8 ± 1.7	36.6 ± 1.3	0.088	26-45
	Follow-up	37.8 ± 2.1	36.9 ± 1.5	0.713	
LVEF (%)	Baseline	62.3 ± 2.5	71.9 ± 2.0	0.005	49-79
	Follow-up	63.3 ± 4.2	65.9 ± 2.0*	0.562	
IVST (mm)	Baseline	12.6 ± 0.9	9.7 ± 0.5	0.011	<13
	Follow-up	11.3 ± 0.8	9.7 ± 0.4	0.066	
PWT (mm)	Baseline	10.3 ± 0.5	9.6 ± 0.4	0.326	<13
	Follow-up	8.6 ± 0.9	9.1 ± 0.2	0.653	
LVMi (g/ $m^{2.7}$)	Baseline	53.8 ± 6.0	42.1 ± 3.9	0.106	49.2 men/ 46.7 women
	Follow-up	45.2 ± 5.2	40.3 ± 3.1	0.418	
LVH (n (%))	Baseline	11 (61)	6 (32)	0.072	
	Follow-up	8 (44)	6 (32)	0.420	

Values are expressed as mean ± SEM. Within the two groups, parameters are compared between baseline and follow-up with paired samples t-tests. The two groups are compared with independent samples t-tests or chi-square tests when appropriate. Reference values are obtained from Ilercil *et al.* (21) and Vitale *et al.* (9). LVEDD Left Ventricular End-Diastolic Diameter; LVESD Left Ventricular End-Systolic Diameter, FS Fractional shortening, LVEF Left Ventricular Ejection Fraction, IVST Inter-Ventricular Septum Thickness, PWT Posterior Wall Thickness, LVMi Left Ventricular Mass Index, LVH Left Ventricular Hypertrophy. *LVEF significantly decreased within inactive patients during follow-up (P=0.038).

Prevalence of valvular regurgitation in the total cohort

At baseline, valvular regurgitation at mitral and aortic sites combined was present in 17 of the 37 patients (46%) and during follow-up in 24 patients (67%, p=0.008 vs. baseline, Table 3). The prevalence of mitral regurgitation significantly increased in the total cohort from 32% at baseline to 60% at follow-up (p=0.002), but the prevalence of aortic regurgitation remained unchanged (27% at baseline and 31% at follow-up, NS).

Table 2/3: Prevalence of mitral and aortic regurgitation in patients with acromegaly at baseline and follow-up.

		None	Trace	Mild	Moderate	Severe
Aortic valve (n (%))	Baseline	27 (73)	4 (11)	5 (14)	0	1* (3)
	Follow-up	25 (69)	4 (11)	7 (19)	0	0
Mitral valve (n (%))	Baseline	25 (68)	8 (22)	4 (11)	0	0
	Follow-up	15 (41)	12 (32)	10 (27)	0	0

Data are presented as number (percentages) of patients. *Valvular replacement surgery after first cardiac assessment.

Influence of active vs. inactive acromegaly on valvular regurgitation

In patients with active acromegaly, the percentage of valvular regurgitation at mitral and aortic sites combined increased significantly (56% at baseline to 88% at follow-up, p=0.031), whereas there were no significant changes in patients with inactive disease (26% at baseline vs. 47% at follow-up, p=0.500). The percentage of active acromegalic patients with mitral regurgitation increased during follow-up from 39% at baseline to 78% (p=0.016), whereas there was no change in inactive acromegalic patients (26% at baseline vs. 42% at follow-up, p=0.250) and no

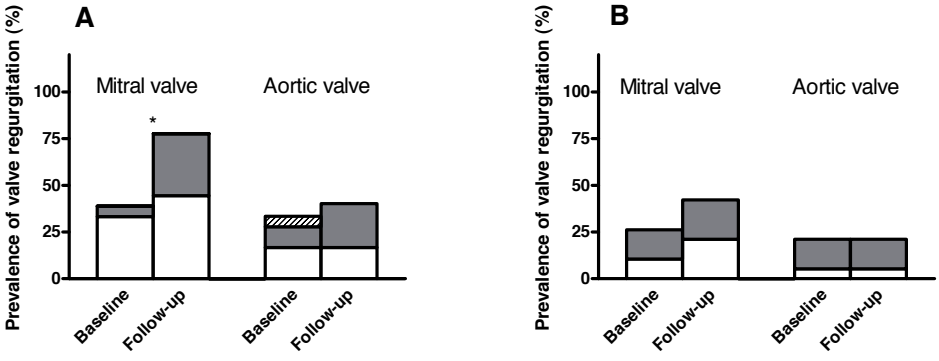

Figure 2/1: The prevalence of mitral or aortic regurgitation at baseline and at follow-up in patients with active and inactive acromegaly.

A: Active acromegaly (n=18); B: Inactive acromegaly (n=19); White bars denote trace regurgitation, gray bars denote mild regurgitation, hatched bars denote severe regurgititation (this patient had valve replacement surgery during follow-up).

*During follow-up the prevalence of trace and mitral regurgitation significantly increases in patients with active acromegaly (P=0.016). The prevalence of mitral regurgitation in patients with inactive disease was 26% at baseline vs. 42% at follow-up, P=0.250.

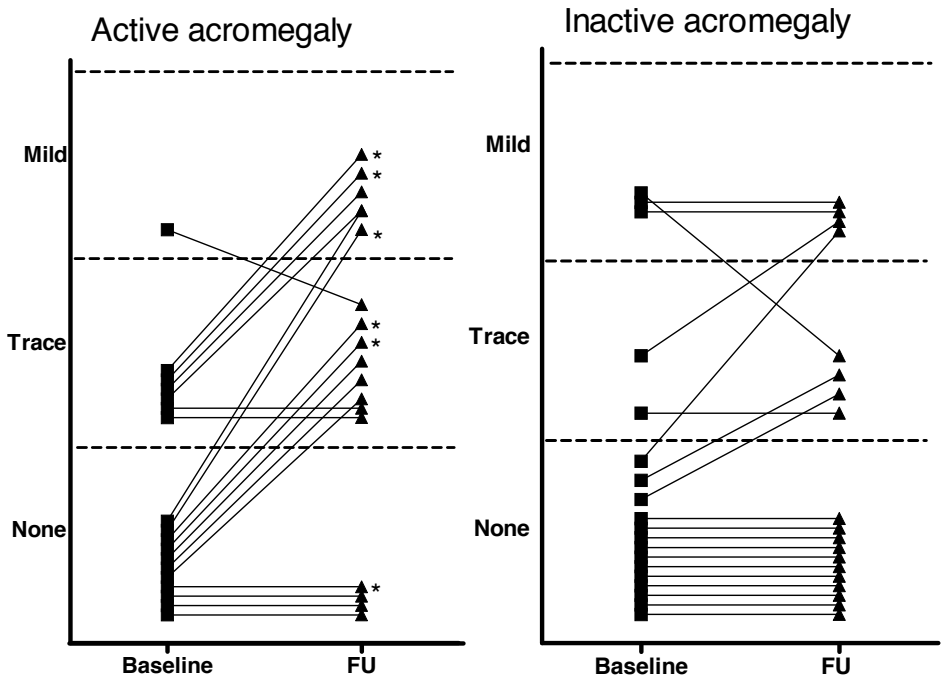

Figure 2/2: Individual course of mitral regurgitation among patients with active acromegaly (left panel) and patients with inactive acromegaly at study entry (right panel). *Patients who were classified as active acromegaly at baseline and were adequately controlled at follow-up (FU).

change in the prevalence of aortic valve regurgitation in both active and inactive patients (Figure 1 and 2). Therefore, the increase in valvular regurgitation in the total cohort of acromegalic patients was explained by an increase in trace and mild mitral valve regurgitation in patients with active acromegaly. In addition, we found no significant difference in the prevalence of mitral/ aortic/ or any valvular regurgitation (mitral and aortic sites combined) between patients with left ventricular hypertrophy and patients without left ventricular hypertrophy, neither in the total cohort, nor when active and inactive patients were analyzed separately.

DISCUSSION

This observational follow-up study demonstrates, that the prevalence of trace and mild mitral valvular regurgitation increased in patients with active acromegaly. These new data reinforce the concept, that acromegaly induces regurgitant valvular disease (5;6). Conversely, adequate control of GH excess is associated with stable valvular function, at least during the follow-up of our study.

The increase in prevalence in valvular regurgitation in active acromegaly in this study was explained by mitral valve involvement, whereas there was no change in aortic valvular regurgitation. This finding is in line with the observed prevalence of mitral and aortic regurgitation in the Framingham heart study, in which mitral regurgitation was more prevalent than aortic regurgitation (12). Prevalences of more than or equal to mild severity mitral regurgitation and more than or equal to trace severity aortic regurgitation were found in approximately 19% and 11%, respectively. In our age- and sex matched controls the prevalences of any aortic regurgitation and any mitral regurgitation were 7% and 32%, respectively (5). One of the major determinants of valvular regurgitation in the general population proved to be increasing age (12). Previously, we could not confirm a significant influence of age in this specific group of acromegalic patients (5). Why the increase in regurgitation in our patients was only observed for the mitral valve might be related to differences in the intrinsic vulnerability between the aortic and mitral valve to exogenous stimuli that promote valvular degenerative changes. For instance, it has been documented that mitral regurgitation, but not aortic regurgitation, is associated with systemic hypertension (12). In addition, it has been postulated that increased afterload may play an important role in the development of minor degrees of mitral regurgitation (12). Given the myxomatous degeneration found in the valves that were removed from several of our acromegalic patients during valvular replacement surgery (5), we postulate that persistent long-term exposure to GH excess predisposes to accelerated degenerative valvular changes. It is also of note that the myxomatous degeneration in acromegaly resembles that found in connective tissue diseases, conditions that are also associated with irreversible valvular disease (13).

Valve regurgitation was asymptomatic except for one patient and varied from only of trace to mild severity. Therefore, the clinical relevance of our findings might be questioned. In this regard recent findings of the impact of asymptomatic valvular regurgitation by Enriquez-Sarano et al. are relevant (14). They found that in the general population, the severity of mitral regurgitation was a powerful predictor of clinical outcome in terms of death from any cause and death from cardiac disease (14). Given the strong correlation between acromegaly and cardiovascular morbidity, at least in active acromegaly (2), asymptomatic valvular regurgitation may contribute to the increased cardiovascular risk profile of these patients. It should be noted that the duration of follow-up in our current study was relatively short and that a longer duration of follow-up could have resulted in more severe valvular changes, because the development of valvular involvement is correlated with the duration of active disease (5).

At the end of the study, several patients of the group with clinically active acromegaly finally had normal GH and IGF-I concentrations, but, nevertheless, in this group the progression of valvular disease took place. At present, it is not known how long disease activity must be controlled to prevent further deterioration of valvular disease. These observations indicate that the duration might be much longer from seen for improvement of myocardial involvement. Myocardial involment is a well recognised complication of acromegaly (2) and seems to be

related to the direct effects of GH and/or IGF-I on the myocardium (15). GH excess leads to the development of myocardial hypertrophy with interstitial fibrosis, resulting in diastolic dysfunction with impaired systolic function during exercise. Reversal of GH and IGF-I excess by surgical removal of the GH secreting pituitary tumour and/or medication attenuates or even reverses abnormal LV measurements and function in acromegalic cardiomyopathy (16;17). Octreotide treatment is known to improve LV function and decrease LV wall thickness in acromegaly (17). These beneficial changes become apparent within 6 months after treatment. The absence of improvement in left ventricular parameters in patients with inactive acromegaly is most likely explained by the fact, that these patients were adequately treated for many years prior to the first echocardiography. The LVEF in inactive patients decreased, which was clinically irrelevant, and within the reference values.

The lack of any effect of persistent inactive disease on valvular regurgitation could suggest that myocardial changes induced by previous acromegaly are partly irreversible. We could not identify any evident differences in clinical parameters between inactive acromegalic patients, who had progression of valvular regurgitation during follow-up, and the other inactive acromegalic patients. However, since the present study is an observational study rather than an intervention study, reversibility of myocardial abnormalities was not studied. The left ventricular indices are presented mainly to demonstrate that the observed changes in valvular heart disease are not caused by changes in left ventricular diameters or function, but probably due to the effects of GH and IGF-I on connective tissue (18-20).

In conclusion, we demonstrated that the prevalence of regurgitant valvular heart disease increases during follow up of patients with active acromegaly. Patients with acromegaly require adequate cardiac evaluation to establish the extent of valvular involvement in acromegalic disease and to install appropriate cardiac monitoring and care.

REFERENCE LIST

1. Clayton RN 2003 Cardiovascular function in acromegaly. Endocr Rev 24:272-277
2. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
3. Sacca L, Cittadini A, Fazio S 1994 Growth hormone and the heart. Endocr Rev 15:555-573
4. Colao A, Cuocolo A, Marzullo P, Nicolai E, Ferone D, Della Morte AM, Pivonello R, Salvatore M, Lombardi G 2001 Is the acromegalic cardiomyopathy reversible? Effect of 5-year normalization of growth hormone and insulin-like growth factor I levels on cardiac performance. J Clin Endocrinol Metab 86:1551-1557
5. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
6. Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, Vitale G, Bonaduce D, Lombardi G 2003 High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 88:3196-3201
7. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, . 1989 Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 2:358-367
8. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N 1986 Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 57:450-458
9. Vitale G, Galderisi M, Pivonello R, Spinelli L, Ciccarelli A, de Divitiis O, Lombardi G, Colao A 2004 Prevalence and determinants of left ventricular hypertrophy in acromegaly: impact of different methods of indexing left ventricular mass. Clin Endocrinol (Oxf) 60:343-349
10. Perry GJ, Helmcke F, Nanda NC, Byard C, Soto B 1987 Evaluation of aortic insufficiency by Doppler color flow mapping. J Am Coll Cardiol 9:952-959
11. Thomas JD 1997 How leaky is that mitral valve? Simplified Doppler methods to measure regurgitant orifice area. Circulation 95:548-550
12. Singh JP, Evans JC, Levy D, Larson MG, Freed LA, Fuller DL, Lehman B, Benjamin EJ 1999 Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). Am J Cardiol 83:897-902
13. Rippe J, Fishbein MC, Carabello B, Angoff G, Sloss L, Collins JJ, Jr., Alpert JS 1980 Primary myxomatous degeneration of cardiac valves. Clinical, pathological, haemodynamic, and echocardiographic profile. Br Heart J 44:621-629
14. Enriquez-Sarano M, Avierinos JF, Messika-Zeitoun D, Detaint D, Capps M, Nkomo V, Scott C, Schaff HV, Tajik AJ 2005 Quantitative determinants of the outcome of asymptomatic mitral regurgitation. N Engl J Med 352:875-883
15. Minniti G, Jaffrain-Rea ML, Moroni C, Baldelli R, Ferretti E, Cassone R, Gulino A, Tamburrano G 1998 Echocardiographic evidence for a direct effect of GH/IGF-I hypersecretion on cardiac mass and function in young acromegalics. Clin Endocrinol (Oxf) 49:101-106
16. Vianna CB, Vieira ML, Mady C, Liberman B, Durazzo AE, Knoepfelmacher M, Salgado LR, Ramires JA 2002 Treatment of acromegaly improves myocardial abnormalities. Am Heart J 143:873-876
17. Colao A, Marzullo P, Ferone D, Spinelli L, Cuocolo A, Bonaduce D, Salvatore M, Boerlin V, Lancranjan I, Lombardi G 2000 Cardiovascular effects of depot long-acting somatostatin analog Sandostatin LAR in acromegaly. J Clin Endocrinol Metab 85:3132-3140
18. Thompson BJ, Shang CA, Waters MJ 2000 Identification of genes induced by growth hormone in rat liver using cDNA arrays. Endocrinology 141:4321-4324

19. Rabkin E, Aikawa M, Stone JR, Fukumoto Y, Libby P, Schoen FJ 2001 Activated interstitial myofibroblasts express catabolic enzymes and mediate matrix remodeling in myxomatous heart valves. Circulation 104:2525-2532
20. Feelders RA, Savelkoul HF, de Herder WW, Hooijkaas H, Lamberts SW, The cytokine network in acromegaly before and after pituitary surgery: growth hormone excess is associated with a reversible shift to the T-helper 1 cytokine response. p 76 (Abstract OR8-5) (Abstract)
21. Ilercil A, O'Grady MJ, Roman MJ, Paranicas M, Lee ET, Welty TK, Fabsitz RR, Howard BV, Devereux RB 2001 Reference values for echocardiographic measurements in urban and rural populations of differing ethnicity: the Strong Heart Study. J Am Soc Echocardiogr 14:601-611

Chapter 3

Increased Aortic Root Diameters in Patients with Acromegaly

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European Journal of Endocrinology, 2008

ABSTRACT

Objective

The clinical manifestations of acromegalic cardiomyopathy include arrhythmias, valvular regurgitation, concentric left ventricular (LV) hypertrophy, and LV systolic and diastolic dysfunction. At present, it is unknown whether acromegaly also affects the aortic root.

Design

Aortic root diameters were prospectively assessed in 37 acromegalic patients (18 patients with active disease, and 19 with controlled disease) by conventional two-dimensional and Doppler echocardiography before, and after, an observation period of 1.9 years (range 1.5-3.0 yr). Baseline parameters were compared to healthy controls.

Results

The diameters of the aortic root at the sino-tubular junction and the ascending aorta were increased in patients with acromegaly: 30 ± 4 vs. 26 ± 3 mm (p=0.0001) and 33 ± 5 vs. 30 ± 4 mm (p=0.006), respectively. The diameter of the aortic root at the aortic annulus and aortic sinus were not different from controls. During follow-up, the aortic root diameters increased at the levels of the annulus and the sinotubular junction (p=0.025 and p=0.024, resp.), whereas there was no change in the diameters at the levels of the sinus and the ascending aorta during follow-up. Baseline aortic root diameters were not influenced by disease duration, current disease activity or blood pressure. When patients with active and patients with inactive disease were analyzed separately, only the diameter of the sinotubular junction increased in patients with inactive acromegaly during follow-up (p=0.031).

Conclusion

Aortic root diameters are increased in patients with acromegaly compared to healthy controls.

INTRODUCTION

Acromegaly is associated with increased cardiovascular morbidity and mortality (1). Active disease leads to a specific acromegalic cardiomyopathy, which involves the myocardium, the conduction system, and the valves (1). Clinical manifestations include arrhythmias, valvular regurgitation, concentric, left ventricular hypertrophy, and left ventricular systolic and diastolic dysfunction. Ten percent of the patients present with overt heart failure. Treatment of GH excess can reduce mortality to that of the normal population (2) and reverse heart failure and myocardial hypertrophy, but valvular regurgitation persists, or even aggravates, when disease activity is insufficiently controlled (3;4). The pathophysiology of these cardiac complications of acromegaly is incompletely understood. On pathological examinations, the myocardium is affected by interstitial fibrosis and the leaflets have the appearance of myxoid degeneration (5). It has been hypothesized, that abnormal extracellular matrix regulation by overproduction of growth hormone (GH) and/or IGF-I in patients with acromegaly may contribute to both systolic and diastolic left ventricular dysfunction. Abnormalities in matrix regulation are associated with cardiac chamber dilation and reduced myocardial tensile strength (6). Abnormal extracellular matrix regulation is also present in Marfan's syndrome and in this syndrome myxoid degeneration extends to the aortic root, which progressively dilates over time, with the need for surgical procedures to avoid dissection or severe aortic regurgitation (7).

It is currently unknown whether the aortic root is also involved in acromegaly, like in Marfan's syndrome. This is of clinical relevance for the appreciation of the extensiveness of the cardiac involvement in patients with acromegaly. Therefore, the aim of the present study was to compare the diameters of the aortic root between patients with acromegaly and healthy controls. In addition, we prospectively evaluated the effects of disease control on aortic root diameters during follow-up of 1.5-3 years.

PATIENTS AND METHODS

Patients

Thirty-seven patients were enrolled in the present study, of whom 35 had participated in a previous cross sectional study on cardiac function and valvular regurgitation in acromegaly, which did not involve assessment of cardiac root parameters (5). The initial diagnosis of acromegaly was based on the characteristic clinical signs and symptoms and confirmed by insufficient suppression of GH during a glucose tolerance test (normal response: GH nadir <0.5 μg/L), elevated age- and gender-adjusted IGF-I levels, and the presence of a pituitary adenoma on radiological imaging. The study was performed prior to the introduction of GH receptor blockade drugs in the Netherlands.

Patients were classified at study entry as having active or inactive acromegaly.

Active acromegaly (n=18) was defined as mean fasting GH concentrations (measured every 30 minutes for 3 hours) >2.5 µg/L, and elevated age- and gender-adjusted IGF-I concentrations. Nineteen patients were classified as having inactive acromegaly which was defined in medically well-controlled patients (n=13) as mean fasting GH concentration (measured for 3 hours with an interval of 30 minutes) <2.5 µg/L, and normal age- and gender-adjusted IGF-I concentrations during treatment with somatostatin analogs (n=13) and in cured acromegaly after surgery and radiotherapy (n=6, n=5 surgery alone, n=1 surgery and radiotherapy) as glucose-suppressed GH <0.5 µg/L, and normal age- and gender-adjusted IGF-I concentration without medical treatment. Of the 13 patients treated with somatostatin analogs, 6 were treated primarily by surgery and 2 with surgery and radiotherapy, whereas in 5 patients somatostatin analogs formed the primary treatment.

One patient presented with severe aortic regurgitation. This patient was excluded from the comparison with healthy controls of the diameter of the aortic root at the level of the aortic annulus and the follow-up data within patients with acromegaly because the patient underwent valve replacement surgery.

Two patients (1 male patient, age 73 yr, active acromegaly throughout follow-up and another male patient, 56 yr, active acromegaly troughout follow-up) were treated for diabetes mellitus. Both did not have aortic regurgitation. Two patients used lipid lowering drugs (1 male patient, age 73 yr, active acromegaly throughout follow-up, without aortic regurgitation and 1 female patient, age 63 yr, cured acromegaly throughout follow-up, no aortic regurgitation). Two patients were using dopamine agonists at the time of the study. Both did not have aortic regurgitation. None of the other patients used dopamine agonists.

None of the patients had hemodynamic instability, previous myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, or connective tissue disease. The medical ethics committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Controls

The patients were compared to 37 healthy age-, body surface area and sex-matched controls. The controls were selected from a database with patients referred to the department of Cardiology, based on age, sex, body surface area, mitral and aortic valvular regurgitation, and left ventricular systolic function. Controls were excluded when referred for echocardiographic evaluation of known valvular heart disease, murmur, congestive heart failure, and cardiac transplantation. Other exclusion criteria were myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, connective tissue disease, carcinoid syndrome, or use of anorectic drugs. We and others have previously demonstrated that recruitment of controls from a large database can also be used as representative controls (5;8).

Echocardiography, Data Acquisition

Aortic root diameters in patients with acromegaly were prospectively evaluated twice: at baseline and after follow-up duration of at least 1.5 years. Baseline parameters were compared to similar parameters in healthy controls. The evaluation of the echocardiographic images was performed blinded for the status of the subjects.

Echocardiography was performed while the patients were in the left lateral decubitus position using a commercially available system (Vingmed Vivid-7, General Electric – Vingmed, Milwaukee, WI, USA). Standard parasternal (long- and short-axis) and apical views (2-, 4-, and 5-chamber) were obtained. M-mode images were obtained from the parasternal long-axis views for quantitative assessment of left ventricular dimensions.

Standard continuous-wave and pulsed-wave Doppler examinations were performed.

The severity of valvular regurgitation was assessed by two independent expert readers blinded to the clinical data on a qualitative scale of trace, mild, moderate, or severe, using previously described methods (9;10). Significant valvular disease was determined using the U.S. Food and Drug Administration (FDA) case definition: mild or greater aortic regurgitation or mitral regurgitation equal to or more than moderate severity (11).

The aortic root was measured in the parasternal long axis view at end-diastole, perpendicular to the long axis of the aorta, according to the leading-edge technique at four levels: 1) annulus aortae, 2) sinuses of Valsalva, 3) sinotubular junction, and 4) proximal ascending aorta.

BSA was calculated by the formula proposed by Dubois et al. (12): 0.007184 x weight $(kg)^{0.425}$ x length $(cm)^{0.725}$. Aortic root diameters were indexed for body surface area (BSA) as proposed by Roman et al. (13).

Hormone Assays

GH concentrations were measured using a sensitive time-resolved immunofluorescent assay (Wallac Oy, Turku, Finland), specific for 22 kDa GH protein. Human biosynthetic GH (Pharmacia and Upjohn, Inc, Uppsala, Sweden) was used as standard, calibrated against WHO-IRP 80-505. The detection limit was 0.012 µg/L. Intra-assay coefficients of variation were 8.4-1.6% and the inter-assay coefficients of variation were interassay 9.0-2.0% in the GH-range 0.1-18 µg/L. Total serum IGF-I concentration was determined by radioimmunoassay (RIA) after extraction and purification on ODS-silica columns (Incstar corp., Stillwater, MN, USA). The intra- and inter-assay coefficients of variation were less than 11%. The detection limit was 1.5 nmol/l. Age- and gender-adjusted IGF-I data were determined in the same laboratory. IGF-I was expressed as a standard deviation (SD) score from age- and gender-related normal levels.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are expressed as the mean ± SD, unless specified otherwise. Independent samples t-tests were used to assess the difference between patients and controls. Non-

parametric Mann-Whitney U-tests and independent samples t-tests after log-transformation of the variables were used to check for consistency of the obtained results of the analysis. Paired samples t-tests were used to assess the difference in aortic root measurements at baseline and at follow-up. Independent samples t-tests or Chi-square tests were used to compare baseline values, follow-up values, and the difference in baseline and follow-up values between patients with active and inactive acromegaly. Linear regression analysis was used to explore relations between aortic root diameters as dependent variables and variables of disease activity (IGF-I/ GH concentration, estimated disease duration) and blood pressure as independent variables. A P-value <0.05 was considered to represent a significant difference.

RESULTS

Clinical characteristics

Patients and controls were matched for age, gender, body surface area, valvular regurgitation and left ventricular dimensions and function (Table 1). The interval between the two echocardiographic studies in patients with acromegaly was 1.9 years, range 1.5 to 3.0 years. During follow-up, mean IGF-SD scores, GH concentrations, and systolic blood pressure decreased.

At baseline as well as at follow-up, mean IGF-I SD scores were significantly higher in patients with active acromegaly compared to patients with inactive acromegaly (8.3 ± 1.9 SD score vs.

Table 3/1: Clinical characteristics of patients with acromegaly compared to healthy age-, sex- and body suface area-matched controls.

	Acromegaly (n=37)	Controls (n=37)	P-value
Age (yrs)	54 ± 14	52 ± 9	NS
Gender (male/ female (n))	15/22	15/22	NS
Body surface area (m^2)	2.0 ± 0.2	1.9 ± 0.1	NS
IGF-I at baseline (SD scores)	4.4 ± 5.3	NA	
IGF-I at follow-up (SD scores)	1.9 ± 2.0*	NA	
GH at baseline (µg/l)	10.7 ± 16.9	NA	
GH at follow-up (µg/l)	3.8 ± 4.6*	NA	
Left ventricular end-diastolic diameter (mm)	51 ± 7	51 ± 6	NS
Left ventricular end-systolic diameter (mm)	34 ± 6	31 ± 6	0.07
Left ventricular ejection fraction (%)	67 ± 11	69 ± 11	NS
Mitral valve regurgitation (n(%) no/ trace/ mild/ moderate/ severe regurgitation)	25(68)/ 8(22)/ 4(10)/ 0/ 0	27(73)/ 8(22)/ 2(5)/ 0/ 0	NS
Aortic valve regurgitation (n (%) no/ trace/ mild/ moderate/ severe regurgitation)	27(73)/ 4(11)/ 5(14)/ 0/ 1(2)	33 (90)/ 2(5) /2(5)/ 0/ 0	NS

Values are expressed as mean ± SD. The two groups are compared with independent samples t-tests or chi-square tests when appropriate. *P<0.05 compared to baseline value of same parameter.

0.8 ± 0.4 SD score, p<0.001 at baseline, 2.9 ± 0.5 vs. 1.0 ± 0.3, p=0.006 at follow-up, respectively). During follow-up, all patients with active acromegaly were treated with somatostatin analogs. At the time of the first echocardiogram, no treatment had been started in 9 of these patients. At the end of follow-up, disease activity in five of these patients was adequately controlled (n=1 after radiotherapy and somatostatin analogs, n=1 after surgery and somatostatin analogs and n=3 after somatostatin analogs alone). Disease activity was still classified as active in the other four patients despite surgery, radiotherapy and somatostatin analogs (n=1), or radiotherapy and somatostatin analogs (n=1), or somatostatin analogs alone (n=2). The other patients in the group of patients with active disease (n=9) had been treated previously with surgery and somatostatin analogs (n=4), somatostatin analogs (n=4 (one of these patients was classified as having adequate disease control at the end of follow-up)), and a combination of surgery, radiotherapy and somatostatin analogs (n=1). GH and IGF-I concentrations significantly decreased at the end of follow-up (GH: 19.0 ± 4.9 µg/L to 5.8 ± 1.4 µg/L, p=0.017 and IGF-1: 8.3 ± 1.9 SD score to 2.9 ± 0.5 SD score, p<0.001, resp). Disease activity in 6 of the 18 patients with active acromegaly at baseline was thus adequately controlled at the end of follow-up. The mean duration of adequate control of acromegaly in patients with inactive disease at study entry was 7.6 years (range 0.8 to 22.7 yrs). Mean estimated disease duration prior to baseline evaluation was 11 years (range 1 to 46 yrs).

Mean systolic blood decreased during follow-up from 144 ± 23 mm Hg at baseline to 134 ± 14 mm Hg at follow-up (p=0.002), whereas diastolic blood pressured remained unchanged (84 ± 8 mm Hg at baseline vs. 84 ± 9 mm Hg at follow-up, p=0.0419).

Comparison with controls

Compared to age, sex and BSA matched controls with similar left ventricular function and valvular regurgitation, the diameters of the aortic root at the sino-tubular junction and the ascending aorta were increased patients with acromegaly (Figure 1). However, the diameter of the aortic root at the aortic sinus and the aortic annulus were not different between patients and controls (Table 2) .

Table 3/2: Aortic root measurements in patients with acromegaly (n=37) compared to healthy controls (n=37).

	Acromegaly (n=37)	Healthy controls (n=37)	P-value
Aortic Annulus (mm)*	22 ± 2	21 ± 2	NS
Sinuses of Valsalva (mm)	32 ± 4	33 ± 4	NS
Sinotubular junction (mm)	30 ± 4	26 ± 3	0.0001
Proximal ascending aorta (mm)	33 ± 5	30 ± 4	0.006

Values are expressed as mean ± SD. Parameters between patients and controls are compared with independent samples T-tests.
*One patient presented with severe aortic regurgitation. This patient was excluded from the comparison with healthy controls of the diameter of the aortic root at the level of the aortic annulus.

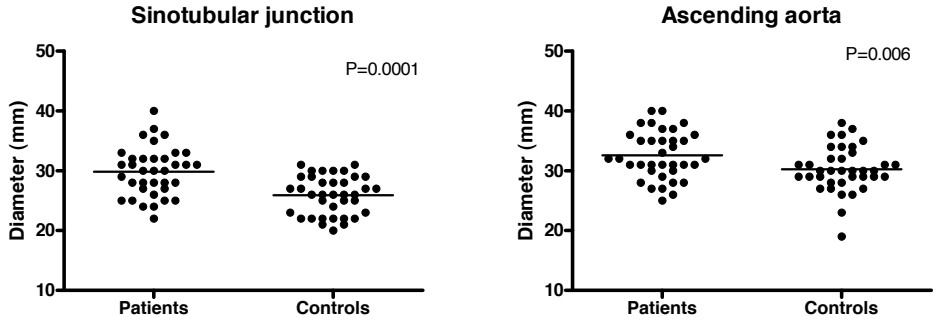

Figure 3/1: The diameter of the aortic root at the level of the sinotubular junction and the ascending aorta in patients with acromegaly compared to controls matched for age, gender, body surface area and aortic regurgitation.

The diameter of the aortic root at the sino-tubular junction and the ascending aorta were increased in patients with acromegaly compared to controls also after exclusion of patients and controls with significant aortic regurgitation (more than trace, Table 3). The diameter of the aortic root at the aortic annulus and the aortic sinus was not different between these two latter groups. Secondly, we excluded all patients and controls with at least trace aortic regurgitation. This analysis again showed an increased aortic root diameter at the level of the sino-tubular junction and the ascending aorta in patients compared to controls (30 ± 4 mm vs. 26 ± 3 mm, p<0.001 and 33 ± 4 vs. 30 ± 4, p=0.027, respectively).

Follow-up of all patients with acromegaly

During follow-up of all patients with acromegaly, the diameter of the aortic root at the annulus and at the sinotubular junction increased. However, the diameters at the level of the sinus and the ascending aorta did not differ between baseline and follow-up (Table 4).

Factors influencing aortic root diameters in patients with acromegaly

Disease activity at baseline

Baseline and follow-up diameters of the aortic root were not different between patients with active and inactive acromegaly. When corrected for BSA or annular size, the ratios of sinuses of

Table 3/3: Aortic root measurements in patients with acromegaly (n=31) compared to healthy controls (n=35) after exclusion of the subjects with significant aortic regurgitation (more than trace).

	Acromegaly (n=31)	Healthy controls (n=35)	P-value
Aortic Annulus (mm)	22 ± 2	21 ± 2	NS
Sinuses of Valsalva (mm)	32 ± 4	33 ± 4	NS
Sinotubular junction (mm)	30 ± 4	26 ± 3	<0.001
Proximal ascending aorta (mm)	32 ± 4	30 ± 4	0.025

Values are expressed as mean ± SD. Parameters between patients and controls are compared with independent samples T-tests.

Table 3/4: Aortic root measurements in patients with acromegaly (n=37) at baseline and during follow-up of 1.9 years (range 1.5-3.0 years).

	Baseline	Follow-up	P-value
Aortic annulus (mm)	22 ± 2	23 ± 3	0.025
Sinuses of Valsalva (mm)	32 ± 4	33 ± 3	NS
Sinotubular junction (mm)	30 ± 4	32 ± 4	0.024
Proximal ascending aorta (mm)	33 ± 4	33 ± 4	NS

Values are expressed as mean ± SD. Baseline and follow-up parameters are compared with paired samples T-tests.

Valsalva and sinutubular junction were not different between patients with active and inactive disease. When patients with active and patients with inactive disease were analyzed separately, only the diameter of the sinotubular junction increased in patients with inactive acromegaly (p=0.031, Table 5).

Estimated disease duration at baseline

No correlations could be detected between estimated disease duration at baseline and any of the aortic root diameters (baseline or follow-up) or the difference between baseline and follow-up.

Blood pressure

No correlations were found between systolic and diastolic blood pressure and aortic root diameters at baseline or follow-up. The aortic root diameters were similar in patients with or without hypertension (systolic blood pressure ≥140 mm Hg and/ or diastolic blood pressure ≥90 mm Hg, n=14 and n=23, respectively). Aortic annulus: 22 ± 2 mm vs. 22 ± 2 mm in patients with and

Table 3/5: Aortic root diameters and disease activity at baseline and during follow up in patients with active *versus* inactive acromegaly.

		Active acromegaly (n=18)	Inactive acromegaly (n=19)	P-value
Annulus aortae (mm)	Baseline	22 ± 2	22 ± 3	0.700
	Follow-up	23 ± 2	23 ± 3	0.440
Sinuses of Valsalva (mm)	Baseline	32 ± 4	32 ± 5	0.963
	Follow-up	33 ± 3	33 ± 4	0.994
Sinotubular junction (mm)	Baseline	31 ± 5	29 ± 4	0.353
	Follow-up	32 ± 3	32 ± 4*	0.702
Proximal ascending aorta (mm)	Baseline	33 ± 5	33 ± 4	0.993
	Follow-up	33 ± 3	33 ± 5	0.581

Values are expressed as mean ± SD. Within the two groups, parameters are compared between baseline and follow-up with paired samples t-tests. The two groups are compared with independent samples t-tests. *P=0.031 compared to baseline in patients with inactive acromegaly.

without hypertension, p=NS; Sinus: 33 ± 5 mm vs. 32 ± 4 mm, p=NS; Sino-tubular junction: 30 ± 4 mm vs. 30 ± 4 mm, p=NS; Ascending aorta: 32 ± 4 mm vs. 34 ± 4 mm, p=NS).

Gender, weight, height and BMI

Diameters of the aortic root at all levels were higher in men than in women (data not shown) as were height and BSA. Weight and height were not correlated with diameters of the aortic root. BMI was positively correlated with the diameter at the aortic sinus (R=0.377, p=0.022).

Left ventricular function and dimensions

There were no correlations between aortic diameters and left ventricular ejection fraction/ fractional shortening/ end-diastolic diameter or end-systolic diameter. Diameters at the level of the aortic annulus and the ascending aorta were higher in patients with left ventricular hypertrophy (n=9, 24%) compared to patients without (22 ± 2 mm vs. 24 ± 3 mm, p=0.043, resp. and 32 ± 4 mm vs. 35 ± 4 mm, p=0.051, resp.). No differences were found in the diameters at the level of the sinus and sino-tubular junction.

DISCUSSION

This case-control and follow-up study evaluated in detail the diameters of the aortic root in patients with acromegaly. In general, the aortic root diameters were increased in patients with acromegaly compared to healthy controls. Disease activity or disease duration did not influence absolute aortic root diameters at baseline. During follow-up, the aortic root diameter at the aortic annulus and at the sinotubular junction increased in patients with acromegaly, irrespective of disease activity. These data indicate that the long-term exposure to GH excess in patients with acromegaly affects the aortic root in addition to previously documented effects on the aortic valve leaflets (5;14;15).

The effects of acromegaly on the diameter of the aortic root have hardly been evaluated. To our knowledge, only one study assessed aortic root diameters in a limited number of patients and aortic root enlargement was found in 1 of 25 patients with active and inactive acromegaly (16). In the present study, we found increased diameters of the aortic root at several levels in patients with acromegaly compared to healthy controls. In the Framingham Heart study, determinants of aortic root size were age, height, weight and sex (17). Therefore, we matched our patients with healthy controls for these factors. To avoid other confounders in this specific comparison, patients and controls were also matched for left ventricular function and valvular regurgitation. The additional influence of increases in blood pressures is limited (17). Accordingly, blood pressure did not influence aortic root diameters or dilatation during follow-up in our study. Moreover, during follow-up, systolic blood pressure decreased due to more stringent disease control in the majority of patients with active disease illustrated by the decrease in GH

and IGF-I standard deviation score. GH has direct effects on potassium reabsorption, hence increasing the extracellular volume. Nonetheless the diameter of the aortic root increased at the level of the aortic annulus and the sinotubular junction during follow-up. We feel that this increase in aortic root diameters despite lowering of blood pressure, suggests that blood pressure is not a major determinant of aortic root diameters in this particular patient group. It is, therefore, unlikely, that uncontrolled hypertension influenced our.

There was no correlation between dilatation of the aortic root and current disease activity or estimated disease duration. Although a similar lack of association was found between valvular regurgitation and current disease activity, valvular regurgitation was strongly associated with disease duration (5), pointing towards direct effects of long-term exposure to increased GH and/or IGF-1 concentrations on cardiac valves. These observations indicate, that the detrimental effects apparently only become manifest after long-term exposure, and that, at best, the reversibility is only partial. However, to evaluate the effects of current disease state on progressive abnormalities of the aortic root a study with a longer follow-up duration and with more patients is needed.

We speculated that the increased diameters of the aortic root are probably due to the same mechanisms that induce the myxomatous degeneration found in the valves that were removed from several of our acromegalic patients during valvular replacement surgery (5). GH is involved in matrix regulation. For example GH increases gene expression of the matrix metalloproteinases (MMPs), that are capable of altering the composition of the extracellular matrix (18). This altered matrix regulation could be responsible for the changes found in the heart valves, as well in the aortic root in patients with acromegaly. The coincidence between valvular regurgitation and aortic root dilatation is also present in Marfan's syndrome, which is also characterized by myxomatous degeneration of cardiac valves and aortic root (7).

We found an increase in the diameter of the aortic root at the sino-tubular junction in patients with inactive acromegaly is somewhat unexpected. The diameter of the aortic root also increased in patients with active acromegaly, although this did not reach statistical significance, and when all patients are analysed together. These data might suggest that possibly the long-standing GH excess has altered the aortic root in such a way that it is more vulnerable to distension even when GH excess is fully reversed.

None of our patients were diagnosed with true thoracic aortic aneurysms. Therefore, the results of the present study do not imply that aortic root diameters should be screened in all patients with acromegaly to detect aneurysms. However, extending the echocardiographic measurements to the aortic root offers a more complete picture of the spectrum of acromegalic cardiomyopathy.

In conclusion, aortic root diameters were increased in patients with acromegaly compared to healthy controls. These abnormalities were not associated with disease duration, current disease activity, or blood pressure. In addition, during follow-up aortic root diameters at the level of the aortic annulus and the sinotubular junction increased. These findings indicate that

in patients with acromegaly an extension of the cardiac evaluation to the aortic root offers a more indebt assessment of the state of the individual acromegalic cardiomyopathy.

REFERENCE LIST

1. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
2. Biermasz NR, Dekker FW, Pereira AM, van Thiel SW, Schutte PJ, van Dulken H, Romijn JA, Roelfsema F 2004 Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. J Clin Endocrinol Metab 89:2789-2796
3. Colao A, Marzullo P, Ferone D, Spinelli L, Cuocolo A, Bonaduce D, Salvatore M, Boerlin V, Lancranjan I, Lombardi G 2000 Cardiovascular effects of depot long-acting somatostatin analog Sandostatin LAR in acromegaly. J Clin Endocrinol Metab 85:3132-3140
4. Colao A, Cuocolo A, Marzullo P, Nicolai E, Ferone D, Della Morte AM, Pivonello R, Salvatore M, Lombardi G 2001 Is the acromegalic cardiomyopathy reversible? Effect of 5-year normalization of growth hormone and insulin-like growth factor I levels on cardiac performance. J Clin Endocrinol Metab 86:1551-1557
5. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
6. Miner EC, Miller WL 2006 A look between the cardiomyocytes: the extracellular matrix in heart failure. Mayo Clin Proc 81:71-76
7. van Karnebeek CD, Naeff MS, Mulder BJ, Hennekam RC, Offringa M 2001 Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child 84:129-137
8. Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G 2007 Valvular heart disease and the use of dopamine agonists for Parkinson's disease. N Engl J Med 356:39-46
9. Perry GJ, Helmcke F, Nanda NC, Byard C, Soto B 1987 Evaluation of aortic insufficiency by Doppler color flow mapping. J Am Coll Cardiol 9:952-959
10. Thomas JD 1997 How leaky is that mitral valve? Simplified Doppler methods to measure regurgitant orifice area. Circulation 95:548-550
11. Weissman NJ, Tighe JF, Jr., Gottdiener JS, Gwynne JT 1998 An assessment of heart-valve abnormalities in obese patients taking dexfenfluramine, sustained-release dexfenfluramine, or placebo. Sustained-Release Dexfenfluramine Study Group. N Engl J Med 339:725-732
12. Dubois D, Dubois E.F. 1916 A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 863-871
13. Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J 1989 Two-dimensional echocardiographic aortic root dimensions in normal children and adults. Am J Cardiol 64:507-512
14. Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, Vitale G, Bonaduce D, Lombardi G 2003 High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 88:3196-3201
15. van der Klaauw AA, Bax JJ, Roelfsema F, Bleeker GB, Holman ER, Corssmit EP, van der Wall EE, Smit JW, Romijn JA, Pereira AM 2006 Uncontrolled acromegaly is associated with progressive mitral valvular regurgitation. Growth Horm IGF Res 16:101-107
16. Savage DD, Henry WL, Eastman RC, Borer JS, Gorden P 1979 Echocardiographic assessment of cardiac anatomy and function in acromegalic patients. Am J Med 67:823-829
17. Vasan RS, Larson MG, Levy D 1995 Determinants of echocardiographic aortic root size. The Framingham Heart Study. Circulation 91:734-740
18. Thompson BJ, Shang CA, Waters MJ 2000 Identification of genes induced by growth hormone in rat liver using cDNA arrays. Endocrinology 141:4321-4324

Chapter 4

Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly

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Clinical Endocrinology, 2008

ABSTRACT

Objective

Cross-sectional studies have shown impaired quality of life in patients in biochemical control of acromegaly. The aim of this study was to assess longitudinal changes in quality of life in a homogenous cohort of patients with sustained biochemical control of acromegaly.

Design

Prospective follow-up study.

Patients and Methods

Quality of life was assessed using four health related quality of life questionnaires (HADS, MFI-20, NHP, SF-36) and one disease-specific quality of life questionnaire (ACRO-QOL) in 82 patients (43 men) with strict biochemical control of acromegaly, aged 56 years (range 29-84 years) at baseline and after 4 years of follow-up. The mean duration of controlled disease was 12 years (range 1-26 years).

Results

During follow-up, scores in 5 of 26 QoL subscales significantly worsened: physical and social functioning (SF-36), physical fatigue (MFI-20), and psychological well-being and personal relations (ACRO-QOL). Using linear regression analysis, baseline item scores predicted the follow-up scores, indicating individual stability over time. Previous radiotherapy (n=27, 33%) negatively influenced several QoL subscales at follow-up: energy, pain, and social isolation (NHP), physical fatigue and reduction in activity and motivation (MFI-20), depression and total anxiety and depression scores (HADS), and physical performance (ACRO-QOL).

Conclusion

During 4 years of follow-up in patients with long-term biochemical control of acromegaly quality of life is subtly, but progressively impaired. Radiotherapy was the predominant indicator of progressive impairment in QoL.

INTRODUCTION

Treatment aims in acromegaly are to relief the symptoms of growth hormone (GH) excess and to decrease increased morbidity and mortality (1;2). Combinations of surgery, radiotherapy, and drug therapy are currently able to control disease activity in most patients (3-6).

Recently, several cross-sectional studies in patients with acromegaly have pointed towards a reduction in quality of life (QoL) (7-11). Some studies revealed a positive influence of adequate control of disease activity on QoL (7-10), whereas in another study no such relationship was found (11). Previous radiotherapy (8;12) and somatostatin treatment (7) were associated with impaired QoL. However, almost all studies were designed as cross-sectional studies with heterogeneous cohorts, including patients with cured and active acromegaly. Recently, a longitudinal study documented an overall unchanged QoL in a cohort consisting of patients with cured and active (66%) acromegaly, using a disease specific questionnaire (13).

Previously, we reported that QoL is impaired in a large cohort of patients with long-term control of acromegaly, which might be due to irreversible effects of previous GH excess and/or treatment (12). The aim of this study was to evaluate whether QoL parameters change and to identify predictors that influence changes in QoL during 4 years of follow-up in this homogeneous cohort of acromegalic patients with sustained biochemical control of acromegaly.

PATIENTS AND METHODS

Protocol

Ninety-six of the 118 patients who participated in the previous study (12) were eligible for participation for the present study. Reasons for non-eligibility were death (n=10), follow-up in other hospitals because of home-moving (n=9), or serious illness (n=3). Identical questionnaires were sent to their homes in prepaid envelopes. To avoid confounding effects of the seasonal effects, both baseline and follow-up questionnaires were sent in February/ March.

Biochemical control (either cure after surgery and/ or radiotherapy or control during somatostatin analogs treatment) was defined by normal serum IGF-I levels for sex and age and serum GH levels below 1.9 µg/l (≈5 mU/l). In addition, in the patients without treatment with somatostatin analogs, control was also confirmed by a normal GH suppression (<0.38 µg/l) during an oral glucose tolerance test performed every year. In patients on somatostatin analogs, mean GH levels were obtained from 5 samples obtained with intervals of 30 minutes between 9.00 and 11.00 h am.

Hypopituitarism was treated with thyroxine, hydrocortisone, testosterone or estrogens (in premenopausal women) according to the following definitions. Premenopausal women were defined as LH/ FSH deficient when secondary amenorrhoea was present for more than 1 year. Postmenopausal women were defined as LH/FSH deficient when gonadotrophin levels

were below the normal postmenopausal range (LH<10 U/l and FSH<30 U/l). In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone test or insulin tolerance test. The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects returning completed questionnaires gave written informed consent for participation in the study.

Questionnaires

HADS (Hospital Anxiety and Depression Scale)

The 14 items of the HADS pertain to anxiety and depression. Each item is measured on a 4-point scale. Scores for the anxiety and depression subscale range from 0-21 and for the total score from 0-42. A high score points to more severe anxiety and depression (14).

MFI-20 (Multidimensional Fatigue Index)

The MFI-20 contains 20 statements to assess fatigue (15). Every statement is measured on a 5-point scale; scores vary from 0 to 20. Higher scores indicate higher experienced fatigue.

NHP (Nottingham Health Profile)

The NHP consists of 38 yes/no questions, which are subdivided in 6 scales assessing impairments (16;17). Subscale scores are calculated as a weighted mean of the associated items and are expressed as a value between 0 and 100. The total score is the mean of the 6 subscales. A high score is related to a worse QoL.

SF-36 (Short Form-36)

The 36 items of the SF-36 record general well being during the previous 30 days (18;19). Scores are expressed on a 0-100 scale. Higher scores are associated with better QoL.

ACRO-QOL (Acromegaly-Quality of Life)

The ACRO-QOL was developed by Webb et al. (20) and is a disease-specific questionnaire. Responses are given as frequency of occurrence or degree of agreement on a five-point scale. Parameters are expressed as percentage, from 0 (very bad) to 100 (very good).

Assays and normal values

GH was measured by a sensitive immunofluorometric assay (Wallac, Turku, Finland). The inter-assay coefficient of variation was 2.0-9.0% en intra-assay coefficient of variation was 1.6-8.4% between 0.1 and 18 µg/l. For conversion of µg/l to mU/l, multiply by 2.6.

At baseline, serum IGF-I (nmol/l) concentrations were measured by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns. The inter-assay coefficient of variation was below 11%. IGF-I is expressed as SD scores (SD score) for age- and gender-related normal levels determined in the same laboratory(21;22). At follow-up, the serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I levels were expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals (23;24).

Statistics

Data are presented as mean ± SD unless specified otherwise. SPSS for windows version 14.0 (SPSS Inc., Chicago, IL) was used for data analysis. We used paired T-tests to compare patient QoL data at baseline and at follow-up. We then calculated the change in the separate subscales of QoL (change QoL=value at follow-up minus value at baseline). Using unpaired t-tests and analysis of variance we explored factors that affected the changes in QoL. In a linear regression model, we further explored relationships between factors that could influence QoL and follow-up QoL scores. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Clinical characteristics

Ninety-one out of 96 questionnaires were returned (95%). Nine patients preferred not to participate, whereas 5 patients did not respond. Thus, 82 completed questionnaires were returned (85%). There were no significant differences in age or gender between the study-population, and patients who did not respond or did not participate. Patient characteristics are detailed in Table 1.

All patients had stable controlled disease activity after (multimodality) treatment (details are provided in Table 1), with sustained biochemical control during the follow-up of 4 years. None of the patients required new treatment for acromegaly. At baseline, the mean duration between the estimated date of disease onset and biochemical control was 9.4 years (range 1 to 45 years). The mean duration of disease control was 11.5 years (range 1 to 26 years). Mean serum GH concentrations were 0.6 ± 0.8 µg/l at baseline and 0.5 ± 0.5 µg/l at follow-up (p=NS). The mean IGF-I SD scores were -0.2 ± 1.5 SD at baseline and 0.1 ± 1.5 SD at follow-up (p=NS).

At baseline, ACTH deficiency and TSH deficiency were present in 23% and 22% of patients, respectively. Sex hormone substitution therapy was given to 13 patients (28% of men required testosterone and 10% of women used estrogens). At follow-up, ACTH deficiency and TSH defi-

Table 4/1: Clinical characteristics of 82 patients with acromegaly, who participated in the long-term quality of life follow-up study.

Age at baseline (mean, range)		55.5 yrs (29-84)
Gender (M/F, n(%))		43 (52)/ 39 (48)
GH (mean ± SD (µg/l))	Baseline	0.6 ± 0.8
	Follow-up	0.5 ± 0.5
IGF-I SD scores	Baseline	-0.2 ± 1.5
	Follow-up	0.1 ± 1.5
Previous treatment (n(%))	Surgery only	37 (45)
	Surgery and radiotherapy	22 (27)
	Surgery and somatostatin analogs	11 (13)
	Surgery, radiotherapy, combined somatostatin analogs	5 (6)
	Somatostatin analogs	6 (7)
	Pituitary apoplexy	1 (1)
Duration of disease control at baseline (mean, range)		11.5 yrs (1-26)

ciency were present in 27% and 24% of patients, respectively. Seventeen patients were treated with sex hormone substitution therapy (33% of men and 8% of women, resp.).

Comparison of baseline and follow-up quality of life

During follow-up, scores in 21 out of 26 QoL subscales remained unaffected. However, physical and social functioning of the SF-36 decreased, physical fatigue increased (MFI-20), and psychological well-being and personal relations were progressively affected during follow-up (ACRO-QOL, Table 2). All subscales of the NHP and HADS remained unaffected.

Factors influencing changes in quality of life during 4 years of follow-up

Gender

We could not identify differences in changes in QoL during 4 years of follow-up between genders, except for more impairment in role functioning due to emotional problems in women (-7.2 ± 40.2 compared to 7.9 ± 31.1 in men, p=0.049).

Age

A higher age was associated with a more negative change in physical functioning (SF-36) during follow-up (R=-0.325, p=0.008). On the other hand, age was also negatively correlated with the change energy (NHP, R=-0.233, p=0.034), whereas a higher score in the NHP is associated with a greater impairment in functioning.

Table 4/2: Baseline and 4-year follow-up evaluation of quality of life in patients with cured acromegaly.

		Baseline	Follow-up	P-value
SF-36	Physical functioning	76.7 ± 23.0	72.1 ± 26.7	0.015
	Social functioning	83.0 ± 21.3	79.0 ± 25.0	0.032
	Role limitations due to physical problems	63.0 ± 42.0	67.4 ± 39.7	NS
	Role limitations due to emotional problems	74.3 ± 38.1	75.1 ± 37.5	NS
	Bodily pain	73.3 ± 22.7	72.6 ± 26.1	NS
	General health perception	60.0 ± 22.3	59.9 ± 23.2	NS
	Change in health	51.6 ± 19.0	46.8 ± 20.3	NS
NHP	Energy	25.5 ± 35.8	26.8 ± 35.2	NS
	Pain	16.9 ± 24.7	17.3 ± 26.5	NS
	Emotional reaction	13.0 ± 20.0	14.0 ± 22.9	NS
	Sleep	16.0 ± 28.0	18.4 ± 29.0	NS
	Physical ability	13.6 ± 20.5	15.5 ± 23.5	NS
	Social isolation	5.4 ± 14.4	6.1 ± 15.2	NS
MFI-20	General fatigue	11.7 ± 4.8	12.2 ± 5.3	NS
	Physical fatigue	10.8 ± 4.5	11.7 ± 5.0	0.030
	Reduced activity	9.8 ± 4.5	10.3 ± 4.7	NS
	Reduced motivation	9.3 ± 3.9	9.7 ± 4.3	NS
	Mental fatigue	9.7 ± 4.5	9.8 ± 4.3	NS
HADS	Anxiety	5.4 ± 4.2	5.6 ± 4.1	NS
	Depression	4.2 ± 3.9	4.8 ± 4.6	NS
	Total	9.5 ± 7.2	10.4 ± 7.9	NS
ACRO-QOL	Total	69.0 ± 17.4	66.8 ± 18.1	NS
	Physical performance	64.7 ± 22.4	64.0 ± 23.6	NS
	Psychological well-being	71.5 ± 16.9	68.5 ± 17.2	0.024
	Appearance	63.2 ± 22.9	61.9 ± 22.4	NS
	Personal relations	80.1 ± 14.4	76.1 ± 14.8	0.006

Data are expressed as mean ± SD. Baseline and follow-up data were compared with a paired samples T-test.

Duration of remisson

No correlations were found between changes in QoL during 4 years of follow-up and duration of remission of acromegaly.

Treatment of acromegaly

Previous treatment with radiotherapy (n=27, 33%) was associated with an increase in anxiety and total anxiety and depression scores (HADS), a reduction in motivation (MFI-20), and a reduction in physical performance (ACRO-QOL) (Table 3 and 4). All observed impairments in QoL during follow-up in these patients except for the HADS anxiety score remained significant after correction for the longer duration of control, lower IGF-I SD scores at baseline and

Table 4/3: Clinical characteristics of 27 patients with acromegaly cured after previous radiotherapy for acromegaly compared to patients who were not treated with radiotherapy (n=55).

		Radiotherapy	No radiotherapy	P-value
Age at baseline (mean ± SD, yrs)		54 ± 13	56 ± 12	NS
Gender (M/F, %)		56/ 44	51/ 49	NS
GH (mean ± SD (µg/l))	Baseline	0.5 ± 0.5	0.6 ± 0.9	NS
	Follow-up	0.4 ± 0.4	0.6 ± 0.6	NS
IGF-I SD scores	Baseline	-0.8 ± 1.7	0.1 ± 1.3	0.005
	Follow-up	-0.3 ± 1.7	0.3 ± 1.4	NS
Duration of control at baseline (mean, range)		14 ± 8	10 ± 7	0.041
Hypopituitarism (%)		67	20	<0.001

a higher prevalence of hypopituitarism in a linear regression model. In an additional analysis we compared the three main groups (surgery only, postoperative radiotherapy, postoperative somatostatin analog treatment). Compared to patients treated with surgery only, patients treated with postoperative radiotherapy experienced more impairment in physical functioning (68.0 ± 29.7 vs. 83.2 ± 20.3, p=0.054) and more pain (64.5 ± 25.0 vs. 80.4 ± 20.3, p=0.019, SF-36), and more loss of energy (39.9 ± 41.0 vs. 15.2 ± 28.2, p=0.023, NHP) at baseline. In addition, at follow-up, these patients had less energy (44.3 ± 38.1 vs. 16.0 ± 29.5, p=0.009) and more pain (23.8 ± 29.4 vs. 8.2 ±18.9, p=0.062, NHP), more reduction in motivation (11.4 ± 4.8 vs. 8.7 ± 3.9, p=0.051, MFI-20), more impairment in physical performance (58.8 ± 27.2 vs. 69.7 ± 19.7, p=0.034) and personal relations (69.8 ± 19.6 vs. 78.9 ± 11.8, p=0.072, ACRO-QOL) compared to patients treated with surgery only.

Hypopituitarism

Hypopituitarism, defined as one or more deficiencies of anterior pituitary hormones, did not influence any of the QoL subscales during follow-up. ACTH deficiency at baseline was associated with increased impairment in physical functioning according the SF-36 (-2.0 ± 14.0 in

Table 4/4: Change in QoL in patients treated with radiotherapy (n=27) vs. patients without radiotherapy (n=55).

		Radiotherapy	No radiotherapy	P-value
MFI-20	Reduced motivation	1.7 ± 4.6	-0.3 ± 3.6	0.041
HADS	Anxiety	1.5 ± 3.6	-0.4 ± 3.8	0.035
	Depression	1.5 ± 3.4	0.2 ± 2.6	0.083
	Total	3.0 ± 6.0	-0.2 ± 5.3	0.016
ACRO-QOL	Physical performance	-5.4 ± 16.1	-1.5 ± 12.2	0.036

Data are expressed as mean ± SD. SF-36 and ACRO-QOL: higher scores, better performance. HADS, NHP, MFI-20 higher scores: more impairment.

patients without ACTH deficiency vs. -11.8 ±19.7 in patients with ACTH deficiency (p=0.023), whereas TSH and LH-FSH deficiency did not influence any QoL subscales during follow-up.

Stepwise linear regression

Regression analysis was performed in a model including baseline scores of the specific subscale, age, gender, previous radiotherapy, and duration of disease control as independent variables and follow-up item scores as dependent variables (Table 5). Baseline QoL scores predicted follow-up scores of all QoL subscales, revealing individual stability over time. Male gender was associated with less impairment in role functioning due to emotional problems and less perceived health change over time. Age negatively influenced physical functioning (SF-36) and reduction in motivation (MFI-20) at follow-up. Radiotherapy negatively influenced several QoL subscale scores at follow-up: energy, pain, and social isolation (NHP), physical fatigue and reduction in activity and motivation (MFI-20), depression and total anxiety and depression scores (HADS), and physical performance (ACRO-QOL). The addition of hypopituitarism as another independent variable did not alter these conclusions with respect to radiotherapy except for pain (NHP) and physical fatigue (MFI-20). Duration of control did not independently influence QoL scores at follow-up.

DISCUSSION

We performed a longitudinal study on parameters of QoL in a homogenous cohort of patients with biochemical control of acromegaly. We found that QoL remained stable in 21 out of 26 QoL subscales, but was progressively impaired with respect to physical and social functioning, physical fatigue, psychological well-being, and personal relations. Previous radiotherapy negatively influenced various subscales of the QoL questionnaires during follow-up, which provides additional evidence for a negative influence of radiotherapy on QoL previously reported in cross-sectional studies. Baseline scores of the various subscales of the health related QoL questionnaires independently predicted follow-up scores showing individual stability during follow-up.

This study was performed in two stages. In the first cross-sectional part, we found that general QoL was severely reduced in patients cured of acromegaly compared to healthy controls (12). In the present study in the same cohort, we found that QoL progressively worsened during follow-up in some subscales, whereas it was relatively stable in the majority of subscales. In none of the subscales improvements were documented. We could identify two longitudinal studies aimed at evaluating the effect of treatment on QoL studying heterogeneous cohorts with active and controlled disease (10;13). These two longitudinal studies have shown positive influences of reduction of disease activity on total ACRO-QOL score during a follow-up of 6 months (10;13). The effects of treatment on reversible manifestations of GH excess can explain,

Table 4/5: Linear regression analysis of factors determining change in quality of life in 82 patients cured after treatment for acromegaly during 4 years of follow-up.

	Baseline score of QoL subscale	Age	Gender (F/M)	Radiotherapy (N/Y)
SF-36				
Physical functioning	0.833 (<0.001)	-0.549 (<0.001)		
Social functioning	0.949 (<0.001)			
Role limitations due to physical problems	0.548 (<0.001)			
Role limitations due to emotional problems	0.532 (<0.001)		15.485 (0.030)	
Bodily pain	0.872 (<0.001)			
General health perception	0.695 (<0.001)			
Change in health	0.363 (0.002)		8.802 (0.044)	
NHP				
Energy	0.584 (<0.001)			18.313 (0.004)
Pain	0.693 (<0.001)			0.277 (0.007)
Emotional reaction	0.721 (<0.001)			
Sleep	0.667 (<0.001)			
Physical ability	0.882 (<0.001)			
Social isolation	0.246 (0.035)			7.169 (0.042)
MFI-20				
General fatigue	0.728 (<0.001)			
Physical fatigue	0.739 (<0.001)			0.202 (0.025)
Reduced activity	0.643 (<0.001)			1.873 (0.030)
Reduced motivation	0.516 (<0.001)	0.066 (0.045)		2.557 (0.003)
Mental fatigue	0.476 (<0.001)			
HADS				
Anxiety	0.550 (<0.001)			
Depression	0.895 (<0.001)			1.339 (0.049)
Total	0.812 (<0.001)			2.905 (0.027)
ACRO-QOL				
Total	0.837 (<0.001)			
Physical performance	0.841 (<0.001)			-7.978 (0.013)
Psychological well-being	0.781 (<0.001)			
Appearance	0.791 (<0.001)			
Personal relations	0.647 (<0.001)			

Stepwise linear regression with the following parameters: baseline QoL subscale score, age, gender, radiotherapy, and duration of control at baseline. Data are expressed as the unstandardized β of independent predictive factors for change in quality of life during 4 years of follow-up with the p-value in brackets. SF-36 and ACRO-QOL: higher scores, better performance. HADS, NHP, MFI-20 higher scores: more impairment.

in part, these observations on QoL parameters. The patients studied in the present study, however, suffered from irreversible, persistent effects of previous GH excess, because they had biochemical disease control between the two assessments. Apparently, these effects negatively affect QoL parameters during prolongation of follow-up.

The question arises to which extent the present observations are influenced by increasing age rather than by acromegaly. In healthy adults, it was found that changes during 5-year follow-up were more pronounced in the older age groups and in the physically oriented domains of the SF-36 (25). This is in accordance with our finding that physical domains of the SF-36 worsened during follow-up in our patients with acromegaly and that age predicted follow-up QoL score with respect to physical functioning. Therefore, it is plausible that our observations are to some extent influenced by the increasing age of the patients at the follow-up study.

Nonetheless, during follow-up, radiotherapy was found to be an independent negative predictor of multiple items such as energy, social isolation, reduction in activity and motivation, physical performance, depression and total anxiety and depression scores. This is in line with the relationship found between radiotherapy and QoL in cross-sectional studies in patients with acromegaly (8;12) and in patients with other pituitary tumors (26). Therefore, increasing age is not the only determinant of the worsening QoL scores in our patients, because previous radiotherapy was independently associated with worsening QoL scores.

The negative impact of radiotherapy on QoL during follow-up might be attributed to different consequences of radiotherapy. First, radiotherapy is associated with anterior pituitary deficiencies, which negatively affects Qol. However, we did not find an increase in anterior pituitary deficiencies during follow-up. Secondly, it can also be due to the long-term negative consequences for neurocognitive functioning, like in long-term survivors of cranial radiation for brain tumors (27). Thirdly, patients treated with combination of surgery and radiotherapy could have the perception of more severe disease and hence a reduced QoL compared to patients treated with surgery only. However, since the introduction of more effective drug therapy for acromegaly, radiotherapy will be applied in fewer patients. Nonetheless, this study could contribute to the clinical assessment of patients with acromegaly treated with radiotherapy since the negative impact on QoL persists and even increases during follow-up.

In summary, during a follow-up of four years, QoL in patients with long-term biochemical control of acromegaly is subtly, but progressively impaired. In addition to increasing age, radiotherapy is the predominant indicator of progressive impairment.

REFERENCE LIST

1. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
2. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP 2008 Mortality in acromegaly: a meta-analysis. J Clin Endocrinol Metab 93:61-67.
3. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F 2005 Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 6:2393-2405
4. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ 2005 Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365:1644-1646
5. Melmed S 2006 Medical progress: Acromegaly. N Engl J Med 355:2558-2573
6. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO 2001 Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358:1754-1759
7. Hua SC, Yan YH, Chang TC 2006 Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. Eur J Endocrinol 155:831-837
8. Kauppinen-Makelin R, Sane T, Sintonen H, Markkanen H, Valimaki MJ, Loyttyniemi E, Niskanen L, Reunanen A, Stenman UH 2006 Quality of life in treated patients with acromegaly. J Clin Endocrinol Metab 91:3891-3896
9. Trepp R, Everts R, Stettler C, Fischli S, Allemann S, Webb SM, Christ ER 2005 Assessment of quality of life in patients with uncontrolled vs. controlled acromegaly using the Acromegaly Quality of Life Questionnaire (AcroQoL). Clin Endocrinol (Oxf) 63:103-110
10. Webb SM, Badia X, Surinach NL 2006 Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study. Eur J Endocrinol 155:269-277
11. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ 2005 Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. J Clin Endocrinol Metab 90:3337-3341
12. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F 2004 Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 89:5369-5376
13. Paisley AN, Rowles SV, Roberts ME, Webb SM, Badia X, Prieto L, Shalet SM, Trainer PJ 2007 Treatment of acromegaly improves quality of life, measured by AcroQol. Clin Endocrinol (Oxf) 67:358-362
14. Zigmond AS, Snaith RP 1983 The hospital anxiety and depression scale. Acta Psychiatr Scand 67:361-370
15. Smets EM, Garssen B, Bonke B, De Haes JC 1995 The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 39:315-325
16. Hunt SM, McKenna SP, McEwen J, Backett EM, Williams J, Papp E 1980 A quantitative approach to perceived health status: a validation study. J Epidemiol Community Health 34:281-286
17. Hunt SM, McEwen J 1980 The development of a subjective health indicator. Sociol Health Illn 2:231-246
18. Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L 1992 Validating the SF-36 health survey questionnaire: new outcome measure for primary care. BMJ 305:160-164
19. Ware JE, Jr., Sherbourne CD 1992 The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 30:473-483
20. Webb SM, Prieto L, Badia X, Albareda M, Catala M, Gaztambide S, Lucas T, Paramo C, Pico A, Lucas A, Halperin I, Obiols G, Astorga R 2002 Acromegaly Quality of Life Questionnaire (ACROQOL) a new health-related quality of life questionnaire for patients with acromegaly: development and psychometric properties. Clin Endocrinol (Oxf) 57:251-258

21. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
22. Janssen YJ, Frolich M, Roelfsema F 1997 A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 82:129-135
23. Cole TJ 1990 The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45-60
24. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-176
25. Hopman WM, Berger C, Joseph L, Towheed T, VandenKerkhof E, Anastassiades T, Adachi JD, Ioannidis G, Brown JP, Hanley DA, Papadimitropoulos EA 2006 The natural progression of health-related quality of life: results of a five-year prospective study of SF-36 scores in a normative population. Qual Life Res 15:527-536
26. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA 2005 Quality of Life in Patients After Long-term Biochemical Cure of Cushing's Disease. J Clin Endocrinol Metab 90:3279-3286
27. Spiegler BJ, Bouffet E, Greenberg ML, Rutka JT, Mabbott DJ 2004 Change in neurocognitive functioning after treatment with cranial radiation in childhood. J Clin Oncol 22:706-71.

Chapter 5

Somatostatin analog treatment is associated with an increased sleep latency in patients with long-term biochemical remission of acromegaly.

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Growth Hormone & IGF Research, 2008

ABSTRACT

Background

Somatostatin analogs induce alterations in sleep in healthy adults. Presently, it is unknown whether somatostatin analog treatment affects sleep parameters in patients with acromegaly.

Design

Case-control study.

Patients and measurements

We assessed sleepiness and sleep patterns in 62 adult patients (32 men, age 61 years (33-88 yrs) controlled by surgery alone or postoperative radiotherapy (69%), and/or somatostatin analogs (31%). We used two validated sleep questionnaires (Epworth sleepiness score and Münchener Chronotype Questionnaire). Patient outcomes were compared to controls.

Results

Sleep duration and timing of sleep were not different in patients compared to controls. However, sleepiness score was increased in all patients compared to controls: 6 (1-20) vs. 4 (0-14), p=0.014 (median (range)), reflecting increased daytime sleepiness. Snoring was reported in 68% of both patients and controls (p=0.996), observed apnoea's and restless legs in 23% and 37% of patients compared to 12% and 21% of controls (p=0.062 and p=0.031, resp.). In addition, sleep latency was increased in patients treated by somatostatin analogs compared to patients cured by surgery and/ or radiotherapy (52 ± 48 min vs. 26 ± 40 min, p=0.005), resulting in a delayed sleep onset (24:08 ± 1:26 h vs. 23:25 ± 0:43 h, p=0.053). Sleep duration was unaffected.

Conclusions

Daytime sleepiness is increased in a homogeneous cohort of patients in long-term remission from acromegaly. In addition, somatostatin analog treatment increases sleep latency and delays sleep onset in patients with long-term biochemical control of growth hormone overproduction without altering total sleep duration.

INTRODUCTION

The aims of treatment in active acromegaly are to relief the symptoms of growth hormone (GH) excess, to decrease mass effects, to restore metabolic alterations, and to reduce the increased mortality risk associated with active acromegaly (1). Somatostatin analog treatment alone or surgical treatment alone can reach these targets in only 50-70% of the patients. Fortunately, combinations of surgery, radiotherapy, and/or drug therapy (somatostatin analogs and/or GH receptor blockade drugs) are able to control disease activity in almost all patients (2-5).

Acromegaly is associated with sleep disorders, including sleep disordered breathing, such as snoring, and sleep apnoea syndrome. The prevalence of the sleep apnoea syndrome was found to be as high as 80% in patients with acromegaly (reviewed in (6)). Treatment of acromegaly by transsphenoidal surgery or somatostatin analogs has been consistently found to reduce this high prevalence of sleep apnoea syndrome (7-12). However, somatostatin per se may also adversely influence sleep, because in experimental studies, somatostatin and its analog, octreotide, altered sleep (13;14). In healthy elderly persons, somatostatin administered during the first half of the night decreased total sleep time and rapid eye movement sleep (REMS) and increased the time spent awake in the first sleep cycle (14). In rats, repeated injections of octreotide caused decreases in non-rapid eye movement sleep (NREMS) time and NREMS intensity (13). In healthy young male subjects octreotide decreased stage 4 NREMS and REMS during the first half of the night (15). Based on these observations, it is possible that somatostatin treatment in patients with acromegaly may also adversely affect sleep.

Therefore, the aim of this study was to compare sleepiness and sleep patterns between patients cured from acromegaly by transsphenoidal surgery and/ or radiotherapy and patients with biochemical control of GH excess during treatment with somatostatin analogs.

PATIENTS AND METHODS

Protocol

For the present study, consecutive patients visiting our outpatient clinic with adequate control of GH excess were screened for participation (n=95).

Inclusion criteria were:

1) biochemical cure after primary treatment by transsphenoidal surgery, and if necessary, by adjuvant postoperative treatment with radiotherapy and/ or somatostatin analogs.

2) biochemical control of GH excess after primary treatment with somatostatin analogs.

Exclusion criteria were:

1) growth hormone deficiency after treatment for acromegaly and treatment with recombinant human growth hormone started within the previous year (n=16).

2) serious illness limiting the completion of the questionnaires (n=3).

Thus, 76 patients were found eligible to participate in this study. Questionnaires were sent to their homes in prepaid envelopes. After 6 weeks, nonresponders received a reminder letter, and, thereafter, were contacted by telephone to encourage completion and return of the questionnaires.

Each patient was also asked to provide a healthy control person of comparable age and sex, who did not use any medication, to serve as a control group with a comparable socio-economic status. The control group was extended with controls derived from other studies in our center who were approached similarly (16).

Cure or biochemical control on somatostatin analogs was defined by normal serum IGF-I levels for sex and age and serum GH levels below 1.9 µg/l (≈5 mU/l) for all patients, and also by a normal GH suppression during oral glucose loading (<0.38 µg/l) only in the patients without somatostatin analog treatment. In patients on somatostatin anologs, mean GH levels were obtained from 5 samples obtained with an interval of 30 minutes between 9.00 and 11.00 h am. Octreotide long-acting repeatable (n=15, Novartis Pharma AG, Basel Switzerland) and lanreotide autogel (n=4, Ipsen Biotech, Paris, France) were used as somatostatin analogs.

Premenopausal women were defined as LH/ FSH deficient when secondary amenorrhoea was present for more than 1 year. Postmenopausal women were defined as LH/FSH deficient when gonadotrophin levels were below the normal postmenopausal range (LH<10 U/l and FSH<30 U/l). In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone test or insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with thyroxine, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided.

Six patients had a GH and prolactin secreting adenoma at diagnosis. Four of these patients were cured after surgery and 1 patient was cured after combined surgery and radiotherapy. One patient was still treated with dopamine agonists at the time of the present study (a 75 year old male patient treated by surgery alone for a GH and prolactin secreting tumor).

The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects returning completed questionnaires gave written informed consent for participation in the study.

Study parameters

Primary study-parameters were the results of the two sleep questionnaires. The results were linked to age and gender of the patients, treatment characteristics (somatostatin analog treatment and duration of cure).

Sleep questionnaires

Epworth sleepiness scale (ESS)

The ESS is a validated eight-item questionnaire. The subject is asked to rate his likelihood of falling asleep in a variety of commonly encountered situations (17). Scores range from 0 (the least sleepy) to 24 (the most sleepy). Scores equal to or above 10 are interpreted as increased daytime sleepiness (18). An additional set of questions evaluating the prevalence of snoring, observed apnoea's, and nocturnal restless legs was added to ensure a standardized clinical assessment.

Münchener chronotype questionnaire (MCQ)

The MCQ is a validated questionnaire aimed at assessing chronotype and sleep patterns (19;20). Patients are explicitly asked to describe their sleep behaviour under normal circumstances. The temporal structure of sleep is assessed separately for workdays and free days. Parameters on free days are regarded to reflect individual sleep patterns without social obligations and are therefore reported in this paper (19).

Sleep duration on free days (SD_F), sleep onset on free days (SO_F), and rise time (RT_F) are calculated from questions concerning sleep onset and awakening on days on which there are no work or social obligations. The midsleep on free days (MS_F: clock time halfway during sleep duration) is calculated from SO_F and RT_F (20). The sleep latency on free days (SL_F) can be calculated by SO_F and time at which a subject went to bed.

Since most chronotypes tend to accumulate a sleep dept on work days, which is compensated for on free days, midsleep on workdays (MS_C) was corrected for the confounder sleep debt as follows: $MS_C=MS_F-(0.5*SD_F-(5*SD_{working\ days}+2*\ SD_F)/7)$ (20). Since only 25 of our patients and 56 of our controls had a daytime job, this correction was performed only for those subjects (20).

Assays and normal values

GH levels were measured with a sensitive immunofluorometric assay (Wallac, Turku, Finland), specific for the 22-kDA GH protein and calibrated against WHO International Reference Preparation 80/505. The detection limit was 0.01 µg/l, and the inter-assay coefficient of variation was 2.0-9.0% en intra-assay coefficient of variation was 1.6-8.4% between 0.1 and 18 µg/l (for conversion of µg/l to mU/l, multiply by 2.6).

Serum IGF-I concentrations (ng/ml) were measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively. IGF-I is expressed as SD score, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals (21;22;22).

Statistics

Data are presented as mean ± SD unless specified otherwise. SPSS for windows version 14.0 (SPSS Inc., Chicago, IL) was used for data analysis. Differences were considered statistically significant at p≤0.05. We used unpaired T-tests in case of normal distribution and non-parametric Mann-Whitney tests for the ESS and in case of skewed distribution. Chi-square tests and linear regression analysis were performed, when appropriate.

RESULTS

Patient and treatment characteristics

Seventy-one of 76 (93%) patients returned the questionnaires on sleep characteristics. Nine patients of these 71 patients preferred not to participate. Thus, 62 completed questionnaires were received. The study-population (32 men) had a mean age of 61 years with a range of 33 to 88 years. No significant differences in age, gender and tumor-characteristics were found between the study-population, and the 14 patients who preferred not to participate or who did not return the questionnaires.

The patients were compared to 98 controls (40 men) were with mean age of 59 years (range of 31 to 83 years). Age and gender in the control group were not different from the studied acromegaly patients (p=0.425 and p=0.152, respectively, Table 1).

All patients were in biochemical remission of GH excess after (multimodality) treatment. Fifty-five patients (89%) were treated with primary transsphenoidal surgery, whereas 6 were primarily treated with somatostatin analogs and 1 patient was in remission after pituitary apoplexy. Thirty-five of those 55 patients (64%) who were treated with primary transsphenoidal

Table 5/1: Clinical characteristics of 62 patients with acromegaly.

		Patients (n=62)	Controls (n=98)	P-value
Age (mean, range (yrs))		60.7 (33-88)	59.3 (31-83)	0.425
Gender (M/F, n)		32 (52)/ 30 (48)	38 (40)/ 57 (60)	0.152
GH (µg/l)		0.6 ± 0.6		
IGF-I SD scores		0.4 ± 1.4		
Disease state (n(%))	Cured after surgery and/ or radiotherapy	43 (69)		
	Well-controlled (current use of somatostatin analogs)	19 (31)		
Radiotherapy (n(%))		11 (18)		
Duration of remission (mean, range (yrs))		15.1 (5-30)		

Data are presented as mean with range or number with percentage in parentheses. M males; F Females; yrs years.

Table 5/2: Clinical characteristics of 43 patients cured of acromegaly versus 19 patients well-controlled by somatostatin analogs.

	Cured (n=43)	Well-controlled (n=19)	P-value
Age (mean, range (yrs))	59.3 (33-88)	64.0 (43-79)	0.140
Gender (M/F, n)	26/ 17	13/ 6	0.036
GH (µg/l)	0.6 ± 0.6	0.6 ± 0.5	0.658
IGF-I SD scores	0.2 ± 1.4	0.8 ± 1.2	0.098
Radiotherapy (n(%))	7 (16)	4 (21)	0.650
Duration of cure (mean, range (yrs))	15.7 (5-29)	20.1 (7-35)	0.077

Data are presented as mean with range or number with percentage in parentheses. M males; F Females; yrs years.

surgery were cured. Eleven patients of those 55 patients had received additional radiotherapy, four of whom were also treated with somatostatin analogs to establish disease control. Nine of the 55 postoperative patients received additional somatostatin analogs without postoperative radiotherapy.

Thus, 43 patients were cured (69%) by surgery only or additional postoperative radiotherapy or apoplexy, whereas 19 patients were biochemically well-controlled (31%) with somatostatin analogs either as primary treatment (n=6) or after surgery (n=13) (Table 2). The mean duration of cure was 15 years (range 5 to 30 years). The mean serum GH concentrations were 0.6 ± 0.6 µg/l and the mean IGF-I SD scores were 0.4 ± 1.4 SD.

Five percent of patients had ADH deficiency, whereas ACTH deficiency and TSH deficiency were present in 11% and 21% of patients, respectively. Seven patients were treated with estrogen or testosterone because of LH-FSH deficiency (19% of men and 14% of premenopausal women).

Comparison between patients with acromegaly and controls

Sleep duration on free days (SD_F) was not different between patients and controls (7:33 ± 1:11 h vs. 7:23 ± 1:01 h, p=0.386). Sleep onset on free days, sleep latency on free days, midsleep on free days, rising time on free days, and corrected midsleep (SO_F, SL_F, MS_F, RT_F and MS_C, respectively) were not different compared to controls as well (Table 3).

However, the Epworth Sleepiness Scale (ESS) score was increased in patients compared to controls: 6 (1-20) vs. 4 (1-14); p=0.014 (median (range)), indicating increased daytime sleepiness in patients.

Sixty-eight percent of both patients and controls reported snoring (p=0.996), whereas 23% of patients reported observed apnoea's compared to 12% of controls (p=0.062). In addition, restless legs were reported in 37% in patients compared to 21% in controls (p=0.031).

In our patients, no correlations could be found between any of the sleep pattern parameters (sleep duration, onset, rise time, latency or midsleep on free days) and sleepiness scores on the ESS.

Table 5/3: Sleepiness scores and subjective sleep patterns in patients cured from acromegaly compared to controls.

		Patients (n=62)	Controls (n=98)	P-value
ESS	Mean score (median (range))	6 (1-20)	4 (0-14)	0.014
	>10 (%)	20	12	0.187
MCTQ	Sleep onset on free days (clock time h:min ± SD)	23:39 ± 1:02	23:44 ± 1:12	0.604
	Sleep latency (minutes ± SD)	34.2 ± 43.8	27.2 ± 22.2	0.185
	Rising time on free days (clock time h:min ± SD)	7:12 ± 1:11	7:08 ± 1:03	0.755
	Sleep duration on free days (duration h:min ± SD)	7:33 ± 1:11	7:23 ± 1:01	0.386
	Midsleep on free days (clock time h:min ± SD)	3:25 ± 0:57	3:26 ± 1:01	0.901
	Corrected midsleep (clock time h:min ± SD, n=25 *vs.* n=56)	3:51 ± 1:03	3:42 ± 0:44	0.456

Data are presented as mean ± SD or as percentages and compared with independent samples T-test, non-parametric Mann-Whitney U-tests or Chi-square test, when appropriate.

Factors influencing sleep in patients with acromegaly

Age

No significant correlations were found between age and ESS score or between age and sleep pattern parameters in all studied patients.

Gender

In patients with acromegaly, sleep duration was longer in women than men (7:52 ± 1:17 h vs. 7:15 ± 1:01 h, p=0.044). Midsleep on free days (MS_F) was significantly different in women compared to men (clock time 3:41 ± 1:00 h vs. clock time 3:11 ± 0:51 h, p=0.009). This difference in MS_F was associated with a later rise time in women (7:37 ± 1:07 h in women vs. 6:49 ± 1:08 h in men, p=0.007). MS_C, SL_P and ESS scores did not differ between men and women.

Duration of remission, GH concentrations and IGF-I SD scores

No significant correlations were found between GH, IGF-I concentrations or duration of remission and ESS score or between GH, IGF-I concentrations or duration of cure and sleep pattern parameters (sleep onset, rising time, sleep duration, and midsleep on free days).

Diabetes mellitus, BMI and smoking

Six patients (10%) suffered from diabetes mellitus. No differences were found between patients with type 2 diabetes mellitus (n=6, 10%) or patients without type 2 diabetes mellitus with respect to ESS score or sleep pattern parameters. Mean BMI was 27.1 ± 4.4 kg/m^2. No correlations between BMI and ESS scores or sleep pattern parameters were found. Twenty-five percent

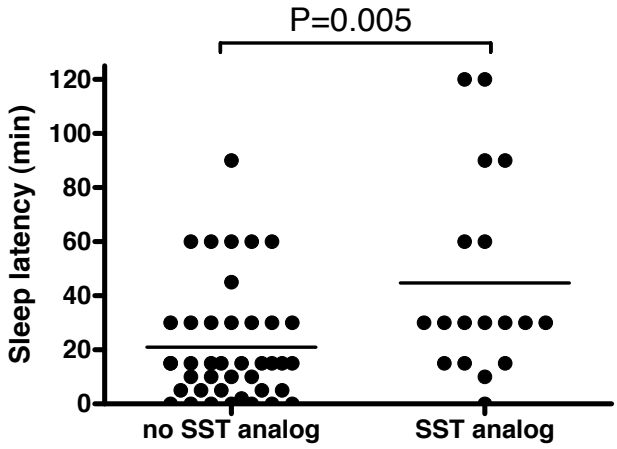

Figure 5/1: Sleep latency is increased in patients treated with somatostatin analogs (SST analog, n=19) compared to patients treated by surgery and or radiotherapy alone (no SST analog, P=0.005).

of patients were smokers. These patients were significantly younger than non-smokers (52.4 ± 10.7 years vs. 63.6 ± 10.4 years, p=0.001). Midsleep on free days, MS_F, was significantly later (4:02 ± 1:18 h in smokers vs. 3:13 ± 0:37 h in non-smokers, P=0.035) due to later sleep onset and rising time.

Cure versus biochemical control by somatostatin analogs

IGF-I SD scores and GH levels did not differ between the cured patients and the patients with biochemical control of acromegaly by somatostatin analogs. The percentage of patients previously treated with radiotherapy or the prevalence anterior pituitary deficiencies did not differ between the two groups as well. Sleep latency on free days was increased in patients biochemical control of acromegaly by somatostatin analogs (52 ± 48 min vs. 26 ± 40 min, p=0.005, Figure 1), which consequently delayed sleep onset (24:08 ± 1:26 h vs. 23:25 ± 0:43 h, p=0.053) and midsleep on free days (MS_F, clock time 3:50 ± 1:17 h vs. clock time 3:14 ± 0:41 h, p=0.022). Sleep duration of free days (SD_F) was unaffected (7:36 ± 1:11 h vs. 7:24 ± 1:11 h, p=0.540, Table 4). After exclusion of patients treated with radiotherapy and patients treated with primary somatostatin analogs, we could assess the difference between patients treated with primary surgery alone (n=36) and patients treated with primary surgery and subsequent somatostatin analogs (n=9). Sleep latency was increased in the latter group (p=0.050 in a non-parametric Mann-Whitney tests) resulting in a delay in sleep onset (24:21 ± 1:41 h vs. 23:24 ± 0:46 h, p=0.081), confirming the above analysis in all patients and pointing towards a somatostatin analog specific effect.

The percentage of smokers did not differ between those patients cured by surgery and/or radiotherapy (26%) and patients with biochemical control of acromegaly by somatostatin analogs (24%, p=09.869).

Table 5/4: Sleepiness scores and subjective sleep patterns in patients cured from acromegaly compared to patients with biochemically well-controlled acromegaly with somatostatin analogs.

		Cured (n=43)	Well-controlled (n=19)	P-value
ESS	Mean score (median (range))	6 (1-20)	7 (0-16)	0.571
	> 10 (%)	17	26	0.405
MCTQ	Sleep onset on free days (clock time h:min ± SD)	23:25 ± 0:43	24:08 ± 1:26	0.053
	Sleep latency (minutes ± SD)	26.2 ± 40.1	51.8 ± 47.5	0.005
	Rising time on free days (clock time h:min ± SD)	7:02 ± 1:03	7:33 ± 1:25	0.151
	Sleep duration on free days (duration h:min ± SD)	7:36 ± 1:11	7:24 ± 1:11	0.540
	Midsleep on free days (clock time h:min ± SD)	3:14 ± 0:41	3:50 ± 1:18	0.022

Data are presented as mean ± SD or as percentages and compared with independent samples T-test, non-parametric Mann-Whitney test or Chi-square test, when appropriate.

Stepwise linear regression analysis

Stepwise linear regression was performed in a model including age, gender, current use of somatostatin analogs, duration of cure, GH, and IGF-I concentrations as independent variables and ESS score, sleep duration on free days (SD_F), sleep onset on free days (SO_F), sleep latency on free days (SL_F), rise time on free days (RT_F), and midsleep on free days (MS_F) as dependent variables. Age, duration of cure, and IGF-I SD scores influenced neither sleep pattern parameters nor ESS scores. Sleep onset on free days (SO_F) was independently predicted by current use of somatostatin analogs (β=2929 (≈49 min), p=0.015) as was sleep latency (β=32.6 min, p=0.028). Rise time on free days (RT_F) was independently influenced by gender (0=Female, 1=Male, β=-3118 (≈-52 min), p=0.018).

DISCUSSION

The data in this study show that patients in long-term remission of active acromegaly experience increased daytime sleepiness compared to controls. Nonetheless, self-reported sleep patterns such as sleep onset or sleep duration did not differ from controls and were not correlated to increased daytime sleepiness scores. In addition, current somatostatin analog treatment induced an increase in self-reported sleep latency and consequently delayed sleep onset. Therefore, this study indicates increased daytime sleepiness despite adequate treatment of acromegaly and demonstrates that somatostatin treatment per se is associated with delayed sleep onset.

The Epworth sleepiness scores indicated increased daytime sleepiness in our patients and are comparable with scores found in another study in 6 patients with acromegaly six months after transsphenoidal surgery (8). In addition, the daytime sleepiness scores in our patients were comparable to scores found in patients treated for other pituitary tumors or cerebral diseases such as non-functioning adenomas (16), craniopharyngeoma (23), hypothalamic tumors (23), subarachnoid haemorrhage (24), or traumatic brain injury (25), indicative for the relationship between cerebral disease in general and increased daytime sleepiness.

In the particular group of patients with biochemical control of acromegaly, sleep apnoea could have contributed to the increased daytime sleepiness. Although not formally excluded, we did not find any significant differences in self-reported snoring or apnoea's. Although many reports have described the amelioration of sleep apnoea's after successful treatment of acromegaly (7-12), only one study in a homogenous cohort of patients cured from acromegaly reported the prevalence of sleep apnoea syndrome to be 20% (26). A detailed assessment of sleep apnoea syndrome in cured acromegaly is warranted even more since the introduction of GH receptor blockade therapy, which could further reduce tissue swelling and subsequently sleep apnoea syndrome in patients with acromegaly.

In addition, increased sleep latency, and consequently a delayed sleep timing, was found in patients on somatostatin analog therapy. In healthy elderly subjects, somatostatin impaired sleep especially by decreasing total sleep time and REMS, and by increasing the time spent awake in the first sleep cycle (14), although it did not influence sleep in young healthy adults (27). In rats, the long-acting somatostatin analog octreotide suppressed NREMS after repeated injections (13). Moreover, octreotide reduced stage 4 NREMS and REMS during the first half of the night and increased intermittent wakefulness during the second half of the night in young healthy adults (15). We could not identify polysomnographic studies of the effects of the depot preparations of octreotide or lanreotide on sleep parameters, which are interesting in the light of our results.

Growth hormone secretion during somatostatin analog treatment in acromegaly is not completely normalized (28), which is associated with altered diastolic function of the heart (29). From the current study, it becomes apparent that somatostatin analog treatment in itself also adversely affects sleep. Additional prospective polysomnographic studies with depot preparations, with or without GH receptor blockade drugs, are needed to further elucidate the effects of these drugs on sleep patterns and sleep quality in acromegaly even more since the primary treatment strategies are likely to change in the future.

Although the mechanism by which somatostatin impairs sleep is unknown, it is postulated to act at the level of the central nervous system. It has been proposed that somatostatin impairs GABAergic neuronal transmission in the sensory thalamus via presynaptic receptors in cats and rats (30), which could contribute to the observed decrease in NREMS after somatostatin administration (31). Growth hormone releasing hormone (GHRH) has been consistently found

to promote NREMS (31). Therefore, sleep seems to be under control of the same reciprocal interaction of GHRH and somatostatin that controls GH secretion.

Some factors may have influenced our results. Sleepiness and sleep patterns were assessed using self-reported questionnaires. The MCQ assesses sleep during free days and working days, but only at one occasion. However, a comparison of the data on sleep habits from the MCQ and data from a sleep log for 5 weeks by Roenneberg et al. demonstrated that sleep times obtained by both questionnaires on both workdays and free days were highly correlated ($p<0.0001$, (19)). Additionally, in the present study patients with acromegaly were compared to controls recruited by the patients. The advantage of using such controls is that they are from the same geographic area and socio-economic class as the patients (32). Although it is known that self-selected controls might be subject to selection bias, because patients might have chosen controls with a supposedly good health status (33), it is not likely that sleeping pattern plays any role in the choice for a specific control. Finally, based on the present results, the next step is to perform objective test of sleepiness and polysomnography to asses sleep quality and patterns in patients treated for acromegaly in more detail.

No relation was found between age and sleepiness scores. This is in contrast with findings in the general healthy population (34;35). This discrepancy is likely due to the limited range in ages of the subjects included in our study due to the generally older age of patients with acromegaly.

In conclusion, we found increased daytime sleepiness in patients cured from acromegaly despite normal sleep patterns and comparable prevalences of self-reported snoring and apnoea's. Treatment with somatostatin analogs is associated with increased sleep latency and, consequently, delayed sleep onset. Since somatostin analogs are the first choice for medical therapy of active acromegaly, additional studies are needed to assess sleep quality in order to identify the optimal treatment regimen not only with respect to therapeutic effects on disease activity, but also on the potential adverse effects on sleep.

REFERENCE LIST

1. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
2. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F 2005 Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 6:2393-2405
3. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ 2005 Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365:1644-1646
4. Melmed S 2006 Medical progress: Acromegaly. N Engl J Med 355:2558-2573
5. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO 2001 Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358:1754-1759
6. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
7. Herrmann BL, Wessendorf TE, Ajaj W, Kahlke S, Teschler H, Mann K 2004 Effects of octreotide on sleep apnoea and tongue volume (magnetic resonance imaging) in patients with acromegaly. Eur J Endocrinol 151:309-315
8. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329
9. Saeki N, Isono S, Tanaka A, Nishino T, Higuchi Y, Uchino Y, Iuchi T, Murai H, Tatsuno I, Yasuda T, Yamaura A 2000 Pre-and post-operative respiratory assessment of acromegalics with sleep apnea--bedside oximetric study for transsphenoidal approach. Endocr J 47 Suppl:S61-S64
10. Buyse B, Michiels E, Bouillon R, Bobbaers H, Demedts M 1997 Relief of sleep apnoea after treatment of acromegaly: report of three cases and review of the literature. Eur Respir J 10:1401-1404
11. Grunstein RR, Ho KK, Sullivan CE 1994 Effect of octreotide, a somatostatin analog, on sleep apnea in patients with acromegaly. Ann Intern Med 121:478-483
12. Leibowitz G, Shapiro MS, Salameh M, Glaser B 1994 Improvement of sleep apnoea due to acromegaly during short-term treatment with octreotide. J Intern Med 236:231-235
13. Beranek L, Obal F, Jr., Taishi P, Bodosi B, Laczi F, Krueger JM 1997 Changes in rat sleep after single and repeated injections of the long-acting somatostatin analog octreotide. Am J Physiol 273:R1484-R1491
14. Frieboes RM, Murck H, Schier T, Holsboer F, Steiger A 1997 Somatostatin impairs sleep in elderly human subjects. Neuropsychopharmacology 16:339-345
15. Ziegenbein M, Held K, Kuenzel HE, Murck H, Antonijevic IA, Steiger A 2004 The somatostatin analogue octreotide impairs sleep and decreases EEG sigma power in young male subjects. Neuropsychopharmacology 29:146-151
16. van der Klaauw AA, Dekkers OM, Pereira AM, van Kralingen KW, Romijn JA 2007 Increased daytime somnolence despite normal sleep patterns in patients treated for non-functioning pituitary macroadenoma. J Clin Endocrinol Metab 92:3898-3903
17. Johns MW 1991 A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545
18. Muller HL, Handwerker G, Wollny B, Faldum A, Sorensen N 2002 Melatonin secretion and increased daytime sleepiness in childhood craniopharyngioma patients. J Clin Endocrinol Metab 87:3993-3996
19. Roenneberg T, Wirz-Justice A, Merrow M 2003 Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms 18:80-90
20. Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A, Merrow M 2004 A marker for the end of adolescence. Curr Biol 14:R1038-R1039

21. Cole TJ 1990 The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45-60
22. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-176
23. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589
24. Schuiling WJ, Rinkel GJ, Walchenbach R, de Weerd AW 2005 Disorders of sleep and wake in patients after subarachnoid hemorrhage. Stroke 36:578-582
25. Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR 2006 Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehabil 87:278-285
26. Rosenow F, Reuter S, Deuss U, Szelies B, Hilgers RD, Winkelmann W, Heiss WD 1996 Sleep apnoea in treated acromegaly: relative frequency and predisposing factors. Clin Endocrinol (Oxf) 45:563-569
27. Steiger A, Guldner J, Hemmeter U, Rothe B, Wiedemann K, Holsboer F 1992 Effects of growth hormone-releasing hormone and somatostatin on sleep EEG and nocturnal hormone secretion in male controls. Neuroendocrinology 56:566-573
28. Biermasz NR, Pereira AM, Frolich M, Romijn JA, Veldhuis JD, Roelfsema F 2004 Octreotide represses secretory-burst mass and nonpulsatile secretion but does not restore event frequency or orderly GH secretion in acromegaly. Am J Physiol Endocrinol Metab 286:E25-E30
29. van Thiel SW, Bax JJ, Biermasz NR, Holman ER, Poldermans D, Roelfsema F, Lamb HJ, van der Wall EE, Smit JW, Romijn JA, Pereira AM 2005 Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 153:231-238
30. Leresche N, Asprodini E, Emri Z, Cope DW, Crunelli V 2000 Somatostatin inhibits GABAergic transmission in the sensory thalamus via presynaptic receptors. Neuroscience 98:513-522
31. Steiger A 2007 Neurochemical regulation of sleep. J Psychiatr Res 41:537-552
32. Grimes DA, Schulz KF 2005 Compared to what? Finding controls for case-control studies. Lancet 365:1429-1433
33. Wacholder S, Silverman DT, McLaughlin JK, Mandel JS 1992 Selection of controls in case-control studies. II. Types of controls. Am J Epidemiol 135:1029-1041
34. Gander PH, Marshall NS, Harris R, Reid P 2005 The Epworth Sleepiness Scale: influence of age, ethnicity, and socioeconomic deprivation. Epworth Sleepiness scores of adults in New Zealand. Sleep 28:249-253
35. Whitney CW, Enright PL, Newman AB, Bonekat W, Foley D, Quan SF 1998 Correlates of daytime sleepiness in 4578 elderly persons: the Cardiovascular Health Study. Sleep 21:27-36.

Chapter 6

Growth hormone (GH) deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the twenty-four hour GH secretion

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European Journal of Endocrinology, 2006

ABSTRACT

Background

Radiotherapy for pituitary adenomas frequently leads to growth hormone (GH) deficiency. The characteristics of GH secretion in GH deficiency induced by postoperative radiotherapy for acromegaly are not known. Hypothesis: In the long-term, stimulated and spontaneous GH release is not different between patients with GH deficiency treated by postoperative radiotherapy for acromegaly or for other pituitary adenomas.

Design/subjects

We compared the characteristics of basal and stimulated GH secretion in patients with GH deficiency, who had previously received adjunct radiotherapy after surgery for GH-producing adenomas (n=10) versus for other pituitary adenomas (n =10). All patients had a maximal GH concentration by insulin tolerance test (ITT) of 3 µg/l or less, compatible with severe GH deficiency. Mean time after radiation was 17 and 18.7 years, respectively. Stimulated GH release was also evaluated by infusion of GHRH, GHRH-arginine and arginine, and spontaneous GH by 10 min. blood sampling for 24h. Pulse analyses were done by Cluster and approximate entropy.

Results

There were no differences between both patient groups in stimulated GH concentrations in any test. Spontaneous GH secretion was not different between both patient groups, including basal GH release, pulsatility and regularity. Pulsatile secretion was lost in 2 acromegalic and 3 non-acromegalic patients. IGF-I was below -2 SD-score in 9 patients in each group.

Conclusion

Acromegalic patients treated by surgery and postoperative radiotherapy with an impaired response to ITT do not differ in the long-term in GH secretory characteristics from patients treated similarly for other pituitary tumors with an impaired response to ITT. The ITT (or the GHRH-arginine test) is therefore reliable in establishing the diagnosis of GHD in patients treated for acromegaly by surgery and radiotherapy.

INTRODUCTION

The aim of treatment in acromegaly is first to relief the symptoms of growth hormone (GH) excess and the mass effects of the pituitary tumor. Additional aims are the restoration of the metabolic changes and the reduction of the increased mortality risk associated with active acromegaly (1). Ideally, the therapy should be directed towards the restoration of physiological GH secretion, which is achieved when responses to dynamic stimuli and the 24h GH production are normalized, including restoration of secretory characteristics such as diurnal rhythm and secretory regularity. At the present time only surgery is capable to fulfill these goals in a limited number of patients, even by expert surgery (2-5). Therefore, additional treatment is required frequently, which may be given as pharmacotherapy (e.g. somatostatin analogs, GH-receptor blockade drugs, dopaminergic drugs or combinations there off) or as radiotherapy.

After pituitary irradiation a decline of ~50% in serum GH levels is observed in the first two years and of ~75% after 5 years (6-8). The normalization of GH and IGF-I levels during follow-up after radiotherapy is mainly dependent on the pre-irradiation serum GH concentrations. Many patients with other pituitary adenomas (e.g. non-functioning adenomas, adrenocorticotrope hormone (ACTH) - or prolactin (PRL)-secreting adenomas) develop GH deficiency after pituitary irradiation (9). Therefore, it seems logical to expect GH deficiency in the long-term in acromegaly after such treatment. In accordance, we have documented a decreased response of GH to insulin-induced hypoglycemia in 36% of the patients with acromegaly, during long-term follow-up of postoperative radiotherapy (7).

In acromegalic patients with GH deficiency after radiotherapy, little information is available on spontaneous 24h GH secretion and other GH-stimulation tests in relation to the insulin tolerance test (ITT), being the most widely used and recommended GH provocative measure for the diagnosis of GH deficiency (10). Therefore, we specifically wished to address whether basal and stimulated GH secretion differed between patients with GH deficiency, who had previously received adjunct radiotherapy after surgery for GH-producing adenomas (n=10) and those who had received adjunct radiotherapy for other pituitary adenomas (n=10). In both groups GH deficiency was defined as a maximal GH concentration during insulin tolerance test (ITT) of 3 µg/l, compatible with severe GH deficiency. We therefore explored various aspects of GH pathophysiology, including spontaneous 24h GH secretion, GH provocative tests aimed at the pituitary gland or acting indirectly, prevailing IGF-I concentrations and the mutual interrelations between these parameters.

METHODS

Patients

Ten previously operated and irradiated patients with a GH-secreting macroadenoma and 10 identically treated patients with a non-functioning pituitary macroadenoma (n=8) or an ACTH-producing microadenoma (n=2), matched for gender and age, were enrolled (pituitary adenoma control group: PT controls). The acromegalic patients were chosen from a cohort of clinically inactive acromegalic patients, previously (>10 years) treated by transsphenoidal surgery and, because of persisting postoperative GH excess by postoperatively conventional radiotherapy (40-45Gy). This cohort of acromegalic patients has previously been described extensively (7). The inclusion criterion was a subnormal GH response to the ITT (short-acting insulin 0.05-0.1 U/kg body weight, blood samples drawn at 0, 20, 30, 45, 60 and 90 min; glucose levels were required to drop below 2.2 mmol / l). The increase in GH concentrations was considered insufficient, when peak GH response was below 3 µg/l (10). The number of pituitary deficiencies other than that of GH were similar for both groups (P = 0.25). Three deficient anterior pituitary functions were established in 6 acromegalic patients and in 7 of the control patient group. Replacement treatment for secondary hypocortisolism was given to 7 acromegaly patients and to 9 control patients (NS), thyroid hormone therapy was given to 7 and 8 patients, respectively (NS), and treatment for secondary hypogonadism to 4 patients of each group (NS). Three of the 10 acromegalic patients and 4 of the 10 control patients used lipid-lowering drugs. None of the patients used dopamine agonists, but 2 of the patients in the PT control group used inhalation β_2-sympathicomimetics and 2 of the patients in the acromegalic group used β-adrenoreceptor blocking medication. The purpose, nature, and possible risks of the study were explained to all subjects and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.

Clinical Protocol

First the GH secretory reserve was assessed by three stimulation tests in addition to the ITT, and subsequently spontaneous GH secretion was measured during 24h with blood sampling intervals of 10 minutes.

The GH stimulation tests were carried out in random order on separate days (during a two-week period) in the fasting condition. The following tests were performed: the GHRH test (Ferring, Hoofddorp, The Netherlands: 1 µg/kg body weight by i.v. bolus injection, blood samples drawn at 0, 20, 30, 45, 60 and 90 min), the l-arginine infusion test (500 mg/kg body weight with a maximum of 30 g, infusion during 30 minutes, blood samples drawn at 0, 30, 45, 60, 90 and 120 min), and the combined GHRH-arginine test as an i.v. bolus injection of GHRH (1 µg/kg body weight) after which l-arginine (500 mg/kg body weight with a maximum of 30 g) was infused during 30 minutes, blood samples drawn at 0, 30, 45, 60, 90 and 120 min. The peak serum response of GH was used as the primary variable for analysis of stimulation tests.

For the 24h sampling study, the patients were admitted to the Clinical Research Center in the morning. An indwelling i.v. cannula was inserted in a forearm vein at least 60 min before sampling began. Blood samples were withdrawn at 10 min. intervals for 24h, starting at 09.00h. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to maintain patency of the i.v. catheter. The subjects were not allowed to sleep during the daytime. Meals were served at 09.00, 12.30 and 17.30h. Lights were turned off between 22.00-24.00h. Plasma samples for GH measurements were collected, centrifuged at 4°C for 7 minutes, and stored at –20°C until later analysis.

Assays

GH concentrations in the samples of the stimulation tests were measured by time resolved immunofluorometric assay (Wallac, Inc, Turku, Finland). Reference values, listed in the tables and main text were obtained with the same assay. Human biosynthetic GH (Pharmacia and Upjohn, Inc, Uppsala, Sweden) was used as standard, calibrated against WHO-IRP 80-505 and the detection limit of this GH assay is 0.01 µg/l with an interassay coefficient of variation of 1.6-8.4%, between 0.1 and 15 µg/l (1 µg/l = 2.6 mU/l). GH concentration in the serum samples of 24h profiles of the patients in this study were measured with the more sensitive automatic immunochemiluminescence assay (Nichols Diagnostics Institute, San Clemente, CA), using 22 kDa rhGH as standard. Cross reactivity with 20kDa GH was 30%. Assay sensitivity (defined as 3 SD above the zero dose level) was 0.005 µg/l. Median intra- and interassay coefficients of variation were 5.2 and 8.3%, respectively. GH concentration was measured in every sample in duplicate. All samples from a single subject were assayed together to eliminate interassay variability,

Serum IGF-I concentrations were determined by Immulite 2000 (DPC, Los Angeles, CA). The assay is calibrated with WHO 2nd International Standard 87/518. Sensitivity of the assay is 20 µg/l. The intra-assay precision is 2.6-4.3 % over the adult operating range. All serial samples in this study were run in the same assay.

Calculations and Statistics

Cluster analysis

For the detection of discrete GH peaks Cluster analysis was used (11). This computerized pulse algorithm is largely model-free, and identifies statistically significant pulses in relation to dose-dependent measurement error in the hormone time series. For the present analysis a 2x1 test cluster configuration was used, two data points for the test nadir and one for the test peak, and a t-statistic of 2.0 for the up- and down-strokes, which minimizes both false positive and false negative peaks. The locations and widths of all significant concentration peaks were identified, the total number of peaks was counted, and the mean peak interval was calculated in minutes. In addition, the following pulse parameters were determined: peak height (highest

value attained within the peak), incremental peak amplitude (the difference between peak height and pre-peak nadir), and area under the peak. Interpulse valleys were identified as regions embracing nadirs with no intervening up-strokes. The total area under the curve was also calculated, as well as the summed pulse areas.

Approximate Entropy

ApEn was used as a scale- and model-independent regularity statistic to quantitate the orderliness or regularity of serial GH serum concentrations over 24h. Normalized ApEn parameters of m = 1 (test range) and r = 20% (threshold) of the intra-series SD were used, as described previously (12). The ApEn metric evaluates the consistency of recurrent subordinate (nonpulsatile) patterns in successive data, and thus yields information distinct from and complimentary to cosinor and deconvolution (pulse) analyses (13). Higher absolute ApEn values denote greater relative randomness of hormone patterns; e.g. as observed for ACTH in Cushing's disease, GH in acromegaly, and PRL in prolactinomas (14-16). Normalized ApEn ratios of observed to 1000 randomly shuffled data series are reported.

Statistical analysis

Results are presented as the mean and 95% confidence interval, unless stated otherwise. Statistical analyses were carried out with the Kolmogorov-Smirnov test. Comparisons between the GH stimulation tests were carried out with General Linear Model (GLM) with appropriate post-hoc contrasts. Associations between variables were quantified by the Spearman's rho test. Statistical calculations were performed with Systat, version 11 (Systat Software, Inc, Richmond, CA) or SPSS version 11 (SPSS Inc, Chicago, Ill). $P<0.05$ was considered significant.

RESULTS

Patients

The age of the patients treated for acromegaly was 56 ± 12 years and of PT control group 62 ± 12 years (p=0.30, Table 1). Body mass index (BMI) of the acromegalic patients was 29.1 ± 2.9 kg/m^2 and 28.3 ± 4.5 kg/m^2 in the PT control group (p=0.66). Conventional radiation therapy with a 8 MeV linear accelerator, with a total tumor dose of 40-45 Gy and fractionated in at least 20 sessions, was given 17.0 ± 7.0 years prior to testing in the acromegalic group, and 18.7 ± 7.6 years in the PT control group (p=0.67).

Stimulation Tests

In Figure 1 the GH response to the 4 stimulation tests are displayed. The peak values reached during these tests are shown in Table 2. No significant differences in GH responses between the two groups were present for any test.

Table 6/1: Clinical characteristics of the patient groups

	Patients treated for acromegaly	Pituitary tumour control patients	P-value
Age (years)	56 (47-65)	62 (53-71)	0.30
Sex (males / females)	4/ 6	5/ 5	0.65
BMI (kg/m^2)	29.1 (27.1-31.2)	28.3 (25.1-31.5)	0.66
IGF-I (µg/l)	59 (36-82)	56 (41-71)	0.96
Interval between stimulation tests and radiotherapy (years)	17.0 (7-25)	18.7 (11-28)	0.67

Data are shown as the mean and the 95% confidence interval. Statistical comparisons were performed with the Kolmogorov-Smirnov test.

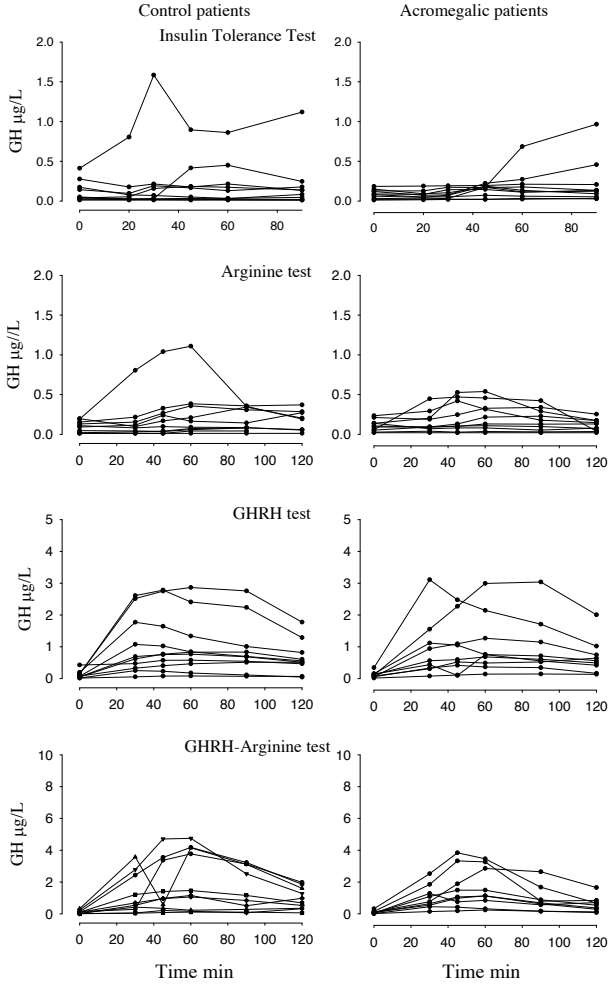

Figure 6/1: GH response to the ITT, arginine, GHRH and the combined GHRH-arginine tests.

Table 6/2: Peak growth hormone response during stimulation tests in patients with acromegaly and pituitary tumor control patients.

	Patients treated for acromegaly	PT control patients	P-value
Insulin tolerance test (µg/l)	0.18 (0.03-0.92)	0.18 (0.01-1.58)	1.00
GHRH test (µg/l)	0.75 (0.15-3.10)	0.80 (0.08-2.86)	0.96
GHRH-arginine test (µg/l)	1.28 (0.24-3.80)	1.30 (0.11-4.75)	0.77
Arginine test (µg/l)	0.28 (0.02-1.36)	0.19 (0.01-1.10)	0.66

Data are shown as median and data limits in parentheses. Statistical comparisons were performed with the Kolmogorov-Smirnov test. Reference values in healthy adults for our laboratory are ITT: > 3 µg/l, GHRH > 3 µg/l; arginine test > 2.9 µg/l, GHRH-arginine test > 8 µg/l.

However, the figure clearly demonstrates the differences in magnitude of GH responses between the tests. Indeed, the univariate ANOVA of the GH peak values applied to the combined patient groups was highly significant ($P < 0.001$). Post-hoc analyses revealed that the GH response to the insulin tolerance test was not different from the arginine test (p=0.39). The GH response in the GHRH and the combined GHRH-arginine tests were significantly higher than in the ITT (p<0.001, Figure 1). The GH response to insulin correlated significantly with that to the combined GHRH-arginine test (R=0.64, p=0.003), and arginine alone (R=0.63, p=0.003), but not with the GH response to GHRH (R=0.42, p=0.07).

Mean IGF-I concentration was 56 µg/l (95% confidence interval 41-71 µg/l) in control patients, and 59 µg /L (95% confidence interval 36-82 µg/l) in acromegalic patients (p=0.96). No relevant correlations were found within the limited range of IGF-I values. IGF-I was below -2 SD-score in 9 patients in each group.

Twenty four hour GH profiles

In Figure 2 representative 24h GH profiles of two acromegalic patients and two controls are shown. The results of the Cluster analysis are listed in Table 3. It should be noted that 2 acromegalic patients and 3 PT control patients had no statistically significant GH pulses, although their GH levels were detectable. For the remaining patients no differences could be demonstrated with respect to integrated area, mean GH concentration, mean pulse height, mean pulse area (mass) and nadir concentration.

The integrated area, reflecting 24h GH secretion correlated with the peak GH of the combined GHRH-arginine test (R=0.78, p=0.001), and also with that of the ITT (R=0.54, p=0.036), but not with the other 2 stimulation tests.

Approximate entropy (ApEn (1, 20%) ratio) was not different between acromegalic patients and PT controls (mean 0.76; 95% confidence interval 0.70 – 0.81 and 0.69; 95 % confidence interval 0.57-0.81, respectively, p=0.25). Reference value for these patients groups is 0.41, 95% confidence interval 0.36 – 0.45.

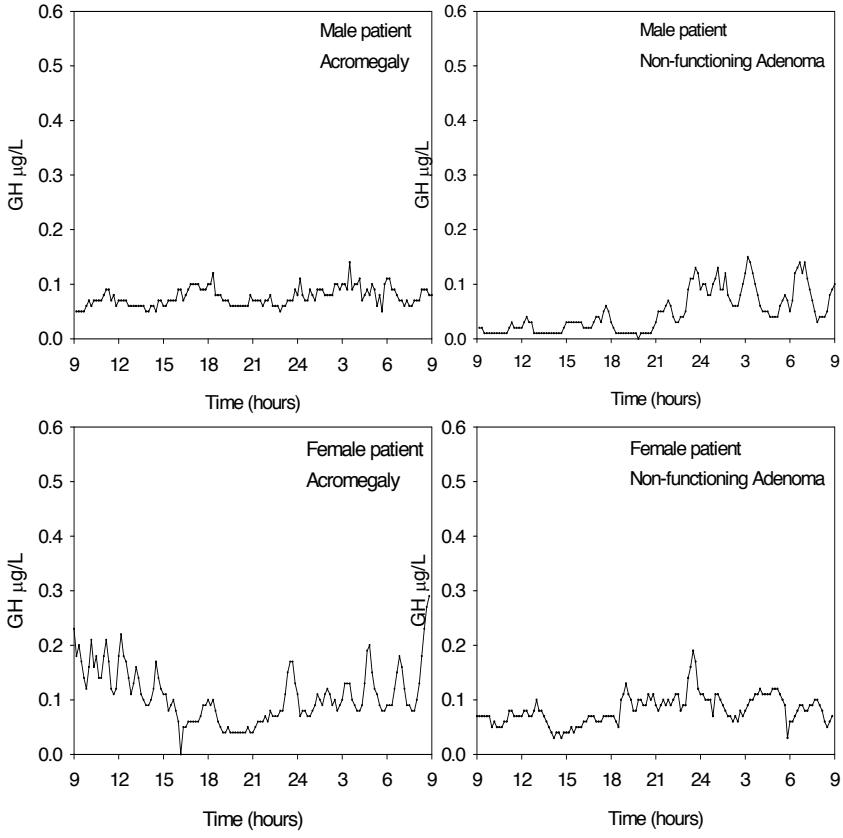

Figure 6/2: Representative 24h GH profiles of two acromegalic patients and two PT control patients

Table 6/3: Cluster analysis of the 24h serum GH profiles in patients with acromegaly and pituitary tumor control patients.

	Patients treated for acromegaly	Pituitary adenoma control patients	P-value
Mean 24h GH concentration (µg/l)	0.12 (0.07-0.16)	0.20 (0.01-0.40)	0.82
Integrated area (µg/l/min)	166 (99-230)	300 (28-590)	0.82
Number of GH pulses/ 24h	15 (11-19)	11 (9-14)	0.37
Mean pulse interval (min)	92 (65-120)	102 (75-130)	0.76
Mean pulse amplitude (µg/l)	0.15 (0.10-0.19)	0.33 (0.02-0.65)	0.51
Mean pulse area (µg/l/min)	2.20 (0.70-3.70)	9.80 (0-21.0)	0.16
Valley mean (µg/l)	0.10 (0.06-0.14)	0.17 (0.05-0.31)	0.87
Nadir (µg/l)	0.09 (0.06-0.13)	0.15 (0.03-0.27)	0.87

Data are given as the mean and 95 % confidence interval. Statistical comparisons were performed with the Kolmogorov-Smirnov test. Reference values for a comparable group of healthy controls are (mean, and 95% CI): 24h mean GH concentration 0.60 µg/l (0.39-0.80), integrated area 850 µg/l/min (550-1165), number of GH pulses/ 24h 9 (8-11), mean pulse interval 155 min (125-185), mean pulse amplitude 1.40µg/l (0.78-2.05), mean pulse area 70 µg/l/min (35-100), valley mean 0.50 µg/l (0.1 -0.90), nadir 0.30 (0.10-0.50).

DISCUSSION

In this study we compared the characteristics of GH secretion between GH deficient acromegalic patients and GH deficient patients with other pituitary adenomas after long-term follow up of postoperative radiotherapy. We found that patients treated by transsphenoidal surgery and additional radiotherapy for acromegaly with an impaired GH response to ITT did not differ with regard to stimulated and spontaneous GH secretion from patients treated analogously for other pituitary adenomas, who had impaired GH response to ITT.

Hypopituitarism is a well-recognized sequel of radiotherapy for pituitary tumors and GH secretion is usually the first hormone affected (9). The ITT is an effective test to define GH deficiency (10), since responses reflect the functional integrity of the hypothalamic-pituitary-GH axis (17;18). The hypothalamus may be more vulnerable to radiation-induced damage than the pituitary gland, since the pituitary remains responsive to hypothalamic releasing- hormones after radiation (19). GH deficiency after radiotherapy for pituitary tumors may therefore occur due to failure of synthesis and/or delivery of endogenous GHRH (or other putative GH-releasing substances, e.g. hypothalamic ghrelin) to the pituitary (20;21). One could hypothesize that the function of the hypothalamic-pituitary-GH axis in acromegalic patients treated by postoperative radiotherapy, as assessed by the ITT, is impaired due to surgical and radiotherapeutical intervention, whereas tumoral activity may persist, thus preventing (temporarily) the emergence of GH deficiency.

GHRH and combined GHRH-arginine infusions resulted in significantly higher GH peak responses than the ITT in both patient groups. This observation is consistent with results obtained by Aimaretti et al. in hypopituitarism due to various etiologies (22). The generally accepted explanation for this difference in the magnitude of the GH responses is that the GHRH-arginine test combines the somatostatin-suppressing effect of arginine (23) with direct stimulation of the somatotroph cell by exogenous GHRH (24), whereas the ITT requires endogenous GHRH (25). Our finding that the GHRH test alone resulted in a higher GH response compared to the ITT (in both groups of patients) might point to hypothalamic dysfunction with diminished endogenous drive to the pituitary. These observations are also in line with the study by Murray et al., in which they investigated patients treated for acromegaly with the arginine test and the GH secretagogue hexarelin (26). They reported loss of response to the arginine test in patients treated by radiotherapy, although the response to the GH secretagogue was retained in about 50% of these patients (26).

The diagnosis of GHD in adults is established by provocative testing, since IGF-I concentrations and mean 24h GH concentrations overlap in adults considered GH deficient (i.e. due to extensive pituitary disease) and healthy subjects (27). The combined GHRH-arginine test is also considered to be a reliable test to detect GHD (28), provided that appropriate cut-off limits related to BMI are defined (29). However, the GH response to arginine alone is found to be less sensitive to the effects of radiotherapy than the GH response to ITT (30). In accordance with

these inferences, GH responses to arginine and insulin in the present cohorts were identical, although greatly diminished, and comparable to those in patients who received cranial irradiation for non-pituitary diseases (30).

There was a moderately positive correlation between peak GH responses to ITT and to the combined GHRH-arginine test and a strongly positive correlation between the peak response to the ITT and arginine. In addition, the peak response to ITT and to combined GHRH-arginine correlated with spontaneous 24h GH secretion. Therefore, from a practical clinical vantage the investigation of GH reserve capacity in acromegalic patients with cardiovascular disease may be equally well explored with the combined test.

GH secretion in active acromegaly is characterized by increased apparent pulse frequency, burst mass and basal (nonpulsatile) secretion (15; 31-32). In contrast, GH burst mass is decreased profoundly in GH deficiency and total 24h secretion is diminished notwithstanding increased pulse frequency (33). In the present study, treated patients with somatotropinomas and other pituitary adenomas clearly fulfilled the criterion of GH deficiency. A remarkable outcome is that there were no differences in mean 24h GH concentration, number of GH pulses per 24h, pulse amplitude or area between the two groups. The spectrum of GH release extended from complete absence of statistically significant GH pulses with low basal concentrations, as observed in 5 patients, to persisting, low amplitude pulsatility (as illustrated in the figures). It is presently unclear, whether residual GH output in treated acromegalic patients is derived from normal somatotrope cells or from tumor remnants.

Peacey et al. described the relationship between 24h GH secretion profiles and IGF-I in cured acromegalic patients (defined as GH levels below 2 µg/l during an oral glucose tolerance test or a GH profile) (34). In that study elevated mean IGF-I concentrations in acromegalic patients treated by radiotherapy compared with healthy controls were inferred to reflect persisting differences in 24h GH secretion (34). The current data extend insights to two more severely compromised groups, in which irrespective of the underlying disorder, IGF-I concentrations were below -2 standard deviations in all but 1 subject and uncorrelated with GH secretory parameters.

Approximate entropy (ApEn), a tool to quantitate the orderliness or regularity of serial GH serum concentrations, did not differ between patients treated for acromegaly and those treated for other pituitary tumors. However, when compared with values reported in normal healthy subjects, ApEn was almost twofold elevated, indicating disorganized GH secretion (5). Elevated ApEn of GH points to increased feed-forward by GHRH or tumoral cells or decreased feedback via somatostatin, GH and IGF-I signaling (35). In active acromegaly disorganized GH secretion is likely attributed to the tumor per se, akin to that of prolactinomas and ACTH-secreting adenomas (14;16). The anatomical substrate for the disorganized secretion might be defective or autonomous cell-cell interactions in the adenoma (36;37). In hypopituitarism of various etiologies, excluding acromegaly, GH secretion is also profoundly irregular and not correlated with pituitary irradiation (33). Such secretory alterations might be attributed to a diminished

somatostatin and/ or GH/IGF-I feedback. Histopathological and controlled feedback studies are required together to elucidate the precise mechanism(s) involved.

We chose to study acromegalic patients with an impaired response to ITT treated by radiotherapy and to compare these patients with those treated likewise without a previous history of acromegaly. The latter group fulfilled the criteria of GH deficiency. Since no differences in spontaneous and stimulated GH secretion between the two groups were found, it is reasonable to conclude that the acromegalic patients became GH-deficient many years after radiation. Another study has suggested that the prevalence of GHD is high in patients treated by surgery alone (38). Direct comparison of those 2 groups would be of interest.

In conclusion, acromegalic patients treated by surgery and postoperative radiotherapy with an impaired response to ITT do not differ in the long-term in GH secretory characteristics from patients treated similarly for other pituitary tumors with an impaired response to ITT. The ITT (or the GHRH-arginine test) is therefore reliable in establishing the diagnosis of GHD in patients treated for acromegaly by surgery and radiotherapy. Irregular GH secretion, low amplitude GH pulses, and reduced IGF-I concentrations thus constitute a final common outcome of combined surgery and radiation treatment of pituitary adenomas.

REFERENCE LIST

1. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
2. Beauregard C, Truong U, Hardy J, Serri O 2003 Long-term outcome and mortality after transsphenoidal adenomectomy for acromegaly. Clin Endocrinol (Oxf) 58:86-91
3. Biermasz NR, van Dulken H, Roelfsema F 2000 Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 85:4596-4602
4. Freda PU, Wardlaw SL, Post KD 1998 Long-term endocrinological follow-up evaluation in 115 patients who underwent transsphenoidal surgery for acromegaly. J Neurosurg 89:353-358
5. van den Berg G, Pincus SM, Frolich M, Veldhuis JD, Roelfsema F 1998 Reduced disorderliness of growth hormone release in biochemically inactive acromegaly after pituitary surgery. Eur J Endocrinol 138:164-169
6. Biermasz NR, Dulken HV, Roelfsema F 2000 Postoperative radiotherapy in acromegaly is effective in reducing GH concentration to safe levels. Clin Endocrinol (Oxf) 53:321-327
7. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
8. Eastman RC, Gorden P, Glatstein E, Roth J 1992 Radiation therapy of acromegaly. Endocrinol Metab Clin North Am 21:693-712
9. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
10. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
11. Veldhuis JD, Johnson ML 1986 Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. Am J Physiol 250:E486-E493
12. Pincus SM, Keefe DL 1992 Quantification of hormone pulsatility via an approximate entropy algorithm. Am J Physiol 262:E741-E754
13. Veldhuis JD, Pincus SM 1998 Orderliness of hormone release patterns: a complementary measure to conventional pulsatile and circadian analyses. Eur J Endocrinol 138:358-362
14. Groote Veldman R, van den Berg BG, Pincus SM, Frolich M, Veldhuis JD, Roelfsema F 1999 Increased episodic release and disorderliness of prolactin secretion in both micro- and macroprolactinomas. Eur J Endocrinol 140:192-200
15. Hartman ML, Veldhuis JD, Vance ML, Faria AC, Furlanetto RW, Thorner MO 1990 Somatotropin pulse frequency and basal concentrations are increased in acromegaly and are reduced by successful therapy. J Clin Endocrinol Metab 70:1375-1384
16. van den Berg G, Pincus SM, Veldhuis JD, Frolich M, Roelfsema F 1997 Greater disorderliness of ACTH and cortisol release accompanies pituitary-dependent Cushing's disease. Eur J Endocrinol 136:394-400
17. Hanew K, Utsumi A 2002 The role of endogenous GHRH in arginine-, insulin-, clonidine- and l-dopa-induced GH release in normal subjects. Eur J Endocrinol 146:197-202
18. Masuda A, Shibasaki T, Hotta M, Yamauchi N, Ling N, Demura H, Shizume K 1990 Insulin-induced hypoglycemia, L-dopa and arginine stimulate GH secretion through different mechanisms in man. Regul Pept 31:53-64
19. Toogood AA 2004 Endocrine consequences of brain irradiation. Growth Horm IGF Res 14 Suppl A:S1-18-S124
20. Ahmed SR, Shalet SM 1984 Hypothalamic growth hormone releasing factor deficiency following cranial irradiation. Clin Endocrinol (Oxf) 21:483-488
21. Blacklay A, Grossman A, Ross RJ, Savage MO, Davies PS, Plowman PN, Coy DH, Besser GM 1986 Cranial irradiation for cerebral and nasopharyngeal tumours in children: evidence for the production of a hypothalamic defect in growth hormone release. J Endocrinol 108:25-29

22. Aimaretti G, Corneli G, Razzore P, Bellone S, Baffoni C, Arvat E, Camanni F, Ghigo E 1998 Comparison between insulin-induced hypoglycemia and growth hormone (GH)-releasing hormone + arginine as provocative tests for the diagnosis of GH deficiency in adults. J Clin Endocrinol Metab 83:1615-1618
23. Alba-Roth J, Muller OA, Schopohl J, von Werder K 1988 Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 67:1186-1189
24. Grossman A, Savage MO, Lytras N, Preece MA, Sueiras-Diaz J, Coy DH, Rees LH, Besser GM 1984 Responses to analogues of growth hormone-releasing hormone in normal subjects, and in growth-hormone deficient children and young adults. Clin Endocrinol (Oxf) 21:321-330
25. Jaffe CA, DeMott-Friberg R, Barkan AL 1996 Endogenous growth hormone (GH)-releasing hormone is required for GH responses to pharmacological stimuli. J Clin Invest 97:934-940
26. Murray RD, Peacey SR, Rahim A, Toogood AA, Thorner MO, Shalet SM 2001 The diagnosis of growth hormone deficiency (GHD) in successfully treated acromegalic patients. Clin Endocrinol (Oxf) 54:37-44
27. Hoffman DM, O'Sullivan AJ, Baxter RC, Ho KK 1994 Diagnosis of growth-hormone deficiency in adults. Lancet 343:1064-1068
28. Valetto MR, Bellone J, Baffoni C, Savio P, Aimaretti G, Gianotti L, Arvat E, Camanni F, Ghigo E 1996 Reproducibility of the growth hormone response to stimulation with growth hormone-releasing hormone plus arginine during lifespan. Eur J Endocrinol 135:568-572
29. Corneli G, Di Somma C, Baldelli R, Rovere S, Gasco V, Croce CG, Grottoli S, Maccario M, Colao A, Lombardi G, Ghigo E, Camanni F, Aimaretti G 2005 The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. Eur J Endocrinol 153:257-264
30. Lissett CA, Saleem S, Rahim A, Brennan BM, Shalet SM 2001 The impact of irradiation on growth hormone responsiveness to provocative agents is stimulus dependent: results in 161 individuals with radiation damage to the somatotropic axis. J Clin Endocrinol Metab 86:663-668
31. Barkan AL, Stred SE, Reno K, Markovs M, Hopwood NJ, Kelch RP, Beitins IZ 1989 Increased growth hormone pulse frequency in acromegaly. J Clin Endocrinol Metab 69:1225-1233
32. van den Berg G, Frolich M, Veldhuis JD, Roelfsema F 1994 Growth hormone secretion in recently operated acromegalic patients. J Clin Endocrinol Metab 79:1706-1715
33. Roelfsema F, Biermasz NR, Veldhuis JD 2002 Pulsatile, nyctohemeral and entropic characteristics of GH secretion in adult GH-deficient patients: selectively decreased pulsatile release and increased secretory disorderliness with preservation of diurnal timing and gender distinctions. Clin Endocrinol (Oxf) 56:79-87
34. Peacey SR, Toogood AA, Veldhuis JD, Thorner MO, Shalet SM 2001 The relationship between 24-hour growth hormone secretion and insulin-like growth factor I in patients with successfully treated acromegaly: impact of surgery or radiotherapy. J Clin Endocrinol Metab 86:259-266
35. Veldhuis JD, Straume M, Iranmanesh A, Mulligan T, Jaffe C, Barkan A, Johnson ML, Pincus S 2001 Secretory process regularity monitors neuroendocrine feedback and feedforward signaling strength in humans. Am J Physiol Regul Integr Comp Physiol 280:R721-R729
36. Fauquier T, Guerineau NC, McKinney RA, Bauer K, Mollard P 2001 Folliculostellate cell network: a route for long-distance communication in the anterior pituitary. Proc Natl Acad Sci U S A 98:8891-8896
37. Mollard P, Functional Cell Networks in the Pituitary Gland; The Endocrine Society's 87th Annual Meeting San Diego; S35-2.
38. Conceicao FL, Fisker S, Andersen M, Kaal A, Jorgensen JO, Vaisman M, Christiansen JS 2003 Evaluation of growth hormone stimulation tests in cured acromegalic patients. Growth Horm IGF Res 13:347-352

Chapter 7

Cardiac Manifestations of Growth Hormone Deficiency after Treatment for Acromegaly: a Comparison to Patients with Biochemical Remission and Controls

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European Journal of Endocrinology, 2008

ABSTRACT

Objective

Both growth hormone (GH) excess and GH deficiency (GHD) lead to specific cardiac pathology. The aim of this study was to evaluate cardiac morphology and function in patients with GHD after treatment for acromegaly.

Design

Cross-sectional study.

Patients and methods

Cardiac parameters were studied by conventional two-dimensional echocardiography and Tissue Doppler imaging in 53 patients with acromegaly (16 patients with GHD, 20 patients with biochemical remission, and 17 patients with active disease). Patients with GHD were also compared to age- and gender-matched controls.

Results

Left ventricular (LV) dimensions, wall thickness, and mass did not differ between the three groups, or between the patients with GHD and healthy controls. Systolic function, assessed by LV ejection fraction, tended to be lower in patients with GHD compared to patients with biochemical remission (65.9 ± 7.3 % vs. 72.4 ± 8.5 %, p=0.070), but was higher when compared to active acromegaly (58.8 ± 9.3 %, p=0.047). No differences were found with healthy controls. Diastolic function, measured with early diastolic velocity (E'), was lower in patients with GHD both when compared to patients with biochemical remission (6.0 ± 2.1 cm/s vs. 8.3 ± 1.5 cm/s, p=0.005) and to healthy controls (8.1 ± 1.9 cm/s, p=0.006).

Conclusion

GHD after acromegaly results in specific decrease in diastolic function compared to patients with biochemical remission of acromegaly and healthy controls. In addition, systolic function tends to be decreased in patients with GHD compared to patients with biochemical remission, but was higher than in patients with active acromegaly.

INTRODUCTION

Acromegaly is associated with increased cardiovascular morbidity and mortality (1). Active disease leads to specific cardiac pathology, which involves the myocardium, the conduction system, and the valves (1). As a consequence, clinical manifestations include biventricular concentric hypertrophy, left ventricular (LV) systolic and diastolic dysfunction, arrhythmias, and valvular regurgitation.

Treatment of growth hormone (GH) excess can normalize mortality (2) and reverse heart failure and myocardial hypertrophy (3;4). However, surgical treatment of GH excess followed by radiotherapy can result in growth hormone deficiency (GHD) (5) and GHD per se is also associated with cardiomyopathy. Cardiac manifestations of GHD include a decrease in left ventricular mass and left ventricular ejection fraction (6-12), which is correlated to the severity of GHD (8). Additionally, impairment in diastolic function has also been observed in patients with GHD (13). Therefore, GHD after successful treatment of acromegaly may be another part of the spectrum of cardiac manifestations of acromegaly.

However, it is presently unknown if, and to what extent, the heart can adapt to prolonged, sequential exposure to GH excess and GHD. Therefore, the aim of this study was to make a detailed assessment of cardiac function and morphology in patients with GHD after treatment for acromegaly, and to compare these data to those obtained in patients with biochemical remission of acromegaly and patients with active acromegaly.

PATIENTS AND METHODS

Patients

We studied 16 patients with GHD after successful treatment of acromegaly (8 men) with a mean age of 56 ± 12 yrs. We compared the parameters of these patients to patients with active acromegaly and patients in biochemical remission of acromegaly, which were previously reported in studies that assessed the prevalence of valvular regurgitation (14) and diastolic dysfunction in acromegaly (15). Since there could be residual cardiac manifestations of previous GH excess in patients in biochemical remission from acromegaly, we also compared the patients with GHD after successful treatment of acromegaly to healthy controls.

Inclusion criteria were:

1. GHD after treatment for acromegaly (n=16): defined as a subnormal GH response to the insulin tolerance test (short-acting insulin 0.05-0.1 U/kg body weight s.c., blood samples drawn at 0, 20, 30, 45, 60 and 90 min; nadir glucose levels were all below 2.2 mmol/l). The increase in GH concentrations was considered insufficient, if peak GH response was below 3 µg/l (5;16). Previous treatment of these patients consisted of surgery and radiotherapy (n=15), or surgery

only (n=1). Radiotherapy was applied 17.9 yrs (range 4 to 29 yrs) prior to inclusion in the present study. Patients were studied just before the start of rhGH replacement.

2. Active acromegaly (n=17): defined as mean fasting GH concentrations (measured every 30 minutes for 3 hours) >2.5 µg/L, and elevated age- and gender-adjusted IGF-I concentrations. These patients consisted of two groups: 1) untreated acromegaly (n=8): no treatment to reduce GH excess had yet been instituted; 2) uncontrolled acromegaly (n=9): elevated mean plasma GH and IGF-I concentrations despite maximal dosages of depot octreotide acetate (30 mg i.m. every 3 weeks).

3. Biochemical remission of acromegaly (n=20): defined as mean fasting GH concentrations (measured for 3 hours with an interval of 30 minutes) <2.5 µg/L, and normal age- and gender-adjusted IGF-I concentrations. These patients consisted of two groups: 1) well-controlled acromegaly (n=14): biochemical control of GH excess during treatment with somatostatin analogs; 2) cured acromegaly (n=6): no GH excess after surgery only (n=5) or primary radiotherapy (n=1).

4. Healthy controls (n=16): the patients with GHD after acromegaly were compared to 16 healthy age-, body surface area and sex-matched controls. The controls were selected from a database with patients referred to the department of Cardiology, based on age, sex, and body surface area. Controls were excluded when referred for echocardiographic evaluation of known valvular heart disease, murmur, congestive heart failure, and cardiac transplantation. Other exclusion criteria were myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, connective tissue disease, carcinoid syndrome, or use of anorectic drugs. We and others have previously demonstrated that recruitment of controls from a large database can also be used as representative controls (14;17).

None of the patients had hemodynamic instability, previous myocardial infarction, thyreotoxicosis, rheumatic fever, endocarditis, or connective tissue disease. The medical ethics committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Echocardiography

Echocardiography was performed while the patients were in the left lateral decubitus position using a commercially available system (Vingmed Vivid-7, General Electric – Vingmed, Milwaukee, WI, USA). Standard parasternal (long- and short-axis) and apical views (2-, and 4-, and long-axis) were obtained.

M-mode images were obtained from the parasternal long-axis views for quantitative assessment of LV dimensions (Inter-Ventricular Septum Thickness (IVST), Posterior Wall Thickness (PWT), LV End-Diastolic Diameter (LVEDD), LV End-Systolic Diameter (LVESD), Fractional Shortening (FS) and LV Ejection Fraction (LVEF) (18).

The following parameters of diastolic function were obtained: diastolic transmitral peak velocities (E and A wave) and the E/A ratio. Quantitative diastolic data were derived from tissue

Doppler imaging (TDI). For TDI analysis, the digital cineloops were analyzed using commercial software (Echopac 6.1; General Electric-Vingmed). The sample volume (4 mm^2) was placed in the LV basal portion of the septum (using the 4-chamber views). The following parameters (mean values calculated from 3 consecutive heartbeats) were derived: early diastolic velocity (E'), late diastolic velocity (A') and the E'/A' ratio.

The severity of valvular regurgitation was assessed by 2 independent expert readers blinded to the clinical data on a qualitative scale of trace, mild, moderate, or severe, using previously described methods (19;20).

LV mass (LVM) was calculated by the cube formula, and using the correction formula proposed by Devereux, et al. (21): 0.8 x {1.04 [(LVEDD + PWT + IVST)^3 - LVEDD^3]}+ 0.6. LVM indexation (LVMi) was corrected for body height (22). LV hypertrophy (LVH) was defined as LVMi exceeding 49.2 g/$m^{2.7}$ for men and 46.7 g/$m^{2.7}$ for women (22).

Assays

GH concentrations were quantitated using a sensitive time-resolved immunofluorescent assay (Wallac Oy, Turku, Finland), specific for 22 kDa GH protein. The detection limit was 0.012 µg/L. Inter-assay coefficients of variation were 8.4-1.6% in the GH-range 0.1-18 µg/L (1 µg/l = 2.6 mU/l). 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 coefficients of variation were less than 11%. The detection limit was 1.5 nmol/l. Age- and gender-adjusted IGF-I data were determined in the same laboratory. IGF-1 was expressed as a standard deviation (SD) score for age- and gender-related normal levels, using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals (23;24).

A Hitachi 800 autoanalyzer (Roche) was used to quantify serum concentrations of glucose, TC, and TG. HDL cholesterol was measured with a homogenous enzymatic assay (Hitachi 911, Roche). LDL concentrations were calculated using the Friedewald formula. Unfortunately, lipid concentrations at the time of echocardiography were only available in 9 out of 17 patients with active acromegaly.

Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are expressed as the mean ± standard deviation (SD), unless specified otherwise. ANOVA analysis with Tukey HSD correction for multiple comparisons was used to compare patients with GHD after acromegaly to patients with biochemical remission and to patients with active acromegaly. We checked all comparisons after log-transformation of the variables. Results were also checked after adjustment for age by ANCOVA. Independent samples T-tests and chi-square tests were used to compare patients with GHD after acromegaly and healthy controls. In addition, regression analysis was performed with systolic and diastolic

function as dependent variables and age, BMI, IGF-I SD scores, hypertension and LVH as independent variables to identify predictors of cardiac function in patients with acromegaly. A P-value <0.05 was considered to represent a significant difference.

RESULTS

Clinical characteristics

Age and gender were not different between the 3 patient groups (Table 1). GH concentrations and IGF-I SD scores were lower in the patients with GHD after acromegaly compared to patients with active acromegaly (p<0.001 and p<0.001, resp.). GH and IGF-I concentrations did not differ between patients with GHD after acromegaly and patients in biochemical remission (p=0.839 and p=0.195, resp.). In patients with GHD after acromegaly, the interval between diagnosis of

Table 7/1: Clinical characteristics of patients with growth hormone deficiency after acromegaly compared to patients with biochemical remission of acromegaly and patients with active acromegaly and healthy controls.

	GHD after acromegaly (n=16)	Biochemical remission of acromegaly (n=20)	Active acromegaly (n=17)	Healthy controls (n=16)#
Age (yrs)	56 ± 12	57 ± 13	54 ± 16	56 ± 6
Gender (male/ female (%))	50/50	50/50	53/47	50/50
BMI (kg/m^2)	30.2 ± 4.5*	26.8 ± 4.1	28.7 ± 3.8	
GH (mU/l)	0.6 ± 0.4**	2.4 ± 0.5	18.7 ± 17.8	
IGF-I (SD scores)	-0.7 ± 1.7**	1.1 ± 1.7	9.1 ± 5.1	
Total cholesterol (mmol/l)	6.1 ± 1.0	5.3 ± 1.1	5.3 ± 1.1	
LDL cholesterol (mmol/l)	4.2 ± 0.9	3.7 ± 1.0	3.7 ± 0.8	
HDL cholesterol (mmol/l)	1.4 ± 0.5	1.8 ± 1.0	1.6 ± 0.4	
Triglycerides (mmol/l)	2.1 ± 1.3	1.3 ± 0.5	1.3 ± 0.4	
Surgery (%)	100	75	29	
Radiotherapy (%)	94	15	12	
Somatostatin analogs (%)	NA	70	53	
No treatment yet (%)	NA	NA	47	

#Healthy controls were age-, gender- and BSA matched to the patients with GHD after acromegaly.

*P<0.05 compared to patients with biochemical remission of acromegaly in an ANOVA with Tukey HSD post-hoc comparison.

**P<0.05 compared to patients with active acromegaly in an ANOVA with Tukey HSD post-hoc comparison.

GHD and this study was 3.2 ± 0.9 years. In addition, the interval between biochemical remission and the ITT was 12.8 ± 7.0 years. The interval between diagnosis and remission was 4.1 ± 5.1 years.

In patients with biochemical remission the interval between disease remission and this study was 6.4 ± 4.9 yrs, between diagnosis and remission 3.8 ± 4.5 yrs.

In patients with active acromegaly, the estimated disease duration was 14.5 ± 10.8 yrs. The number of patients with hypertension in the active disease group was (n=7, 41%), in the biochemical remission group (n=3, 15%) and GHD (n=6, 38%, overall P-value 0.168). None of the patients with GHD after acromegaly compared to two patients with active acromegaly (12%) and 2 with biochemical remission (10%) suffered from diabetes mellitus (overall P-value=0.384). Two of the patients with GHD after acromegaly (13%) compared to 1 with active acromegaly (5%) and 1 with biochemical remission (5%) used lipid lowering drugs (overall P-value=0.665).

Table 7/2: Left ventricular dimensions, systolic function, and diastolic function in patients with growth hormone deficiency (GHD) after acromegaly compared to patients with biochemical remission after acromegaly and patients with active acromegaly.

	GHD after acromegaly (n=16)	Biochemical remission acromegaly (n=20)	P-value*	Active acromegaly (n=17)	P-value*
LVEDD (mm)	51.6 ± 6.1	53.3 ± 6.7	NS	54.1 ± 10.2	NS
LVESD (mm)	33.5 ± 5.0	34.2 ± 6.6	NS	37.5 ± 10.5	NS
IVST (mm)	12.3 ± 3.5	10.2 ± 2.4	NS	13.5 ± 3.9	NS
PWT (mm)	10.3 ± 1.8	9.7 ± 1.7	NS	10.7 ± 2.3	NS
FS (%)	36.8 ± 5.8	36.9 ± 5.8	NS	30.4 ± 7.3	0.014
LVEF (%)	65.9 ± 7.3	72.4 ± 8.5	0.070	58.8 ± 9.3	0.047
E (mm/s)	50.9 ± 11.7	56.0 ± 15.0	NS	56.0 ± 16.2	NS
A (mm/s)	59.6 ± 17.7	56.3 ± 15.4	NS	63.6 ± 16.0	NS
E/A ratio	0.9 ± 0.2	1.0 ± 0.5	NS	0.92 ± 0.38	NS
E′ (cm/s)	6.0 ± 2.1	8.3 ± 1.5	0.005	6.0 ± 2.4	NS
A′ (cm/s)	7.4 ± 1.8	7.8 ± 1.8	NS	8.1 ± 2.9	NS
E′/A′ ratio	0.8 ± 0.4	1.1 ± 0.4	0.079	0.77 ± 0.26	NS
LVM (g)	235.2 ± 68.4	211.8 ± 84.5	NS	289.8 ± 158.3	NS
LVMi (g/m$^{2.7}$)	50.9 ± 15.3	45.8 ± 18.1	NS	65.8 ± 38.4	NS
LVH (%)	50%	30%	NS**	71	NS**

LVEDD: Left Ventricular End-Diastolic Diameter; LVESD: Left Ventricular End-Systolic Diameter, FS: Fractional shortening, LVEF: Left Ventricular Ejection Fraction, E: E wave (early filling phase), A: A wave (atrial contraction), E′: Tissue Doppler E wave, A′: Tissue Doppler A wave, IVST: Inter-Ventricular Septum Thickness, PWT: Posterior Wall Thickness, LVMi: Left Ventricular Mass Index, LVH: Left Ventricular Hypertrophy.

* ANOVA analysis with Tukey HSD correction for multiple comparisons was used to compare patients with GHD after acromegaly to patients with biochemical remission and to patients with active acromegaly.

**Chi-square test.

ACTH deficiency was present and substituted in 9 patients with GHD after acromegaly (56%), 3 patients with biochemical remission (15%) and 2 patients with active acromegaly (5%). TSH deficiency was present and substituted in 5 patients with GHD after acromegaly (31%), 1 patient with biochemical remission (5%) and 1 patient with active acromegaly (6%). Three male patients and 2 female patients with GHD after acromegaly were treated with testosterone and estrogen substitution, respectively. Three male patients with biochemical remission of acromegaly and 3 male patients with active acromegaly were treated with testosterone substitution. None of the female patients with biochemical remission or active acromegaly needed estrogen substitution.

GHD after acromegaly compared to biochemical remission of acromegaly

Left ventricular size and mass

LV size (LVESD, LVEDD), wall thickness (IVST, PWT), and mass (LVM, LVMi) did not differ between the 2 groups (Table 2). LVH (defined as LVMi exceeding 49.2 g/m$^{2.7}$ for men and 46.7 g/m$^{2.7}$ for women (22)) was present in 50% of patients with GHD after acromegaly compared to 30% of patients with biochemical remission of acromegaly (p=0.226). These results were confirmed after adjustment for age.

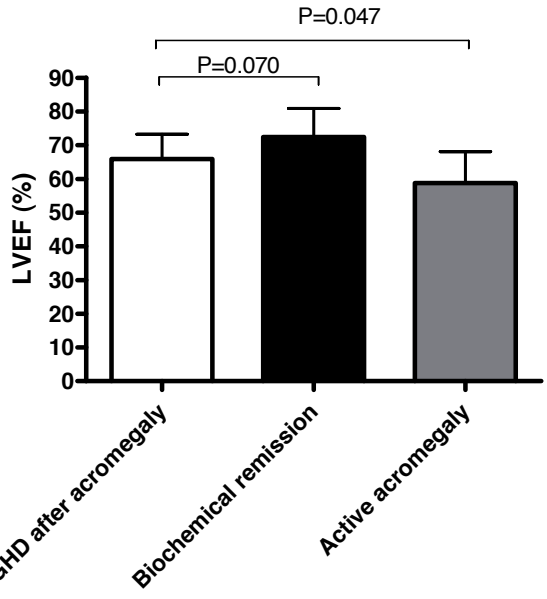

Figure 7/1: Systolic function, reflected by left ventricular ejection fraction (LVEF), was lower in patients with GHD acromegaly compared to patients with biochemical remission of acromegaly and increased compared with patients active acromegaly.

Left ventricular systolic function

FS did not differ between the two groups. LVEF tended to be lower in patients with GHD after acromegaly compared to patients with biochemical remission (p=0.070, Figure 1). These results were not affected after adjustment for age (p=0.030).

Left ventricular diastolic function

No differences were noted in diastolic parameters (E and A wave velocities, E/A ratio) between the 2 groups. Additional data on diastolic function, as assessed by TDI, revealed that E′ was lower in patients with GHD after acromegaly compared to patients with biochemical remission (p=0.005, Figure 2). Accordingly, E′/ A′ ratio tended to be decreased (p=0.079). These results were even more marked after adjustment for age (p=0.001 and p=0.018 for the E′ and E′/A′ ratio, respectively).

Heart valves

Mitral regurgitation was absent in 81% of patients with GHD, whereas 13% had trace, and 6% mild regurgitation, compared to 70%, 15%, and 15%, respectively, of patients with biochemical remission (p=NS). Aortic regurgitation was absent in 88% of patients with GHD, whereas 13% had trace regurgitation, compared to 70%, 10% trace, and 20% mild regurgitation of patients with biochemical remission (p=NS).

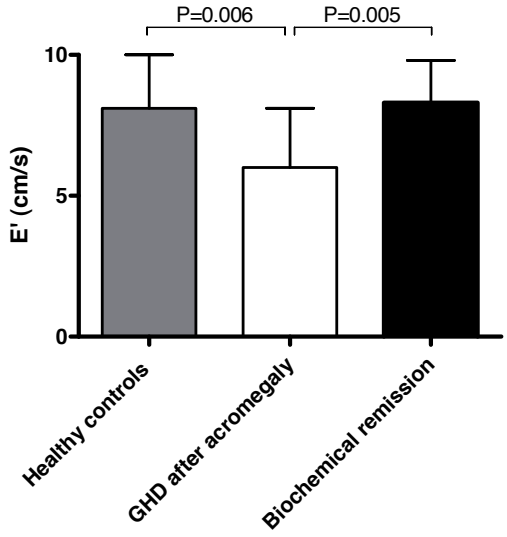

Figure 7/2: E′ as a marker of diastolic function (assessed by tissue Doppler imaging was significantly lower in patients with growth hormone deficiency (GHD) after acromegaly compared to healthy controls and patients with biochemical remission of acromegaly.

Left ventricular size and mass

LV size (LVESD, LVEDD), wall thickness (IVST, PWT), and mass (LVM, LVMi) did not differ between the 2 groups (Table 3). LVH (defined as LVMi exceeding 49.2 g/m$^{2.7}$ for men and 46.7 g/m$^{2.7}$ for women (22)) was present in 50% of patients with GHD after acromegaly compared to 38% of the healthy controls (p=NS).

Left ventricular systolic function

FS and LVEF did not differ between the two groups.

Left ventricular diastolic function

E wave velocity was lower in patients with GHD after acromegaly compared to healthy controls without any differences in A wave velocity and E/A ratio. Additional data on diastolic function, as assessed by TDI, revealed that E' was also lower in patients with GHD after acromegaly compared to healthy controls (p=0.006, Figure 2). Accordingly, E'/ A' ratio tended to be decreased (p=0.079).

Table 7/3: Left ventricular dimensions, systolic function, and diastolic function in patients with growth hormone deficiency (GHD) after acromegaly compared to healthy controls.

	GHD after acromegaly (n=16)	Healthy controls (n=16)	P value*
LVEDD (mm)	51.6 ± 6.1	50.0 ± 6.3	NS
LVESD (mm)	33.5 ± 5.0	30.6 ± 4.4	NS
IVST (mm)	12.3 ± 3.5	10.8 ± 1.9	NS
PWT (mm)	10.3 ± 1.8	10.6 ± 1.5	NS
LVEF (%)	65.9 ± 7.3	68.8 ± 5.5	NS
FS (%)	36.8 ± 5.7	38.8 ± 4.4	NS
E (mm/s)	50.9 ± 11.7	62.5 ± 17.4	0.035
A (mm/s)	59.6 ± 17.7	68.2 ± 17.9	NS
E/A ratio	0.90 ± 0.24	0.99 ± 0.55	NS
E' (mm/s)	6.0 ± 2.1	8.1 ± 1.9	0.006
A' (mm/s)	7.4 ± 1.8	7.8 ± 2.4	NS
E'/A'ratio	0.85 ± 0.36	1.27 ± 0.85	0.084
LVM (g)	235.2 ± 68.4	207.9 ± 55.4	NS
LVMi (g/m$^{2.7}$)	50.9 ± 15.3	44.3 ± 9.8	NS
LVH, n (%)	8 (50)	6 (38)	NS**

LVEDD: Left Ventricular End-Diastolic Diameter; LVESD: Left Ventricular End-Systolic Diameter, FS: Fractional shortening, LVEF: Left Ventricular Ejection Fraction, E: E wave (early filling phase), A: A wave (atrial contraction), E': Tissue Doppler E wave, A': Tissue Doppler A wave, IVST: Inter-Ventricular Septum Thickness, PWT: Posterior Wall Thickness, LVMi: Left Ventricular Mass Index, LVH: Left Ventricular Hypertrophy.

*Independent samples T-test **Chi-square test.

Heart valves

Mitral regurgitation was absent in 81% of patients with GHD, whereas 13% had trace, and 6% mild regurgitation, compared to 73%, 25%, and 6%, respectively, in healthy controls (p=NS). Aortic regurgitation was absent in 88% of patients with GHD, whereas 13% had trace regurgitation, compared to 94% no and 13% trace regurgitation of healthy controls (p=NS).

GHD after acromegaly compared to active acromegaly

Left ventricular size and mass

LVESD and LVEDD did not differ between the 2 groups (Table 2). Remarkably, IVST, PWT, and LVM were not different in patients with GHD after acromegaly compared to patients with active acromegaly. LVH (defined as LVMi exceeding 49.2 g/m$^{2.7}$ for men and 46.7 g/m$^{2.7}$ for women (22)) was present in 50% of patients with GHD after acromegaly compared to 71% of patients with active acromegaly (p=NS). These results were confirmed after adjustment for age.

Left ventricular systolic function

FS and LVEF were significantly higher in patients with GHD after acromegaly compared to patients with active acromegaly (p=0.014 and p=0.047, Figure 1). These results were confirmed after adjustment for age (p=0.005 and p=0.021 for FS and LVEF, respectively).

Left ventricular diastolic function

No differences were observed in diastolic parameters (E and A wave velocities, E/A ratio) between the 2 groups. Diastolic function, assessed by TDI, did not reveal differences between the two groups. These results were confirmed after adjustment for age.

Heart valves

The prevalence of mitral regurgitation was not different between the two groups (GHD: 81% absent, 13% trace, and 6% mild vs. active acromegaly: 60% absent, 24% trace, 6% mild, 12% severe (2 patients), p=NS). The prevalence of aortic regurgitation was also not different between the groups (GHD: 88% absent, 13% trace vs. 71% absent, 6% trace, 18% mild, 6% severe, in active acromegaly, p=NS).

Multiple linear regression analysis

Regression analysis was performed with systolic and diastolic function as dependent variables and age, BMI, IGF-I SD scores, hypertension and LVH as independent variables. All patients with acromegaly were included as one group in this analysis. Age was found to influence diastolic function as measured with conventional echocardiography (β=0.386, p=0.021 for A and β=-0.010, p=0.007 for E/A ratio). LVH and IGF-I SDS influenced diastolic function as measured

with TDI (β=-1.9, p=0.010 for LVH on E' and β=0.135, p=0.032) for IGF-I SDS on A'). We did not find any predictors for systolic function.

DISCUSSION

In this study, we characterized cardiac function and morphology in patients with GHD after treatment for acromegaly. Because both acromegaly per se and GHD per se lead to specific structural and functional cardiac alterations we wanted to assess to which extend GHD after previous exposure to GH excess influences cardiac parameters. This study indicates that GHD after acromegaly results in specific cardiac changes in diastolic function and that normal cardiac function is dependent on normal GH and IGF-I regulation.

To our knowledge, data on the cardiac manifestations of GHD after treatment for acromegaly have not been reported previously. In active acromegaly, a specific cardiomyopathy develops characterized by concentric, LV hypertrophy, and LV systolic and diastolic dysfunction. Adequate treatment with stringent control of GH and IGF-I levels ameliorates signs and symptoms of acromegalic cardiomyopathy. Successful transsphenoidal surgery tends to reverse LV hypertrophy and to improve diastolic function (25). A recent meta-analysis, that evaluated the impact of this treatment on the heart in acromegaly, demonstrated that somatostatin analog treatment (with a duration ranging from a few days to 18 months) consistently improved markers of LV hypertrophy (left ventricular mass index, interventricular septum thickness, left ventricular posterior wall thickness) and diastolic function (26). The findings in our patients with active acromegaly compared to those with biochemical remission are in line with the data from these intervention studies.

Treatment of acromegaly, however, can result in GHD in some patients, especially after previous radiotherapy (5). Several parameters of cardiac morphology and function were altered in our patients with GHD after acromegaly.

First, systolic function at rest tended to be decreased compared to patients with biochemical remission. However, when compared to healthy controls systolic function was not affected in GHD after acromegaly. Therefore, we should be careful in interpreting this trend, since previous acromegaly might have influenced systolic function in patients with biochemical remission. Systolic function was found to be decreased in patients with adult-onset GHD not previously exposed to GH excess. This was found to be correlated with both age and the severity of GHD (7;8). In addition, we noted that systolic function was lower in patients with active acromegaly than in those with GHD after acromegaly. Hypertension and left ventricular hypertrophy are major determinants of systolic function. Forty-one percent of patients with active acromegaly suffered from hypertension and 71% had left ventricular hypertrophy compared to 38% and 50% in patients with GHD, respectively. Apparently, most probably among many others, these

factors result in cardiac systolic function being more affected in states of GH excess than in GH deficiency.

Second, TDI revealed a decrease in parameters reflecting diastolic function in patients with GHD after acromegaly compared to those with biochemical remission and to healthy controls. To our knowledge, only one study assessed diastolic function in adults with GHD with TDI (13). In that study, E' was decreased compared to controls (13), in line with the observed decrease in E' in our patients. In active acromegaly, however, diastolic function was also affected (15). Indeed, there was no difference in diastolic function in patients with GHD after acromegaly compared to patients with active acromegaly.

Indices of LVM, wall thickness, and LV diameters were unaltered in patients with GHD after acromegaly compared to patients with biochemical remission of acromegaly and to healthy controls. Indeed, in patients with adult-onset GHD due to other diseases, IVST does not differ from healthy controls (7). However, in adults with childhood-onset GHD it was found to be decreased (9;10). LVM was unaffected in our patients with GHD after acromegaly compared to patients with biochemical remission of acromegaly and compared to patients with active acromegaly. Several studies in patients with childhood-onset GHD revealed a decreased LVM (6;9;10), whereas it was unaffected in patients with adult-onset GHD, as was the case in our patients (7).

The effects of rhGH on the myocardium in adults with GHD without previous exposure to acromegaly have been reported in a meta-analysis (27). RhGH replacement with a maximum duration of 18 months increased LVM and IVST, whereas diastolic function was not affected (27). Additionally, a trend in improvement in FS was observed (27). It is unknown, however, whether these beneficial changes can also occur in patients with GHD induced by previous treatment for active acromegaly.

In conclusion, GHD after acromegaly results in specific cardiac alterations in diastolic function. In addition, systolic function tended to be decreased in patients with GHD after acromegaly compared to patients with biochemical remission but not when compared to healthy controls, but was higher than in patients with active acromegaly. This study shows that normal cardiac function is dependent on normal GH and IGF-I regulation. It remains to be determined whether these specific cardiac changes after previous prolonged exposure to GH excess followed by GHD affect the response to rhGH replacement.

REFERENCE LIST

1. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
2. Biermasz NR, Dekker FW, Pereira AM, van Thiel SW, Schutte PJ, van Dulken H, Romijn JA, Roelfsema F 2004 Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. J Clin Endocrinol Metab 89:2789-2796
3. Colao A, Marzullo P, Ferone D, Spinelli L, Cuocolo A, Bonaduce D, Salvatore M, Boerlin V, Lancranjan I, Lombardi G 2000 Cardiovascular effects of depot long-acting somatostatin analog Sandostatin LAR in acromegaly. J Clin Endocrinol Metab 85:3132-3140
4. Colao A, Cuocolo A, Marzullo P, Nicolai E, Ferone D, Della Morte AM, Pivonello R, Salvatore M, Lombardi G 2001 Is the acromegalic cardiomyopathy reversible? Effect of 5-year normalization of growth hormone and insulin-like growth factor I levels on cardiac performance. J Clin Endocrinol Metab 86:1551-1557
5. van der Klaauw AA, Pereira AM, van Thiel SW, Smit JW, Corssmit EP, Biermasz NR, Frolich M, Iranmanesh A, Veldhuis JD, Roelfsema F, Romijn JA 2006 GH deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the 24 h GH secretion. Eur J Endocrinol 154:851-858
6. Amato G, Carella C, Fazio S, La Montagna G, Cittadini A, Sabatini D, Marciano-Mone C, Sacca L, Bellastella A 1993 Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J Clin Endocrinol Metab 77:1671-1676
7. Colao A, Cuocolo A, Di Somma C, Cerbone G, Della Morte AM, Nicolai E, Lucci R, Salvatore M, Lombardi G 1999 Impaired cardiac performance in elderly patients with growth hormone deficiency. J Clin Endocrinol Metab 84:3950-3955
8. Colao A, Di Somma C, Cuocolo A, Filippella M, Rota F, Acampa W, Savastano S, Salvatore M, Lombardi G 2004 The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. J Clin Endocrinol Metab 89:5998-6004
9. Merola B, Cittadini A, Colao A, Longobardi S, Fazio S, Sabatini D, Sacca L, Lombardi G 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab 77:1658-1661
10. Sartorio A, Ferrero S, Conti A, Bragato R, Malfatto G, Leonetti G, Faglia G 1997 Adults with childhood-onset growth hormone deficiency: effects of growth hormone treatment on cardiac structure. J Intern Med 241:515-520
11. Colao A, Cuocolo A, Di Somma C, Cerbone G, Morte AM, Pivonello R, Nicolai E, Salvatore M, Lombardi G 2000 Does the age of onset of growth hormone deficiency affect cardiac performance? A radionuclide angiography study. Clin Endocrinol (Oxf) 52:447-455
12. Erdogan D, Tukek T, Aral F, Oflaz H, Ozaydin M, Kocaman O, Akkaya V, Goren T, Molvalilar S 2004 Structural, functional and autonomic changes in the cardiovascular system in growth hormone deficient patients. Ann Noninvasive Electrocardiol 9:19-23
13. Yurci A, Oflaz H, Meric M, Ozbey N 2005 Mitral and tricuspid annular velocities determined by Doppler tissue imaging in hypopituitary, growth hormone-deficient patients. Horm Res 64:107-114
14. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
15. van Thiel SW, Bax JJ, Biermasz NR, Holman ER, Poldermans D, Roelfsema F, Lamb HJ, van der Wall EE, Smit JW, Romijn JA, Pereira AM 2005 Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 153:231-238

16. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
17. Zanettini R, Antonini A, Gatto G, Gentile R, Tesei S, Pezzoli G 2007 Valvular heart disease and the use of dopamine agonists for Parkinson's disease. N Engl J Med 356:39-46
18. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, . 1989 Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 2:358-367
19. Perry GJ, Helmcke F, Nanda NC, Byard C, Soto B 1987 Evaluation of aortic insufficiency by Doppler color flow mapping. J Am Coll Cardiol 9:952-959
20. Thomas JD 1997 How leaky is that mitral valve? Simplified Doppler methods to measure regurgitant orifice area. Circulation 95:548-550
21. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N 1986 Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 57:450-458
22. Vitale G, Galderisi M, Pivonello R, Spinelli L, Ciccarelli A, de Divitiis O, Lombardi G, Colao A 2004 Prevalence and determinants of left ventricular hypertrophy in acromegaly: impact of different methods of indexing left ventricular mass. Clin Endocrinol (Oxf) 60:343-349
23. Cole TJ 1990 The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45-60
24. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-176
25. Jaffrain-Rea ML, Minniti G, Moroni C, Esposito V, Ferretti E, Santoro A, Infusino T, Tamburrano G, Cantore G, Cassone R 2003 Impact of successful transsphenoidal surgery on cardiovascular risk factors in acromegaly. Eur J Endocrinol 148:193-201
26. Maison P, Tropeano AI, Macquin-Mavier I, Giustina A, Chanson P 2007 Impact of somatostatin analogs on the heart in acromegaly: a metaanalysis. J Clin Endocrinol Metab 92:1743-1747
27. Maison P, Chanson P 2003 Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 108:2648-2652.

Chapter 8

Limited Effects of Growth Hormone Replacement n Patients with GH deficiency During Long-Term Cure of Acromegaly

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Submitted for publication

ABSTRACT

Objective

The aim of this study was to assess the effects of replacement with recombinant human growth hormone (rhGH) in patients with GH deficiency (GHD) after treatment of acromegaly.

Design

Intervention study.

Patients and methods

Sixteen patients (8 men, age 56 yrs), treated for acromegaly by surgery and radiotherapy, with an insufficient GH response to insulin-induced hypoglycaemia, were treated with 1 year of rhGH replacement. Study parameters were assessed at baseline and after 1 year of rhGH replacement. Study parameters were cardiac function, body composition, bone mineral density (BMD), fasting lipids, glucose, bone turnover markers, and Quality of Life (QoL).

Results

During rhGH replacement IGF-I concentrations increased from –0.4 ± 0.7 SD to 1.0 ± 1.5 SD (p=0.001), with a mean daily dose of 0.2 ± 0.1 mg in men and 0.3 ± 0.2 mg in women. Nonetheless, rhGH replacement did not alter cardiac function, lipid and glucose concentrations, body composition or QoL. Bone turnover markers (PINP and β crosslaps) levels increased (p=0.005 and p=0.021, resp.), paralleled by a small, but significant decrease in BMD of the hip.

Conclusion

The beneficial effects of rhGH replacement in patients with GHD during cure from acromegaly are limited in this study.

INTRODUCTION

Growth hormone deficiency (GHD) in adults is characterized by an adverse cardiovascular metabolic profile, altered body composition (reflected in reduced muscle strength and mass, and visceral obesity), decreased bone mass, decreased cardiac function and decreased quality of life (reviewed in (1)). Treatment with recombinant human growth hormone (rhGH) ameliorates symptoms and signs of the GHD syndrome in the short (2) and in the long-term (3;4). GHD is a well-known sequel of pituitary radiotherapy, e.g. for non-functioning adenomas, adrenocorticotrope hormone- or prolactin-secreting adenomas (5). Remarkably, GHD can also be induced by treatment of active acromegaly. We have documented a diminished GH increase to insulin-induced hypoglycemia during long-term follow-up in 36% of the patients with acromegaly after postsurgical radiotherapy (6).

Almost all randomized controlled studies on the efficacy of rhGH replacement in adult GHD have excluded patients previously treated for acromegaly. Nonetheless, two intervention studies have reported on the effects of rhGH replacement in patients with GHD after previous treatment of acromegaly. In a subanalysis of acromegalic patients with GHD extracted from the large KIMS database, 6 months of rhGH replacement had no significant beneficial effects in the acromegalic patients (7). In addition, a recent study compared the effects of 2 years of rhGH replacement on body composition, muscle strength, bone mass and metabolic parameters between 10 patients previously treated for acromegaly and 10 patients treated for non-functioning pituitary disease (8). At baseline, patients with acromegaly had decreased muscle endurance and increased LDL concentrations compared to the other patients, but after two years of rhGH replacement there were no differences between both groups (8).

The aim of this study was to evaluate 1 year of rhGH replacement on heart function, quality of life, glucose and lipid metabolism, body composition and bone mass and turnover in order to extend the exploration whether GH replacement is beneficial in acromegalic patients with GHD during long-term biochemical cure.

PATIENTS AND METHODS

Patients

We enrolled 16 acromegalic patients (8 men and 8 women), who developed GHD after combined pituitary surgery and radiotherapy in the study. Inclusion criteria were previous treatment for acromegaly by surgery and/or radiotherapy and an insufficient GH increase to insulin-induced hypoglycemia (short-acting insulin 0.05-0.1 U/kg body weight, blood samples drawn at 0, 20, 30, 45, 60 and 90 min; nadir glucose levels below 2.2 mmol/l) (9). The increase in GH concentrations was considered insufficient, when the peak GH response was below 3 µg/l (10).

Fifteen patients had been treated with primary surgery and secondary conventional radiotherapy (mean interval after radiotherapy 18 years (range 4-29 years). The other patient was diagnosed with pituitary apoplexy of a GH producing adenoma. Because GH concentrations remained elevated, he underwent surgery and subsequently developed complete anterior pituitary failure. Clinical details were published previously (11).

Additional hormone replacement therapy was kept stable for at least 3 months prior to study inclusion, and was only adjusted thereafter when necessary. The purpose, nature, and possible risks of the study were explained to all subjects and written informed consent was obtained. The study protocol was approved by the ethics committee of the Leiden University Medical Center.

Study design

Study parameters were assessed both at baseline and after 1 year of rhGH replacement. The following variables were measured: fasting concentrations of lipoproteins, glucose, and IGF-I, body composition, bone turnover markers and bone mass, echocardiography, and Quality of Life parameters.

Growth hormone vials of 1 ml were manufactured and provided by Novo Nordisk Pharma, Denmark. Growth hormone replacement dose was started at 0.2 mg/ day and, subsequently, titrated in the first 12 weeks of the study to obtain an IGF-I concentration within the age- and gender-adjusted reference range, according to Growth Hormone Research Society guidelines (10).

The mean age of the patients was 56 years (range 34 to 75 years). The interval between radiotherapy and the start of the study was 18 yrs (range 4-29 yrs). TSH deficiency was present in 5 patients, ACTH deficiency in 9 patients, and LH-FSH deficiency in 8 patients (see Table 1).

Body composition

Body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured. Waist-hip (WH) ratio was calculated. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. Lean body mass and fat mass were measured with DXA (Hologic 4500; Hologic Inc., Waltham, MA, USA).

Markers for bone turnover and bone mass

The following serum markers of bone turnover were measured: N-terminal propeptides of type I collagen (PINP), as a marker for bone synthesis, and β-crosslaps as a marker for bone resorption. Bone mineral density (BMD) was measured by DXA (Hologic 4500; Hologic Inc., Waltham, MA, USA). Sites measured were the lumbar spine (L1-L4) and the femoral neck (left and right). Mean BMD of the left and right femoral neck was calculated. Mean T and Z scores were calculated for

Table 8/1: Clinical characteristics of the 16 patients with growth hormone deficiency after acromegaly.

	Age (yr)	Gender	Substitution therapy
1	65	Male	None
2	65	Male	None
3	59	Male	Thyroxine, Testosterone, Hydrocortisone
4	75	Female	Hydrocortisone
5	66	Female	None
6	62	Female	Hydrocortisone
7	66	Female	Thyroxine, Estradiol, Hydrocortisone
8	40	Female	Thyroxine, Estradiol, Hydrocortisone
9	43	Male	Testosterone
10	56	Male	Thyroxine, Testosterone, Hydrocortisone
11	48	Male	Thyroxine, Testosterone, Hydrocortisone
12	51	Female	None
13	34	Female	Hydrocortisone
14	74	Male	Testosterone
15	45	Male	Testosterone
16	50	Female	Hydrocortisone

total left and right hip using the NHANES reference values. The CV of BMD measurements was 1% and the machine was cross-calibrated at regular interval.

Echocardiography

Echocardiography was performed while the patients were in the left lateral decubitus position using a commercially available system (Vingmed Vivid-7, General Electric – Vingmed, Milwaukee, WI, USA). Standard parasternal (long- and short-axis) and apical views (2-, and 4-, and long-axis) were obtained. M-mode images were obtained from the parasternal long-axis views for quantitative assessment of LV dimensions (Inter-Ventricular Septum Thickness (IVST), Posterior Wall Thickness (PWT), LV End-Diastolic Diameter (LVEDD), LV End-Systolic Diameter (LVESD), Fractional Shortening (FS) and LV Ejection Fraction (LVEF) (12).

The following parameters of diastolic function were obtained: diastolic transmitral peak velocities (E and A wave) and the E/A ratio. Quantitative diastolic data were derived from tissue Doppler imaging (TDI). For TDI analysis, the digital cine loops were analyzed using commercial software (Echopac 6.1; General Electric-Vingmed). The sample volume (4 mm^2) was placed in the LV basal portion of the septum (using the 4-chamber views). The following parameters (mean values calculated from 3 consecutive heartbeats) were derived: early diastolic velocity (E'), late diastolic velocity (A') and the E'/A' ratio. The severity of valvular regurgitation was assessed by 2 independent expert readers blinded to the clinical data on a qualitative scale of trace, mild, moderate, or severe, using previously described methods (13;14). Left ventricular mass (LVM) was calculated by the cube formula, and using the correction formula proposed by Devereux,

et al. (15): 0.8 x {1.04 [(LVEDD + PWT + IVST)^3 - LVEDD^3]}+ 0.6. The data were assessed by two independent observers, blinded for the clinical data of the patients.

Quality of life

Quality of Life was assessed using four different validated health-related quality of life questionnaires:

HADS (Hospital Anxiety and Depression Scale)

The HADS consists of 14 items pertaining to anxiety and depression. Each item is measured on a 4-point scale. Scores for the anxiety and depression subscale range from 0-21 and for the total score from 0-42. A high score points to more severe anxiety and depression (16).

MFI-20 (Multidimensional Fatigue Index)

The MFI-20 contains 20 statements to assess fatigue (17). Five different dimensions of fatigue (four items each) are calculated from these statements; 1) general fatigue; 2) physical fatigue; 3) reduced activity; 4) reduced motivation and 5) mental fatigue. Every statement is measured on a 5-point scale; scores vary from 0 to 20. Higher scores indicate higher experienced fatigue.

NHP (Nottingham Health Profile)

The NHP is frequently used in patients with pituitary disease to assess general well-being and QoL. The survey consists of 38 yes/no questions, which are subdivided in 6 scales assessing impairments, i.e. pain (8 items), energy level (3 items), sleep (5 items), emotional reactions (9 items), social isolation (5 items) and disability/functioning, i.e. physical mobility (8 items) (18;19). Subscale scores are calculated as a weighted mean of the associated items and are expressed as a value between 0 and 100. The total score is the mean of the 6 subscales.

QoL-AGDHA (Quality of Life-Assessment of Growth Hormone Deficiency in Adults)

This disease specific quality of life questionnaire has been developed specifically for the detection of deficits in needs achievements in areas which have shown to be commonly affected in adults with GHD (20). The questionnaire comprises 25 items, which are summed to form a total score. Higher numerical scores (to a maximum of 25) denote poorer quality of life.

Assays and dynamic tests

Growth hormone reserve was evaluated by the insulin tolerance test in fasting conditions (short-acting insulin 0.05-0.1 U/kg body weight, blood samples drawn at 0, 20, 30, 45, 60 and 90 min; the nadir glucose concentration should drop below 2.2 mmol/l) (9). The increase in GH concentration was considered insufficient, when the peak GH concentration was below 3 µg/l (10).

Serum IGF-I concentration was measured with the Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, USA). The intra-assay variation was 5.0 and 7.5% at mean serum levels of 8 and 75 nmol/l, respectively. IGF-I levels are expressed as standard deviation-scores (SDS), using lambda-mu-sigma (LMS) smoothed reference curves based on measurements in 906 healthy individuals (21;22;22).

IGFBP-3 was measured using an immunometric technique on an IMMULITE Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 0.02 mg/l and inter-assay variation was 4.4 and 4.8% at 0.91 and 8.83 mg/l. A Hitachi P800 auto analyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose, total cholesterol and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany). LDL cholesterol concentrations (LDL) were calculated using the Friedewald formula. C-crosslinking terminal telopeptide of type I collagen (β -crosslaps) and procollagen type I aminoterminal propeptide (PINP) by chemoluminescence immunoassay with the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands).

Statistics

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are scored as the mean ± standard deviation (SD), unless specified otherwise. The data were analyzed with the paired samples Student's t-test. Statistical significance was set at $P<0.05$.

Table 8/2: Metabolic and antroprometric parameters before and after 1 year of rhGH replacement.

	Before	After	P-value
Total cholesterol (mmol/l)	6.0 ± 1.0	5.7 ± 1.2	NS
TG (mmol/l)	1.8 ± 1.0	2.1 ± 1.3	NS
HDL-cholesterol (mmol/l)	1.4 ± 0.5	1.4 ± 0.4	NS
LDL-cholesterol (mmol/l)	4.1 ± 0.8	3.9 ± 1.0	NS
Glucose (mmol/l)	4.6 ± 0.6	4.7 ± 0.5	NS
SBP (mm Hg)	138 ± 17	135 ± 10	NS
DBP (mm Hg)	87 ± 9	88 ± 8	NS
Waist circumference (cm)	102.8 ± 11.4	103.6 ± 12.5	NS
WH ratio	0.9 ± 0.1	0.9 ± 0.1	NS
LBM (kg)	57.1 ± 13.0	61.7 ± 13.4	NS
Fat mass (kg)	34.8 ± 16.0	31.3 ± 14.7	NS

TG: triglycerides; HDL High-Density Lipoprotein; LDL Low-Density Lipoprotein; SBP: Systolic Blood pressure; DBP: Diastolic blood pressure; WH ratio: Waist-to-Hip ratio; LBM: Lean Body Mass.

Table 8/3: Bone markers and biochemical parameters of bone turnover before and after 1 year rhGH replacement.

	Before	After	P-value
PINP (ng/ml)	29.1 ± 19.5	44.3 ± 33.4	0.005
β crosslaps (ng/ml)	0.2 ± 0.1	0.3 ± 0.2	0.021
BMD lumbar spine (g/cm^2)	1.1 ± 0.2	1.1 ± 0.2	NS
BMD femoral neck (g/cm^2)	0.85 ± 0.17	0.81 ± 0.15	<0.001
T score total hip	-0.30 ± 1.5	-0.24 ± 1.4	NS
Z score total hip	0.26 ± 1.6	0.37 ± 1.5	NS

PINP: N-terminal propeptides of type I collagen; BMD: Bone Mineral Density.

RESULTS

IGF-1 and IGFBP-3 concentrations

One year of rhGH replacement increased IGF-I SD scores and IGF-BP3 levels (baseline IGF-I SD score: –0.4 ± 1.7 and 1.0 ± 1.5 at 1 year, p<0.001; baseline IGFBP-3: 4.2 ± 1.2 mg/l and 5.2 ± 1.4 mg/l after 1 year, p<0.001).

Cardiovascular risk parameters and body composition

During rhGH replacement lipid profiles did not change. In addition, blood pressure (systolic and diastolic) and fasting glucose concentrations did not change (Table 2). Mean lean body mass increased by almost 4 kg and total fat mass decreased by approximately 3 kg, but these differences did not reach statistical significance.

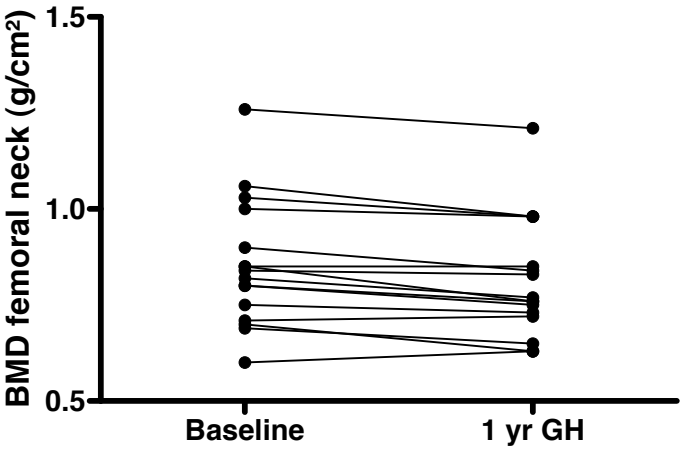

Figure 8/1: Bone mineral density decreased in all patients during1 year rhGH replacement (n=16, P<0.001).

Table 8/4: Cardiac parameters before and after 1 year rhGH replacement.

	Before	After	P-value
LVEDD (mm)	51.6 ± 5.8	47.0 ± 15.5	NS
LVESD (mm)	34.3 ± 4.4	32.6 ± 7.5	NS
IVST (mm)	12.2 ± 3.4	12.4 ± 3.9	NS
PWT (mm)	10.2 ± 1.6	10.0 ± 1.3	NS
FS (%)	35.1 ± 6.7	37.0 ± 6.1	NS
LVEF (%)	63.5 ± 8.4	66.3 ± 7.5	NS
E (mm/s)	0.5 ± 0.2	0.5 ± 0.1	NS
A (mm/s)	0.6 ± 0.2	0.6 ± 0.1	NS
E/A ratio	1.0 ± 0.3	0.9 ± 0.3	NS
E' (cm/s)	0.6 ± 0.2	0.5 ± 0.3	NS
A' (cm/s)	0.7 ± 0.1	0.6 ± 0.4	NS
E'/A' ratio	0.8 ± 0.4	0.9 ± 0.3	NS
LVM (g)	233 ± 64	215 ± 105	NS

LVEDD: Left Ventricular End-Diastolic Diameter; LVESD: Left Ventricular End-Systolic Diameter; FS: Fractional shortening; LVEF: Left Ventricular Ejection Fraction; IVST: Inter-Ventricular Septum Thickness; PWT: Posterior Wall Thickness; LVM: Left-Ventricular Mass.

Bone parameters

RhGH replacement increased plasma concentrations of bone turnover markers (PINP and β-crosslaps) in all patients (Table 3, Figure 1). During rhGH replacement bone mass at the lumbar spine remained unchanged in all patients, but decreased significantly at the femoral neck by 4% (Table 3, Figure 1).

Cardiac parameters and quality of life parameters

During rhGH replacement there were no significant changes in cardiac parameters or QoL parameters (Table 4 and 5).

DISCUSSION

In this prospective study, we evaluated the effect of rhGH treatment on a range of relevant parameters in GHD patients, previously treated for acromegaly. During rhGH replacement IGF-I concentrations increased into the age- and gender-adjusted normal range, but neither cardiac parameters, nor any of the cardiovascular risk parameters or quality of life parameters changed during rhGH tretament. Bone turnover markers increased during rhGH replacement, which was associated with a decrease of bone mineral density at the femoral neck of 4%, whereas the bone mass of the lumbar spine remained unchanged. These data indicate that the effects of rhGH treatment of GHD patients previously treated for acromegaly are limited.

Table 8/5: Quality of life parameters at baseline and after 1 year rhGH replacement in patients with GHD after previous treatment for acromegaly.

		Before	After	P-value
QoL NHP	Energy	55.2 ± 38.2	41.8 ± 35.4	NS
	Pain	19.4 ± 21.8	14.1 ± 18.8	NS
	Emotional reaction	12.0 ± 16.4	13.2 ± 20.0	NS
	Sleep	13.6 ± 26.9	14.1 ± 24.4	NS
	Physical mobility	20.1 ± 20.8	16.0 ± 23.7	NS
	Social isolation	12.0 ± 17.1	10.6 ± 17.1	NS
QoL MFI-20	General fatigue	15.4 ± 4.5	14.2 ± 4.7	NS
	Physical fatigue	12.9 ± 5.2	13.2 ± 5.2	NS
	Reduction in activity	11.4 ± 4.9	11.8 ± 4.9	NS
	Reduction in motivation	10.8 ± 4.4	10.1 ± 4.5	NS
	Mental fatigue	9.3 ± 4.6	9.8 ± 4.3	NS
QoL HADS	Anxiety	4.6 ± 2.4	4.9 ± 2.3	NS
	Depression	6.0 ± 4.4	6.1 ± 4.3	NS
	Total score	10.6 ± 5.0	11.0 ± 5.3	NS
QoL-AGDHA		7.6 ± 6.1	7.7 ± 5.9	NS

HADS, NHP, MFI-20, QoL-AGDHA higher scores: more impairment.

Data on the manifestations of GHD after treatment for acromegaly are limited. Two previous studies reported several clinical manifestations in this particular patient group (7;8). The first study compared patients with GHD after treatment for acromegaly and Cushing's disease with patients with GHD due to other etiologies (7). No differences in body mass index, waist-hip ratio, serum lipid concentrations, bone mineral density (at the lumbar spine and femoral neck), or IGF-I SD score were found in patients with GHD after acromegaly compared with patients with GHD due to other etiologies (7). In the second study, muscle strength, bone mass and metabolic indices were compared between 10 patients previously treated for acromegaly and 10 patients treated for non-functioning pituitary disease (8). Although there were no differences between both groups after two years of rhGH replacement, at baseline, patients with acromegaly had a decreased muscle endurance and increased LDL concentrations compared to the other patients, which points towards differences in their response to the treatment (8). For instance, body fat decreased and lean body mass increased in that study in the patients with non-functioning pituitary disease, whereas it did not change in the same number of patients with GHD after acromegaly simultaneously studied (8), in complete agreement with our findings.

In adult patients with GHD, rhGH replacement increases bone mineral density (23), left ventricular mass and stroke volume (24), lean body mass (1), and quality of life (25), whereas it improves the serum lipid profile (26). These effects are apparent within 6-12 months and are maintained during continued treatment with rhGH in the long-term (4;24;26-29). However, it appears that these abnormalities associated with GHD in adults are not always reversed

completely solely by rhGH replacement (29;30) and that some patients might benefit more from combined treatment of rhGH with, for instance, lipid-lowering agents and bisfosfonates (31;32).

In our study, parameters of both bone resorption and bone formation increased, paralleled by a net decrease in bone mineral density at the femoral neck, in agreement with the observed increase in bone turnover found during 2 years of rhGH replacement in these patients by Norrman et al. (8). In this latter study, however, no treatment differences in the response of bone mineral density between patients previously treated for acromegaly and patients previously treated for non-functioning pituitary disease were found (8). We could not find any data showing increase or decrease of bone mineral density in both patients treated for acromegaly and patients treated for non-functioning pituitary disease. It is important to note, however, that there are several small differences with our study. The patients in the study of Norrman et al. were included between 1991 and 1997.Hence, the initial dose previously applied before the consensus statement of the Growth Hormone Research Society in 1998 was first based on weight in some patients, but was subsequently gradually lowered when the weight based dose regime was abandoned. In addition, almost all patients studied (90%) were female (8). These differences could explain the discrepant effects of rhGH replacement found on bone mineral density between the present study and the study by Norrman et al. (8).

The decrease in BMD found in our study could point towards a different response to rhGH replacement in patients previously exposed to persistently increased GH concentrations. Alternatively, this observation may indicate that the possible beneficial effect of rhGH replacement on bone in these patients is insufficient to compensate the ongoing bone loss after previous GH excess in these specific patients. In active acromegaly, bone mineral density is increased (33) and this favorable effect seems to persist after successful biochemical cure (34). However, in patients with biochemical cure of acromegaly, radiotherapy was an independent negative predictor of bone mineral density at the femoral neck, which is probably related to the diminished GH secretion frequently observed after this treatment modality (34). Almost all patients in our cohort had been treated previously by radiotherapy. On the other hand, in patients with adult-onset GHD due to other etiologies, some, but not all, studies have found a decreased bone mass at the lumbar spine (reviewed in (1)). Replacement with rhGH in those patients seems to modestly increase bone mineral density after 1 year (27). However, the lack of the increase in BMD, usually seen during rhGH replacement but absent in our specific patients, is in accordance with the only other study performed in patients with GHD after treatment of acromegaly (7). Further longer-term studies are needed to clarify this issue.

Body composition did not change during rhGH replacement, whereas it has been consistently found to be altered by rhGH replacement in patients with GHD due to other diseases (an increase in lean body mass and a decrease in body fat (1)). However, the trends seen in our study point towards similar changes in body composistion in patients previously treated for acromegaly. Interestingly, body fat decreased and lean body mass increased in the study of

Norrman et al. in the patients with non-functioning pituitary disease, whereas it did not change in the same number of patients with GHD after acromegaly simultaneously studied (8). Replacement with rhGH did not improve QoL parameters. Various aspects of QoL seem to improve slightly during rhGH replacement in adults with GHD due to other diseases (25). Therefore, it is likely that other factors in our patients with GHD after treatment for acromegaly explain the lack of effect on QoL during rhGH replacement, such as persisting joint related complaints (35) and/or unfavorable late effects of previous radiotherapy (36).

Considering the beneficial effects of rhGH replacement in patients with GHD due to other causes than acromegaly, substitution in this particular subgroup is warranted. We did not find many marked beneficial effects of rhGH replacement in these patients. Higher, non-physiological, doses of rhGH could possibly result in detectable changes in the targeted parameters, as has been extensively documented in patients with GHD not pre-exposed to acromegaly. In clinical practice, however, current treatment guidelines from the Growth Hormone Research Society, advocate to titrate rhGH dose to target IGF-I concentrations within the normal age-related reference range, to ensure a therapeutic dose also in those patients with severe GHD, and avoid side effects of rhGH replacement.

In conclusion, the effects of rhGH replacement in patients with GHD after treatment for acromegaly seem to be limited..The observed effect on bone resorption and in bone mineral density might be affected by ongoing bone loss despite rhGH replacement seen in acromegaly after radiotherapy, or by the response to rhGH of bone after previous long-term exposure to GH excess. Larger long-term studies in this specific patient group are warranted to clarify the issue whether the effects of rhGH replacement in GHD might be altered by previous acromegaly. However, these patients will most probably become increasingly rare since the introduction of effective drug treatment for acromegaly.

REFERENCE LIST

1. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
2. Maison P, Demolis P, Young J, Schaison G, Giudicelli JF, Chanson P 2000 Vascular reactivity in acromegalic patients: preliminary evidence for regional endothelial dysfunction and increased sympathetic vasoconstriction. Clin Endocrinol (Oxf) 53:445-451
3. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG 2000 Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 85:3762-3769
4. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
5. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
6. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
7. Feldt-Rasmussen U, Abs R, Bengtsson BA, Bennmarker H, Bramnert M, Hernberg-Stahl E, Monson JP, Westberg B, Wilton P, Wuster C 2002 Growth hormone deficiency and replacement in hypopituitary patients previously treated for acromegaly or Cushing's disease. Eur J Endocrinol 146:67-74
8. Norrman LL, Johannsson G, Sunnerhagen KS, Svensson J 2008 Baseline Characteristics and the Effects of Two Years of Growth Hormone (GH) Replacement Therapy in Adults with GH Deficiency Previously Treated for Acromegaly. J Clin Endocrinol Metab 93:2531-2538.
9. van der Klaauw AA, Pereira AM, van Thiel SW, Smit JW, Corssmit EP, Biermasz NR, Frolich M, Iranmanesh A, Veldhuis JD, Roelfsema F, Romijn JA 2006 GH deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the 24 h GH secretion. Eur J Endocrinol 154:851-858
10. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
11. Roelfsema F, van den BG, van Dulken H, Veldhuis JD, Pincus SM 1998 Pituitary apoplexy in acromegaly, a long-term follow-up study in two patients. J Endocrinol Invest 21:298-303
12. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I, . 1989 Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 2:358-367
13. Perry GJ, Helmcke F, Nanda NC, Byard C, Soto B 1987 Evaluation of aortic insufficiency by Doppler color flow mapping. J Am Coll Cardiol 9:952-959
14. Thomas JD 1997 How leaky is that mitral valve? Simplified Doppler methods to measure regurgitant orifice area. Circulation 95:548-550
15. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N 1986 Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 57:450-458
16. Zigmond AS, Snaith RP 1983 The hospital anxiety and depression scale. Acta Psychiatr Scand 67:361-370
17. Smets EM, Garssen B, Bonke B, De Haes JC 1995 The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 39:315-325
18. Hunt SM, McEwen J 1980 The development of a subjective health indicator. Sociol Health Illn 2:231-246
19. Hunt SM, McKenna SP, McEwen J, Backett EM, Williams J, Papp E 1980 A quantitative approach to perceived health status: a validation study. J Epidemiol Community Health 34:281-286

20. McKenna SP, Doward LC, Alonso J, Kohlmann T, Niero M, Prieto L, Wiren L 1999 The QoL-AGHDA: an instrument for the assessment of quality of life in adults with growth hormone deficiency. Qual Life Res 8:373-383
21. Cole TJ 1990 The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45-60
22. Rikken B, van Doorn J, Ringeling A, Van den Brande JL, Massa G, Wit JM 1998 Plasma levels of insulin-like growth factor (IGF)-I, IGF-II and IGF-binding protein-3 in the evaluation of childhood growth hormone deficiency. Horm Res 50:166-176
23. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 156:55-64
24. Maison P, Chanson P 2003 Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 108:2648-2652
25. Deijen JB, Arwert LI, Witlox J, Drent ML 2005 Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes 3:63
26. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
27. Davidson P, Milne R, Chase D, Cooper C 2004 Growth hormone replacement in adults and bone mineral density: a systematic review and meta-analysis. Clin Endocrinol (Oxf) 60:92-98
28. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 92:1442-1445
29. van der Klaauw AA, Romijn JA, Biermasz NR, Smit JW, van Doorn J, Dekkers OM, Roelfsema F, Pereira AM 2006 Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 155:701-708
30. van der Klaauw AA, Biermasz NR, Feskens EJ, Bos MB, Smit JW, Roelfsema F, Corssmit EP, Pijl H, Romijn JA, Pereira AM 2007 The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. Eur J Endocrinol 156:455-462
31. Monson JP, Jonsson P, Koltowska-Haggstrom M, Kourides I 2007 Growth hormone (GH) replacement decreases serum total and LDL-cholesterol in hypopituitary patients on maintenance HMG CoA reductase inhibitor (statin) therapy. Clin Endocrinol (Oxf) 67:623-628
32. Biermasz NR, Hamdy NA, Janssen YJ, Roelfsema F 2001 Additional beneficial effects of alendronate in growth hormone (GH)-deficient adults with osteoporosis receiving long-term recombinant human GH replacement therapy: a randomized controlled trial. J Clin Endocrinol Metab 86:3079-3085
33. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
34. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F 2005 Long-term maintenance of the anabolic effects of GH on the skeleton in successfully treated patients with acromegaly. Eur J Endocrinol 152:53-60
35. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F 2005 Morbidity after Long-term Remission for Acromegaly; Persisting Joint-Related Complaints Cause Reduced Quality of Life. J Clin Endocrinol Metab
36. van der Klaauw AA, Biermasz N, Hoftijzer HC, Pereira AM, Romijn JA 2008 Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly. Clin Endocrinol (Oxf), In press.

Chapter 9

Postoperative radiotherapy for acromegaly delays the circadian phase of melatonin secretion

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Submitted for publication

ABSTRACT

Objective

Postoperative radiotherapy for acromegaly is associated with a considerable increase in pituitary insufficiencies. In general, the notion is that this is due to side effects of radiotherapy on the pituitary, but the hypothalamus may also be involved. Circadian variations in melatonin secretion are under the control of endogenous clock signals arising from the suprachiasmatic nucleus of the hypothalamus. Therefore, the aim of this study was to assess the effects of postoperative radiotherapy on characteristics of diurnal melatonin secretion in patients cured from acromegaly.

Design

cross-sectional study in 3 groups of 8 subjects (4 men in each group) matched for age, gender and BMI.

Patients and methods

The groups consisted of: 1) patients treated with postoperative radiotherapy 14 ± 2 years prior to this study, 2) patients treated with transsphenoidal surgery, and 3) healthy controls. Melatonin concentrations were measured each hour during 24h and circadian rhythmicity was appraised by a skewed baseline cosine curve fit procedure.

Results

Mean serum melatonin concentrations were highest during nighttime and lowest during the afternoon compared to the morning. Mean morning, afternoon, nighttime or total melatonin concentrations did not differ between the groups. The peak level and the onset and offset of melatonin did not differ between the groups. The acrophase, however, was delayed in patients treated with postoperative radiotherapy compared to healthy controls.

Conclusion

There is a delayed acrophase in melatonin circadian rhythmicity in patients treated by postoperative radiotherapy for acromegaly, potentially reflecting altered timing of the suprachiasmatic nucleus.

INTRODUCTION

Postoperative radiotherapy for acromegaly is effective in reducing persistent growth hormone (GH) excess. A decline of ~50% in serum GH levels is observed within the first two years after radiotherapy and of ~75% within 5 years (1-3). On the other hand, radiotherapy for pituitary tumors can lead to deficiencies in anterior pituitary hormones either due to direct effects on healthy pituitary tissue and/or alterations in hypothalamic functioning. Despite advances in modern radiation treatment there is still uncertainty with respect to the dosimetric accuracy of potential radiation damage to the hypothalamic nuclei. Moreover, the hypothalamus is considered more vulnerable to radiation damage than the pituitary (4). In addition, radiotherapy negatively influences quality of life in these patients, especially with respect to general and physical fatigue (5).

Within the hypothalamus, the suprachiasmatic nucleus (SCN) is considered to be the central circadian pacemaker of the body. Altered regulation of endogenous rhythms could contribute to the increased fatigue observed during long-term follow-up in patients previously treated by postoperative radiotherapy (5;6). For instance, cranial radiation therapy in childhood is associated with objective and subjective changes in the sleep–wake rhythm in adulthood (7). Unfortunately, diurnal variations of pituitary hormones can not be used for assessment of the diurnal regulation by the SCN in patients previously treated for pituitary adenomas, because anterior pituitary deficiencies are frequently seen after radiotherapy due to the combined effects on healthy pituitary and hypothalamic tissue (8). Another circadian output regulated by the SCN is the diurnal variation of melatonin secretion by the pineal gland (9). We, therefore, hypothesized that radiation damage to the hypothalamus, especially to the SCN, could be reflected in altered diurnal variation of melatonin secretion. Therefore, the aim of the present study was to compare the 24h circadian variation of melatonin secretion in patients, who had been cured from GH excess by combined surgery and radiotherapy, to patients cured by surgery only and healthy controls.

PATIENTS AND METHODS

Patients

Patients were recruited from a cohort of patients with biochemical remission after acromegaly, who have been described extensively (2;10). We selected 3 groups that were carefully matched for age, gender and body mass index.

The first group consisted of acromegalic patients who had been treated and cured by postoperative radiotherapy (n=8), because of persisting postoperative disease activity. The second group consisted of patients who had been treated by transsphenoidal surgery only (n=8) and who were in long-term remission. The third group consisted of healthy controls (n=8).

Exclusion criteria for patients and healthy controls were hypertension, diabetes mellitus, pregnancy and recent transatlantic flights. Healthy controls did not use any medication. All patients had normal glucose-suppressed GH concentrations (<0.38 µg/L) and normal IGF-1 levels corrected for sex and age at the time of inclusion. Radiation had been given with a conventional linear accelerator (8 MeV) in a rotating field; total tumor dose was 40-45 Gy, fractionated in 20 sessions over a period of 4-5 weeks.

The patients who had been by postoperative radiotherapy underwent an insulin tolerance test (ITT) for establishing GH reserve. To be included for this study a subnormal GH-peak response of less than 3 µg/L was required in the presence of glucose nadir <2.2 mmol/L (11), because that would mean that due to postoperative radiotherapy at least 1 hypothalamic-pituitary axis was affected. We have previously shown that an insufficient GH response to the ITT after postoperative radiotherapy is diagnostic for GHD after acromegaly (12). All patients in the surgery only group had normal GH reserve. All subjects underwent identical 24h blood sampling studies (see below).

Premenopausal women were defined as LH/ FSH deficient when secondary amenorrhoea was present for more than 1 year. In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after an insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with thyroxine, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. All patients with pituitary deficiencies were on stable conventional substitution therapy, for at least 12 months prior to the study. None of the postmenopausal women used estrogens.

Written informed consent was obtained from all the patients and control subjects. The study was approved by the ethical committee of the Leiden University Medical Center.

Sampling protocol

Patients and controls were admitted to the hospital on the day of study. An indwelling i.v. cannula was inserted into a vein of the forearm 60 min before hourly sampling began starting at 09.30h A.M. for the next 24h. A slow infusion of 0.9% NaCl and heparin (1 U/ml) was used to keep the line patent. Meals were served at 0800h, 1230h and 1730h. The subjects were free to ambulate, but not to sleep during daytime. Lights were turned off between 2200h and 2400h, when patients indicated they wanted to go sleep to ensure similarity with normal out-of-hospital sleeping patterns. Plasma samples were collected on ice in heparinized tubes. The samples were centrifuged at 4°C for 7 minutes; the plasma was then separated, frozen and stored at -20°C until the assays were performed.

Assay

Plasma melatonin concentrations were determined by RIA (LDN Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany). Sensitivity of the assay is 2 pg/ml. The intra-assay coefficient of variation is 12.1-12.3 % in the range of 15.1-157 pg/ml. All serial samples in this study were run in the same assay.

Data analysis

First, the mean concentrations of melatonin in the morning, afternoon and night were obtained from the average values in samples obtained during the morning (samples of 9.30h, 10.30h, 11.30h, 12.30h), during the afternoon (samples of 14.30h, 15.30h, 16.30h, 17.30h), and during the night (1.30h, 2.30h, 3.30h, 4.30h). The area under the curve (AUC) was estimated using trapezoidal integration.

Subsequently, diurnal variations in melatonin concentrations were appraised with a skewed baseline cosine function proposed by van Someren and Nagtegaal (13). This method has been validated to best describe the typical range of melatonin profiles and combines a fixed baseline with skewness of the cosine function, allowing differences in the steepness of the rising and falling limb of the melatonin peak. Outcome measures for statistical analyses were the peak level of melatonin and the corresponding acrophase (clock-time of the maximal melatonin concentration), a melatonin onset marker (up-cross time: the time after which the curve exceeds one-quarter of its amplitude) and a melatonin offset marker (down-cross time: the time after which the curve drops below one quarter of its amplitude).

Statistics

Results are presented as the mean ± standard error of the mean (SEM), unless stated otherwise. Statistical contrasts between groups were evaluated with the non-parametric Mann-Whitney U-test. SPSS for windows version 14.0 (SPSS Inc., Chicago, IL) was used for data analysis. $P<0.05$ was considered significant.

RESULTS

Patients

Age, gender, BMI and IGF-I concentrations did not differ between the three groups (Table 1). Radiotherapy was applied 13.9 ± 2.1 years prior to this study in the patients treated by postoperative radiotherapy. Two patients in the postoperative radiotherapy group had TSH deficiency, 1 patient had ACTH deficiency and 2 premenopausal female patients were treated with estrogens in combination with progesterone or and 2 male patients with testosterone. In contrast, none of the patients who were treated by surgery only suffered from anterior pituitary deficiencies.

Table 9/1: Clinical characteristics of patients with acromegaly treated by postoperative radiotherapy, patients treated by surgery only, and healthy controls.

	Radiotherapy (n=8)	Surgery (n=8)	Controls (n=8)
Age (years, mean (range))	52 (37-62)	52 (43-69)	53 (37-77)
Gender (n)	4/4	4/4	4/4
Body mass index (kg/m^2)	29.2 ± 1.8	30.2 ± 1.2*	25.6 ± 1.2
IGF-I (µg/l)	90 ± 14	125 ± 16	122 ± 9

Data are shown as mean ± SEM unless stated otherwise.
*P=0.038 *vs.* controls in the Mann-Whitney U-test.

Mean melatonin concentrations during the morning, afternoon, and night and total melatonin secretion

Mean serum melatonin concentrations were highest during nighttime (85.2 ± 10.1 pg/ml, p<0.001 compared to morning and afternoon) and lowest during the afternoon (27.0 ± 2.2 pg/ml, p=0.021 compared to morning and p<0.001 compared to nighttime) and in between these values during the morning (35.3 ± 3.0 pg/ml). Mean morning, afternoon, and nighttime concentrations did not differ between the three groups (Figure 1). Mean AUC did not differ between patients treated with postoperative radiotherapy (68653 ± 10391 pg/ml/24h), patients treated by surgery only (60524 ± 10550 pg/ml/24h), and healthy controls (68019 ± 9286 pg/ml/24h, p=NS).

Diurnal analysis

Representative examples of 24h melatonin secretion profiles in patients treated by postoperative radiotherapy and surgery only are shown in Figure 2. The peak level was not different between the three groups. Onset (up-cross time) and offset (down-cross time) were both later in patients treated with postoperative radiotherapy compared to surgery only and controls although these differences did not reach statistical significance. However, the acrophase was

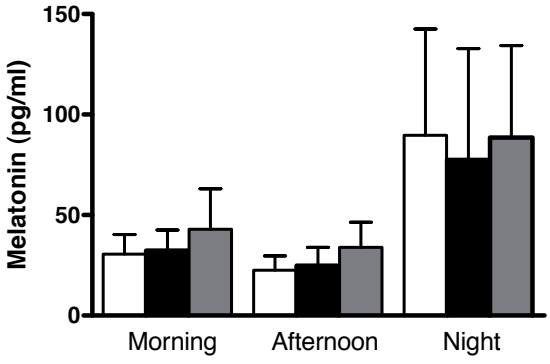

Figure 9/1: Mean morning, afternoon, and night melatonin concentrations in patients treated with postoperative radiotherapy (white bars), patients treated with surgery alone (black bars), and healthy controls (grey bars).

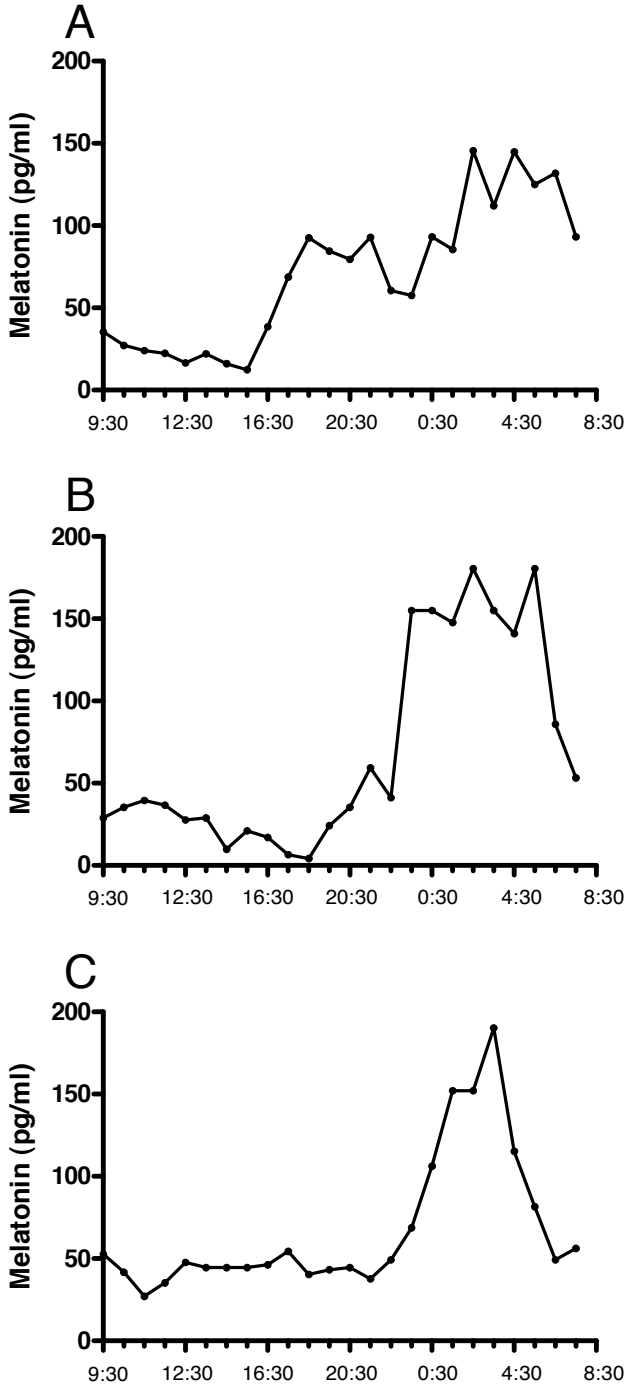

Figure 9/2: Serum melatonin time series in three representative subjects. A 62-yr old male patient cured by surgery and postoperative radiotherapy; B a 47-yr old male patient cured by surgery; C a 43-yr old healthy control male subject.

Table 9/2: Melatonin diurnal phase marker parameters in patients treated by postoperative radiotherapy, patients treated by surgery only, and healthy controls.

	Radiotherapy (n=8)	Surgery (n=8)	Controls (n=8)
Peak (pg/ml)	134.1 ± 30.0	91.5 ± 19.6	102.1 ± 14.9
Acrophase (h:min before or after midnight)	4:38 ± 1:11*	3:25 ± 1:37	1:38 ± 1:35
Down-cross time	7:00 ± 1:05	6:04 ± 1:44	5:20 ± 0:56
Up-cross time	18:42 ± 2:09	17:28 ± 2:37	16:31 ± 2:43

*P=0.038 *vs.* controls in the Mann-Whitney U-test. Data are shown as mean ± SEM.

significantly delayed in patients treated with postoperative radiotherapy compared to healthy controls (p=0.038, Table 2). In addition, the acrophase was also later in patients treated with postoperative radiotherapy compared to patients treated with surgery only, but this difference did not reach statistical significance. The point-estimation of the acrophase in patients treated with surgery only was in between patients treated with postoperative radiotherapy and healthy controls.

DISCUSSION

Radiotherapy for acromegaly is effective in lowering GH and IGF-I concentrations in acromegalic patients (1-3), whereas on the other hand it is associated with hypopituitarism and impairment in quality of life in these patients (5). The hypothalamus is considered to be even more vulnerable to radiation damage than the pituitary gland (4). Within the hypothalamus, the suprachiasmatic nucleus (SCN) regulates various circadian rhythms, including those of pituitary hormones and of melatonin by the pineal gland (9). Therefore, damage to the hypothalamus could translate into alterations in the diurnal profiles of behavior and physiology. Indeed, cranial radiation therapy in childhood is associated with objective and subjective changes in the sleep–wake rhythm in adulthood (7). The data presented here give additional support to the contention of SCN alterations by radiotherapy by showing that previous radiotherapy is associated with a shift in acrophase timing in diurnal melatonin secretion in acromegalic patients.

In our study, total melatonin secretion and diurnal timing of melatonin secretion were unaffected in patients treated for acromegaly by surgery only. Only a few studies have addressed circadian melatonin rhythms in patients with acromegaly. Data on melatonin secretion in active acromegaly are conflicting. In one study, total melatonin secretion was decreased compared to healthy controls, whereas the acrophase occured earlier (14). In contrast, another study reported an increased average 24h melatonin secretion, without any evidence of disturbed circadian timing (15). Additional studies showed increased melatonin levels during daytime

in patients with acromegaly (16;17) and patients with other intrasellar pituitary region tumors (18).

In our present study, radiotherapy did not affect total melatonin secretion, which contrasts with a previous study reporting diminished nocturnal melatonin secretion in 8 patients who had been treated with radiotherapy for acromegaly (19). However, in contrast to our study patients, those 8 patients still showed elevated growth hormone levels with failure of suppression during an oral glucose suppression test and two of these patients had elevated prolactin concentrations. To our knowledge, there are no other studies of the effects of radiotherapy on the pituitary gland on melatonin circadian secretion.

We found that radiotherapy was associated with a delayed acrophase of the circadian melatonin secretion profile, even though total melatonin secretion was unaffected. Melatonin secretion is under the control of endogenous cyclic signals arising from the SCN, the main circadian pacemaker (9). The close proximity of the pituitary gland to the ventromedial hypothalamic location of the SCN may make the latter vulnerable for scattered radiation aimed at the pituitary. Additional leads to support this hypothesis were found in the altered timing of sleep in patients during long term follow up after treatment for large non-functioning macroadenomas (20). In those patients, mid-sleep timing was clearly delayed. Interestingly, mid-sleep timing is highly correlated with the melatonin phase (21), in accordance with the delay in acrophase of melatonin concentrations observed in the current study. It is likely that with evaluation of radiotherapeutical precision techniques the scattering of radiation to the hypothalamus could be limited.

Alternatively, rather than direct radiation damage to the SCN per se, other hypothalamic nuclei that control SCN function could be damaged by radiotherapy resulting in altered excitatory/ inhibitory input to the SCN. GHRH, which is predominantly produced by the paraventricular nucleus, mediates specific feed back signals to the SCN (22) . There are indications that acromegalic patients previously treated by postoperative radiotherapy have a diminished GHRH tone (12;23). In this respect, it is interesting, that intra-SCN injection of GHRH during daytime was found to advance circadian phase in hamsters (22).

Circadian melatonin rhythms are relatively stable over time under controlled conditions like in our experiment (24). Indeed, it takes several days for the external factors to shift the phase of the body clock (24), which makes it unlikely that possible changes in the time that the lights were turned off in our experiment compared to the home situation influenced our findings. Melatonin secretion are also influenced by other factors such as environmental factors (transatlantic flights, night shift work), aging, alcohol consumption, and depression (24). However, the patients were matched for age and patients or controls who had recently undertaken transatlantic flights were excluded.

During long-term follow-up of patients with strict biochemical control of acromegaly, persisting complaints of general and physical fatigue have been observed, especially in relation to radiotherapy (5;6). Indeed, adequate sleep quality and quantity is obtained only when aligned

with the most favorable circadian timing window for sleep, e.g. during the high nocturnal levels of melatonin (25). In addition, melatonin is suggested to be important for optimal functioning of other human circadian systems (25). From this perspective, a phase delay in melatonin acrophase could thus not only be a consequence of alterations in hypothalamic circadian timing, but could in itself contribute to the complex persisting morbidity seen in patients treated for acromegaly especially in case of applied postoperative radiotherapy.

In conclusion, this study indicates that radiotherapy for acromegaly induces a delay in the acrophase of melatonin circadian rhythmicity, which could be a reflection of altered hypothalamic circadian timing.

REFERENCE LIST

1. Biermasz NR, Dulken HV, Roelfsema F 2000 Postoperative radiotherapy in acromegaly is effective in reducing GH concentration to safe levels. Clin Endocrinol (Oxf) 53:321-327
2. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
3. Eastman RC, Gorden P, Glatstein E, Roth J 1992 Radiation therapy of acromegaly. Endocrinol Metab Clin North Am 21:693-712
4. Toogood AA 2004 Endocrine consequences of brain irradiation. Growth Horm IGF Res 14 Suppl A:S1-18-S124
5. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F 2004 Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 89:5369-5376
6. van der Klaauw AA, Biermasz N, Hoftijzer HC, Pereira AM, Romijn JA 2008 Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly. Clin Endocrinol (Oxf)In press
7. Van Someren EJ, Swart-Heikens J, Endert E, Bisschop PH, Swaab DF, Bakker PJ, Romijn JA, Fliers E 2004 Long-term effects of cranial irradiation for childhood malignancy on sleep in adulthood. Eur J Endocrinol 150:503-510
8. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
9. Mignot E, Taheri S, Nishino S 2002 Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5 Suppl:1071-1075
10. Biermasz NR, van Dulken H, Roelfsema F 2000 Ten-year follow-up results of transsphenoidal microsurgery in acromegaly. J Clin Endocrinol Metab 85:4596-4602
11. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
12. van der Klaauw AA, Pereira AM, van Thiel SW, Smit JW, Corssmit EP, Biermasz NR, Frolich M, Iranmanesh A, Veldhuis JD, Roelfsema F, Romijn JA 2006 GH deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the 24 h GH secretion. Eur J Endocrinol 154:851-858
13. Van Someren EJ, Nagtegaal E 2007 Improving melatonin circadian phase estimates. Sleep Med 8:590-601
14. Sinisi AA, Pasquali D, D'Apuzzo A, Esposito D, Venditto T, Criscuolo T, De Bellis A, Bellastella A 1997 Twenty-four hour melatonin pattern in acromegaly: effect of acute octreotide administration. J Endocrinol Invest 20:128-133
15. Terzolo M, Piovesan A, Ali A, Codegone A, Pia A, Reimondo G, Torta M, Paccotti P, Borretta G, Angeli A 1995 Circadian profile of serum melatonin in patients with Cushing's syndrome or acromegaly. J Endocrinol Invest 18:17-24
16. Lissoni P, Mainini E, Rovelli F, Mazzi C, Ardizzoia A, Gelosa M, Capra M, Rivolta MR 1992 A clinical study of the pineal hormone melatonin in patients with growth hormone or prolactin secreting pituitary tumours. Eur J Med 1:407-410
17. Piovesan A, Terzolo M, Borretta G, Torta M, Buniva T, Osella G, Paccotti P, Angeli A 1990 Circadian profile of serum melatonin in Cushing's disease and acromegaly. Chronobiol Int 7:259-261
18. Touitou Y 1993 Melatonin in patients with pituitary tumours. Eur J Med 2:63
19. Zwirska-Korczala K, Ostrowska Z, Kos-Kudla B, Buntner B, Zych F 1994 The lack of melatonin rhythm in active acromegaly after radiotherapy. Endocr Regul 28:97-103
20. van der Klaauw AA, Dekkers OM, Pereira AM, van Kralingen KW, Romijn JA 2007 Increased daytime somnolence despite normal sleep patterns in patients treated for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 92:3898-3903

21. Burgess HJ, Savic N, Sletten T, Roach G, Gilbert SS, Dawson D 2003 The relationship between the dim light melatonin onset and sleep on a regular schedule in young healthy adults. Behav Sleep Med 1:102-114
22. Vaccarino FJ, Sovran P, Baird JP, Ralph MR 1995 Growth hormone-releasing hormone mediates feeding-specific feedback to the suprachiasmatic circadian clock. Peptides 16:595-598
23. van der Klaauw AA, Pereira AM, van Thiel SW, Frolich M, Iranmanesh A, Veldhuis JD, Roelfsema F, Romijn JA 2007 Attenuated pulse size, disorderly growth hormone and prolactin secretion with preserved nyctohemeral rhythm distinguish irradiated from surgically treated acromegaly patients. Clin Endocrinol (Oxf) 66:489-498
24. Selmaoui B, Touitou Y 2003 Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: a study of three different 24-h cycles over six weeks. Life Sci 73:3339-3349
25. Arendt J 2006 Melatonin and human rhythms. Chronobiol Int 23:21-37

Chapter 10

Recombinant human growth hormone replacement increases CD34+ cells and improves endothelial function in adults with growth hormone deficiency

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European Journal of Endocrinology, 2008

ABSTRACT

Objective

Adult patients with growth hormone deficiency (GHD) are at increased risk for cardiovascular morbidity and mortality. Endothelial function, vascular stiffness and loss of circulating CD34+ cells are considered biomarkers for cardiovascular disease. The aim of this study was to assess vascular structure and function in relation to circulating CD34+ cells in adults with GHD before and during 1 year of recombinant human growth hormone (rhGH) replacement.

Design

1 year intervention with rhGH.

Patients and methods

Vascular function (flow-mediated dilatation (FMD)) and structure (pulse wave velocity (PWV) and analysis) were assessed in 14 adult patients (9 men) with GHD (mean age 57 yrs, range 27-71 year). In addition, the number of CD34+ cells was evaluated using flow cytometric analysis. Study parameters were analyzed at baseline, and after 6 months and 1 year of rhGH replacement.

Results

RhGH replacement increased IGF-I levels from 10.4 ± 4.5 mmol/l at baseline to 18.4 ± 10.1 mmol/l, and 20.5 ± 8.0 mmol/l, at 6 months, and 1 year, resp. (p=0.001). FMD increased from 3.5 ± 1.8% to 6.0 ± 2.5% and 5.1 ± 2.5% during 1 yr rhGH replacement, p=0.008). There was no beneficial effect on PWV, central pulse pressure, central systolic pressure and augmentation index. The number of CD34+ cells increased from 794.9 ± 798.8 cells/ml to 1270.7 ± 580.1 cells/ml and to 1356.9 ± 759.0 cells/ml (p=0.010).

Conclusion

One year of rhGH replacement in adults with GHD improves endothelial function and increases the number of circulating CD34+ cells.

INTRODUCTION

Growth hormone deficiency (GHD) is associated with an increased prevalence of cardiovascular risk factors, such as central obesity, hypertension, dyslipidemia, a, decrease in lean body mass and an increase in insulin resistance (1;2). In addition, abnormalities in vascular function and structure have been described in GHD (3-5). Recombinant human growth hormone (rhGH) replacement in GHD is aimed at reversing these abnormalities (6-10).

Since a decade, bone marrow-derived endothelial progenitor cells have been proposed to play an important role in maintenance and repair of the vasculature. Both re-endothelialization and angiogenic capacity have been put forward as mechanisms by which these cells are involved in vascular repair (11). We, and others, have shown that the number of these cells are reduced in patients with type 1 diabetes (12), in patients with other cardiovascular risk factors and with established cardiovascular disease (13;14).

There is, however, a continuing debate on the phenotypic characteristics of endothelial progenitor cells (11;15;16). Many groups perform flow cytometric analysis using CD34, CD133 and/or VEGFR as cell surface markers to characterize the cells. Of these, CD34-positive cells (without VEGFR expression) show a stronger inverse correlation with the presence and number of cardiovascular risk factors than CD34/VEGFR+ cells or cells with other combinations of positive surface markers believed to be endothelial progenitors (17).

Thus, CD34+ cells are a bone-marrow derived biomarker for cardiovascular risk, but there is no information on the effects of rhGH replacement on these cells in adults with GHD. Therefore, the aim of our study was to evaluate the effects of rhGH replacement on the number of circulating CD34+ cells and vascular function and structure in adults with GHD.

METHODS

Patients

Fourteen patients with GHD were included in this prospective, open-label intervention study. GHD was confirmed in all patients by an insulin tolerance test (nadir blood glucose <2.2 mmol/) with a peak GH concentration <3 µg/l. An additional inclusion criteria was stable hormonal substitution of dysfunctional hormonal axes at least 3 months prior to study start. Exclusion criteria were a hormonally active pituitary tumor, history of cancer, presence of chronic inflammatory disease, diabetes mellitus, and a history of cardiovascular disease. The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and written informed consent was obtained from all subjects.

Treatment protocol

Patients were treated with recombinant-human growth hormone for 12 months. After initial measurements were obtained, all patients were treated with subcutaneous injections of rhGH (Genotropin® Pharmacia/Pfizer or Zomacton® Ferring, Norditropin® NovoNordisk, or Humatrope® Lilly), given every evening. The initial dose of 0.2 mg/day rhGH was individually adjusted each month in the first half year to achieve physiological serum IGF-I concentrations, within the age-dependent laboratory reference range (IGF-I standard deviation scores (SD scores)). The patients were studied at baseline, and 6 and 12 months after growth hormone replacement. During the study, no antihypertensive or lipid-lowering drugs were prescribed.

Study parameters

Endothelial function

Nitric oxide-dependent flow mediated dilatation (FMD), expressed as percentage diameter change in the brachial artery after reactive hyperemia, was measured non-invasively by ultrasonography using standard procedures in our Vascular Reseach Unit (18). Measurements were performed at the elbow of the right arm using a vessel wall movement system (Wall Track System, Pie Medical, Maastricht, The Netherlands), which consists of an ultrasound imager with a 10 MHz linear array transducer connected to a data acquisition system and a personal computer. Three measurements were averaged to calculate a baseline diameter of the brachial artery. By inflation of a blood pressure cuff for 5 min at a pressure of 200 mm Hg, ischemia was applied to the forearm distal to the location of the transducer. Ultrasonography continued for 5 min after cuff release with measurements at 30 sec intervals. The widest lumen diameter was taken as a measurement for maximal vasodilatation. Nitroglycerin spray (400µg) was administered to determine endothelium-independent vasodilatation. All measurements were performed by the same technician with patients supine in a quiet temperature controlled environment after at least 15 minutes of rest. All patients were requested to refrain from smoking on the morning of vascular measurements. Control values for FMD were obtained from healthy age-, gender-matched subjects (9 men) with a BMI of 26.6 ± 2.9 kg/m^2 (age 49.8 ± 12.4 yrs, p=NS compared to patients). Three control subjects smoked.

Pulse wave velocity

Arterial stiffness was assessed non-invasively by aortic PWV using standard procedures in our Vascular Reseach Unit (19). In short, sequential tonometry was performed at the common carotid artery and the femoral artery using a Sphygmocor device (Sphygmocor, Actor Medical, Sydney, Australia) to record the arterial pulse waveform. Pulse transit time between the two sites was determined by the system software from the average of 10 consecutive heartbeats. The distance between the two recording sites was measured and aortic PWV was calculated as the distance traveled by the pulse wave divided by the transit time (in cm/s). The validation

of this automatic method and its reproducibility have been published previously (20). The measurements were performed twice in each patient and then averaged to obtain the mean aortic PWV, which was used for statistical analysis. The same control subjects as for the FMD were used.

Central pressure and augmentation index

Central pulse pressure was determined by measuring the brachial blood pressure, determining the pulse waveform at the brachial and carotid artery by applanation tonometry using a Millar probe (Millar Instruments, Houston, Texas) and applying the calibration method according to Kelly and Fitchett (21) to determine central systolic blood pressure and central pulse pressure (22). This method assumes that the mean arterial pressure and diastolic blood pressure remain constant from the aorta to the large peripheral arteries which allows central pulse pressure calculation. The same control subjects as for the FMD were used.

CD34-positive cells

For enumeration of CD34-positive circulating (CD34+ cells), flow cytometric analysis was performed using a multi-parametric gating strategy based on the International Society of Hematotherapy and Graft Engineering (ISHAGE). This lyse/no wash method uses Trucount tubes (Becton Dickinson, Franklin Lakes, NJ, USA) that contain a defined number of brightly fluorescent microbeads, permitting the acquisition of absolute counts of cells, even at very low numbers. Circulating CD34+ cells are defined as cells with low-expression for CD45, positive for CD34, and located in the lympho-gate on a side- and forward-scatter plot. Within 2h of blood-withdrawal, 50 µl of EDTA-anticoagulated whole blood was added per Trucount tube (two per subject) by reverse pipetting and directly labeled antibodies were added: CD45-PerCP, CD34-FITC (BD Biosciences, Erembodegem, Belgium). After 30 min incubation on ice and in the dark, cells were fixed using FACS-lysing solution (BD Biosciences) and the samples were measured within 24h using a fluorescence-activated cell sorter (FACS)-Calibur (BD Biosciences). A total of 500.000 CD45+ cells were measured (excluding the beads) and the number of CD34+ cells per microliter blood was calculated. Reference values were obtained from 9 healthy men with a mean age of 61 ± 5 yrs and a BMI of 24.2 ± 1.0 kg/m^2 obtained in our center.

Biochemical parameters

IGF-I, IGFBP-3, fasting levels of glucose, total cholesterol, HDL cholesterol (HDL), and triglycerides (TG) were measured at baseline, after 6 months, and after 1 year of follow-up. LDL cholesterol concentrations (LDL) were calculated using the Friedewald formula. Patients were requested to fast overnight before blood samples were taken for laboratory measurements of lipid profiles and glucose.

Assays

Serum IGF-I concentrations (ng/ml) were measured using an immunometric technique on an Immulite 2500 system (Diagnostic Products Corporation, Los Angeles, USA). The intra-assay variation was 5.0 and 7.5% at mean plasma levels of 8 and 75 nmol/l, respectively.

IGFBP-3 was measured using an immunometric technique on an IMMULITE Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 0.02 mg/l and inter-assay variation was 4,4 and 4.8% at 0.91 and 8,83 mg/l.

A Hitachi P800 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose, total cholesterol and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany).

Statistics

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are scored as the mean ± standard deviation (SD), unless specified otherwise. ANOVA repeated measurements with Sidak correction for multiple comparisons were used. A P-value <0.05 was assumed to represent a significant difference.

RESULTS

Patients

Fourteen patients (9 men) were included in this prospective, open-label intervention study with a mean age of 51 years (range 27-71 years) and a mean BMI of 29.4 ± 3.9 kg/m^2. GHD was secondary to a non-functioning pituitary macroadenoma in eleven patients, to an enlarged

Table 10/1: Clinical characteristics of the included patients.

		N=14
Age (yrs, mean (range))		50.8 (27-71)
Gender (M/F (n(%)))		9 (64)/ 5 (36)
Etiology of GHD (n(%))	Non-functioning pituitary adenoma	11 (79)
	Other	3 (21)
Treatment of pituitary tumor	Surgery (n(%))	11 (79)
	Radiotherapy (n(%))	4 (29)
Pituitary deficiencies	TSH deficiency (n(%))*	8 (57)
	ACTH deficiency (n(%))*	6 (43)
	LH-FSH deficiency (n(%))*	7 (50)
	ADH deficiency (n(%))	1 (7)
Smoking (n(%))		4 (29)

*TSH deficiency was treated with thyroid hormone substitution in all TSH deficient patients. ACTH deficiency was treated with hydrocortisone substitution in all ACTH deficient patients as was LH-FSH deficiency with either testosterone and estrogen.

pituitary stalk in 2 patients, and was idiopathic in 1 patient. Additional clinical characteristics are detailed in Table 1.

Effects of 1 year rhGH replacement

During rhGH replacement, IGF-I and IGFBP-3 concentrations increased within 6 months after the start of treatment (p=0.006 and p=0.053, resp., Table 2), and remained unchanged between 6 months and 1-year rhGH replacement (p=1.0 and p=1.0, respectively). Total, LDL and HDL cholesterol remained unchanged as well as fasting glucose and triglycerides during 1 yr rhGH replacement.

Table 10/2: Vascular endothelial function, PWV and PWA during 1 year rhGH replacement in adult patients with growth hormone deficiency.

	Baseline	6 months rhGH replacement	1 yr rhGH replacement	Overall P-value	Control values*
IGF-I (nmol/l)	10.4 ± 4.5	18.4 ± 10.1	20.5 ± 8.0	0.001	
IGFBP-3 (mg/l)	2.7 ± 1.2	4.0 ± 1.7	4.0 ± 1.1	0.003	
Glucose (mmol/l)	5.1 ± 0.6	5.1 ± 0.7	4.9 ± 0.7	0.062	
Total cholesterol (mmol/l)	5.5 ± 0.1	5.2 ± 1.1	5.2 ± 0.8	0.335	
LDL cholesterol (mmol/l)	3.7 ± 0.8	3.6 ± 0.4	3.5 ± 0.8	0.545	
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.3 ± 0.4	1.4 ± 0.4	0.122	
Triglycerides (mmol/l)	1.6 ± 0.7	1.3 ± 0.6	1.6 ± 0.7	0.064	
FMD (%)	3.5 ± 1.8	6.0 ± 2.5	5.1 ± 2.5	0.008	9.1 ± 4.7
NTG (%)	13.5 ± 5.2	14.9 ± 6.5	13.1 ± 4.8	0.427	18.0 ± 5.9
Aortic PWV (cm/s)	7.9 ± 1.9	7.7 ± 1.9	7.7 ± 2.2	0.777	8.1 ± 1.3
Brachial systolic pressure (mm Hg)	136.2 ± 11.9	131.6 ± 17.2	136.6 ± 15.0	0.302	133.0 ± 7.0
Brachial diastolic pressure (mm Hg)	84.1 ± 8.1	80.7 ± 8.1	81.2 ± 8.1	0.056	83.8 ± 5.6
Brachial pulse pressure (mm Hg)	52.1 ± 11.3	50.9 ± 17.6	55.4 ± 10.2	0.374	49.2 ± 6.9
Central systolic pressure (mm Hg)	135.6 ± 13.5	135.1 ± 22.6	138.1 ± 17.5	0.812	129.8 ± 7.5
Central pulse pressure (mm Hg)	51.8 ± 12.1	54.8 ± 22.3	57.0 ± 13.7	0.569	46.8 ± 8.1
Augmentation index	26.6 ± 10.6	24.9 ± 11.6	25.7 ± 11.4	0.483	32.9 ± 11.9

Data were compared with ANOVA with repeated measurements. *Reference values of age- and gender matched healthy controls. FMD, flow-mediated vasodilatation. NTG, nitroglycerin. PWV, pulse wave velocity.

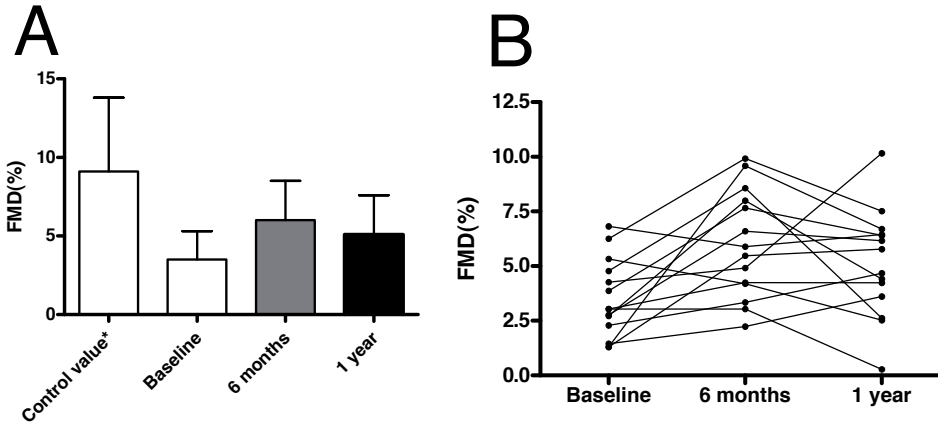

Figure 10/1: Percentage flow-mediated dilatation (FMD) during 1 year rhGH replacement in adult patients with growth hormone deficiency increases significantly, predominantly in the first 6 months of rhGH replacement (overall P=0.008). Upper panel (A): The first white bar represents the control value* of FMD in age- and gender-matched healthy subjects obtained in our center. The second white bar represents baseline, grey bar 6 months of rhGH replacement, and black bar 1 year of rhGH replacement. Lower panel (B): Individual FMD during 1 year of rhGH replacement.

Vascular assessment and CD34+ cells during rhGH

FMD increased during 1 yr rhGH replacement (p=0.008, Table 2), most markedly during the first half year of rhGH replacement (Figure 1). No change in PWV, brachial systolic and pulse pres-

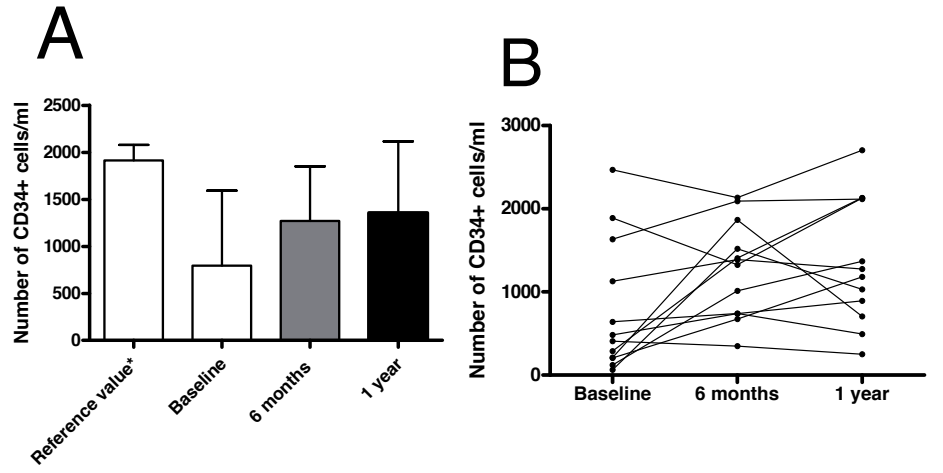

Figure 10/2: Number of circulating CD34+ haematopoietic stem cells during 1 year of rhGH replacement in adult patients with growth hormone deficiency increases (overall P=0.010) Upper panel (A): The first white bar represents the reference value* of the number of circulating CD34+ cells in 9 men with a mean age of 61 ± 5 yrs and a BMI of 24.2 ± 1.0 kg/m^2 obtained in our center. Second white bar represents baseline, grey bar 6 months of rhGH replacement, and black bar 1 year of rhGH replacement. Lower panel (B): Individual concentrations of circulating CD34+ haematopoietic stem cells during 1 year of rhGH replacement.

sure, central pulse pressure, central systolic pressure and augmentation index were observed during GH therapy (Table 2).

The number of circulating CD34+ cells increased from 794.9 ± 798.8 cells/ml to 1270.7 ± 580.1 cells/ml and, 1356.9 ± 759.0 cells/ml, at 6 and 12 months, respectively, after treatment (p=0.010, Figure 2). The reference values of the number of circulating CD34+ cells in 9 men with a mean age of 61 ± 5 yrs and a BMI of 24.2 ± 1.0 kg/m^2 obtained in our center was 1913.6 ± 1640.2 cells/ml. The number of erythrocytes, lymphocytes, and leukocytes (CD45 positive cells) remained unchanged.

There were no correlations between the change in FMD and the change in number of CD34+ cells (R=0.217, p=0.499), or between the change in IGF-I and the change in FMD (R=0.080, p=0.785) and the change in CD34+ cells (R=0.425, p=0.169). Smoking habits and gender were not related to either change in FMD or change in number of CD34+ cells. Age was correlated with change in CD34+ cells after 1 months (r^2=0.367, p=0.04, Figure 3).

DISCUSSION

The novel finding in this study is the beneficial effect of treatment with rhGH both on the number of circulating CD34+ cells and on endothelial function, which was manifest within 6 months after the start of treatment and maintained 6 months thereafter.

Hypopituitarism in general is associated with increased mortality, predominantly due to cardiovascular diseases (23), which has been ascribed to untreated GHD (24). These observations in patients with GHD are related to an increased prevalence of cardiovascular risk factors, such as hypertension, dyslipidemia, and alterations in body composition (2). Indeed, intima-media thickness (IMT) is increased in patients with GHD compared to control subjects (25). However,

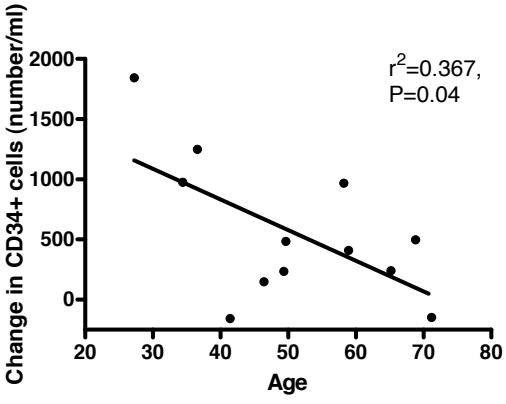

Figure 10/3: Correlation between change number of circulating CD34+ haematopoietic stem cells during 1 year of rhGH replacement in adult patients with growth hormone deficiency and age.

the effect of rhGH replacement on cardiovascular health is still subject to debate. IMT decreased during rhGH replacement in one study (25), whereas others reported that intima-media thickness was not different compared to controls (3;26) and remained unchanged during rhGH replacement (6).

In our study, the number of circulating CD34+ cells in adults with GHD increased within 6 months of rhGH replacement and remained stable thereafter. These results are in line with the very recently observed potential of rhGH treatment to increase the number of circulating endothelial progenitor cells (classified as CD133/VEGFR2 cells and colony-forming units) in healthy volunteers (27). In addition, the potential of rhGH to positively influence hematopoiesis has previously been shown in another clinical setting, i.e. harvesting of CD34+ cells destined for autologous hematopoietic stem cell transplantation in patients with relapsed or refractory hematologic malignancies (28).

Endothelial function was measured in our study before and during rhGH replacement by assessing flow-mediated vasodilatation (FMD). Indeed, at baseline FMD was decreased compared to reference values obtained in our vascular unit. The observed improved FMD after rhGH replacement was also observed within 6 months and continued until the end of the study. These data are in agreement with earlier other reports assessing the effects of rhGH replacement on endothelial function (6;25;29). A putative mechanism by which rhGH replacement improves vascular function is IGF-I mediated stimulation of nitric oxide synthesis in endothelial cells (30;31).

Since we intended to use circulating bone marrow-derived cells as biomarkers for cardiovascular health, we focused on CD34+ cells only, which are closely linked to cardiovascular risk, even more closely than CD34+/VEGFR+ cells (17). The potential mechanisms, responsible for the increase in the number of CD34+ cells in our study, are not clear. Improvement in endothelial function is associated with increased nitric oxide bio-availability, in particular in the bone marrow (32), which is associated with increased mobilization of CD34+ cells. Indeed, growth hormone treatment was found to induce markers of nitric oxide bio-availability in health volunteers (27). In addition, CD34+ cells express both GH and IGF-I receptors (33) as is the case for several other cell types that could be involved. Indeed, studies in rodents and on fetal bone marrow demonstrate direct effects of GH and IGF-I on hematopoiesis (33;34). It is likely that complex interactions between circulating IGF-I, IGFBP-3 and their effects on nitric-oxide bioavalability result in the increase in CD34+ cells in our study. Indeed, a recent study reported that IGFBP-3 also promotes migration, tube formation of CD34+ cells and differentiation of these cells into endothelial cells, leading to increased vessel stabilization and quicker blood vessel development (35) illustrating the complexity of potential mechanisms involved in rhGH effects.

In addition, we also determined several measures of arterial stiffness before and during one year of rhGH replacement. Pulse wave velocity, as a direct measure of arterial stiffness, did not change during the study. This is in contrast with an earlier report by McCallum et al.

(36). In that study PWV decreased from 8.1 to 6.7 m/s during 6 months of rhGH replacement in 16 patients with GHD (36). In our study no change in PWV was found after 6 months or 1 year of rhGH replacement. The discrepancies between the two studies might be related to a more disadvantageous cardiovascular risk profile in our patients group, since they were older (average 7 years), included more men (64% versus 37%) and had a higher BMI (29.4 vs. 27.8 kg/m^2). The small improvement in central systolic blood pressure and augmentation index in a previous report (9) was not observed in our study possibly due the differences in patient groups or the limited number of patients studied.

Although we did not find a statistically significant decrease in lipid concentrations in our limited number of patients, LDL cholesterol decreased by 0.2 mmol/l and total cholesterol by 0.3 mmol/l. In a metaanalysis of short-term trials (treatment up to 18 months) with rhGH replacement in GHD (7), the weighted mean differences for LDL and total cholesterol were –0.53 mmol/l and –0.34 mmol/l respectively. Thus, the changes in lipid concentrations in our study move in a similar direction to the changes noted in rhGH replacement in general.

The major study limitation is the low number of patients that were included due to the fact that GHD is a rare disease and that our study design excluded subject with a history of cardiovascular disease, which could influence our measurements. In addition, the beneficial effects of rhGH replacement have been widely accepted which limits the possibilities to study the natural course of this disease with respect to CD34+ cells. Nonetheless, the differences in CD34+ cell numbers found in this group with a wide age range are relatively major which supports a role of GH in the regulation of this cell type. Thus, this study provides new data into the relationship of circulating endothelial progenitor cells and growth hormone which can be used as a basis for additional larger studies. . Interestingly, the change in CD34+ cells showed an inverse relationship with advancing age indicating that the effect of rhGH on CD34+ cells is age-dependent.

In conclusion, one year of rhGH replacement in adult patients with GHD improved endothelial function and increased the number of CD34+ cells. Since these outcome parameters are strong biomarkers for cardiovascular disease risk, our data indicate that growth hormone replacement in adults with GHD may have beneficial effects on the vasculature.

ACKNOWLEDGEMENTS

We thank Jos op 't Roodt for skilled performance of vascular function tests and Jacques Duijs for expert technical assistance.

REFERENCE LIST

1. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
2. van der Klaauw AA, Biermasz NR, Feskens EJ, Bos MB, Smit JW, Roelfsema F, Corssmit EP, Pijl H, Romijn JA, Pereira AM 2007 The prevalence of the metabolic syndrome is increased in patients with GH deficiency, irrespective of long-term substitution with recombinant human GH. Eur J Endocrinol 156:455-462
3. Elhadd TA, Abdu TA, Oxtoby J, Kennedy G, McLaren M, Neary R, Belch JJ, Clayton RN 2001 Biochemical and biophysical markers of endothelial dysfunction in adults with hypopituitarism and severe GH deficiency. J Clin Endocrinol Metab 86:4223-4232
4. Evans LM, Davies JS, Goodfellow J, Rees JA, Scanlon MF 1999 Endothelial dysfunction in hypopituitary adults with growth hormone deficiency. Clin Endocrinol (Oxf) 50:457-464
5. Lanes R, Gunczler P, Lopez E, Esaa S, Villaroel O, Revel-Chion R 2001 Cardiac mass and function, carotid artery intima-media thickness, and lipoprotein levels in growth hormone-deficient adolescents. J Clin Endocrinol Metab 86:1061-1065
6. Abdu TA, Elhadd TA, Buch H, Barton D, Neary R, Clayton RN 2004 Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk. Clin Endocrinol (Oxf) 61:387-393
7. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
8. Pfeifer M, Verhovec R, Zizek B, Prezelj J, Poredos P, Clayton RN 1999 Growth hormone (GH) treatment reverses early atherosclerotic changes in GH-deficient adults. J Clin Endocrinol Metab 84:453-457
9. Smith JC, Evans LM, Wilkinson I, Goodfellow J, Cockcroft JR, Scanlon MF, Davies JS 2002 Effects of GH replacement on endothelial function and large-artery stiffness in GH-deficient adults: a randomized, double-blind, placebo-controlled study. Clin Endocrinol (Oxf) 56:493-501
10. van der Klaauw AA, Romijn JA, Biermasz NR, Smit JW, van Doorn J, Dekkers OM, Roelfsema F, Pereira AM 2006 Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 155:701-708
11. Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ 2004 Endothelial progenitor cells: more than an inflammatory response? Arterioscler Thromb Vasc Biol 24:834-838
12. Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC, Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ 2004 Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 53:195-199
13. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T 2003 Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 348:593-600
14. Werner N, Kosiol S, Schiegl T, Ahlers P, Walenta K, Link A, Bohm M, Nickenig G 2005 Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 353:999-1007
15. Yoder MC, Mead LE, Prater D, Krier TR, Mroueh KN, Li F, Krasich R, Temm CJ, Prchal JT, Ingram DA 2007 Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 109:1801-1809
16. Urbich C, Dimmeler S 2004 Endothelial progenitor cells: characterization and role in vascular biology. Circ Res 95:343-353
17. Fadini GP, de Kreutzenberg SV, Coracina A, Baesso I, Agostini C, Tiengo A, Avogaro A 2006 Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. Eur Heart J 27:2247-2255
18. van Wijk JP, de Koning EJ, Cabezas MC, op't RJ, Joven J, Rabelink TJ, Hoepelman AI 2005 Comparison of rosiglitazone and metformin for treating HIV lipodystrophy: a randomized trial. Ann Intern Med 143:337-346

19. van Wijk JP, de Koning EJ, Cabezas MC, Joven J, op't RJ, Rabelink TJ, Hoepelman AM 2006 Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. J Am Coll Cardiol 47:1117-1123
20. Wilkinson IB, Fuchs SA, Jansen IM, Spratt JC, Murray GD, Cockcroft JR, Webb DJ 1998 Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. J Hypertens 16:2079-2084
21. Kelly R, Fitchett D 1992 Noninvasive determination of aortic input impedance and external left ventricular power output: a validation and repeatability study of a new technique. J Am Coll Cardiol 20:952-963
22. Verbeke F, Segers P, Heireman S, Vanholder R, Verdonck P, Van Bortel LM 2005 Noninvasive assessment of local pulse pressure: importance of brachial-to-radial pressure amplification. Hypertension 46:244-248
23. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM 2001 Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 357:425-431
24. Rosen T, Bengtsson BA 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285-288
25. Pfeifer M, Verhovec R, Zizek B 1999 Growth hormone (GH) and atherosclerosis: changes in morphology and function of major arteries during GH treatment. Growth Horm IGF Res 9 Suppl A:25-30
26. Lanes R, Soros A, Flores K, Gunczler P, Carrillo E, Bandel J 2005 Endothelial function, carotid artery intima-media thickness, epicardial adipose tissue, and left ventricular mass and function in growth hormone-deficient adolescents: apparent effects of growth hormone treatment on these parameters. J Clin Endocrinol Metab 90:3978-3982
27. Thum T, Fleissner F, Klink I, Tsikas D, Jakob M, Bauersachs J, Stichtenoth DO 2007 Growth hormone treatment improves markers of systemic nitric oxide bioavailability via insulin-like growth factor-1. J Clin Endocrinol Metab 92: 4172-4179
28. Carlo-Stella C, Di Nicola M, Milani R, Guidetti A, Magni M, Milanesi M, Longoni P, Matteucci P, Formelli F, Ravagnani F, Corradini P, Gianni AM 2004 Use of recombinant human growth hormone (rhGH) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the mobilization and collection of CD34+ cells in poor mobilizers. Blood 103:3287-3295
29. Evans LM, Davies JS, Anderson RA, Ellis GR, Jackson SK, Lewis MJ, Frenneaux MP, Rees A, Scanlon MF 2000 The effect of GH replacement therapy on endothelial function and oxidative stress in adult growth hormone deficiency. Eur J Endocrinol 142:254-262
30. Tsukahara H, Gordienko DV, Tonshoff B, Gelato MC, Goligorsky MS 1994 Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. Kidney Int 45:598-604
31. Boger RH, Skamira C, Bode-Boger SM, Brabant G, von zur MA, Frolich JC 1996 Nitric oxide may mediate the hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. J Clin Invest 98:2706-2713
32. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S 2003 Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 9:1370-1376
33. Hanley MB, Napolitano LA, McCune JM 2005 Growth hormone-induced stimulation of multilineage human hematopoiesis. Stem Cells 23:1170-1179
34. Merchav S 1998 The haematopoietic effects of growth hormone and insulin-like growth factor-I. J Pediatr Endocrinol Metab 11:677-685
35. Chang KH, Chan-Ling T, McFarland EL, Afzal A, Pan H, Baxter LC, Shaw LC, Caballero S, Sengupta N, Li CS, Sullivan SM, Grant MB 2007 IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor cell function during vascular development. Proc Natl Acad Sci U S A 104:10595-10600

36. McCallum RW, Sainsbury CA, Spiers A, Dominiczak AF, Petrie JR, Sattar N, Connell JM 2005 Growth hormone replacement reduces C-reactive protein and large-artery stiffness but does not alter endothelial function in patients with adult growth hormone deficiency. Clin Endocrinol (Oxf) 62:473-479

Chapter 11

Sustained effects of recombinant growth hormone replacement after 7 years of treatment in adults with growth hormone deficiency

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European Journal of Endocrinology, 2006

ABSTRACT

Context

The goal of replacement with recombinant human growth hormone (rhGH) is to ameliorate symptoms, signs and complications of adult growth hormone deficiency (GHD) in the long-term. To determine whether the observed short-term beneficial effects of rhGH treatment are sustained in the long-term, we evaluated biochemical and anthropometric parameters after seven years of rhGH replacement.

Patients and methods

Sixty-three adult GHD patients (30 men, 52 adult-onset GHD) were assessed after 2, 5 and 7 years of rhGH replacement. IGF-I increased during rhGH replacement, and a stable dose of rhGH was reached within 1 year of rhGH substitution. Thereafter, this individualized dose was continued.

Results

Plasma levels of total cholesterol and LDL cholesterol decreased even after 5 years of rhGH replacement (11% decrease, $p<0.001$ and 22% decrease, $p<0.001$, respectively). HDL cholesterol levels increased during 7 years of rhGH replacement (1.4 ± 0.5 mmol/l at baseline vs. 1.7 ± 0.5 mmol/l after 7 years, $p<0.001$), whereas triglyceride concentrations remained unchanged. Fasting glucose levels increased during follow-up, mainly during the first two years of rhGH replacement (4.4 ± 0.7 mmol/l to 5.0 ± 1.0 mmol/l, $p<0.001$). BMI increased during follow-up, whereas waist circumference and waist-to-hip ratio remained unchanged. Diastolic blood pressure decreased ($p=0.002$), but when patients using antihypertensive medication were excluded this decrease did not reach significance ($p=0.064$). Systolic blood pressure remained unchanged.

Conclusion

The beneficial effects of rhGH replacement, described after short-term rhGH replacement, are sustained in the long-term up to seven years.

INTRODUCTION

Adult GHD is characterized by an adverse cardiovascular metabolic profile: increased serum concentrations of serum total cholesterol (TC), low-density lipoprotein (LDL) and triglycerides (TG), and decreased serum concentrations of high-density lipoprotein cholesterol (HDL), and an altered body composition reflected in reduced muscle strength and mass, visceral obesity, and decreased bone mass (1). The goal of recombinant human growth hormone (rhGH) replacement is to ameliorate symptoms and signs of the adult growth hormone deficiency (GHD) syndrome. Short-term (up to 24 months) replacement therapy with rhGH decreases the plasma concentrations of LDL cholesterol, total cholesterol, as well as fat mass and diastolic blood pressure, and increases lean body mass, fasting glucose and insulin concentrations (2;2). Because GHD is in general an irreversible condition which requires long term replacement, the question arises, whether these short term changes are sustained during long-term rhGH replacement.

Götherström et al. reported results of 5 years of rhGH replacement in 118 patients, and documented decreases in TC, LDL, TG, glycosylated hemoglobin, and body fat, and increases in HDL and lean body mass (3). However, only 3 small studies have reported a follow-up duration of more than 5 years (maximum number of 12 patients, summarized in Table 3, (4-6)). To determine whether the beneficial effects of rhGH treatment are sustained in the long-term in a larger cohort, we evaluated the effects of seven-years of rhGH replacement on biochemical parameters and anthropometric parameters in our cohort of GHD adults.

SUBJECTS AND METHODS

Patients

We prospectively enrolled 88 consecutive patients with GHD. After the initiation of treatment, twenty patients discontinued after a mean duration of rhGH treatment of 3.2 years (range 0.7-6.3 years), 2 patients died (acute cardiac arrest and acute stroke), and 3 were lost to follow-up (Figure 1). Sixty-three patients completed 7 years of rhGH replacement. Baseline characteristics of the patients, who did not complete the study, did not differ from the patients, who completed the study (gender/ age/ BMI/ age at onset of GHD/ etiological diagnosis/ surgery/ radiotherapy/ pituitary deficiencies). Biochemical and anthropometric efficacy parameters were studied in these 63 patients who completed the 7 years of rhGH replacement. During follow-up 1 patient; a 29-year old male patient with idiopatic GHD and a BMI of 37.5 kg/m^2 developed diabetes 7 years after the start of rhGH replacement. Biochemical and anthropometric efficacy parameters were studied separately in 7 patients who stopped after completion of 2 years of rhGH replacement and in 5 patients who stopped after completion of 5 years of rhGH replacement (8 patients dropped out before the first efficacy evaluation). There were no differences in responses to rhGH replacement for any of the efficacy parameters between the 7 patients

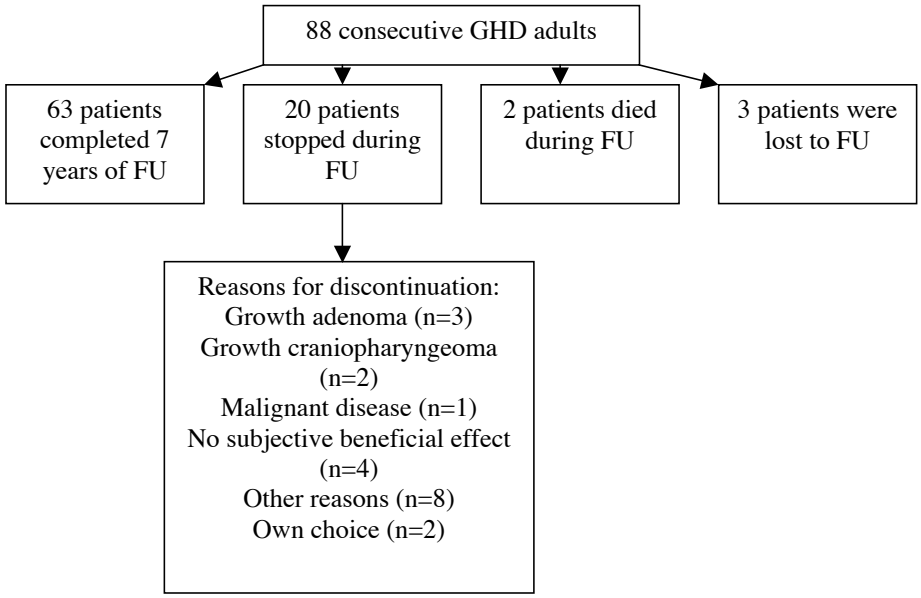

Figure 11/1: Flow sheet of 88 consecutive GHD adults enrolled in the open label observational study. Other reasons for discontinuation consisted of nonspecific complaints, pregnancy, no coverage of insurance, patient's mistake, severe depression, unknown (n=3). GHD: growth hormone deficiency, FU: follow-up

who stopped after 2 years and the patients who continued after 2 years. The response to rhGH replacement during 5 years was also the same for the 5 patients who stopped after 5 years and patients who continued after 5 years.

Treatment Protocol

Prior to the start of rhGH treatment, the diagnosis of growth hormone deficiency (GHD) was established by a peak GH concentration <3 µg/l during an insulin tolerance test (nadir blood glucose <2.2 mmol). Patients were then prospectively enrolled in an open label treatment protocol.

All patients were treated with subcutaneous injections of rhGH (Genotropin® Pharmacia/Pfizer, Zomacton® Ferring, or Norditropin® NovoNordisk, Humatrope® Lilly), given subcutaneously every evening. The initial dose of rhGH was 0.2 mg/day, which was individually adjusted each month in the first half year to achieve serum IGF-I concentrations within the age-dependent laboratory reference range, aimed at SDS scores between 0 and + 2 SDS. Thereafter, this individualized dose was continued for the duration of the study (see Figure 2).

Mean basal IGF-I concentration was 9.1 ± 4.6 nmol/l and increased to 16.4 ± 6.4 nmol/l after 2 years ($p<0.0001$). At 5 years IGF-I was 22.2 ± 9.4 nmol/l ($p<0.0001$ vs. basal and 2 years) and at 7 years 25.5 ± 9.9 nmol/l ($p=0.05$ vs. 5 years). Fluid related side-effects were noticed in 9 patients (14% of patients) only during the first and second year of rhGH replacement.

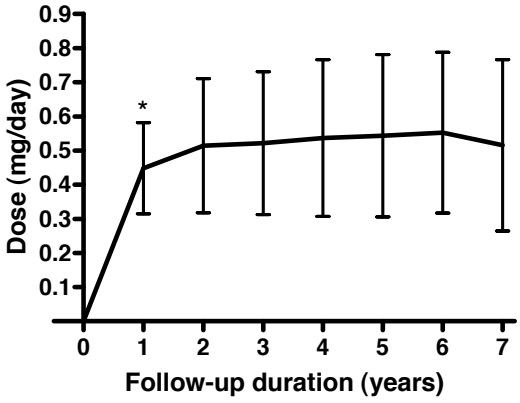

Figure 11/2: Dose of rhGH replacement used during 7 years. After 1 year a stable daily dose was reached and after 7 years mean daily dose was 0.5 ± 0.3 mg. *p<0.001 compared to baseline.

When secondary amenorrhoea was present for more than 1 year premenopausal women were defined as being LH/FSH deficient. In men, LH/FSH deficiency was defined, by a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as total T_4 or free T_4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value < 0.55 µmol/l) after a corticotrophin releasing hormone stimulation test or the insulin tolerance test. Patients received replacement therapy with hydrocortisone, L-thyroxine, testosterone in men and / or estrogen (15 females on oral estrogen replacement therapy and 2 on transdermal estrogens) in combination with prostagens in premenopausal women only. There were 17 premenopausal women and 17 postmenopausal women. Substitution therapy was monitored during replacement with rhGH and the respective dosages were adjusted as required. Thyroid hormone replacement dosing was started with 0.1 µg/day and dose titration was based on clinical response and serum free T4 concentrations within the reference range. In all patients with childhood-onset GHD, rhGH replacement was stopped for at least 3 months prior to retesting GH-reserve, applying adult cut-off values.

Patients were treated with lipid-lowering medication and antihypertensive medication according to the discretion of their attending physicians. Two patients were already treated with lipid lowering drugs at baseline and in 7 additional patients treatment with lipid lowering drugs was initiated during follow-up. All patients used statins. Four patients were already treated with antihypertensive medication at baseline and in 7 additional patients antihypertensive treatment was initiated during follow-up. The antihypertensive medication consisted of diuretics, β-adreno-receptor blockage drugs, calcium-antagonists, ACE inhibitors, angiotensin type II receptor antagonist and central acting antihypertensive medication, and combinations thereof.

Efficacy parameters

The following efficacy parameters were assessed before the start, after 2, 5, and 7 years of rhGH replacement:

1. Biochemical parameters: fasting levels of glucose, total cholesterol, HDL cholesterol (HDL), and triglycerides (TG). LDL cholesterol concentrations (LDL) were calculated using the Friedewald formula. Patients were requested to fast overnight before blood samples were taken for laboratory measurements of lipid profiles and glucose.
2. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP, respectively) were measured. Body-mass index (BMI) and waist-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. The BMI was calculated as weight in kilograms divided by the square of height in meters.

Assays

At baseline, during the first year, at 2 years and at 5 years serum IGF-I (nmol/l) concentration was measured by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns. The detection limit of this assay is 1.5 nmol/l, and the inter-assay coefficient of variation was below 11%.

Table 11/1: Baseline characteristics of the total cohort and the 63 patients who completed 7 years of rhGH replacement

		Total cohort (n=88)	Patients that completed 7 years (n=63)
Gender (M/F (n))		44/ 44	30/ 33
Age (years ± SD)		46.8 ± 14.0	46.7 ± 14.3
Age at onset (AO/CO (n))		71/ 17	52/ 11
BMI (kg/ m^2)		25.9 ± 3.4	25.5 ± 3.3
Etiological diagnosis of GHD (n)			
	Non-functioning adenoma	28	19
	Functioning adenoma	17	14
	Craniopharyngeoma	13	8
	Cerebral malignancy	6	4
	Sheehan's syndrome	4	4
	Idiopathic	13	10
	Other causes	5	4
Surgery (TS/ TC (n))		37/ 25	30/ 14
Radiotherapy (n)		36	26
Anterior pituitary deficiencies (n)	TSH/ LH-FSH/ ACTH/ ADH	75/ 76/ 69/ 23	52/ 53/ 47/ 15

M male; F female; AO adult-onset; CO childhood-onset; TS transsphenoidal; TC transcranial.

During the last two years of the study, a new assay was introduced. The serum IGF-I concentration (ng/ml) was measured using an immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, USA). The lower limit of detection was 6.0 ng/ml and intra-assay variation (n=250) was 8.0 and 6.0% at mean plasma levels of 30 and 450 ng/ml, respectively. Inter-assay variation was 8.7, 5.8 and 6.5% at mean IGF-I plasma levels of 33, 174 and 445 ng/l, respectively (n=115). The concentrations were converted to SI units by dividing by 7.65.

A Hitachi 747 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose, total cholesterol and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany). In 2003 the Hitachi 747 was replaced by a modular P 800 with no change in the chemistry components.

Statistics

Statistical analysis was performed using SPSS for Windows, version 12.0 (SPSS Inc. Chicago, Illinois, USA). Results are scored as the mean ± standard deviation (SD), unless specified otherwise. Independent samples t-tests and ANOVA repeated measurements with Bonferroni correction for multiple comparisons were used, when appropriate. Logarithmic transformation of the data was used to limit dispersion of the variance. P-value < 0.05 was assumed to represent a significant difference.

RESULTS

Fasting plasma glucose levels significantly increased during follow-up by 15% ($p<0.001$, Table 2) due to a significant increase within the first two years (Figure 3).

Total cholesterol levels significantly decreased during follow-up by 11% ($p<0.001$, Table 2). Compared to baseline, this decrease became significant after 5 years of rhGH replacement ($p=0.006$) and decreased further thereafter (Figure 3). LDL concentrations significantly decreased during follow-up ($p<0.001$, Table 2, Figure 3). HDL significantly increased during follow-up compared to baseline ($p<0.001$, Table 2), due to an increase between 2 and 5 years (Figure 3). After 5 years of treatment, the increase of HDL was only borderline statistically significant ($p=0.052$).

Because a subset of the patients (9/63=14%) were on lipid-lowering medication at any time-point during follow-up, the data are also analyzed for the cohort free of lipid-lowering medication at any time-point. The pattern of change remained unaffected for total cholesterol, HDL cholesterol and triglycerides. For LDL cholesterol the decrease became significant only after 5 years, and continued to decrease thereafter. In those patients using lipid-lowering medication the degree of change for total cholesterol was significantly greater compared to those patients who were not (-0.2 ± 0.1 mmol/l vs. -0.1 ± 0.1 mmol/l, respectively, $p=0.007$).

Table 11/2: Biochemical and anthropometric parameters in GHD adults before, and after 7 years of rhGH replacement.

	Baseline	7 years or rhGH replacement	P-value
IGF-I (nmol/l)	9.1 ± 4.6	25.5 ± 9.9	<0.001
Fasting Glucose (mmol/l)	4.4 ± 0.7	5.0 ± 1.0	<0.001
TC (mmol/l)	6.4 ± 1.2	5.6 ± 1.0	<0.001
LDL (mmol/l)	4.7 ± 1.1	3.5 ± 0.9	<0.001
HDL (mmol/l)	1.4 ± 0.5	1.7 ± 0.5	<0.001
TG (mmol/l)	1.6 ± 0.9	1.7 ± 1.2	NS
BMI (kg/m^2)	25.5 ± 3.3	27.1 ± 3.9	<0.001
Waist circumference (cm)	92.2 ± 11.8	94.8 ± 11.9	NS
WH ratio	0.9 ± 0.08	0.9 ± 0.08	NS
SBP (mm Hg)	131.3 ± 16.4	132.9 ± 19.3	NS
DBP (mm Hg)	84.0 ± 9.2	80.1 ± 8.2	0.002

TC Total Cholesterol; TG Triglycerides; WH ratio Waist-to-Hip ratio; SBP Systolic Blood Pressure; DBP Diastolic Blood Pressure.

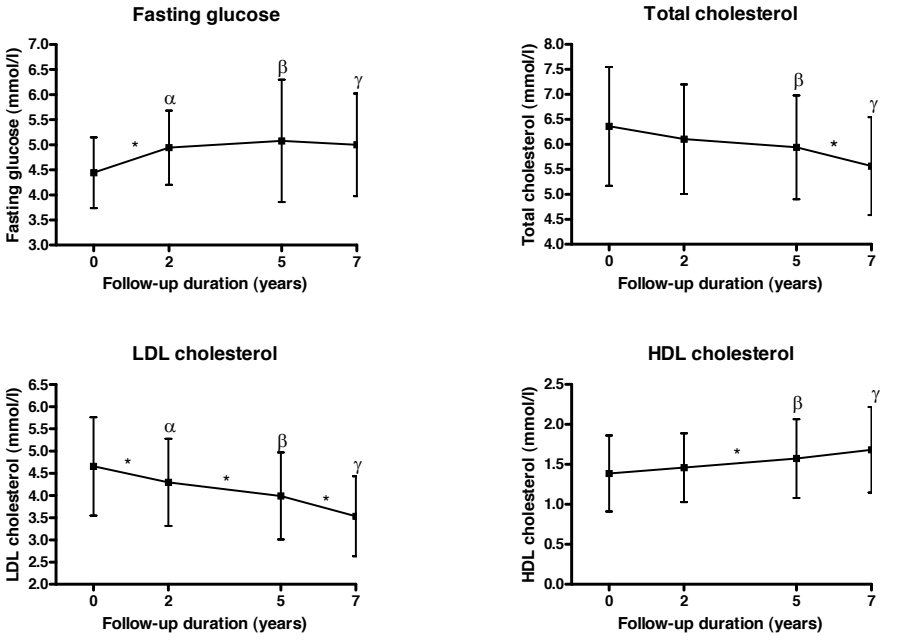

Figure 11/3: Fasting glucose levels significantly increased when baseline and 7 years were compared ($p<0.001$). Plasma levels of total cholesterol, LDL cholesterol (LDL), and HDL cholesterol (HDL) during 7 years of rhGH replacement in all patients. Total cholesterol and LDL cholesterol significantly decreased after 7 years ($p<0.001$). HDL significantly increased ($p<0.001$). $^{\alpha}$ $p<0.05$ compared to baseline. $^{\beta}$ $p<0.05$ compared to baseline. $^{\gamma}$ $p<0.05$ compared to baseline. * $p<0.05$ compared to previous time-point.

Body mass index (BMI) significantly increased by 6% (p<0.001, Table 2), mainly due to an increase between 2 and 5 years (Figure 4). Waist circumference and waist-to-hip (WH) ratio remained unchanged (Table 2). SBP did not change during follow-up (Table 2, Figure 4). Exclusion of the patients using antihypertensive medication (n=11) did not affect the conclusions. DBP decreased during follow-up in all patients (p=0.002, Table 2), but this decrease failed to reach statistical significance when patients using antihypertensive medication were excluded (p=0.064).

Influence of gender

The dose of rhGH was significantly higher in women compared with men at all time points. At the 2 years evaluation, women used 0.42 ± 0.9 mg and men 0.60 ± 0.22 mg (p=0.002). At 5 years the doses were 0.42 ± 0.10 mg and 0.66 ± 0.26 mg (p<0.001) and at 7 years 0.36 ± 0.10 and 0.66 ± 0.26 mg (p<0.001), respectively. We found no differences in treatment effects of any of the biochemical and anthropometric parameters between men and women.

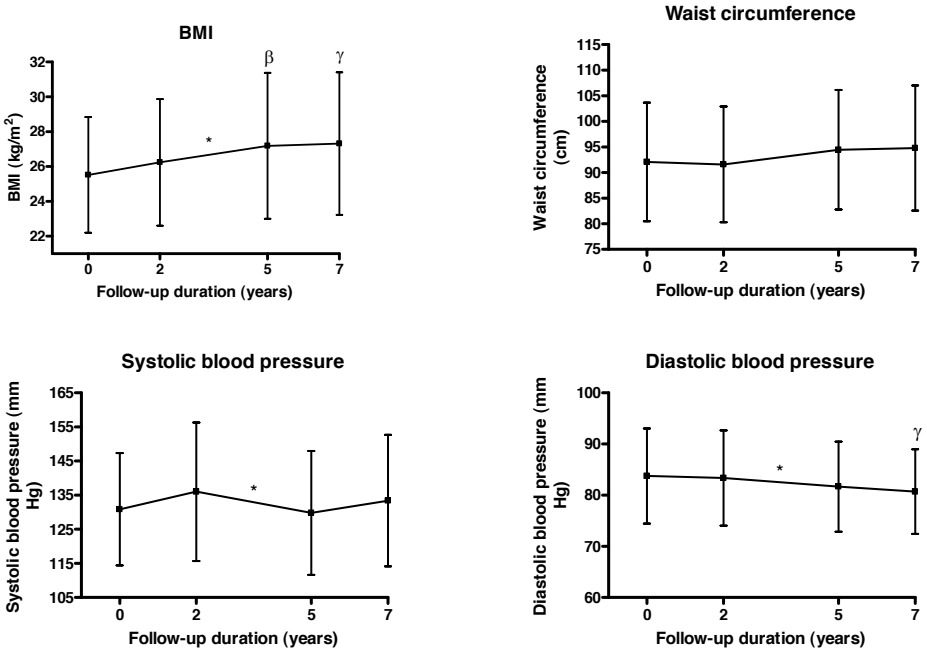

Figure 11/4: Body mass index (BMI) significantly increased during follow-up when baseline and 7 years were compared (p<0.001, respectively). Waist circumference remained unchanged. SBP remained unchanged when baseline and 7 years were compared (p=0.483). DBP significantly decreased when baseline and 7 years were compared (p=0.002), but the decrease failed to reach significance when patients using antihypertensives were excluded (p=0.064). $^{\alpha}$ p<0.05 compared to baseline. $^{\beta}$ p<0.05 compared to baseline. $^{\gamma}$ p<0.05 compared to baseline. * p<0.05 compared to previous time-point.

Influence of age

Thirty-five patients (56%) were younger than 50 years at the start of the study and 28 patients (44%) were between 50 and 75 years of age. As was expected, older patients had a higher BMI, waist circumference, WH ratio, LDL and total cholesterol, triglycerides, fasting glucose, SBP, and DBP compared to younger patients (data not shown). The individualized rhGH dose used in older patients was significantly lower compared to younger patients (0.4 ± 0.2 mg/day compared to 0.6 ± 0.3 mg/day, p=0.012). Age was not a significant covariate of the IGF-I response to treatment. The differences in LDL and total cholesterol concentrations after 7 years of rhGH replacement were higher in the older patients compared with the younger patients (-1.4 ± 0.9 mmol/l compared with -0.9 ± 1.0 mmol/l for LDL cholesterol, p=0.020, and -1.3 ± 1.0 mmol/l compared to -0.4 ± 0.9 mmol/l for total cholesterol, p=0.001). The mean change in triglycerides was -0.4 ± 0.9 mmol/l in older patients compared with 0.5 ± 1.5 mmol/l in younger patients (p=0.006). Younger patients had a larger increase in BMI compared with older patients (2.2 ± 2.7 kg/m^2 compared to 0.9 ± 1.7 kg/m^2, respectively, p=0.036). The mean change in DBP was larger in older patients compared with younger patients (-6.7 ± 8.0 mm Hg compared with -1.7 ± 9.2 mm Hg, p=0.033). There were no age differences in the response of HDL cholesterol, glucose, waist circumference, WH ratio, or SBP to 7 years of rhGH replacement.

DISCUSSION

In this large single-center study, we described the effects of 7 years of rhGH replacement with a stable individualized dose in adult GHD patients on biochemical and anthropometric parameters. We demonstrated that the beneficial changes on total cholesterol, HDL cholesterol levels and diastolic blood pressure were sustained even after 7 years of treatment, whereas the anthropometric parameters, except BMI, remained unchanged.

Short-term effects of rhGH on biochemical cardiovascular risk factors have been reported in many studies, including a recent meta-analysis, restricted to placebo-controlled trials. These studies conclude that rhGH replacement therapy was beneficial for total and HDL cholesterol levels, but had a negative influence on glucose and insulin concentrations (2). Long-term observational studies are rather scarce. Götherström et al. published their results of rhGH replacement therapy during 5 years in a cohort of 118 patients (3). We identified only three other studies with a follow-up of more than 5 years, comprising 33 patients in total, which described biochemical and anthropometric changes after GH substitution (4;5;7).

Fasting glucose increased in our study in accordance with a weighted mean increase of 0.2 mmol/l demonstrated in the meta-analysis of short-term studies (2), and the increase in fasting glucose found in the largest observational study (3). However, during longer-term studies in a limited number of patients, only a transient increase in fasting glucose was during the first year of the study was observed (7;8). This discrepancy with the increase in the meta-analysis and

the increase in the largest observational study might be explained by the number of patients included. The increase of fasting glucose is probably due to the direct GH-induced insulin resistance (9).

In our study, the decrease in total and LDL cholesterol levels was manifest during the first 5 years of rhGH replacement and continued even to decrease thereafter. This is consistent the mean weighted changes during short-term rhGH replacement calculated in the meta-analysis and with the changes found by Götherström et al. during 5 years of rhGH replacement (2). In the studies of 7 years or longer ,summarized in table 3, LDL cholesterol concentrations persistently decreased, but total cholesterol decreased in only one study (4). HDL cholesterol levels significantly increased after 5 years of rhGH replacement, mainly due to the increase after 2 years. However, no increase was found in short-term studies included in the meta-analysis, which is consistent with our findings that HDL increased mainly after 3 years of rhGH (2). Similar to the present findings, HDL cholesterol levels increased in 3 out of the 5 long-term studies summarized in Table 3 (3;5;6). Triglycerides concentrations remained unchanged in our study and all long-term studies, except for a minimal decrease observed by Götherström et al. (3).

The effects on blood pressure were minimal. Diastolic blood pressure decreased after 7 years of rhGH replacement, but this effect was abolished when patients on antihypertensive medication were excluded. Systolic blood pressure remained unchanged. In line with these findings, only one other long-term study reported beneficial changes on diastolic blood pressure with systolic blood pressure remaining unaffected (4).

BMI significantly increased during follow-up, but waist circumference and waist-to-hip ratio remained unchanged. The increase in BMI in patients receiving rhGH replacement for 7 years is consistent with a previous report in only 12 adults, in which both treated and untreated GHD adults showed an increase in BMI after 7 years (4). It appears that increasing age of GHD adults is associated with an increase in BMI, irrespective of rhGH replacement, like in the normal population (10). However, favorable changes in body composition (increase in fat free mass and decrease in body fat mass) were reported in 4 of 5 long-term studies (3-6). Waist circumference, a more sensitive parameter reflecting visceral adipose tissue remained unaffected.

The question to be addressed is whether our observations were affected by the subgroup of patients using lipid-lowering and/ or antihypertensive medication. Excluding patients using lipid-lowering drugs did not affect our observations for total cholesterol, HDL cholesterol and triglyceride levels. For LDL cholesterol the decrease manifested after 5 years, and thereafter. However, as expected, the decrease in total cholesterol was significantly greater in patients using lipid-lowering drugs. Conversely, when patients using antihypertensive medication were excluded, the decrease in diastolic blood pressure was no longer significant (P=0.064). The favourable clinical effects on the lipid profile might be attributed to the patients who were withdrawn from the study. However, such effect was not demonstrable in a detailed subanalysis. Patients became substantially older during this study which may have affected negatively various studied parameters. On the other hand IGF-I continued to increase during this study ,

Table 11/3: Summary of long-term studies (longer than 4 years) of effectivity of rhGH replacement therapy in GHD adults.

Authors	Numbers treated with rhGH (men)	Mean age at baseline	Mean dose	Duration (years)	Outcome
Al-Shoumer (8)	13 (7)	47 (24-65)	0.7 mg/ day	4	TC ↓, LDL ↓, Fasting insulin ↑. TG and HDL unchanged. Fasting glucose ↑ in 1st year, thereafter return to pretreatment values.
Götherström (3)	118 (70)	49.3 ± 1.0 SEM	Initially 0.98 mg/ day, lowered to 0.48 mg/day	5	TC ↓, LDL ↓, HDL ↑, TG ↓, Fasting glucose ↑. LBM ↑, Body fat ↓.
Svensson (6)	11 (7)	48.0	Initially 1.1 mg/ day, lowered to 0.61 mg/ day	7	HDL ↑, LDL ↓. TG and TC unchanged. Fasting glucose ↑ transiently during 1st year. BF ↓, FFM ↑.
Chrisoulidou (4)	12 (6)	52 ± 10	0.7 mg/ day	7	TC ↓, LDL ↓. Fasting glucose, fasting insulin, TG, and HDL unchanged. Subscapular skinfold ↓, Total body water ↑, FFM ↑, BF ↓, weight ↑, BMI ↑. Resting SBP unchanged, resting DBP ↓.
Gibney (5)	10 (7)	38	0.0075 mg/ kg/ day	10	LDL ↓, HDL ↑. TG and TC unchanged. LBM ↑.
Our study	63 (30)	46.7 ± 14.0	0.5 mg/ day	7	Fasting glucose ↑, TC ↓, LDL ↓, HDL ↑. TG unchanged. BMI ↑, waist ↑, DBP ↓.

TC Total Cholesterol; HDL HDL cholesterol; LDL LDL cholesterol; TG Triglycerides; BF Body fat; FFM Fat-free mass; LBM Lean Body mass; SBP Systolic blood pressure; DBP Diastolic blood pressure; BMI Body Mass Index; Waist Waist circumference; ↓ Significant decrease; ↑ Significant increase.

which might be due to age-related increased responsiveness to GH replacement. It is therefore conceivable that part of the ongoing favourable effects which we describe here are due to the effect of IGF-I.

The consequence of the increase in fasting glucose in terms of cardiovascular morbidity of mortality remains to be determined, but it has been established that there is a graded positive correlation between fasting glucose levels and the subsequent 12-year occurrence of cardiovascular events, even apparent for glucose levels below the diabetic threshold (11;12).

The clinical relevance of these long-term effects of rhGH on lipids can best be discussed in view of the previously documented beneficial changes of lipid-lowering drugs (especially statins). In our study, total and LDL cholesterol levels decreased with a mean change of 0.8 mmol/l (11% decrease) and 1.0 mmol/l (22% decrease), respectively. In patients treated for hypercholesterolemia, cardiovascular mortality risk reduces by 15% for every 10-percentage points of cholesterol lowering by conventional lipid-lowering drugs (13). Whether lowering of total cholesterol levels in GHD adults is associated with the same magnitude of reduction in cardiovascular mortality remains to be established. In patients with risk factors for cardiovascular disease, lipid-lowering should be targeted at a LDL cholesterol below 2.6 mmol/l, a total cholesterol below 5.2 mmol/l, and a HDL cholesterol above 1.6 mmol/l (14). Since patients with hypopituitarism are at increased risk for cardiovascular mortality (15;16), we applied these targets to our population on rhGH replacement alone (n=53). Target goals were reached on rhGH replacement alone in 8 of 49 patients with elevated LDL cholesterol concentrations for LDL cholesterol, in only 11 of 44 patients with elevated total cholesterol concentrations for total cholesterol, and in 17 of 40 patients with low HDL cholesterol for HDL cholesterol. It should be noted that the recently published clinical practice guideline for evaluation and treatment of adult GHD did not incorporate guidelines for cholesterol or blood pressure (17).

In conclusion, persistent beneficial changes are present even after 7 years of rhGH treatment. However, given the magnitude of these changes on lipid concentrations by rhGH alone and the fact that adults with panhypopituitarism are at high risk of (cardiovascular) mortality, we propose that these patients should be carefully monitored according to a multimodality approach, in addition to rhGH replacement.

REFERENCE LIST

1. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
2. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
3. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
4. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG 2000 Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 85:3762-3769
5. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D 1999 The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 84:2596-2602
6. Svensson J, Stibrant SK, Johannsson G 2003 Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 88:2061-2069
7. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO 2002 Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 87:2121-2127
8. al Shoumer KA, Gray R, Anyaoku V, Hughes C, Beshyah S, Richmond W, Johnston DG 1998 Effects of four years' treatment with biosynthetic human growth hormone (GH) on glucose homeostasis, insulin secretion and lipid metabolism in GH-deficient adults. Clin Endocrinol (Oxf) 48:795-802
9. Smith TR, Elmendorf JS, David TS, Turinsky J 1997 Growth hormone-induced insulin resistance: role of the insulin receptor, IRS-1, GLUT-1, and GLUT-4. Am J Physiol 272:E1071-E1079
10. van Lenthe FJ, Droomers M, Schrijvers CT, Mackenbach JP 2000 Socio-demographic variables and 6 year change in body mass index: longitudinal results from the GLOBE study. Int J Obes Relat Metab Disord 24:1077-1084
11. Gerstein HC 1997 Dysglycemia, not just diabetes, is a continuous risk factor for cardiovascular disease. Evid Based Cardiovasc Med 1:87-88
12. Coutinho M, Gerstein HC, Wang Y, Yusuf S 1999 The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 22:233-240
13. Gould AL, Rossouw JE, Santanello NC, Heyse JF, Furberg CD 1998 Cholesterol reduction yields clinical benefit: impact of statin trials. Circulation 97:946-952
14. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) 2001 JAMA 285:2486-2497
15. Rosen T, Bengtsson BA 1990 Premature mortality due to cardiovascular disease in hypopituitarism. Lancet 336:285-288
16. Tomlinson JW, Holden N, Hills RK, Wheatley K, Clayton RN, Bates AS, Sheppard MC, Stewart PM 2001 Association between premature mortality and hypopituitarism. West Midlands Prospective Hypopituitary Study Group. Lancet 357:425-431
17. Molitch ME, Clemmons DR, Malozowski S, Merriam GR, Shalet SM, Vance ML 2006 Evaluation and treatment of adult growth hormone deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 91:1621-1634

Chapter 12

The prevalence of the metabolic syndrome is increased in patients with growth hormone deficiency, irrespective of long-term substitution with recombinant human growth hormone

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European Journal of Endocrinology, 2007

ABSTRACT

Objectives

Many reports demonstrate improvements in cardiovascular risk factors during growth hormone replacement (rhGH) in adult growth hormone deficiency (GHD). However, it remains to be determined to what extent these changes translate into a reduction of increased cardiovascular morbidity and mortality. The aim of this study was to evaluate the effects of long-term rhGH replacement on the prevalence of the metabolic syndrome.

Design, settings, main outcome measures

The metabolic syndrome was scored using the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) definition in 50 consecutive GHD patients (age 45 ± 9 years), before, after 2, and after 5 years of rhGH replacement and the data of untreated patients were compared to the general population using data from a Dutch population based study (n=1062, age 44 ± 8 years).

Results

Hypertriglyceridemia (46.0% vs. 18.5%, p<0.0001), hypertension (66.0% vs. 35.5%, p<0.0001), and abdominal obesity (38.0% vs. 23.4%, p=0.0178) were more prevalent in untreated patients compared to controls, resulting in a higher prevalence of the metabolic syndrome in patients (38.0% vs. 15.7%, p<0.0001). During rhGH replacement at a mean dose of 0.5 mg ± 0.2 mg/day resulting in IGF-I concentrations in the normal age-adjusted reference range, mean HDL cholesterol increased compared to baseline (p<0.001). However, the prevalence of (components of) the metabolic syndrome did not change after 2 or 5 years of treatment with rhGH.

Conclusion

In this study, the prevalence of the metabolic syndrome in patients with GHD is increased compared to healthy controls, irrespective of rhGH replacement.

INTRODUCTION

Many reports documented the beneficial effects of short-term (1) and long-term (Table 1) recombinant human growth hormone (rhGH) on cardiovascular risk factors in adults with growth hormone deficiency (GHD). In placebo-controlled studies, with a duration ranging from 1 week to 18 months, rhGH treatment was beneficial for lean and fat body mass, total and HDL cholesterol levels and diastolic blood pressure, whereas rhGH negatively influenced plasma glucose and insulin levels (1). Long-term studies have revealed a increase in HDL cholesterol (2-4) and fasting glucose levels (3;5;6), and a decrease in triglyceride levels (3;7). Systolic blood pressure remained unchanged (3;7;8), whereas diastolic blood pressure decreased only in one study (8). The actual data reported in these studies indicate that despite the beneficial effects cardiovascular risk factors remain increased in many patients.

The metabolic syndrome is a cluster of metabolic abnormalities, that identifies persons at high risk for cardiovascular disease (9-11). The aim of this study was to characterize the baseline characteristics of the metabolic syndrome in a cohort of adults with GHD and to evaluate the effect of subsequent rhGH replacement during 5 years on the prevalence of the metabolic syndrome. We hypothesized that long-term rhGH replacement in adults with GHD would ultimately lead to an improved cardiovascular risk profile as assessed by the criteria of the metabolic syndrome.

METHODS

Patients

From October 1994 to April 2000, sixty-four consecutive patients with adult-onset GHD aged 30 to 59 years were enrolled. In 50 patients we could score the presence of the metabolic syndrome at baseline and after 5 years of rhGH replacement, according to the NCEP-ATP III definition (12). The other 14 patients were excluded from analysis, because insufficient data were available to score the prevalence of the metabolic syndrome (n=7) or because rhGH treatment was ended earlier by the patients because of subjective lack of benefit (n=4) or side effects (n=3). No differences were present between the 14 excluded patients and the other 50 patients with respect to age, gender, and BMI.

Treatment Protocol

Patients were prospectively enrolled in an open label treatment protocol. Growth hormone deficiency (GHD) was confirmed in all patients by an insulin tolerance test (nadir blood glucose < 2.2 mmol/) with a peak GH concentration < 3 µg/l. After initial measurements were obtained, all patients were treated with subcutaneous injections of rhGH (Genotropin® Pharmacia/Pfizer or Zomacton® Ferring, Norditropin® NovoNordisk, or Humatrope® Lilly), given subcutaneously

Table 12/1: Observational studies on the effect of recombinant human growth hormone on cardiovascular parameters with a duration of at least 2 year.

Authors	Duration (years)	Numbers (men)	Age	Daily dose	HDL	TG	Glucose	SBP	DBP	Waist circ.	BMI	WH ratio
Colao (7)	2	20 women	18-45	0.077 mg/ kg		↓		=	=		=	
Colao (7)	2	18 men	19-45	0.065 mg/ kg		↓		=	=		↓	
Florakis (5)	2	24 (10)	48	0.4 mg		=	↑					
O'Neal (6)	2	22 (16)	42	0.01 mg/kg	=	=	↑				↑	↓
Garry (27)	3	21 (16)	45.9	0.007 mg/ kg	=	=	=					
Al-Shoumer (28)	4	13 (7)	Median 47	0.008 mg/ kg	=	=	=					
Götherström (3)	5	118 (70)	49	0.48 mg	↑	↓	↑	=	=		↑	
Present study	5	50 (24)	45	0.5 mg	↑	=	=	=	=	=	↑	=
Svensson (4)	7	11 (7)	48	0.61 mg	↑	=	=				=	
Chrisoulidou (8)	7.1	12 (6)	52	0.7 mg	=	=	=	=	↓	=	↑	
Gibney (1;2)	10	10 (7)	38	0.008 mg/ kg	↑	=						

Observational studies on the effect of rhGH on cardiovascular parameters with a duration of at least 2 years. HDL High-density lipoprotein cholesterol; TG Triglycerides; SBP Systolic blood pressure; DBP Diastolic blood pressure; Waist circ. Waist circumference; TC Total cholesterol; BMI Body mass index; WH ratio Waist-to-hip ratio; ↓ Significant decrease; ↑ Significant increase; = No change.

every evening. The initial dose of rhGH was 0.2 mg/day, which was individually adjusted each month in the first half year to achieve physiological serum IGF-I concentrations, within the age-dependent laboratory reference range (IGF-I standard deviation scores (SD scores)). Thereafter, this individualized dose was continued in each patient and adjusted, if necessary, to maintain a normal IGF-I concentration for the duration of the study.

Patients with a functioning adenoma (8 patients with Cushing's disease, 2 patients with acromegaly and 7 patients with prolactinoma) were in long-term remission before entering the study. When secondary amenorrhoea was present for more than 1 year premenopausal women were defined as LH/FSH deficient. In men, LH/FSH deficiency was defined, as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone stimulation test or insulin tolerance test. Patients were then treated with hydrocortisone (n=39, mean dosage 25 ± 8 mg/ day, range 10-40 mg/day), L-thyroxine (n=44), testosterone (n=21), and/ or estrogen in combination with prostagens in premenopausal women only (n=18). Conventional substitution therapy was monitored during substitution with rhGH and the respective dosages were adjusted, as required for normalization of clinical and biochemical parameters of pituitary deficiencies. Regular screening for pituitary deficiencies was continued during follow-up. After baseline, additional L-thyroxine substitution was started in 1 patient and additional hydrocortisone was started in 1 patient. Patients were treated with antihypertensive and lipid-lowering medication when needed following standard patient care procedures. During follow-up, lipid-lowering treatment was initiated by the treating physician in 4 of the 50 patients, and antihypertensive medication in 6 of the 50 patients because of additional cardiovascular risk factors.

The study protocol was approved by the local Ethics Committees. All adult patients gave written informed consent to participation in the study.

Study Parameters

The following study parameters were assessed before, after 2 and 5 years of, substitution with rhGH: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP, respectively), and fasting serum levels of glucose, HDL and TG were measured. Body-mass index (BMI) and waist-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. The BMI was calculated as weight in kilograms divided by the square of height in meters. SBP and DBP were measured using the sphygmomanometric cuff method.

Patients were requested to fast overnight before blood samples were taken for laboratory measurements of lipid profiles, glucose and IGF-I concentrations.

The serum IGF-I (nmol/l) concentration was measured by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns. The detection limit of this assay is 1.5

nmol/l, and the intra- and inter-assay coefficients of variation were below 11%. Age and gender-adjusted IGF-I data were determined in the same laboratory (13;14). IGF-I was expressed as SD scores for age- and gender-related normal levels.

GH concentrations in the samples of the insulin tolerance test were measured by time resolved immunofluorometric assay (Wallac, Inc, Turku, Finland). Human biosynthetic GH (Pharmacia and Upjohn, Inc, Uppsala, Sweden) was used as standard, calibrated against WHO-IRP 80-505 and the detection limit of this GH assay is 0.01 µg/l with an interassay coefficient of variation of 1.6-8.4%, between 0.1 and 15 µg/l.

A Hitachi 747 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany). In 2003, the Hitachi 747 was replaced by a modular P 800 with no change in the chemistry components.

Control subjects

Control subjects were participants from the Monitoring Project on Risk Factors for Chronic Disease (MORGEN study) organized by he National Institute for Public Health and the Environment (RIVM) (15;16). From 1993 to 1997 age- and sex-stratified random samples were drawn from the municipality registries from three towns in the Netherlands in a cross-sectional study design. The patients were of the same geographical area as the controls. Anthropometry, blood pressure and total and HDL-cholesterol were measured during the survey. Stored plasma samples from subjects participating fasting in the period 1993-1995 were retrieved in 1996 and analyzed for triglyceride concentrations (n=1378). All participants aged 30 years and older were included in the present study (n=1062). Since some of our patients were recruited somewhat later than controls were studied, we investigated whether time-frame of recruitment in our patients influenced the prevalence of the metabolic syndrome. We compared the prevalence of the metabolic syndrome in patients, who started rhGH replacement exactly during the time in which controls were selected (n=31), to patients, who started after the time in which controls were selected (n=19) and found no significant difference in the prevalence of the metabolic syndrome (32% vs. 43%, P=0.285).

Standard enzymatic methods were used to measure HDL-cholesterol and glucose levels. Triglyceride concentrations were measured with Abbott Spectrum clinical analyzer (Abbott Laboratories, Chicago, IL, USA). Blood pressure was measured by Random Sphygmomanometer.

Definition of the Metabolic Syndrome

The metabolic syndrome was scored according to definition of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATPIII) (NCEP-ATPIII) published in 2001 (12), which defines the metabolic syndrome as the presence of three or more of the following criteria:

(1) Fasting plasma glucose concentration ≥ 6.1 mmol/l

(2) TG concentration ≥1.69 mmol/l

(3) HDL concentration <1.04 mmol/l in men and <1.29 mmol/l in women
(4) BP ≥130/85 mmHg
(5) Waist circumference >102 cm in men and >88 cm in women

Statistical analysis

Statistical analysis was performed using SAS for Windows, version 9.1 and SPSS for Windows, version 12.0 (SPSS Inc. Chicago, Illinois, USA). Results are expressed as the mean ± SD, unless specified otherwise. Paired samples t-tests or ANOVA with repeated measurements with Bonferroni correction for multiple comparisons were used to assess the differences of continuous variables, when appropriate. Chi-square tests were used to assess the difference in prevalence of the metabolic syndrome between patients and controls. Friedman test for related fractions was used to assess the effect of treatment on the prevalence of the metabolic syndrome. Logistic regression was used to identify indicators of the prevalence of the metabolic syndrome at baseline. General linear model for repeated measurements were used to assess the prevalence of the metabolic syndrome at baseline and after 5 years of treatment taking trend in BMI into account. A p-value of <0.05 was considered to be significant.

RESULTS

Untreated GHD adults compared to controls

Characteristics of patients and controls

Gender was equally distributed in both cohorts (Table 2). There were no differences in age, gender and BMI between controls and patients. The mean maximal GH response during insulin induced hypoglycemia was only 0.3 ± 0.4 µg/l (range 0.1-1.6 µg/l), confirming the diagnosis of severe GHD (17).

Cardiovascular risk factors

Patients had significantly lower fasting glucose concentrations and significantly higher mean TG, SBP, DBP, waist circumference in men, and WH ratio in men and women compared to controls (Table 3).

Comparison of components of the metabolic syndrome

Hypertriglyceridemia (46.0% vs. 18.5%, p<0.0001), hypertension (66.0% vs. 35.5%, p<0.0001) and abdominal obesity (38.0% vs. 23.4%, p=0.0178) were significantly more prevalent in patients, compared to controls (Figure 1).

Table 12/2: Characteristics of patients with adult-onset growth hormone deficiency and controls.

		Patients (n=50)	Controls (n=1062)	P-value
Gender (%)	Male/ Female	48/ 52	52/ 48	NS
Age (years (mean ± SD))		45.2 ± 9.1	43.8 ± 8.0	NS
BMI (kg/ m^2 (mean ± SD))		26.7 ± 4.2	25.7 ± 4.0	NS
Antihypertensive medication (%)		12	17	NS
Lipid-lowering drugs (%)		10	13	NS
Etiology of GHD (%)	Non-functioning pituitary adenoma	24		
	Functioning pituitary adenoma*	34		
	Craniopharyngioma	14		
	Other**	28		
Surgery (%)		78		
Radiotherapy (%)		56		
ADH deficiency (%)		30		
ACTH deficiency (%)		78		
TSH deficiency (%)		88		
LH/ FSH deficiency (%)	Men	88		
	Women	100		

Data are presented as percentages unless specified otherwise. *Prolactinoma, ACTH producing adenoma, GH producing adenoma. **Trauma, hypophysitis, germinoma, epidermoidcyst, Sheehan's syndrome, unknown cause.

Comparison of the metabolic syndrome

The prevalence of the metabolic syndrome was 38% (19/50 patients) vs. 15.7% in controls (p<0.0001). In patients, BMI was a significant indicator of the presence of the metabolic syndrome (OR 1.2, 95% CI [1.0-1.4], p=0.031, Figure 1). The presence of the metabolic syndrome was not dependent on age, gender, etiological diagnosis of GHD, surgery, or radiotherapy (p=0.823, p=0.729, p=0.340, p=0.704, p=0.093, respectively). There were no correlations between calendar time of diagnosis of GHD and the prevalence of the metabolic syndrome or between interval between diagnosis of GHD and start of rhGH replacement.

Effects of long-term substitution with rhGH

RhGH substitution characteristics

After 1 year of rhGH treatment a stable dose of rhGH was maintained and the mean dose after 5 years was 0.5 ± 0.2 mg/day. Mean IGF-I SD scores were –2.0 ± 0.8 at baseline, -0.2 ± 1.8 after 2 years, and 0.8 ± 2.0 after 5 years of rhGH replacement (p<0.001, Table 3).

Table 12/3: Metabolic and anthropometric parameters in adult-onset growth hormone deficient patients after 5 years treatment.

		Baseline controls (n=1062)	Baseline patients (n=50)	2 years treatment patients	5 years treatment patients	P-values between patients (baseline) and controls	P-values patients between baseline and 2 years treatment	P-values between baseline and 5 years treatment
IGF-I SD scores			-2.0 ± 0.8	-0.2 ± 1.8	0.8 ± 1.9		<0.001	0.001
Glucose (mmol/l)		5.4 ± 1.1	4.8 ± 1.2	5.0 ± 0.7	5.3 ± 1.3	<0.0001	NS	NS
HDL (mmol/l)	Men	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	1.3 ± 0.4	NS	NS	0.081
	Women	1.5 ± 0.4	1.5 ± 0.6	1.6 ± 0.5	1.7 ± 0.6	NS	NS	0.020
TG (mmol/l)		1.3 ± 1.1	1.9 ± 1.1	2.0 ± 1.0	2.1 ± 1.4	<0.0001	NS	NS
Systolic BP (mm Hg)		121 ± 16	128 ± 12	137 ± 19	129 ± 14	0.0004	0.017	NS
Diastolic BP (mm Hg)		79 ± 10	83 ± 8	86 ± 8	82 ± 9	0.0045	NS	NS
Waist circumference (cm)	Men	94.2 ± 10.8	101.5 ± 8.8	98.9 ± 8.3	104.3 ± 9.8	0.0017	NS	0.062
	Women	82.7 ± 11.3	86.8 ± 12.0	87.8 ± 13.3	88.4 ± 12.3	NS	NS	NS
WH ratio	Men	0.91 ± 0.07	0.98 ± 0.05	0.95 ± 0.05	0.99 ± 0.06	<0.0001	0.008	0.042
	Women	0.80 ± 0.07	0.86 ± 0.07	0.85 ± 0.06	0.87 ± 0.06	<0.0001	NS	NS
BMI (kg/ m^2)		25.7 ± 4.0	26.7 ± 4.2	26.7 ± 4.1	27.8 ± 4.7	NS	NS	0.002

Values are expressed as mean ± SD. IGF-I SD scores, IGF-I Standard-Deviation scores; HDL, High-Density-Lipoprotein Cholesterol; TG, Triglycerides; BP, Blood Pressure; WH ratio, waist to hip ratio; BMI Body Mass Index.

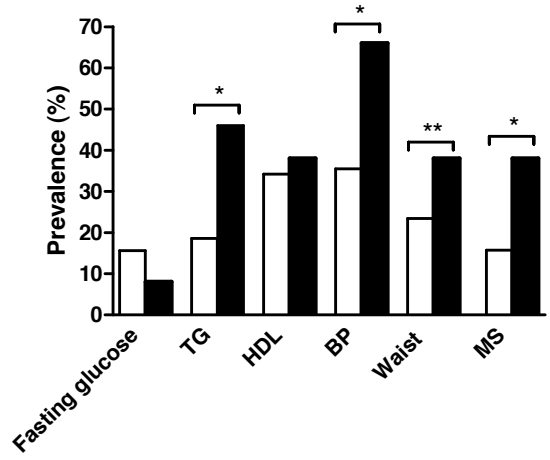

Figure 12/1: The prevalence of (components of) the metabolic syndrome in patients with growth hormone deficiency at baseline compared to healthy controls.
Black bars denote GHD patients at baseline and white bars denote controls; The prevalence of hypertriglyceridemia, hypertension and abdominal obesity were significantly higher in GHD adults compared to controls (*p<0.0001, **p<0.02). The prevalence of the metabolic syndrome was significantly higher in the total cohort (38.0% vs. 15.7%, *p<0.0001). TG, Triglycerides; HDL, High-Density-Lipoprotein cholesterol; BP, Blood Pressure; Waist, Waist circumference; MS, Metabolic syndrome.

Effects of rhGH on cardiovascular risk factors

Fasting glucose and TG levels remained unchanged during follow-up, whereas mean HDL levels increased during follow-up from 1.3 ± 0.5 mmol/l at baseline, to 1.4 ± 0.5 mmol/l after 2 years and to 1.5 ± 0.5 mmol/l after 5 years of rhGH treatment (p<0.001, Table 3). However, when men and women were analysed seperately the increase in HDL in men failed to reach statistical significance (p=0.081). SBP and DBP remained unchanged, except for a transient increase in SBP during the first two years of treatment (128 ± 12 mm Hg at baseline, 137 ± 19 mm Hg after 2 years (p=0.017), and 129 ± 14 mm Hg after 5 years of treatment). Mean BMI increased from 26.7 ± 4.2 kg/ m^2 to 27.8 ± 4.7 kg/ m^2 (p=0.002). Mean waist circumference remained unchanged, except for a slight increase in men after 5 years of treatment which failed to reach statistical significance (p=0.062). This increase was also reflected in the increase in waist-to-hip ratio only seen in men during follow-up (0.98 ± 0.05 at baseline to 0.99 ± 0.06 after 5 years of treatment, p=0.042).

Effects of rhGH on components of the metabolic syndrome

After 2 and after 5 years of rhGH replacement, the prevalence of the components of the metabolic syndrome remained unchanged (Figure 2, Table 4). The decrease in prevalence of low HDL levels, (38% at baseline, 42% after 2 years to 20% after 5 years) failed to reach statistical significance (p=0.068).

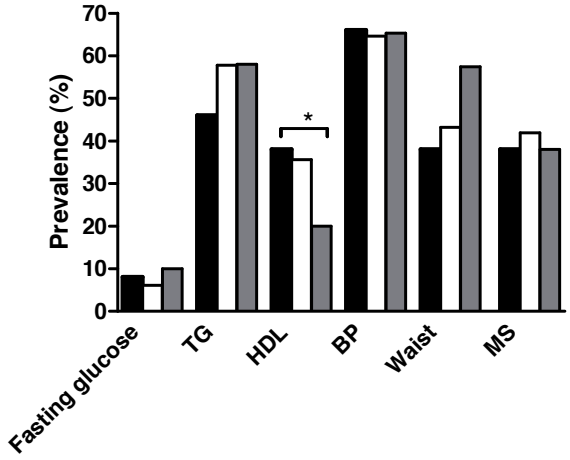

Figure 12/2: The prevalence of (components of) the metabolic syndrome in patients with growth hormone deficiency at baseline, after 2 and after 5 years of substitution of rhGH. Black bars denote GHD patients at baseline; White bars denote patients after 2 years of substitution with rhGH and grey bars denote patients after 5 years of substitution with rhGH. The prevalence of an abnormal low HDL decreases after 5 years of substitution with rhGH (*p=0.068).

Effects of rhGH on the prevalence of the metabolic syndrome

After 2 years of rhGH treatment the prevalence of the metabolic syndrome was 42% and after 5 years the metabolic syndrome was still present in 19 patients (38.0%, NS in a Friedman test for related fractions, Figure 2).

Influence of age, gender, BMI and etiological diagnosis of GHD

When adjusted for the increase in BMI during the period, the prevalence estimate for the metabolic syndrome did not change during 5 years of treatment with rhGH (39.6% at baseline vs. 36.4% after 5 years; p=0.71).

There were no differences in age, gender, BMI, etiological diagnosis of GHD, and applied treatments (radiotherapy or surgery), mean dose of rhGH after 5 years, and mean change in

Table 12/4: Prevalence of components of the metabolic syndrome in adult onset growth hormone deficient adults before, after 2 years and after 5 years treatment.

	Baseline	2 years treatment	5 years treatment	P-values treatment*
Increased fasting glucose (%)	8.0	6.1	10.0	0.717
Increased BP (%)	66.0	64.6	65.3	0.913
Decreased HDL cholesterol (%)	38.0	35.6	20.0	0.068
Increased TG levels (%)	46.0	57.8	58.0	0.084
Increased waist circumference (%)	38.0	43.2	57.4	0.741
Metabolic syndrome (%)	38.0	41.9	38.0	0.846

Values are expressed as percentages. BP, Blood pressure; HDL, High-Density-Lipoprotein Cholesterol; TG, Triglycerides; *Friedman test for related fractions.

IGF-I levels between patients who acquired the metabolic syndrome (n=9), who were relieved from the metabolic syndrome (n=9), or who were stable during follow-up (metabolic syndrome present (n=10) or absent (n=22)).

One of the 8 patients with Cushing's disease had the metabolic syndrome at baseline and after 5 years none of the patients treated for Cushing's disease had the metabolic syndrome.

There were no significant differences in the prevalence of the metabolic syndrome at any time-point between patients with or without ACTH deficiency and patients with or without TSH deficiency, or between patients with or without LH/ FSH deficiency. Logistic regression revealed no statistically significant influence of increasing number of anterior pituitary deficiency or increasing hydrocortisone dose on the prevalence of the metabolic syndrome at any time point during follow-up.

DISCUSSION

In this study, patients with GHD have a more than twofold increased prevalence of the metabolic syndrome, when compared to the general population. This increase is predominantly due to increased prevalences of hypertension, abdominal obesity and hypertriglyceridemia among patients with GHD. Long-term rhGH treatment increased HDL cholesterol concentrations, whereas fasting glucose and triglyceride concentrations remained unchanged in accordance with previous observations (references summarized in Table 1). However, rhGH treatment did not reduce the high prevalence of other cardiovascular risk factors assessed by the criteria of the metabolic syndrome.

The rationale for treatment with rhGH is to improve the metabolic abnormalities and psychological well-being, with the expectation to, ultimately, normalize life expectancy. The results of placebo-controlled trials with rhGH on cardiovascular risk factors were recently evaluated in a meta-analysis (1). In those studies (with a duration ranging from 1 week to 18 months) rhGH treatment was beneficial for lean and fat body mass, total and HDL cholesterol levels and diastolic blood pressure, but negatively influenced plasma glucose and insulin levels (1). Moreover, the weighted mean change, especially in total cholesterol, LDL cholesterol levels and diastolic blood pressure, was restricted to a maximum of only 0.3 mmol/l, 0.5 mmol/l, and 1.8 mm Hg, respectively (1). It remains unclear, to what extend these relative small changes would translate into a net beneficial effect on cardiovascular risk.

Some factors may have influenced the effect of rhGH in our study. The data obtained after 5 years of follow-up were not only affected by rhGH treatment, but also by age and BMI. In our control population an increase of 5 years in age was associated with a significant increase in prevalence of the metabolic syndrome from 15.7% to 20.9% (data not shown). However, this does not affect our conclusions with respect to the limited effects of rhGH treatment, since the prevalence of the metabolic syndrome remained much higher in GHD patients, irrespective of

the duration of follow-up and rhGH treatment.Additional adjustment for the trend in BMI did not affect our conclusions. Moreover, after two years of rhGH replacement, in which the BMI did not increase, the prevalence also remained unaffected by rhGH replacement. However, we cannot exclude the possibility that rhGH replacement was able to stabilize the prevalence of the metabolic syndrome and prevented an increase in prevalence of cardiovascular risk factors, associated with the observed increase in BMI and age. Although BMI significantly increased during follow-up, but waist circumference and waist-to-hip ratio remained unchanged. The increase in BMI in patients receiving rhGH replacement for 5 years is consistent with a previous reports in GHD adults (3;6;8). Moreover, with linear regression in our control population, an increase in BMI of 0.5 kg/ m^2 after a 5-year age increase was estimated. Thus, it appears that increasing age of GHD adults is associated with an increase in BMI, irrespective of rhGH replacement, just like in the normal population.

Waist circumference transiently decreased in men during follow-up, but remained unchanged after 5 years of rhGH replacement compared to baseline. This is in line with findings of a large short-term studies by Filipsson et al. and of Abs et al. in which waist circumference decreased after 1 year and 2 years of rhGH treatment, respectively (18;19), as well as with findings of one long-term study in which waist circumference was unchanged after 7 years of rhGH replacement (8). Thus, it seems that short-term rhGH replacement is able to induce favorable changes in waist circumference but unable to prevent the increase in waist circumference due to ageing as is seen in healthy adults.

The high prevalence of the metabolic syndrome could also be related to the complex syndrome of anterior pituitary deficiencies. All patients received adequate replacement therapies for pituitary insufficiencies prior to the start of study except for GHD. Moreover, conventional substitution therapy was carefully monitored during substitution with rhGH and the respective dosages were adjusted, as required for normalization of clinical and biochemical parameters of pituitary deficiencies. The prevalence of the anterior pituitary deficiencies in our cohort is comparable to the prevalence found in a large study of long-term rhGH replacement in GHD adults by Götherström et al. (3). In our study, 94% of patients had a deficiency of at least one anterior pituitary hormone compared to 92% in the study of Götherström et al. and 78% in our study had at least three other deficiencies besides GHD compared to 68% in the study of Götherström et al. (3). Although the prevalence of pituitary deficiencies might influence the prevalence of the metabolic syndrome in these patients, we did not find any differences between patients with or without various deficiencies. This might be related to the limited number of patients included in the present study.

Recently, it has been shown by Filipsson et al. that dose of glucocorticoids in the treatment of ACTH deficiency influences body mass index, triglycerides, LDL and total cholesterol in a dose-dependent manner, whereas it did not affect treatment response to rhGH replacement (20). In our limited number of patients and limited range of hydrocortisone doses used, we

were unable to show such a relationship between the metabolic syndrome and hydrocortisone dose.

The underlying diseases that caused GHD could also influence our results. Patients with Cushing's disease are known to have a high prevalence of cardiovascular risk factors, even after successful long-term cure of Cushing's disease (21). On the other hand, Feldt-Rasmussen et al. found no differences in waist-to-hip ratio, body mass index, triglycerides, and total, LDL and HDL cholesterol between patients with GHD due to previous treatment for Cushing's disease compared to patients with GHD due to other aetiologies (22). In our limited number of patients, we were unable to demonstrate differences in the prevalence of the metabolic syndrome between patients treated for Cushing's disease or acromegaly and patients treated for non-functioning pituitary adenomas, or between patients with producing pituitary adenomas compared to patients with non-functioning adenomas.

In our study, in four patients lipid-lowering treatment was started during follow-up and in six patients antihypertensive medication. The exclusion of these patients from the analysis of lipid parameters or systolic and diastolic blood pressure measurements, respectively, did not affect our observations. Recently, Grundy et al. proposed to take the medication use into account (23). We therefore chose to perform a second analysis in which we also scored antihypertensive and lipid-lowering medication in patients and controls. The comparison with controls remained unchanged (38% vs. 16.5%, $p<0.0001$) as well as the prevalence after 5 years of substitution with rhGH (38% at baseline vs. 43% after 5 years, NS vs. baseline).

The concept of the metabolic syndrome is subject to debate, because the pathophysiological basis of the proposed syndrome is unclear (24), and because the combination of cardiovascular risk factors does not add to the risk related to the individual risk factors (25). Nonetheless, this current debate does not affect our conclusions, because we also focus on the prevalence of the individual, well-recognized cardiovascular risk factors, which all individually have been associated with increased cardiovascular morbidity and mortality in the general population (9;10;25).

It needs to be established whether adult GHD patients with the metabolic syndrome have the same risks for cardiovascular morbidity and mortality compared with those with the metabolic syndrome in the general population. It remains to be studied whether the prognostic significance of the metabolic syndrome in these patients with GHD is the same as in the healthy general population. Moreover, the pathogenesis of the metabolic syndrome might be different in our patients compared to the general population. For example the effect of GHD and rhGH replacement on insulin sensitivity might influence the prevalence of the metabolic syndrome in our patients. Data so far on insulin resistance during rhGH replacement are conflicting, but some studies have pointed towards an improved insulin sensitivity during long-term rhGH replacement (4), which could be attributed to favourable changes in body composition (26). Furthermore, it remains to be studied in prospective trials if GHD adults may benefit from more

aggressive antihypertensive and lipid-lowering therapy and life style intervention to reverse the metabolic abnormalities seen in the adult GHD syndrome.

In conclusion, the prevalence of the metabolic syndrome in our GHD adults is significantly higher compared to the general population, irrespective of rhGH treatment. Apparently, appropriate substitution of rhGH and other hormones in adult patients with GHD is insufficient to improve this adverse cardiovascular risk profile.

ACKNOWLEDGMENTS

The MORGEN project was financially supported by the Ministry of Public Health, Welfare and Sports of The Netherlands and the National Institute for Public Health and the Environment. The sponsors did not have a role in the study design, collection/ analysis/ interpretation of the data, writing of the report, or the decision to submit the paper for publication.

REFERENCE LIST

1. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
2. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D 1999 The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 84:2596-2602
3. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
4. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO 2002 Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 87:2121-2127
5. Florakis D, Hung V, Kaltsas G, Coyte D, Jenkins PJ, Chew SL, Grossman AB, Besser GM, Monson JP 2000 Sustained reduction in circulating cholesterol in adult hypopituitary patients given low dose titrated growth hormone replacement therapy: a two year study. Clin Endocrinol (Oxf) 53:453-459
6. O'Neal DN, Hew FL, Best JD, Alford F 1999 The effect of 24 months recombinant human growth hormone (rh-GH) on LDL cholesterol, triglyceride-rich lipoproteins and apo [a] in hypopituitary adults previously treated with conventional replacement therapy. Growth Horm IGF Res 9:165-173
7. Colao A, Di Somma C, Cuocolo A, Spinelli L, Acampa W, Spiezia S, Rota F, Savanelli MC, Lombardi G 2005 Does a gender-related effect of growth hormone (GH) replacement exist on cardiovascular risk factors, cardiac morphology, and performance and atherosclerosis? Results of a two-year open, prospective study in young adult men and women with severe GH deficiency. J Clin Endocrinol Metab 90:5146-5155
8. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG 2000 Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 85:3762-3769
9. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L 2001 Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 24:683-689
10. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT 2002 The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. JAMA 288:2709-2716
11. Reaven GM 1988 Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37:1595-1607
12. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III) 2001 JAMA 285:2486-2497
13. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
14. Janssen YJ, Frolich M, Roelfsema F 1997 A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 82:129-135
15. Balkau B, Charles MA, Drivsholm T, Borch-Johnsen K, Wareham N, Yudkin JS, Morris R, Zavaroni I, van Dam R, Feskins E, Gabriel R, Diet M, Nilsson P, Hedblad B 2002 Frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of an insulin resistance syndrome. Diabetes Metab 28:364-376
16. Lean ME, Han TS, Seidell JC 1998 Impairment of health and quality of life in people with large waist circumference. Lancet 351:853-856

17. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
18. Filipsson H, Monson JP, Koltowska-Haggstrom M, Mattsson A, Johannsson G 2006 The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients. J Clin Endocrinol Metab 91:3954-3961
19. Abs R, Feldt-Rasmussen U, Mattsson AF, Monson JP, Bengtsson BA, Goth MI, Wilton P, Koltowska-Haggstrom M 2006 Determinants of cardiovascular risk in 2589 hypopituitary GH-deficient adults - a KIMS database analysis. Eur J Endocrinol 155:79-90
20. Filipsson H, Monson JP, Koltowska-Haggstrom M, Mattsson A, Johannsson G 2006 The impact of glucocorticoid replacement regimens on metabolic outcome and comorbidity in hypopituitary patients. J Clin Endocrinol Metab 91:3954-3961
21. Colao A, Pivonello R, Spiezia S, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G 1999 Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. J Clin Endocrinol Metab 84:2664-2672
22. Feldt-Rasmussen U, Abs R, Bengtsson BA, Bennmarker H, Bramnert M, Hernberg-Stahl E, Monson JP, Westberg B, Wilton P, Wuster C 2002 Growth hormone deficiency and replacement in hypopituitary patients previously treated for acromegaly or Cushing's disease. Eur J Endocrinol 146:67-74
23. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr., Spertus JA, Costa F 2005 Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 112:2735-2752
24. Gale EA 2005 The myth of the metabolic syndrome. Diabetologia 48:1679-1683
25. Dekker JM, Girman C, Rhodes T, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ 2005 Metabolic syndrome and 10-year cardiovascular disease risk in the Hoorn Study. Circulation 112:666-673
26. Giavoli C, Porretti S, Ronchi CL, Cappiello V, Ferrante E, Orsi E, Arosio M, Beck-Peccoz P 2004 Long-term monitoring of insulin sensitivity in growth hormone-deficient adults on substitutive recombinant human growth hormone therapy. Metabolism 53:740-743
27. Garry P, Collins P, Devlin JG 1996 An open 36-month study of lipid changes with growth hormone in adults: lipid changes following replacement of growth hormone in adult acquired growth hormone deficiency. Eur J Endocrinol 134:61-66
28. al Shoumer KA, Gray R, Anyaoku V, Hughes C, Beshyah S, Richmond W, Johnston DG 1998 Effects of four years' treatment with biosynthetic human growth hormone (GH) on glucose homeostasis, insulin secretion and lipid metabolism in GH-deficient adults. Clin Endocrinol (Oxf) 48:795-802.

Chapter 13

Administration route-dependent effects of estrogens on IGF-I levels during fixed growth hormone replacement in women with hypopituitarism

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European Journal of Endocrinology, 2007

ABSTRACT

Objective

Growth hormone (GH)-deficient women using oral estradiol treatment require higher doses of recombinant human GH (rhGH) to achieve similar insulin-like growth factor (IGF)-I levels compared with men and women on transdermal estradiol replacement. The aim of this study was to evaluate the effects of oral versus transdermal estrogen administration at similar plasma estradiol levels on IGF-I, IGF binding protein-3 and SHBG concentrations.

Design

Parallel cross-over study in which two groups of hypogonadal and GH-deficient women with fixed and stable rhGH replacement passed through four different estradiol treatment schemes (2 and 4 mg oral and 50 and 100 µg transdermal estradiol) with a duration of 4 cycles each to ensure a new steady state. Group I (18 patients using oral estradiol prior to the study) was treated with oral followed by transdermal estradiol and group II (5 patients with transdermal estradiol prior to inclusion) with transdermal followed by oral estradiol.

Results

Estradiol concentrations were lowest during 50 µg transdermal and highest during 4 mg oral estradiol. Estradiol concentrations did not differ during 100 µg transdermal and 2 mg oral treatment. Nevertheless, IGF-I levels were significantly higher during 100 µg transdermal compared with 2 mg oral treatment (p=0.005 in group I and 0.02 in group II), while SHBG levels were significantly lower (p=0.002 in group I and p=0.004 in group II). SHBG and IGF-I concentrations were negatively correlated (R=-0.41, p=0.0001).

Conclusion

During fixed growth hormone replacement, the route of estrogen administration is a determinant of IGF-I levels in hypogonadal, GH-deficient women.

INTRODUCTION

Clinical studies have demonstrated clear effects of estrogens on GH production and IGF-I levels. Estrogens amplify GH releasing hormone (GHRH)- and insulin-stimulated GH release and spontaneous 24h GH production is threefold higher in healthy premenopausal women than in healthy matched men (1;2). Women on estrogen replacement require higher doses of rhGH replacement than eugonadal women and men to achieve similar IGF-I concentrations (3-5).

Conversely, discontinuation of oral estrogen substitution increases IGF-I levels during continued substitution with rhGH in female patients with hypopituitarism (6). The route of estrogen administration also affects IGF-I levels. A switch from oral to transdermal estrogen therapy increases IGF-I levels and amplifies the IGF-I response during incremental doses of rhGH (7;8). In adult women with growth hormone deficiency (GHD) on a stable rhGH replacement dose, IGF-I levels increased during a switch from oral to transdermal 17β-estradiol therapy, however, together with a decrease in serum levels of estradiol (7).

Although it has been suggested that these differential effects of transdermal and oral estradiol on the GH/ IGF-I axis are due to a first-pass effect of oral estradiol, prior studies in GH-deficient women on stable rhGH replacement were never aimed at identical serum estradiol concentrations. In order to differentiate between the effects of serum estradiol concentrations per se and the route of estrogen administration on IGF-I levels in hypogonadal GH-deficient women, we designed a study to investigate the effects of different doses of oral estradiol (2mg/day and 4 mg/day) and different doses of transdermal estradiol (50 µg/day and 100 µg/day) aimed at identical serum estradiol concentrations on serum concentrations of IGF-I, IGFBP-3, and sex hormone binding globulin (SHBG) during rhGH replacement with a fixed dose.

PATIENTS AND METHODS

Patients

Twenty-three women with GHD and gonadotropin deficiency were included. Patients were recruited at the Outpatient Clinic of the Leiden University Medical Center and the University Medical Center in Utrecht, the Netherlands.

Inclusion criteria were:

1) GHD defined by a peak GH concentration <3 µg/l during the insulin tolerance test (nadir blood glucose <2.2 mmol/l).
2) stable rhGH replacement during at least 3 months.
3) oral or transdermal estradiol treatment because of gonadotropin deficiency.
4) written informed consent.

Patients were enrolled in the two study arms based on their pretreatment (oral estradiol and transdermal estradiol), with a individualized dose of GH replacement aimed at achieving a

normal IGF-I for age. Eighteen patients started with oral estradiol (of whom 15 completed the study), and 5 patients with transdermal estradiol. Clinical details are shown in Table 1. The mean dose of rhGH replacement during the study was 0.75 ± 0.28 mg/day (range 0.3 to 1.3 mg/day). Mean duration of rhGH replacement prior to the start of the study was 6 years (range 1 to 13 years). Conventional substitution therapy was monitored and held stable during the study. The study protocol was approved by the local Ethics Committees.

Study design

The study was designed as a parallel cross-over study (Figure 1). Patients were divided into two groups based on the route of administration of estrogens prior to study start. The dose of rhGH had been individually titrated aimed at achieving a normal serum IGF-I for age at the estrogen treatment used prior to inclusion, and during the study this rhGH replacement dose was kept stable.

To avoid carry-over effects and to ensure a new steady state, the duration of each estradiol treatment was four cycles of 28 days. In group I, the baseline oral estradiol treatment of 2 mg was first increased to 4 mg, and thereafter patients passed through the following treatments: 2 mg oral, 100 µg and 50 µg transdermal estradiol. In group II, the baseline treatment of 50 µg transdermal estradiol was sequentially increased to 100 µg transdermal, and 2 and 4 mg oral estradiol. Because of the necessity to achieve a new steady state at the highest oral estradiol dose, the protocol was 4 cycles longer in group I. Study parameters during the two periods of estradiol administration (cycles 1 and 9) did not differ, excluding a carry-over effect.

Study parameters were measured on day 12 of cycle 1, before dydrogesteron was added, and during each fourth cycle of 28 days of stable estrogen therapy during the subsequent cycles 5, 9, 13 in group I and II and cycle 17 in group I only.

Study medication

Estradiol (Estrofem 2 mg and 4 mg, (Novo Nordisk Farma BV, Alphen aan den Rijn, The Netherlands), Dermestril 50 µg and 100 µg (Sigma Tau Ethifarma BV, Assen, The Netherlands)) was given with additional dydrogesteron (Duphaston 10 mg, Solvay Pharmaceuticals, Weesp, The Netherlands) from days 15 to 28. Transdermal estrogen patches were used every Monday and Thursday at fixed time points. Tablets were taken every day at fixed time points (8:00 a.m. and 6:00 p.m.). The fasting serum estradiol concentrations were assumed to reflected the 24h concentrations, because the patients received the Estrofem tablets twice daily, and because of the long apparent half-life of the drug (about 16h) due to the extended resorption phase (9).

Study parameters and assays

Study parameters were serum levels of IGF-I, IGF-BP3, estradiol, and SHBG. All serum samples were obtained in the fasting state. The serum samples were immediately centrifuged and

Table 13/1: Characteristics of patients with GH and gonadotrophin deficiency included in the study.

	Group	Age	Etiology	Treatment	Pituitary deficiencies besides GH and gonadotropin deficiency	rhGH replacement dose (mg/day)	Completed study
1	OR	21	Craniopharyngeoma	TSS	ACTH, TSH, ADH	1.30	Yes
2	OR	45	M. Sheehan		ACTH, TSH	1.00	No
3	OR	33	M. Cushing	TSS	ACTH, TSH, ADH	1.00	Yes
4	OR	36	Craniopharyngeoma	TCS	ACTH, TSH, ADH	0.53	Yes
5	OR	46	Prolactinoma	TSS	TSH	1.00	Yes
6	OR	64	Arachnoidal cyst of pituitary stalk		ACTH, TSH	0.80	No
7	OR	51	M. Cushing	TSS	ACTH, TSH, ADH	1.07	Yes
8	OR	38	Prolactinoma	TSS & RT	ACTH, TSH	0.50	Yes
9	OR	50	Non-functioning adenoma	TSS	ACTH	0.30	Yes
10	OR	48	Non-functioning adenoma	TSS & RT	ACTH, TSH, ADH	1.00	No
11	OR	37	Prolactinoma	TSS & RT	ACTH, TSH, ADH	0.80	Yes
12	OR	50	M. Sheehan		ACTH, TSH	0.60	Yes
13	OR	32	M. Cushing	TSS & RT	ACTH, TSH, ADH	0.80	Yes
14	OR	52	Non-functioning adenoma	TSS	ACTH, TSH	0.40	Yes
15	OR	40	Prolactinoma	RT	ACTH, TSH	0.67	Yes
16	OR	27	Prolactinoma	TSS & RT	ACTH, TSH, ADH	0.50	Yes
17	OR	49	M. Sheehan		ACTH, TSH	1.20	Yes
18	OR	50	M. Cushing	TSS	ACTH, TSH	0.53	Yes
19	TD	45	Non-functioning adenoma	TSS & RT	ACTH, TSH	0.80	Yes
20	TD	41	Prolactinoma	TSS & RT	ACTH, TSH	0.80	Yes
21	TD	54	M. Cushing	TSS	ACTH, TSH	0.80	Yes
22	TD	49	Epidermoid cyst	TSS	ACTH, TSH	0.33	Yes
23	TD	48	M. Sheehan		ACTH	0.50	Yes

Legend to Table 1: OR: patients starting with oral estrogen treatment; TD: patients starting with transdermal estrogen treatment; TSS Transsphenoidal surgery; RT radiotherapy; TCS transcranial surgery.

stored at -20 ºC until analysis. All samples of all subjects were analyzed simultaneously at the end of the study.

Serum IGF-I was measured using an immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The lower limit of detection was 6.0 ng/ml and the intra-assay variation (n=250) was 8.0 and 6.0% at mean plasma levels of 30 and 450 ng/ml, respectively. The inter-assay variation was 8.7, 5.8 and 6.5% at mean IGF-I plasma levels of 33, 174 and 445 ng/l, respectively (n=115). The conversion factor (ng/ml to mmol/l) was 7.65. Serum IGFBP-3 was measured with an in-house RIA, as described previously (10). The lower limit of detection was 0.002 mg/l (absolute concentration) and the inter-assay variation was 7.5, 5.7 and 7.4% at mean plasma IGFBP-3 levels of 0.97, 2.0, and 3.0 mg/l, respectively (n = 44). Estradiol was measured after diethyl ether extraction and Sephadex chromatography using an in house competitive radio-immunoassay. The lower limit of detection for estradiol was 20 pmol/l (2 mL sample). The inter-assay variation was 12% and 3% at 80 and 660 pmol/l, respectively (n = 45, resp. 25). We measured SHBG with an immunometric technique on an Immulite Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 5 nmol/l and inter-assay variation was 5.5, 4.1 and 5.3% at 14, 34 and 91 nmol/l respectively (n = 23).

Statistics

Statistical analysis was performed using Systat, version 11 (Systat Software, Richmond, CA). Results are shown as the mean ± standard error of the mean (SEM), unless specified otherwise. A p-value < 0.05 was assumed to represent a significant difference.

Patients were divided into two groups based on prior oral or transdermal estrogen treatment (see study design) and groups were analyzed separately. Raw data were logarithmic-transformed and equality of the variances at each time period was verified with the Bartlett test and the Levene test. The serial data of both groups were analyzed by ANOVA with repeated measures with a General Linear Model. The statistical significance between the contrasts was corrected with the Bonferroni procedure for multiple comparisons in the post-hoc tests in group I.

RESULTS

Patients

Twenty-three patients were included of whom 20 patients completed the four different treatments (Fig. 1). Mean age was 44.2 ± 9.6 years and mean BMI was 28.5 ± 6.7 kg/m^2. Reasons for withdrawal were aggravated menstrual blood loss (n=1) and fluid retention (n=1), both during 2 mg OR, and personal reasons (n=1) during 100 µg TD estradiol.

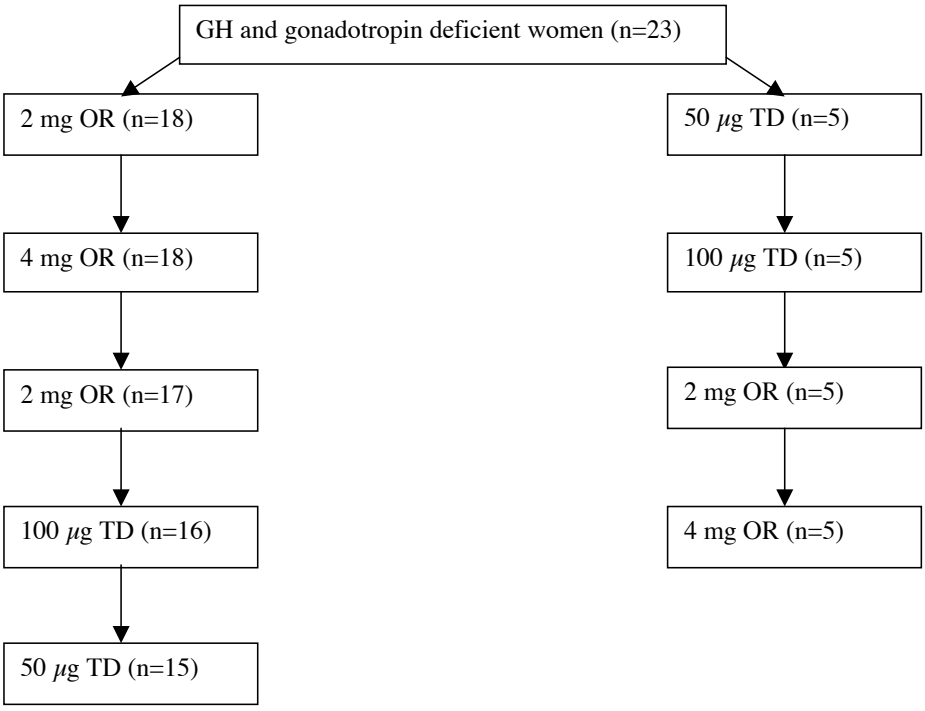

Figure 13/1: Study design of dose and route of estrogen administration in patients with GH and gonadotropin deficiency on stable dose rhGH replacement.

High versus low dose estrogen administration

The individual hormone concentrations in the two groups are plotted in Figure 2 and the relevant statistical details are listed in Table 2. The lowest estradiol concentrations were measured during 50 µg TD estradiol administration and the highest during 4 mg OR. Estradiol concentrations were approximately two-fold higher during the higher OR and TD doses compared with lower OR and TD doses. The mean serum estradiol concentrations were not statistically different between the 2 mg OR and 100 µg TD treatment period (P=1.0).

During the high (4 mg) compared to the low (2 mg) oral estrogen dose, serum IGF-I was significantly lower (97 ± 8.4 vs. 140 ± 13.8 µg/l, P=0.003 in group I and P=0.04 in group II), while SHBG concentration was higher (p<0.002) and serum IGFBP-3 was not significantly different.

Although during the transdermal dose of 50 µg estradiol the lowest mean estradiol and SHBG concentrations and higher mean IGF-I and IGF-BP3 concentrations were measured compared with those obtained during the 100 µg TD dose, the differences were not statistically significant.

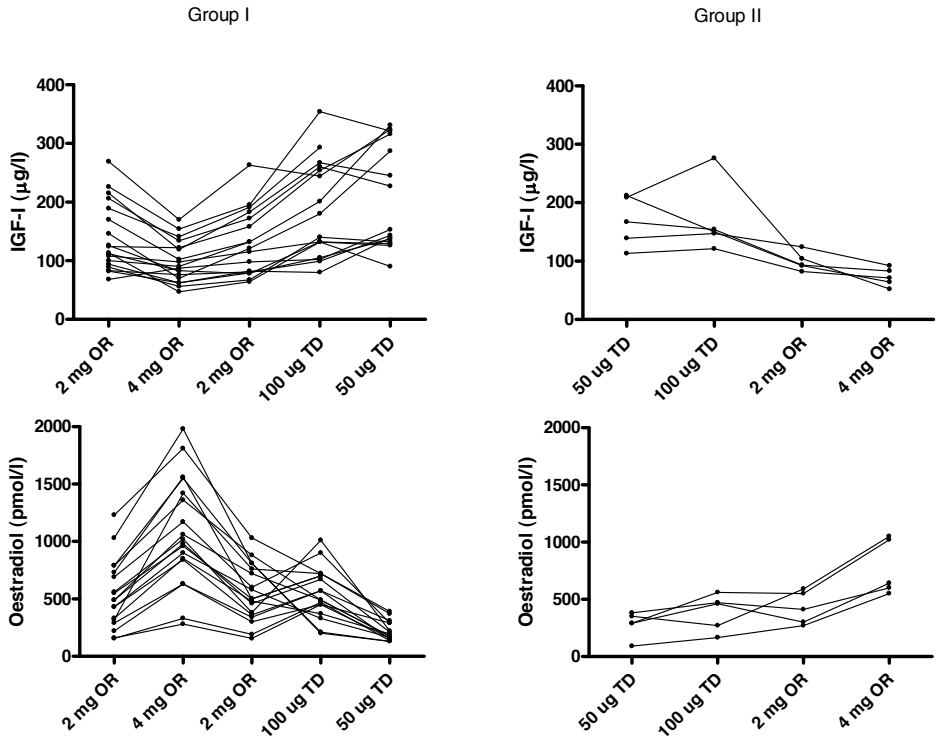

Figure 13/2: Individual plots of IGF-I (upper panels) and estradiol (lower panels) in hypogonadal GH-deficient females during a fixed dose of GH replacement and different doses of oral and transdermal estradiol replacement. Statistical details are listed in Table 2.

Oral (2 mg) versus transdermal (100 µg) estrogen administration

Estradiol concentrations measured during 2 mg OR and 100 µg TD estradiol were not statistically different. Despite comparable estradiol concentration, serum IGF-I was significantly lower in the 2 mg OR compared to the 100 µg TD period (P=0.005 in group I and P=0.02 in group II), while SHBG concentrations were significantly higher (P=0.002 in group I and P=0.004 in group II). Serum SHBG and IGF-I concentrations were negatively correlated in a linear model (R=0-.41,P=0.0001).

Side effects

Patients of group I especially had physical complaints during the use of the 50 µg estradiol dermal patch. Eight patients had muscle pains, two had arthralgias and four carpal tunnel syndrome. The complaints quickly disappeared when the patients were switched again to oral estradiol after completion of the trial. During 100 µg TD and during 50 µg TD, IGF-I concentrations were above 2 SD scores, indicating clear over-treatment in 3 and 5 cases, respectively. In

Table 13/2: Serum hormone concentrations in female growth hormone-deficient patients during GH and estrogen replacement.

	2 mg OR	4 mg OR	50 µg TD	100 µg TD
OR to TD group I				
IGF-I (µg/l)	140 ± 13.8 [a,c]	97 ± 8.4 [b]	206 ± 23	186 ± 21
Estradiol (pmol/l)	540 ± 70 [d,f]	1070 ± 111	221 ± 23	552 ± 57 [e]
IGFBP-3 (mg/l)	2.1 ± 0.1	1.9 ± 0.1	2.4 ± 0.2 [g]	2.3 ± 0.2
SHBG (nmol/l)	135 ± 13 [h,k]	174 ± 14 [k]	65 ± 6	95 ± 12
TD to OR group II				
IGF-I (µg/l)	99 ± 7.1 [m,l]	72 ± 7	168 ± 19	170 ± 27
Estradiol (pmol/l)	424 ± 64 [n,o]	772 ± 108	280 ± 51	385 ± 72
IGFBP-3 (mg/l)	1.8 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	2.1 ± 0.1
SHBG (nmol/l)	120 ± 26 [q,r]	153 ± 28	70 ± 13	88 ± 21

Legend to Table 2: Data are shown as mean ± SEM. Data were analyzed by ANOVA for repeated measures. Significance was tested with the Bonferroni correction for multiple comparisons. [a] : p=0.005 vs. 100 µg TD ; [b] : p<0.0001 vs. 50 µg and 100 µg TD; [c] : p=0.003 vs. 4 mg OR; [d] : p<0.0001 vs. 4 mg OR; [e] : p<0.0001 vs. 50 µg TD; [f] : p=0.01 vs. 50 µg TD; [g]: p<0.001 vs. 4 mg OR; [h]: p<0.002 vs. 4 mg OR; [k]: p=0.002 vs. 50 and 100 µg TD; [l] : p=0.04 vs. 4 mg OR; [m] : p=0.02 vs. 100 µg TD ; [n:] p=0.007 vs. 4 mg OR; [o]: p=0.07 vs. 50 µg TD; [q] : p=0.004 vs. 100 µg TD; [r] : p=0.02 vs. 4 mg OR.

the other group the most important complaints were increase in weight and emotional lability during the high dose estradiol period.

DISCUSSION

This study was designed to differentiate between the effects of serum estradiol concentrations per se and the route of estrogen administration on IGF-I levels in women with GH- and gonadotropin-deficiency. Each subject was kept on a fixed dose of rhGH during the whole study. Oral administration of estradiol resulted in lower IGF-I levels compared with transdermal administration, in accordance with previous studies that were not aimed at achieving comparable estradiol concentrations (7;8). IGF-I levels were higher during transdermal administration of 100 µg 17β-estradiol compared with oral administration of 2 mg 17β-estradiol while circulating estradiol concentrations were similar. Therefore, we conclude that the route of estradiol administration is a determinant of IGF-I levels during fixed recombinant human growth hormone replacement.

Serum IGF-I concentrations decreased during increasing oral dose of estrogen treatment or increased after discontinuation of estrogen replacement as shown by studies in GH-deficient women (Table 3 (6;11;12)). In two other cross-over studies aimed at unraveling the effects of different routes of estrogen administration on IGF-I concentrations, patients received both transdermal and oral estrogen replacement (Table 3 (7;8)). In the first cross-over study, IGF-I

levels increased in women on a fixed rhGH replacement dose during the switch from oral to transdermal estrogen therapy, but after the switch serum levels of estradiol decreased, which could have contributed to the IGF-I increase (7). The second cross-over study compared IGF-I concentrations with baseline values. In that study GH administration increased IGF-I levels in a stepwise dose-dependent manner during both estrogen administration routes, but IGF-I concentrations were lower during oral estradiol than transdermal administration at all used GH doses (8). Also in healthy postmenopausal women with an intact GH-IGF-I axis, oral estrogen administration reduces IGF-I concentrations, whereas transdermal estrogen administration has a variable effect (13-15).

The hypothalamic-somatotrope-IGF-I axis is primarily driven by GHRH, ghrelin and somatostatin and restrained by the negative feedback of liver-derived IGF-I, which acts upon the somatotrope and hypothalamic centers in a complex interplay (1). In addition, other hormones and metabolic signals modulate this system. Pathophysiological studies in postmenopausal women have revealed a central GH-stimulating role for estradiol (16;17). Therefore, for precise studies of IGF-I modulation by estrogens it is crucial that GH is fixed, as applied in the present study.

Estradiol is rapidly absorbed from the gastro-intestinal tract, but undergoes extensive first-pass effects resulting in the conversion into various metabolites. In pigs, only 6% of orally administered estradiol is present as such in the portal vein, whereas the remainder is metabolized into estrone and glucuronide and sulphate conjugates of estradiol and estrone (18). Most of the portal estradiol is rapidly cleared into the systemic circulation (19). Therefore, similar plasma estradiol concentrations can only be reached during oral estradiol treatment compared with transdermal estradiol treatment at the expense of high estrogen exposure to the liver. In accordance with this notion, we found that SHBG concentrations, a reflection of estrogen exposure of the liver (20), were highest during oral estrogen treatment compared with transdermal treatment. Nevertheless, the inhibitory effect on serum IGF-I concentration can also be accomplished by using large (nonphysiological) doses of transdermal estradiol, thus underscoring that the hepatic estradiol effects on IGF-I, SHBG, CBG, clotting factors, and CRP are liver-specific rather than route-specific (21). In the model we used, the differential effects of oral and transdermal estrogen administration on IGF-I levels were explained by different degrees of estrogen exposure of the liver. We found the well known dose dependent estrogen effects on serum IGF-I, but also a ~25% mean reduction of IGF-I by the first pass effect of oral administration.

Several animal studies have shown the relationship between estrogen treatment and hepatic IGF-I RNA expression. In ovariectomized rats, replacement with estradiol dose-dependently suppressed hepatic IGF-I liver mRNA expression and plasma IGF-I concentrations (22;23). Recently, the molecular mechanism underlying the hepatic effect of estrogen on IGF-I synthesis was discovered. Growth hormone signaling via the JAK-STAT pathway is inhibited by the suppression of JAK2 phosphorylation through stimulation of SOCS-2 (24). These basal mechanisms

Table 13/3: Summary of intervention studies with estrogens in GH and gonadotropin deficient women.

Author	Year of publication	Number of subjects	Design	Outcome
Kam (12)	2000	9	Single intervention study, 4 weeks treatment with oral estradiol valerate, 2 mg/day	Compared to baseline: IGF-I ↓, IGFBP-3 ↓, ALS ↓
Wolthers (8)	2001	8	Crossover study, randomized sequence, 8 weeks oral estradiol valerate 2 mg/day and 8 weeks of transdermal 17β-estradiol 100 µg/day, during 2nd month of each estrogen phase rhGH administration in a stepwise incremental regimen.	Compared to baseline: during OR IGF-I ↓, during TD IGF-I ↔. RhGH administration: during OR IGF-I and increment in IGF-I lower compared to TD.
Christiansen (6)	2005	26	Withdrawal study, patients with oral estrogens were studied between tablet 2 and 10 in the estrogen replacement cycle and after 28 days of estrogen discontinuation	Discontinuation: SHBG ↑, IGF-I ↑, IGFBP-3 ↑. Regression: change in SHBG and change in IGFBP-3 were main contributors in change in IGF-I (86% of variation)
Janssen (7)	2000	6	Crossover study, 2 cycles oral estradiol (2mg/day), then switch 3 cycles of transdermal estradiol (50 µg/day)	Switch from OR to TD estrogen: IGF-I ↑, IGFBP-3 ↔, estradiol ↔ (P=0.067), estrone ↓, SHBG ↓
Gibney (11)	2005	12	Crossover randomized study, 4 wk treatment with 17β-estradiol (2mg, followed by 4 mg) or raloxifene (slective estrogen receptor modulator with tissue-specific estrogen agonistic and antagonistic effects, 60 mg, followed by 120 mg)	Estradiol: IGF-I ↓, IGFBP-3 ↓ Raloxifene: IGF-I ↓, IGFBP-3 ↑

Legend to table 3: Summary of intervention studies in GH and gonadotrophin deficient women. ↓ significant decrease, ↔ no change, ↑ significant increase.

explain the gender difference that is observed in GH sensitivity for example in the treatment of GH-deficient patients (25).

IGF binding protein (BP) 3 concentrations were lowest during 4 mg oral estrogen therapy, but the changes in this study were limited and also other studies report conflicting data on the estrogen effect on IGFBP-3. Either no effect (in normal pre- and postmenopausal women) (13;15) or a decrease was observed (in postmenopausal women and hypogonadal GH-deficient patients after oral estrogen administration) (11;12), while no changes were seen after transdermal estrogen administration. Although the limited changes in IGFBP-3 may be caused by the decrease of IGF-I, direct inhibition of IGFBP-3 synthesis and release from Kupfer cells is also possible (24). It is important to note that IGFBP-3 is of limited use in clinical practice to detect changes in disease activity of GH disorders.

The changes in SHBG levels after estrogen treatment were found to be dose dependent, with the highest SHBG concentrations during oral estrogen treatment. Serum SHBG concentrations correlated negatively with IGF-I concentrations, as reported by others in healthy subjects and in GHD (6;26). Moreover, SHBG levels were lower during transdermal administration of 100 µg estradiol compared with oral administration of 2 mg estradiol, despite similar circulating estradiol concentrations. Therefore, the route of estrogen administration is also a determinant of SHBG levels.

In this study, we could not include naïve untreated gonadotropin and GH-deficient premenopausal women. Consequently, a randomization procedure was not possible and the preference of patients and physicians for oral estradiol substitution is reflected in the unbalanced number of patients between the two groups. Because of the limited number of patients (in group II) and the four different estrogen treatments that had to be compared, the groups were separately analyzed, although no group effects or carry-over effects were present. Although probably due to the low number of patients in group II some of the changes did not reach significance, it is interesting to note that the changes in this group were comparable to the changes observed in group I. The additive value of group II is that the results of this study are both valid for interventions leading to IGF-I concentrations in the supraphysiological range (group I) as in the subphysiological range (group II). When the two groups were analyzed together, the conclusions remained unaltered (data not shown), particularly for the comparisons of IGF-I, IGFBP-3, and SHBG during 2 mg oral estrogen treatment and 100 µg transdermal estrogen treatment.

From a cost-effective point of view of GH substitution transdermal estrogen replacement is preferred. Patients who are switched from oral to transdermal estrogen replacement require ~ 0.3 mg GH less per day which on a nation wide scale is a considerable cost reduction (27). Leung et al. have calculated a cost reduction for the USA population of $110 billion or approximately $4400 per patient (27).

In summary, the route of estrogen administration is a determinant of serum IGF-I concentrations in adult women with GH and gonadotropin deficiency during fixed rhGH replacement.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Prof. dr. F.M. Helmerhorst from the Department of Gynecology, Leiden University Medical Center, and Mrs. I. Maitimu from the Laboratory of Endocrinology, University Medical Center Utrecht.

REFERENCE LIST

1. Giustina A, Veldhuis JD 1998 Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 19:717-797
2. van den BG, Veldhuis JD, Frolich M, Roelfsema F 1996 An amplitude-specific divergence in the pulsatile mode of growth hormone (GH) secretion underlies the gender difference in mean GH concentrations in men and premenopausal women. J Clin Endocrinol Metab 81:2460-2467
3. Cook DM, Ludlam WH, Cook MB 1999 Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 84:3956-3960
4. Johansson AG, Engstrom BE, Ljunghall S, Karlsson FA, Burman P 1999 Gender differences in the effects of long term growth hormone (GH) treatment on bone in adults with GH deficiency. J Clin Endocrinol Metab 84:2002-2007
5. Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG 2000 Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab 85:1121-1125
6. Christiansen JJ, Fisker S, Gravholt CH, Bennett P, Svenstrup B, Andersen M, Feldt-Rasmussen U, Christiansen JS, Jorgensen JO 2005 Discontinuation of estrogen replacement therapy in GH-treated hypopituitary women alters androgen status and IGF-I. Eur J Endocrinol 152:719-726
7. Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F 2000 A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. J Clin Endocrinol Metab 85:464-467
8. Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK 2001 Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. Am J Physiol Endocrinol Metab 281:E1191-E1196
9. Fink BJ, Christensen MS 1981 Bioavailability of oestradiol and oestriol administered orally to oophorectomized women. Maturitas 3:289-294
10. de Vries BB, Robinson H, Stolte-Dijkstra I, Tjon Pian Gi CV, Dijkstra PF, van Doorn J, Halley DJ, Oostra BA, Turner G, Niermeijer MF 1995 General overgrowth in the fragile X syndrome: variability in the phenotypic expression of the FMR1 gene mutation. J Med Genet 32:764-769
11. Gibney J, Johannsson G, Leung KC, Ho KK 2005 Comparison of the metabolic effects of raloxifene and oral estrogen in postmenopausal and growth hormone-deficient women. J Clin Endocrinol Metab 90:3897-3903
12. Kam GY, Leung KC, Baxter RC, Ho KK 2000 Estrogens exert route- and dose-dependent effects on insulin-like growth factor (IGF)-binding protein-3 and the acid-labile subunit of the IGF ternary complex. J Clin Endocrinol Metab 85:1918-1922
13. Lissett CA, Shalet SM 2003 The impact of dose and route of estrogen administration on the somatotropic axis in normal women. J Clin Endocrinol Metab 88:4668-4672
14. Paassilta M, Karjalainen A, Kervinen K, Savolainen MJ, Heikkinen J, Backstrom AC, Kesaniemi YA 2000 Insulin-like growth factor binding protein-1 (IGFBP-1) and IGF-I during oral and transdermal estrogen replacement therapy: relation to lipoprotein(a) levels. Atherosclerosis 149:157-162
15. Bellantoni MF, Vittone J, Campfield AT, Bass KM, Harman SM, Blackman MR 1996 Effects of oral versus transdermal estrogen on the growth hormone/insulin-like growth factor I axis in younger and older postmenopausal women: a clinical research center study. J Clin Endocrinol Metab 81:2848-2853
16. Veldhuis JD, Evans WS, Bowers CY 2003 Estradiol supplementation enhances submaximal feedforward drive of growth hormone (GH) secretion by recombinant human GH-releasing hormone-1,44-amide in a putatively somatostatin-withdrawn milieu. J Clin Endocrinol Metab 88:5484-5489
17. Veldhuis JD, Anderson SM, Kok P, Iranmanesh A, Frystyk J, Orskov H, Keenan DM 2004 Estradiol supplementation modulates growth hormone (GH) secretory-burst waveform and recombinant

human insulin-like growth factor-I-enforced suppression of endogenously driven GH release in postmenopausal women. J Clin Endocrinol Metab 89:1312-1318
18. Ruoff WL, Dziuk PJ 1994 Absorption and metabolism of estrogens from the stomach and duodenum of pigs. Domest Anim Endocrinol 11:197-208
19. Scharl A, Beckmann MW, Artwohl JE, Kullander S, Holt JA 1991 Rapid liver metabolism, urinary and biliary excretion, and enterohepatic circulation of 16 alpha-radioiodo-17 beta-estradiol. Int J Radiat Oncol Biol Phys 21:1235-1240
20. Stomati M, Hartmann B, Spinetti A, Mailand D, Rubino S, Albrecht A, Huber J, Petraglia F, Genazzani AR 1996 Effects of hormonal replacement therapy on plasma sex hormone-binding globulin, androgen and insulin-like growth factor-1 levels in postmenopausal women. J Endocrinol Invest 19:535-541
21. Friend KE, Hartman ML, Pezzoli SS, Clasey JL, Thorner MO 1996 Both oral and transdermal estrogen increase growth hormone release in postmenopausal women--a clinical research center study. J Clin Endocrinol Metab 81:2250-2256
22. Borski RJ, Tsai W, DeMott-Friberg R, Barkan AL 1996 Regulation of somatic growth and the somatotropic axis by gonadal steroids: primary effect on insulin-like growth factor I gene expression and secretion. Endocrinology 137:3253-3259
23. Krattenmacher R, Knauthe R, Parczyk K, Walker A, Hilgenfeldt U, Fritzemeier KH 1994 Estrogen action on hepatic synthesis of angiotensinogen and IGF-I: direct and indirect estrogen effects. J Steroid Biochem Mol Biol 48:207-214
24. Leung KC, Doyle N, Ballesteros M, Sjogren K, Watts CK, Low TH, Leong GM, Ross RJ, Ho KK 2003 Estrogen inhibits GH signaling by suppressing GH-induced JAK2 phosphorylation, an effect mediated by SOCS-2. Proc Natl Acad Sci U S A 100:1016-1021
25. Janssen YJ, Frolich M, Roelfsema F 1997 A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 82:129-135
26. Pfeilschifter J, Scheidt-Nave C, Leidig-Bruckner G, Woitge HW, Blum WF, Wuster C, Haack D, Ziegler R 1996 Relationship between circulating insulin-like growth factor components and sex hormones in a population-based sample of 50- to 80-year-old men and women. J Clin Endocrinol Metab 81:2534-2540
27. Leung KC, Johannsson G, Leong GM, Ho KK 2004 Estrogen regulation of growth hormone action. Endocr Rev 25:693-721

Chapter 14

Influence of the d3-growth hormone receptor isoform on short-term and long-term treatment response to growth hormone replacement in growth hormone deficient adults

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Journal of Clinical Endocrinology and Metabolism, 2008

ABSTRACT

Objective

Recombinant human growth hormone (rhGH) replacement in adults is aimed at improving signs and symptoms of the adult GH deficiency (GHD) syndrome. In children, a common polymorphism of the GH receptor (exon-3 deletion, d3GHR) increases the response to rhGH replacement. The aim of this study was to assess the effects of this polymorphism on the response to rhGH replacement in adults.

Design

Prospective intervention with rhGH during 1 year (n=99) and in a subset during 5 years (n=53).

Patients and methods

The presence of the d3GHR variant was established in GHD patients and linked to short-term and long-term effects of rhGH replacement on IGF-I, lipid metabolism, anthropometric parameters, and bone mineral density.

Results

Fifty-five patients had two wildtype alleles (56%), whereas 38 patients (38%) had one allele and 6 patients (6%) had two alleles coding the d3GHR isoform. During short-term rhGH replacement, the increase in IGF-I was higher in patients bearing at least one d3GHR allele compared to those with two wildtype alleles (at an identical mean dose of rhGH). The decrease in total cholesterol and LDL cholesterol was lower in the group bearing at least one d3GHR allele, whereas the increase in HDL cholesterol was higher compared to patients with the wildtype genotype. In contrast, these differential responses of GHR genotype could not be demonstrated during long-term rhGH replacement.

Conclusion

The d3GHR genotype contributes, at least for some parameters, to the interindividual differences in efficacy of short-term, but not of long-term, rhGH replacement in adults with GHD.

INTRODUCTION

The aim of recombinant human growth hormone (rhGH) replacement in adults is to ameliorate symptoms and signs of the adult growth hormone deficiency (GHD) syndrome. In adults with GHD these effects include beneficial effects in lipid concentrations, body composition, and bone mineral density (1-5).

Recently, a polymorphism in the growth hormone receptor, a genomic deletion of exon 3 (d3GHR), has been described to increase growth velocity during rhGH replacement in children with GHD (6) and idiopathic short stature or children who were short for gestational age (7). Due to this polymorphism GH signal transduction is enhanced despite unaltered GH receptor binding (8). The allele-prevalence is estimated to be 25-32% with a frequency of homozygosity of 9-14% (8;9). Consequently, this polymorphism might also contribute to inter-individual variability of the clinical response to rhGH replacement in adults with GHD.

Therefore, the aim of this study was to assess the effects of this common polymorphism on the response to short-term replacement (1 year) and, in a subset of patients, long-term rhGH replacement (5 years) with rhGH on IGF-I levels, anthropometric and metabolic parameters, and bone mineral density.

PATIENTS AND METHODS

Patients

All patients with GHD, visiting our outpatient clinic and in whom efficacy parameters of rhGH replacement were collected prospectively in a standardized manner, could be included in the present study (n=145). The exclusion criteria were known genetic defects in GH-IGF-I pathways (for example known mutations in the GHR).

All eligible patients were sent an extensive letter regarding the present protocol. Subsequently, the patients were contacted by telephone to ask consent for participation. Eleven patients declined participation, and 23 patients did not respond at all to repeated telephone calls or did not have updated telephone number. Consequently, saliva collection kits were sent to the home of the 111 remaining patients in prepaid envelopes. Ten of these 111 samples were not returned, and 2 saliva samples were not collected in a proper manner. Thus, genotyping could be performed in 99 patients. No differences were found in clinical characteristics between patients of whom DNA samples were received (n=101) and the remaining patients (n=44). Short-term effects could be evaluated in all 99 patients (Table 1), whereas in 53 of these 99 patients long-term effects could be evaluated due to an earlier starting date of rhGH treatment (Table 2).

Study design and treatment protocol

Patients were prospectively enrolled in an open label treatment protocol. Growth hormone deficiency (GHD) was confirmed in all patients by an insulin tolerance test (nadir blood glucose <2.2 mmol/l) with a peak GH concentration <3 µg/l. After initial measurements were obtained, all patients were treated with subcutaneous injections of rhGH (Genotropin® Pharmacia/Pfizer or Zomacton® Ferring, Norditropin® NovoNordisk, or Humatrope® Lilly), administered subcutaneously every evening. The initial dose of rhGH was 0.2 mg/day, which was individually adjusted each month in the first half year to achieve physiological serum IGF-I concentrations, within the age-dependent laboratory reference range (IGF-I standard deviation scores (SD scores)). Thereafter, this individualized dose was continued in each patient and adjusted, if necessary, to maintain a normal IGF-I concentration for the duration of the study.

Patients with a functioning adenoma (short-term group: 18 patients with Cushing's disease, 4 patients with acromegaly and 12 patients with prolactinoma and long-term subset: 7 patients with Cushing's disease, 2 patients with acromegaly and 7 patients with prolactinoma) were in remission before entering the study. The diagnosis of GHD after acromegaly was suspected in patients treated with postoperative radiotherapy and now suffering from panhypopituitarism and confirmed with an insulin-tolerance test (10). When secondary amenorrhoea was present for more than 1 year premenopausal women were defined as LH/FSH deficient. In men, LH/FSH deficiency was defined, as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone stimulation test or insulin tolerance test. Conventional substitution therapy was monitored during substitution with rhGH and the respective dosages were adjusted, as required for normalization of clinical and biochemical parameters of pituitary deficiencies.

The study protocol was approved by the local Ethics Committee. All patients gave written informed consent to participation in the study.

DNA collection and genetic analysis

All patients, who consented on participation, received a saliva collection kit (Oragene kit Westburg, Leusden, The Netherlands). Participants were requested to provide 2 ml of saliva. DNA extraction was done 4 to 6 weeks after saliva collection and storage at room temperature. DNA concentrations and purity (OD260/280) were determined spectrophotometrically using the nanodrop (Isogen, IJsselstein, The Netherlands). The exon 3 deletion in the GHR gene was detected as described previously (10). Briefly, 25 ng DNA was PCR amplified using hotstart PCR mastermix (Qiagen, Venlo, The Netherlands) and primers G1, G2 and G3 (genbank asseccionnumber AF155912) as follows: initial denaturation of 15 minutes at 94°C, 35 cycles of 30s at 95°C, 30 s at 60°C and 2min at 72°C, followed by a final extension of 10min at 72°C. PCR

products were separated and visualized on an ethidiumbromide stained agarose gel. Expected allele frequencies were calculated by Hardy-Weinberg equilibrium.

Study parameters

Based on the genotype patients could be divided into two groups (group 1: homozygote wild type: two wildtype alleles, group 2: heterozygote (one d3GHR isoform, and one wildtype allele) and homozygote (two d3GHR isoforms).

Genotype was linked to:

1. Insulin-like growth factor-I (IGF-I) concentrations
2. Biochemical metabolic parameters: fasting levels of glucose, total cholesterol, HDL cholesterol (HDL), and triglycerides (TG). LDL cholesterol concentrations (LDL) were calculated using the Friedewald formula. Patients were requested to fast overnight before blood samples were taken for laboratory measurements of lipid profiles and glucose.
3. Anthropometric parameters: body weight and height, waist circumference, hip circumference, systolic and diastolic blood pressure (SBP and DBP respectively.) were measured. Body-mass index (BMI) and waist-hip (WH) ratio were calculated. Body weight was measured to the nearest 0.1 kg, and body height was measured barefoot to the nearest 0.001 m. The BMI was calculated as weight in kilograms divided by the square of height in meters.
4. Bone mineral density (BMD): BMD of the lumbar spine (L1-L4) was measured using dual-photon X-ray absorptiometry (DXA: Hologic QDR 1000 and 4500, Waltham, MA). During follow-up, the Hologic QDR 1000 was replaced by the Hologic QDR 4500. To be able to properly compare BMD scores, a conversion formula was developed based on data from an in–house comparison of both methods in 300 subjects (BMD LWK: Hologic 4500 (g/cm^2) =(0.9736*BMD Hologic QDR 1000)+0.0109).

Assays

Serum IGF-I (nmol/l) concentration was measured by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns. The detection limit of this assay is 1.5 nmol/l, and the inter-assay coefficient of variation was below 11%. Age-adjusted IGF-I data were determined in the same laboratory (11;12). IGF-I concentrations were also expressed as SD scores for age-related normal levels.

A Hitachi 747 autoanalyzer (Roche, Mannheim, Germany) was used to quantify serum concentrations of glucose, total cholesterol and TG. HDL was measured with a homogenous enzymatic assay (Hitachi 911, Roche, Mannheim, Germany). In 2003 the Hitachi 747 was replaced by a modular P 800 with no change in the chemistry components.

Statistics

Statistical analysis was performed using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, Illinois, USA). Results are scored as the mean ± standard error of the mean, unless specified otherwise. Paired samples Student's t-test were used to compare baseline and 1 year (short-term) and baseline and 5 year rhGH replacement (long-term).

Patients were divided into two groups: 1) patients with two wildtype alleles and 2) patients carrying one allele coding for the d3GHR isoform and patients with two alleles coding for the d3GHR isoform combined. Differences for short-term and long-term rhGH replacement were calculated for all study parameters. Independent samples T-test were used to compare both groups. A p-value of <0.05 was assumed to represent a significant difference.

RESULTS

Patients

Short-term effects (1 year, Table 1): Ninety-nine patients (43 men) with a mean age of 51 years (range: 19-81 years) were studied. Fifty-five patients had two wildtype alleles (56%), whereas 38 patients (38%) had one allele coding the d3GHR isoform, and 6 patients (6%) had two alleles coding the d3GHR isoform. There were no differences in clinical characteristics between the two patient groups. Four patients were previously treated for acromegaly (n=2 wildtype, n=2 heterozygotes) and 18 patients were previously treated for Cushing's disease (n=10 wildtype, n=7 heterozygotes and n=1 homozygote).

At baseline, 9 patients with two wildtype alleles and 12 patients bearing at least one d3GHR allele used antihypertensive drugs (p=0.220), compared to 11 patients with two wildtype alleles and 14 patients bearing at least one d3GHR allele at follow-up (p=0.196). At baseline, 6 patients with two wildtype alleles and 5 patients bearing at least one d3GHR allele used lipid-lowering drugs (p=0.943), compared to 7 patients with two wildtype alleles and 7 patients bearing at least one d3GHR allele at follow-up (p=0.652).

Long-term effects (5 years, Table 1): Fifty-three patients (23 men) with a mean age of 49.4 years (range 19-70 years) were included in the long-term rhGH replacement pharmacogenetic analysis. Thirty-one patients had two wildtype alleles (58%), whereas 17 patients (32%) had one allele coding the d3GHR isoform, and 5 patients (9%) had two alleles coding the d3GHR isoform. There were no differences in clinical characteristics between the two patient groups. Two patients were previously treated for acromegaly (n=1 wildtype and n=1 heterozygote) and 7 patients were previously treated for Cushing's disease (n=4 wildtype, n=2 heterozygote and n=1 homozygote). At baseline, 4 patients with two wildtype alleles and 4 patients bearing at least one d3GHR allele used antihypertensive drugs (p=0.670), compared to 6 patients with two wildtype alleles and 7 patients bearing at least one d3GHR allele at follow-up (p=0.331). At baseline, 3 patients with two wildtype alleles and 2 patients bearing at least one d3GHR allele

Table 14/1: Clinical characteristics replacement in adults with growth hormone deficiency who completed short-term(n=99) and long-term (n=53) recombinant human growth hormone replacement: comparison between patients with two wildtype alleles (WT-WT), patients bearing one allele coding for the d3GHR isoform (d3GHR-WT), and patients bearing two d3GHR alleles (d3GHR-d3GHR).

		WT-WT (n=55)	d3GHR-WT and d3GHR-d3GHR (n=44)	P-value	WT-WT (n=31)	d3GHR-WT and d3GHR-d3GHR (n=22)	P-value
Gender (%)	Male/ Female	46/54	41/59	NS	45/ 55	41/59	NS
Age (years (mean ± SEM))		49.6 ± 1.5	52.7 ± 1.9	NS	50.6 ± 2.2	47.8 ± 2.9	NS
Etiology of GHD (%)	Non-functioning pituitary adenoma	29	36	NS	35	27	NS
	Functioning pituitary adenoma*	33	36		26	36	
	Craniopharyngioma	11	14		7	23	
	Other**	27	14		32	14	
Onset of disease (%)	Adult onset/ Childhood onset	96/4	95/5	NS	94/ 7	91/9	NS
Surgery (%)		78	80	NS	81	82	NS
Radiotherapy (%)		44	36	NS	42	41	NS
ADH deficiency (%)		31	23	NS	26	32	NS
ACTH deficiency (%)		78	75	NS	84	82	NS
TSH deficiency (%)		89	71	0.019	97	86	NS
LH/ FSH substitution therapy(%)	Men	80	67	NS	93	78	NS
	Women	53	42	NS	59	54	NS

*Prolactinoma, Cushing's disease, acromegaly.

**Cerebral malignancies and their treatment, traumatic brain injury, sheehan's syndrome, hypophysitis, midline defect, empty sella.

NS: non significant.

used lipid-lowering drugs (p=0.943), compared to 3 patients with two wildtype alleles and 4 patients bearing at least one d3GHR allele at follow-up (p=0.368).

Effects of rhGH replacement

Short-term effects

IGF-I concentrations significantly increased during 1 year of rhGH replacement. Glucose and HDL cholesterol concentrations increased during 1 year rhGH replacement, whereas total and LDL cholesterol concentrations decreased (Table 2). Triglyceride concentrations tended to increase during 1 year rhGH replacement. There were no changes in anthropometric parameters (SBP, DBP, waist circumference, WH ratio, BMI) or BMD.

To study variables that independently influenced short-term treatment effects, linear regression analysis with treatment endpoints which were influenced by short-term rhGH replacement (glucose, total cholesterol, LDL and HDL cholesterol) as dependent variables and the baseline value of the specific treatment parameter, age, gender (female (=0) vs. male (=1)), BMI at baseline, rhGH replacement dose, and diagnosis (no pituitary tumor (=0) vs. pituitary tumor (=1)) was performed. Glucose concentrations after 1 year rhGH replacement were affected by baseline glucose concentration (β=0.454, $p<0.001$) and baseline BMI (β=0.055, p=0.010). Total cholesterol after 1 year rhGH replacement was affected by baseline cholesterol (β=0.767, $p<0.001$), gender (β=-0.443, p=0.014) and diagnosis (β=0.479, p=0.019). HDL cholesterol after 1 year rhGH replacement was affected by baseline HDL (β=0.856, $p<0.001$), gender (β=-0.119, p=0.020), rhGH dose (β=0.425, p=0.007) and diagnosis (β=0.149, p=0.009). LDL cholesterol after 1 year rhGH replacement was only affected by LDL at baseline (β=0.741,$p<0.001$).

Long-term effects

IGF-I concentrations were significantly increased during 5 years rhGH replacement. Glucose levels tended to increase, although this difference did not reach statistical significance. Total and LDL cholesterol levels decreased significantly, whereas HDL increased despite a significant increase in BMI during long-term follow-up. Waist circumference and WH ratio, however, remained unchanged. In addition, DBP decreased, whereas SBP remained unaffected. BMD increased during 5 years of rhGH replacement. To study variables that independently influenced long-term treatment effects, linear regression analysis with treatment endpoints which were influenced by long-term rhGH replacement (total cholesterol, LDL and HDL cholesterol, DBP and BMD) as dependent variables and the baseline value of the specific treatment parameter, age, gender (female (=0) vs. male (=1)), BMI at baseline, rhGH replacement dose, and diagnosis (no pituitary tumor (=0) vs. pituitary tumor (=1)) was performed. None of these independent parameters apart from the individual baseline values of the parameters influenced the long-term endpoints (data not shown).

Table 14/2: Effects of recombinant human growth hormone (rhGH) replacement during short-term (1 year) and long-term (5 years) treatment in adults with growth hormone deficiency.

	Baseline	1 yr rhGH replacement	P-value*
Short-term (n=99)			
IGF-I (nmol/l)	10.1 ± 0.6	23.3 ± 1.0	<0.001
IGF-I SD score	-1.6 ± 0.1	1.0 ± 0.2	<0.001
Glucose (mmol/l)	4.7 ± 0.09	4.9 ± 0.09	0.007
Total cholesterol (mmol/l)	6.4 ± 0.1	6.1 ± 0.1	<0.001
LDL cholesterol (mmol/l)	4.7 ± 0.1	4.2 ± 0.1	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.05	1.5 ± 0.05	0.009
Triglycerides (mmol/l)	1.8 ± 0.1	1.9 ± 0.1	0.056
SBP (mm Hg)	133.8 ± 1.6	136.5 ± 2.1	NS
DBP (mm Hg)	84.0 ± 0.9	85.2 ± 1.0	NS
Waist circumference (cm)	95.3 ± 1.4	94.6 ± 1.4	NS
WH ratio	0.9 ± 0.009	0.9 ± 0.009	NS
BMI (kg/m^2)	27.1 ± 0.5	27.4 ± 0.5	NS
BMD (g/cm^2)	1.0 ± 0.1	1.0 ± 0.2	NS
Long-term (n=53)	Baseline	5 yr rhGH replacement	
IGF-I (nmol/l)	9.3 ± 0.7	22.6 ± 1.5	<0.001
IGF-I SD score	-1.8 ± 0.1	0.9 ± 0.3	<0.001
Glucose (mmol/l)	4.6 ± 0.1	4.9 ± 0.1	0.073
Total cholesterol (mmol/l)	6.8 ± 0.2	5.9 ± 0.2	<0.001
LDL cholesterol (mmol/l)	5.1 ± 0.2	4.0 ± 0.2	<0.001
HDL cholesterol (mmol/l)	1.4 ± 0.1	1.6 ± 0.1	<0.001
Triglycerides (mmol/l)	1.8 ± 0.1	2.0 ± 0.2	NS
SBP (mm Hg)	132.3 ± 2.0	129.7 ± 2.2	NS
DBP (mm Hg)	84.3 ± 1.2	81.5 ± 1.4	0.029
Waist circumference (cm)	92.7 ± 2.0	93.6 ± 2.1	NS
WH ratio	0.9 ± 0.01	0.9 ± 0.01	NS
BMI (kg/m^2)	26.0 ± 0.6	27.0 ± 0.7	0.006
BMD (g/cm^2)	0.9 ± 0.02	1.0 ± 0.03	0.011

Data are presented as mean ± SEM. *Baseline and 1 yr as well as baseline and 5 yr recombinant human growth hormone (rhGH) replacement data were compared with a paired samples T-test.
NS: non significant.

Pharmacogenetics during short-term rhGH replacement

No differences were found in the baseline values of the different metabolic parameters between the two groups (glucose, total/ LDL/ HDL cholesterol, and triglycerides).

However, the decrease in total cholesterol during 1 year rhGH replacement was markedly reduced in patients bearing at least one allele coding the d3GHR isoform compared to patients with two wildtype alleles (-0.08 ± 0.1 mmol/l in the d3GHR group vs. -0.5 ± 0.1 mmol/l in patients

Table 14/3: Changes in IGF-I, metabolic parameters, anthropometric parameters, and BMD during 1 year recombinant growth hormone replacement (short-term) and during 5 years in a subset of patients (long-term): comparison between patients with two wildtype alleles (WT-WT) and patients bearing at least one allele coding for the d3GHR isoform (d3GHR-WT and d3GHR-d3GHR).

	Short term treatment			Long-term treatment		
	WT-WT (n=55)	d3GHR-WT and d3GHR-d3GHR (n=44)	P-value	WT-WT (n=31)	d3GHR-WT and d3GHR-d3GHR (n=22)	P-value
IGF-I (nmol/l)	10.9 ± 1.1	15.9 ± 1.7	0.012	12.7 ± 2.0	14.1 ± 2.0	NS
IGF-I SD score	2.1 ± 0.2	3.2 ± 0.3	0.010	2.6 ± 0.4	2.9 ± 0.4	NS
Dose rhGH 1 yr (mg/day)	0.4 ± 0.02	0.4 ± 0.03	NS	0.5 ± 0.04	0.5 ± 0.05	NS
Glucose (mmol/l)	0.3 ± 0.1	0.2 ± 0.1	NS	0.1 ± 0.1	0.4 ± 0.3	NS
Total cholesterol (mmol/l)	-0.5 ± 0.1	-0.08 ± 0.1	0.010	-0.9 ± 0.3	-0.4 ± 0.2	NS
LDL cholesterol (mmol/l)	-0.6 ± 0.1	-0.2 ± 0.1	0.028	-1.0 ± 0.2	-0.7 ± 0.3	NS
HDL cholesterol (mmol/l)	0.01 ± 0.03	0.1 ± 0.03	0.012	0.1 ± 0.05	0.2 ± 0.1	NS
Triglycerides (mmol/l)	0.2 ± 0.1	0.002 ± 0.1	NS	0.1 ± 0.2	0.3 ± 0.2	NS
SBP (mmHg)	0.9 ± 3.1	4.9 ± 2.8	NS	-4.8 ± 2.0	0.6 ± 3.3	NS
DBP (mmHg)	0.6 ± 1.2	1.9 ± 1.8	NS	-3.1 ± 1.5	-2.4 ± 2.1	NS
Waist circumference (cm)	-1.1 ± 0.7	-0.2 ± 0.8	NS	1.2 ± 1.2	0.5 ± 1.4	NS
WH ratio	-0.004 ± 0.008	-0.002 ± 0.009	NS	0.02 ± 0.01	-0.03 ± 0.02	0.030
BMI (kg/m^2)	0.2 ± 0.2	0.4 ± 0.3	NS	0.9 ± 0.5	1.2 ± 0.3	NS
BMD (g/cm^2)	0.0004 ± 0.007	0.005 ± 0.007	NS	0.05 ± 0.02	0.04 ± 0.01	NS

Data are presented as mean ± SEM. NS: non significant.

with two wildtype alleles, p=0.010, Table 3, Figure 1). The reduction in LDL cholesterol was also lower in patients bearing at least one allele coding the d3GHR isoform compared to patients with two wildtype alleles (-0.2 ± 0.1 mmol/l vs. -0.6 ± 0.1 mmol/l, p=0.028). HDL increased more in patients bearing one allele coding the d3GHR isoform compared to patients with two wild-type alleles (0.1 ± 0.03 mmol/l vs. 0.01 ± 0.03 mmol/l, p=0.012). An additional analysis excluding all patients who used lipid-lowering drugs during follow-up, did not alter the conclusions.

No differences were found in baseline parameters or change during 1 year rhGH replacement in serum triglycerides or the anthropometric parameters (SBP, DBP, waist circumference, WH ratio, and BMI) between the two groups.

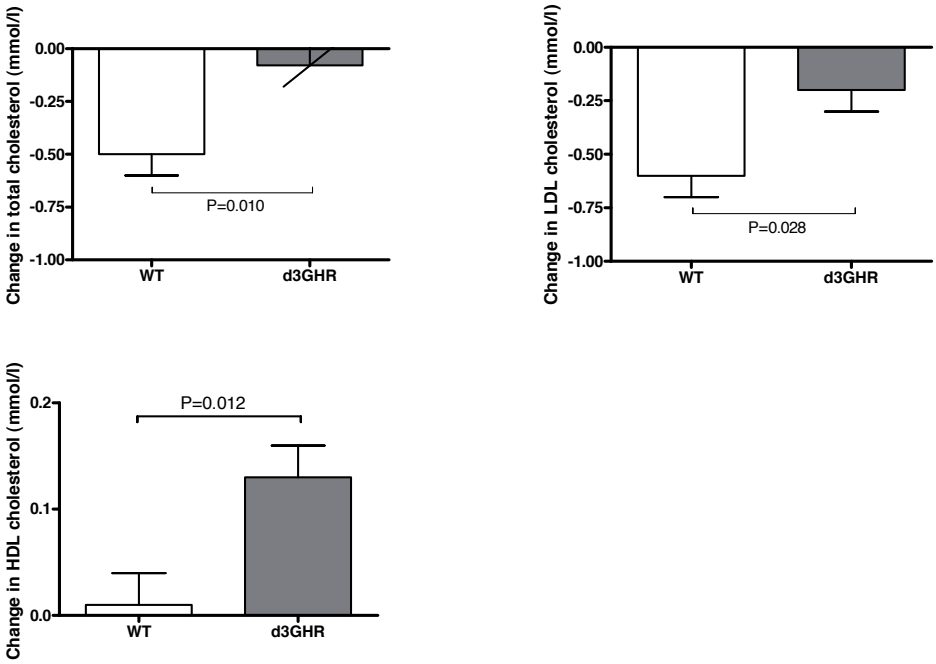

Figure 14/1: Change in IGF-I standard deviation score (SDS) total cholesterol, LDL cholesterol and HDL cholesterol levels during short-term rhGH replacement: comparison between patients with two wildtype alleles (WT-WT) and patients bearing at least one allele coding for the d3GHR isoform (d3GHR-WT and d3GHR-d3GHR).

No differences were found in BMD at the lumbar spine at baseline between the three groups, or during 1-year rhGH replacement.

Pharmacogenetics of long-term rhGH replacement

During long-term follow-up, no differences in treatment efficacy could be discerned between the two groups (Table 3). IGF-I concentrations and IGF-I SD scores were increased to a similar extent during long-term follow-up with no difference between rhGH replacement dose at 5 years. WH ratio decreased in patients bearing at least one allele of the d3GHR whereas it increased slightly in patients with two WT alleles, without any differences in waist circumference at baseline or follow-up or change in waist circumference. None of changes in the other biochemical metabolic parameters, anthropometrics measurements, and BMD differed between the two groups.

DISCUSSION

In this study, we evaluated the pharmacogenetics of rhGH replacement in adult patients with GHD according to d3GHR genotype. We found that the d3GHR genotype was associated with differences in efficacy during short-term, but not during long-term, rhGH replacement. These results suggest that the d3GHR genotype could contribute, at least for some parameters, to the inter-individual differences seen in efficacy of rhGH replacement in adults with GHD.

Various studies have assessed the interaction between efficacy of rhGH replacement and d3GHR polymorphisms in various groups of GHD and non-GHD children (6;13-18). The results in GHD children are conflicting. One study found a higher growth velocity in the group bearing at least one d3-GHR allele (6), whereas two other studies did not show this relationship (15;18).

The efficacy parameters of rhGH replacement in children with GHD are clear cut: growth velocity and final height. In adults with GHD the effects of rhGH replacement include more subtle effects in lipid concentrations, body composition, and bone mineral density (1-5). In this study, we focused on biochemical and anthropometric data and bone mineral density during short-term (1 year) and long-term (5 years) rhGH replacement in GHD adults. The increase in IGF-I levels was remarkably higher during short-term rhGH replacement in heterozygous patients bearing at least one allele of the d3GHR compared to patients bearing two wildtype alleles despite the fact that patients were treated with exactly the same dose of rhGH. This enhanced IGF-I generation upon stimulation with rhGH is in line with the higher IGF-I increment during an IGF-I generation test in children with idiopathic short stature bearing the d3GHR allele (19). In addition, in patients with acromegaly, a lower GH concentration was required in carriers of the d3GHR allele to produce a given increase in serum IGF-I concentrations (20). After long-term rhGH replacement, however, we did not observe such a pharmacogenetic effect of rhGH on IGF-1 levels. We speculate that downregulation of the GH-IGF-I system via negative feedback mechanisms might be involved to explain this discrepancy between short- and long term treatment with rhGH. Additionally, the fact that rhGH doses were individualized to achieve normal IGF-I levels could explain the lack of correlation between the GHR polymorphism and specific long-term endpoints.

In addition to these pharmacogenetic differences in IGF-I increase during short-term rhGH replacement between the two different genotypes, lipid parameters were differentially influenced by short-term rhGH replacement. The decrease in total cholesterol and LDL cholesterol during short-term rhGH replacement, was significantly lower in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles. Moreover, the increase in HDL cholesterol during rhGH replacement, was significantly higher in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles.

GH and IGF-I both have effects on lipid metabolism. In addition to stimulating lipolysis and thereby increasing plasma free fatty acid availability, GH increases the number and activity of hepatic LDL receptors, which enables LDL catabolism (21). In accordance, GH treatment in

mice with genetic LDL receptor defects does not lower plasma LDL concentrations (22). GH also increases the activity of cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (23). These effects contribute to the decrease of total cholesterol and LDL cholesterol seen during short-term as well as long-term rhGH replacement. On the other hand, growth hormone enhances the expression of mRNA of sterol regulatory element-binding protein 1c (SREBP-1c), involved in hepatic lipogenesis (24). Furthermore, IGF-I suppresses scavenger receptor of class BI (SR-BI) (25). The SR-BI is expressed in liver and steroidogenic tissues and clears the HDL cholesterol from the circulation (25). These two latter effects of GH and IGF-I on lipogenesis and HDL expression in light of the enhanced signal transduction of the d3GHR variant and the increased IGF-I response in our patients, might thus contribute to the differential effects of the genotype on short-term rhGH replacement effects on lipid metabolism. In the long-term, these effects might be overruled by negative feedback signals due to changes in fat mass during rhGH replacement (2;26). Detailed studies on lipid metabolism in adult patients with the d3GHR genotype compared to patients with the wildtype genotype are warranted.

Some factors may have influenced our results. Only 6 patients were homozygous for the d3GHR variant, in accordance with the estimated prevalence in the general population (8;10). Therefore, we chose to evaluate those patients and patients heterozygous for the d3GHR variant together. Larger, probably multicenter studies are warranted to tease out the additional differences between patients heterozygous and homozygous for the d3GHR allele.

In conclusion, the d3GHR genotype is associated with differences in efficacy of short-term rhGH replacement with respect to IGF-I and lipid metabolism. These results suggest that the d3GHR genotype could contribute, at least for some parameters, to the inter-individual differences seen in efficacy of rhGH replacement in adults with GHD.

REFERENCE LIST

1. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
2. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
3. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 92:1442-1445
4. van der Klaauw AA, Romijn JA, Biermasz NR, Smit JW, van Doorn J, Dekkers OM, Roelfsema F, Pereira AM 2006 Sustained effects of recombinant GH replacement after 7 years of treatment in adults with GH deficiency. Eur J Endocrinol 155:701-708
5. Janssen YJ, Hamdy NA, Frolich M, Roelfsema F 1998 Skeletal effects of two years of treatment with low physiological doses of recombinant human growth hormone (GH) in patients with adult-onset GH deficiency. J Clin Endocrinol Metab 83:2143-2148
6. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ 2006 Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91:1076-1080
7. Dos SC, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
8. Dos SC, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
9. Pantel J, Machinis K, Sobrier ML, Duquesnoy P, Goossens M, Amselem S 2000 Species-specific alternative splice mimicry at the growth hormone receptor locus revealed by the lineage of retroelements during primate evolution. J Biol Chem 275:18664-18669
10. van der Klaauw AA, Pereira AM, van Thiel SW, Smit JW, Corssmit EP, Biermasz NR, Frolich M, Iranmanesh A, Veldhuis JD, Roelfsema F, Romijn JA 2006 GH deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the 24 h GH secretion. Eur J Endocrinol 154:851-858
11. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
12. Janssen YJ, Frolich M, Roelfsema F 1997 A low starting dose of genotropin in growth hormone-deficient adults. J Clin Endocrinol Metab 82:129-135
13. Dos SC, Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
14. Audi L, Esteban C, Carrascosa A, Espadero R, Perez-Arroyo A, Arjona R, Clemente M, Wollmann H, Fryklund L, Parodi LA 2006 Exon 3-deleted/full-length growth hormone receptor polymorphism genotype frequencies in Spanish short small-for-gestational-age (SGA) children and adolescents (n = 247) and in an adult control population (n = 289) show increased fl/fl in short SGA. J Clin Endocrinol Metab 91:5038-5043
15. Blum WF, Machinis K, Shavrikova EP, Keller A, Stobbe H, Pfaeffle RW, Amselem S 2006 The growth response to growth hormone (GH) treatment in children with isolated GH deficiency is independent of the presence of the exon 3-minus isoform of the GH receptor. J Clin Endocrinol Metab 91:4171-4174

16. Carrascosa A, Esteban C, Espadero R, Fernandez-Cancio M, Andaluz P, Clemente M, Audi L, Wollmann H, Fryklund L, Parodi L 2006 The d3/fl-growth hormone (GH) receptor polymorphism does not influence the effect of GH treatment (66 microg/kg per day) or the spontaneous growth in short non-GH-deficient small-for-gestational-age children: results from a two-year controlled prospective study in 170 Spanish patients. J Clin Endocrinol Metab 91:3281-3286
17. Binder G, Baur F, Schweizer R, Ranke MB 2006 The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children. J Clin Endocrinol Metab 91:659-664
18. Pilotta A, Mella P, Filisetti M, Felappi B, Prandi E, Parrinello G, Notarangelo LD, Buzi F 2006 Common polymorphisms of the growth hormone (GH) receptor do not correlate with the growth response to exogenous recombinant human GH in GH-deficient children. J Clin Endocrinol Metab 91:1178-1180
19. Toyoshima MT, Castroneves LA, Costalonga EF, Mendonca BB, Arnhold IJ, Jorge AA 2007 Exon 3-deleted genotype of growth hormone receptor (GHRd3) positively influences IGF-1 increase at generation test in children with idiopathic short stature. Clin Endocrinol (Oxf) 67:500-504
20. Schmid C, Krayenbuehl PA, Bernays RL, Zwimpfer C, Maly FE, Wiesli P 2007 Growth hormone (GH) receptor isoform in acromegaly: lower concentrations of GH but not insulin-like growth factor-1 in patients with a genomic deletion of exon 3 in the GH receptor gene. Clin Chem 53:1484-1488
21. Rudling M, Olivecrona H, Eggertsen G, Angelin B 1996 Regulation of rat hepatic low density lipoprotein receptors. In vivo stimulation by growth hormone is not mediated by insulin-like growth factor I. J Clin Invest 97:292-299
22. Lind S, Rudling M, Ericsson S, Olivecrona H, Eriksson M, Borgstrom B, Eggertsen G, Berglund L, Angelin B 2004 Growth hormone induces low-density lipoprotein clearance but not bile acid synthesis in humans. Arterioscler Thromb Vasc Biol 24:349-356
23. Rudling M, Parini P, Angelin B 1997 Growth hormone and bile acid synthesis. Key role for the activity of hepatic microsomal cholesterol 7alpha-hydroxylase in the rat. J Clin Invest 99:2239-2245
24. Ameen C, Linden D, Larsson BM, Mode A, Holmang A, Oscarsson J 2004 Effects of gender and GH secretory pattern on sterol regulatory element-binding protein-1c and its target genes in rat liver. Am J Physiol Endocrinol Metab 287:E1039-E1048
25. Cao WM, Murao K, Imachi H, Yu X, Dobashi H, Yoshida K, Muraoka T, Kotsuna N, Nagao S, Wong NC, Ishida T 2004 Insulin-like growth factor-i regulation of hepatic scavenger receptor class BI. Endocrinology 145:5540-5547
26. Svensson J, Stibrant SK, Johannsson G 2003 Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 88:2061-2069

Chapter 15

Disease specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas

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Clinical Endocrinology, 2008

ABSTRACT

Objective

Quality of life (QoL) is impaired in patients treated for pituitary adenomas. However, differences in age and gender distributions hamper a proper comparison of QoL. Therefore, we compared age- and gender-specific standard deviations scores (Z scores) of QoL parameters in patients treated for pituitary adenomas.

Patients and Methods

We determined Z scores for health-related questionnaires (HADS, MFI-20, NHP, SF-36) in patients during long-term follow-up (13 ± 8 yrs) after treatment for pituitary adenomas. Z scores were calculated by comparing the data of 403 patients (acromegaly, n=118), Cushing's disease (n=58), prolactinoma (n=128), non-functioning macroadenoma (n=99)) with a control population (n=440) for each subscales of the questionnaires and for total QoL score.

Results

All subscales of the questionnaires and the total QoL score were negatively affected in patients compared to controls. Comparing the Z scores, patients treated for acromegaly reported more impairment in physical ability and functioning and more bodily pain compared to patients treated for non-functioning macroadenoma and patients treated for prolactinoma. Patients with Cushing's disease reported impairment in physical functioning compared to patients treated for non-functioning macroadenoma. Linear regression analysis, with correction for age and gender, confirmed these findings. Additionally, Cushing's disease was associated with increased anxiety. Hypopituitarism negatively influenced multiple aspects of QoL.

Conclusion

QoL is impaired in patients during long-term follow-up after treatment of pituitary adenomas. Patients with pituitary adenomas should be informed on these persistent adverse effects of their disease on QoL to prevent inappropriate expectations with respect to the long term results of treatment.

INTRODUCTION

Quality of life (QoL) is impaired during long-term follow-up of patients treated for pituitary adenomas (1-7). Different factors are related to this decrease in self-reported health-related parameters: radiotherapy (2;8;9), pituitary surgery (4), and pituitary deficiencies (10). In addition, there may be disease-specific effects of the different pituitary adenomas on QoL. This is supported by the only study, that compared QoL in patients with different pituitary adenomas by the questionnaires of the Short Form-36 (SF-36) (11). In that study, patients with acromegaly had the greatest impairment in measures of physical function and patients with Cushing's disease had the most severe impairment in all measures of the SF-36 compared to patients with other pituitary adenomas (11).

There are major differences in age and gender distributions between the different pituitary adenomas. For instance, patients with Cushing's disease are predominantly female and are relatively young. In contrast, patients with a non-functioning pituitary macroadenoma have a more or less equal gender distribution and a higher mean age than patients with Cushing's disease. Because age and gender per se are major determinants of QoL (12-14), a proper comparison of QoL parameters between patients with different pituitary adenomas can only be performed after adjustment for these differences in age and gender distributions. This issue can be addressed by calculating age- and gender-specific standard deviation scores for each pituitary disease using a large group of healthy controls. Therefore, the aim of this study was to compare age- and gender-specific standard deviation scores of general health-related QoL questionnaires in patients during long-term follow-up after treatment for different pituitary adenomas to determine whether it is possible to identify disease-specific impairments of QoL. For this purpose, we assessed QoL in patients with acromegaly, Cushing's disease, prolactinoma, non-functioning pituitary macroadenoma, and in a large group of healthy controls.

PATIENTS AND METHODS

Patients

We included all consecutive patients visiting our out-patient clinic during long-term follow-up for acromegaly, Cushing's disease, prolactinoma and non-functioning macroadenoma. Primary study parameters were the results of the four health-related QoL questionnaires. Patients were asked to return questionnaires, which were sent to their home address in prepaid envelopes. After 6 weeks non-responders received a reminder letter, and, thereafter, they were contacted by telephone to encourage completion and return of the questionnaires.

Acromegaly

All patients previously treated for acromegaly who were now considered cured or biochemically well-controlled, based on recent biochemical evaluation were identified and sent QoL questionnaires (n=131) (8). The response rate was 90% (n=118). In patients without treatment with somatostatin analogs cure of acromegaly was defined by a normal suppression of GH levels (GH nadir <0.38 µg/l) during oral glucose loading and normal IGF-1 levels for age and gender. For conversion of GH concentration from µg/l to mU/l, multiply by 2.6. In patients with treatment of somatostatin analogs cure of acromegaly was defined by normal serum IGF-I levels for age and gender and mean serum growth hormone (GH) levels below 1.9 µg/l for all patients (obtained from 5 consecutive samples taken in the postabsorptive state with intervals of 30 min from 9.00 until 11.00 a.m.). None of the patients was treated by Pegvisomant, at the time of the current study.

Cushing's disease

All patients previously treated for Cushing's disease who were considered cured on recent biochemical evaluation were identified and sent QoL questionnaires (n=63) (9). The response rate was 92% (n=58). Cure of Cushing's disease was defined by normal 24h urinary cortisol excretion rates (<220 nmol/24h) in two consecutive samples and by normal overnight suppression of plasma cortisol levels (<99.4 nmol/l) after 1 mg dexamethasone.

Prolactinoma

All patients treated for prolactinoma were identified and sent QoL questionnaires (n=190). The response rate was 67% (n=128). Criteria for prolactinoma were serum prolactin levels above 50 µg/l (1 µg/l =36 mU/l) and evidence on MRI of a pituitary tumor without evidence of primary hypothyroidism or drugs that increase prolactin levels. To diagnose a macroprolactinoma, a tumor diameter on MRI of more than 1 cm and serum prolactin levels five times above reference values were required. Patients were treated with a combination of dopamine agonists, surgery or radiotherapy.

Non-functioning macroadenoma

All patients previously treated for non-functioning macroadenoma by transsphenoidal surgery were identified and sent questionnaires (n=128) (10). The response rate was 77% (n=99).

Controls

A control group was formed by healthy persons of comparable age and gender distribution from the direct social environment of the patients (8-10;15;15-17). The control group existed of 440 persons (138 men (31%), with a mean age of 51 years, range 17 to 89 years).

Paragangliomas

To compare the effects of pituitary adenomas on Qol parameters with another disease, we also included the assessment of a similar analysis in paraganlioma patients described in detail in a previous study (16). This group consisted of 82 patients (age 49 ± 12 yrs, 42 men) treated in our department because of paragangliomas.

The study protocol was approved by the medical ethics committee of the Leiden University Medical Center, and all subjects returning completed questionnaires gave written consent for participation in the study.

General follow-up of all patients

All patients were seen at least twice yearly by an endocrinologist, with appropriate evaluation and treatment of recurrent disease or of possible deficits of pituitary hormones. GH deficiency was defined as an IGF-I level below the reference range for age and gender and/or an insufficient rise in GH levels (absolute value <3 µg/l) after stimulation during an insulin tolerance test (glucose nadir <2.2 mmol/l). Prior studies have demonstrated that patients with multiple pituitary hormone deficiencies, including two or more pituitary hormone deficiencies other than GH-deficiency, had a likelihood of approximately 95% of harboring GH-deficiency (18-20). Based on these data, we classified patients, in whom GH-stimulation test data were not obtained, but who were deficient in 3 other pituitary axes, as GH-deficient. When secondary amenorrhea was present for more than 1 year, premenopausal women were defined as LH/FSH deficient. In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone test or during an insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with growth hormone, thyroxin, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided.

Quality of life questionnaires

HADS (Hospital Anxiety and Depression Scale)

The HADS consists of 14 items pertaining to anxiety and depression. Each item is measured on a 4-point scale. Scores for the anxiety and depression subscale range from 0-21 and for the total score from 0-42. A high score points to more severe anxiety and depression (21).

MFI-20 (Multidimensional Fatigue Inventory)

The MFI-20 contains 20 statements to assess fatigue (22). Five different dimensions of fatigue (four items each) are calculated from these statements: 1) general fatigue, 2) physical fatigue, 3)

reduced activity, 4) reduced motivation, and 5) mental fatigue. A higher score points to higher experienced fatigue.

NHP (Nottingham Health Profile)

The NHP is frequently used in patients with pituitary disease to assess general well-being and QoL. The survey consists of 38 yes/no questions, which are subdivided in 6 scales assessing impairments, i.e. pain (8 items), energy level (3 items), sleep (5 items), emotional reactions (9 items), social isolation (5 items), and disability/functioning, i.e. physical mobility (8 items). A higher score is associated with more impairment (23;24).

SF-36 (Short Form-36)

The SF-36 questionnaire comprises 36 items and records general well being during the previous 30 days. The items are formulated as statements or questions to assess eight health concepts: 1) physical functioning, 2) social functioning, 3) limitations in usual role activities because of physical health problems, 4) pain, 5) general mental health (psychological distress and well-being), 6) limitations in usual role activities because of emotional problems, 7) vitality (energy and fatigue), and 8) general health perceptions and change in health (25;26). Because the HADS and the MFI-20 are more specific questionnaires for mental health and fatigue, the vitality and general mental health items were left out in this evaluation. Higher scores are associated with better QoL.

Total QoL score

For an integral comparison of the QoL parameters addressed in the 4 questionnaires, we developed a total QoL score which is the sum of all different QoL questionnaire subscales. First, all subscales of the questionnaires were converted to a 100-point score, in which a higher score is a worse quality of life. The SF-36 subscale scores were inverted. The HADS total score was not included, since this score is obtained by simply adding the HADS anxiety and depression scores. Subsequently, all 20 subscale scores were added and divided by 20, generating a total QoL score (minimal value 0, maximal value 100). Therefore, a higher score indicates a greater impairment of QoL.

Statistics

Statistical analysis was performed using SPSS for Windows, version 12.0 (SPSS Inc. Chicago, Illinois, USA). Results are expressed as the mean ± SD or mean with 95% CI, unless specified otherwise. Normal distribution of data was verified by the Kolmogorov-Smirnov test.

Calculation of Z scores: Gender specific mean and standard deviation values could be calculated per decade of age, because all QoL data obtained in the healthy controls were approximately normally distributed. Using these values, age- and gender-specific Z scores could be calculated for each individual patient in the different groups of pituitary adenomas.

The Z score reveals how many units of the standard deviation a case is above or below the mean. The Z score is calculated by the following formula: $Z=(x-\mu)/\sigma$, where x=individual QoL value, μ=mean QoL value of controls of equal gender and age, and σ=standard deviation of QoL value of controls of equal gender and age. The Z score could be calculated for all subscales of the four different questionnaires (21 subscales) and the total QoL score. Since a higher score is a worse QoL at the HADS, MFI-20, NHP, and the total QoL score, a positive Z score denotes a decreased QoL compared to healthy controls. In contrast, in the SF-36 scales a higher score reflects a better QoL and, consequently, negative Z scores in the SF-36 denote a decreased QoL compared to healthy controls.

Hypothesis testing: First, absolute QoL scores were compared between all patients with pituitary adenomas and controls by independent samples T-tests. Subsequently, the Z scores of each Qol score were compared between the patients groups with the different pituitary adenomas by analysis of variance with post-hoc comparisons with Tukey's HSD correction for multiple comparisons. Finally, linear regression analysis was performed in a model using the absolute scores of questionnaire subscales and total QoL score as dependent variables and age, gender, patient group, radiotherapy, follow-up duration, and hypopituitarism as independent variables. A p-value of <0.05 was considered to be significant.

RESULTS

Clinical characteristics of the patients

Acromegaly

The mean age of the patients with acromegaly (61 men) was 59 ± 13 years (Table 1). Treatment for acromegaly consisted of transsphenoidal surgery in 92%, radiotherapy in 28% (postoperative treatment in 31 patients and primary treatment in 2 patients), somatostatin analog therapy in 22% of the patients (postoperative treatment in 17 patients and primary treatment in 7 patients). At the time of evaluation, 22% of the patients were using somatostatin analogs. The duration of cure of biochemical control of the disease at the time of evaluation was 12 ± 7 years.

Cushing's disease

The mean age of the patients with Cushing's disease (10 men) was 52 ± 15 years. Treatment for Cuhsing's disease consisted of transsphenoidal surgery in all patients, additional radiotherapy in 19%, and additional bilateral adrenalectomy in 5% of the patients. The duration of remission at the time of evaluation was 13 ± 7 years.

Table 15/1: Clinical characteristics of the 403 patients with pituitary adenomas.

		Acromegaly (n=118)	Cushing's disease (n=58)	Prolactinoma (n=128)	Non-functioning macroadenoma (n=99)
Age (years)		58.6 ± 12.9	51.7 ± 15.2	48.3 ± 12.7	61.9 ± 11.7
Follow-up duration (years)		12.0 ± 7.4	13.4 ± 6.7	15.1 ± 8.7	9.9 ± 6.6
Gender n (%)	Men	61 (52)	10 (17)	29 (23)	54 (55)
	Women	57 (48)	48 (83)	99 (77)	45 (45)
Surgery, n (%)		108 (92)	58 (100)	34 (27)	99 (100)
Radiotherapy, n (%)		33 (28)	11 (19)	13 (10)	37 (37)
ACTH deficiency, n (%)		30 (25)	28 (48)	15 (12)	61 (63)
TSH deficiency, n (%)		28 (24)	21 (36)	28 (22)	59 (62)
GH deficiency, n (%)		2 (2)	13 (22)	9 (7)	81 (83)
LH/FSH deficiency, n (%)	Men n (% of men)	16 (26)	1 (10)	10 (35)	43 (80)
	Premenopausal women n(% of premenopausal women)	4 (14)	7 (25)	3 (4)	8 (80)
ADH deficiency, n(%)		4 (4)	11 (19)	3 (2)	9 (9)

Data are presented as mean ± SD or as number (percentage).

Prolactinoma

The mean age of the patients with prolactinoma (29 men) was 48 ± 13 years. Sixty-nine percent of women and 24% of men had a microadenoma (total n=75). Treatment of the prolactinoma consisted of primary dopamine agonist drug therapy in 84%, additional surgery in 11% of patients, radiotherapy in 6% of patients, primary surgery in 12% or a combination of surgery and radiotherapy in 4% of patients. Fifty-eight patients (45%) used dopamine agonist drugs at the time of evaluation. Mean prolactin concentrations were 21.2 ± 39.8 µg/l in those patients compared to 40.9 ± 51.4 µg/l in those without dopamine agonist drugs. At the time of the assessment of quality of life parameters, mean prolactin concentrations were 37.8 ± 46.5 µg/l in patients with microprolactinoma and 19.1 ± 44.4 µg/l in patients with macroadenoma. The mean follow-up period after initial diagnosis was 15 ± 9 years.

Non-functioning macroadenoma

The mean age of the patients with non-functioning macroadenoma (54 men) was 62 ± 12 years. All patients were treated primarily by transsphenoidal surgery. Twenty-two patients had received prophylactic postoperative radiotherapy (22%). Tumor recurrence was treated by radiotherapy in 11 patients (11%) and combined surgery and radiotherapy in 4 (4%). The mean follow-up period after primary treatment was 10 ± 7 years.

Comparison of absolute QoL scores between patients with pituitary adenomas and healthy controls

We compared the results of the questionnaires from 403 patients with pituitary adenomas (154 men (38%), mean age of 55 years, range 22 to 89 years) with those obtained from 440 control subjects (138 men (31%), mean age of 51 years, range 17 to 89 years, Table 2). All subscales of the HADS, MFI-20, SF-36, and NHP were negatively affected in the patients compared to the controls. Total QoL score was significantly higher compared to the healthy controls, indicative for an impaired QoL in patients with pituitary adenomas.

Table 15/2: Absolute QoL scores of patients with pituitary adenomas and healthy controls.

Questionnaire		Patients (n=403)	Healthy controls (n=440)	P-value
Total QoL score				
		31.1 ± 18.0	20.2 ± 11.7	<0.001
SF-36				
	Physical functioning	75.2 ± 26.1	88.2 ± 16.6	<0.001
	Social functioning	76.4 ± 26.0	88.3 ± 18.8	<0.001
	Role limitations due to physical problems	62.1 ± 41.7	84.3 ± 31.3	<0.001
	Role limitations due to emotional problems	70.0 ± 40.7	86.5 ± 29.5	<0.001
	Bodily pain	76.7± 24.0	85.7 ± 18.6	<0.001
	General health perception	57.9 ± 24.1	71.6 ± 18.7	<0.001
	Change in health	49.6 ± 22.0	53.6 ± 17.9	0.004
NHP				
	Energy	29.2 ± 38.4	6.2 ± 18.7	<0.001
	Pain	12.6 ± 23.7	4.9 ± 14.6	<0.001
	Emotional reaction	15.5± 23.9	5.5 ± 13.7	<0.001
	Sleep	17.4 ± 26.9	8.9 ± 19.2	<0.001
	Physical ability	13.5 ± 22.2	4.0 ± 10.3	<0.001
	Social isolation	10.2 ± 21.6	2.4 ± 8.5	<0.001
MFI-20				
	General fatigue	11.8 ± 5.3	8.5 ± 4.0	<0.001
	Physical fatigue	11.1 ± 5.0	7.6 ± 3.7	<0.001
	Reduced activity	10.1 ± 4.9	7.2 ± 3.4	<0.001
	Reduced motivation	9.5 ± 4.7	7.3 ± 3.4	<0.001
	Mental fatigue	10.0 ± 5.1	7.8 ± 3.9	<0.001
HADS				
	Anxiety	5.7 ± 4.2	4.0 ± 3.2	<0.001
	Depression	4.8 ± 4.4	2.8 ± 2.9	<0.001
	Total	10.5 ± 7.8	6.8 ± 5.3	<0.001

Data are expressed as mean ± SD and compared by independent samples T-test.

Comparison of Z scores between patient groups with different pituitary adenomas

Perceived quality of life is significantly different between the patient groups (p=0.003) assessed by the total QoL Z score and is especially decreased in patients treated for acromegaly compared to patients treated for non-functioning macroadenoma (p=0.006) and to patients treated for prolactinoma (p=0.011, Table 3).

There were no disease specific differences in the Z scores for the subscales of the HADS and MFI-20. The Z scores for energy, pain, emotional reaction, sleep, and social isolation according to the NHP did not differ between the patient groups. The Z scores for physical ability, however, did differ significantly between the patient groups (p=0.002). Patients previously treated for acromegaly had a larger impairment in physical ability compared to patients treated for non-functioning macroadenoma (p=0.004) and to patients treated for prolactinoma (p=0.008).

According to the SF-36, the Z scores for social functioning, role limitations due to physical problems or emotional problems, general health perception, and change in health did not differ between the different patients groups. However, patients with acromegaly had increased bodily pain compared to patients treated for non-functioning macroadenoma (p=0.010) and impairment in physical functioning compared to patients treated for non-functioning macroadenoma (p=0.002) and prolactinoma (p=0.037). Patients with Cushing's disease also experienced impairment in physical functioning compared to patients treated for non-functioning macroadenoma (p=0.043).

Comparison of Z scores between patients with pituitary adenomas and patients with paraganglioma

We performed an additional analysis to compare QoL in patients with pituitary adenomas to QoL in patients with another chronic disease: paraganglioma (Table 4). The comparison between these patients and controls has been published previously (16). Total QoL Z score was not different between the two groups. Patients with pituitary adenomas had experienced more impairment in role functioning due to emotional and physical problems (SF-36), more pain and impairment in physical ability (NHP) and more general and physical fatigue (MFI-20) compared to patients with paraganglioma.

Influence of disease specific characteristics on absolute QoL scores within subgroups

Acromegaly

We did not find any significant differences in QoL SD scores between the patients treated with somatostatin analogs and patients cured after surgery and/or radiotherapy (data not shown).

Prolactinoma

QoL parameters did not differ between patients with a macro- and a microadenoma. QoL parameters did not differ between patients using dopamine agonists and those who did not (data not shown).

Table 15/3: Z-scores (mean, 95% CI) of QoL in patients treated for acromegaly, Cushing's disease, prolactinoma, and non-functioning macroadenoma.

Questionnaire		Acromegaly (n=118)	Cushing's disease (n=58)	Prolactinoma (n=128)	Non-functioning macroadenoma (n=99)	ANOVA P-value
Total QoL score						
		1.4 (1.0, 1.7)	1.1 (0.6, 1.5)	0.7 (0.4, 1.0)	0.5 (0.1, 0.9)	0.003
SF-36						
	Physical functioning	-1.4 (-1.8, -0.6)	-1.3 (-1.9, -0.6)	-0.7 (-1.0, -0.3)	-0.3 (-0.7, -0.02)	0.001
	Social functioning	-0.6 (-0.8, -0.3)	-0.8 (-1.2, -0.3)	-0.8 (-1.1, -0.5)	-0.6 (-0.9, -0.3)	0.677
	Role limitations due to physical problems	-1.0 (-1.3, -0.7)	-0.7 (-1.1, -0.2)	-0.8 (-1.0, -0.5)	-0.7 (-1.1, -0.4)	0.333
	Role limitations due to emotional problems	-0.9 (-1.3, -0.7)	-0.6 (-1.0, -0.2)	-0.5 (-0.8, -0.2)	-0.8 (-1.2, -0.4)	0.454
	Bodily pain	-0.8 (-1.0, -0.5)	-0.6 (-1.0, -0.2)	-0.4 (-0.7, -0.2)	-0.2 (-0.4, 0.04)	0.015
	General health perception	-0.8 (-1.1, -0.6)	-0.9 (-1.2, -0.5)	-0.6 (-0.9, -0.3)	-0.6 (-1.0, -0.3)	0.536
	Change in health	-0.2 (-0.4, 0.04)	-0.1 (-0.4, 0.3)	-0.3 (-0.5, -0.1)	-0.04 (-0.3, 0.2)	0.392
NHP						
	Energy	1.3 (0.9, 1.7)	1.3 (0.7, 1.8)	1.2 (0.4, 1.4)	1.2 (0.6, 1.7)	0.982
	Pain	1.4 (0.6, 2.1)	1.2 (-0.005, 2.5)	0.4 (-0.04, 0.8)	1.0 (-0.2, 2.1)	0.255
	Emotional reaction	1.5 (0.3, 2.7)	0.9 (0.4, 1.5)	0.6 (0.2, 1.0)	1.5 (0.4, 2.5)	0.398
	Sleep	0.8 (0.5, 1.2)	0.4 (0.03, 0.9)	0.4 (0.1, 0.7)	0.8 (0.3, 1.3)	0.249
	Physical ability	1.6 (1.0, 2.1)	1.1 (0.5, 1.8)	0.6 (0.2, 0.9)	0.4 (0.04, 0.8)	0.002
	Social isolation	1.1 (0.4, 1.7)	1.1 (0.2, 1.9)	0.9 (0.4, 1.4)	1.4 (0.5, 2.3)	0.677

Table 15/3 continued

Questionnaire		Acromegaly (n=118)	Cushing's disease (n=58)	Prolactinoma (n=128)	Non-functioning macroadenoma (n=99)	ANOVA P-value
MFI-20						
	General fatigue	1.1 (0.9, 1.4)	0.9 (0.5, 1.2)	0.7 (0.4, 1.0)	0.8 (0.5, 1.2)	0.279
	Physical fatigue	1.1 (0.8, 1.4)	1.0 (0.7, 1.4)	0.9 (0.6, 1.2)	0.7 (0.4, 1.0)	0.290
	Reduced activity	0.9 (0.7, 1.2)	0.8 (0.4, 1.2)	0.8 (0.5, 1.0)	0.8 (0.5, 1.2)	0.830
	Reduced motivation	0.7 (0.5, 1.0)	0.7 (0.3, 1.1)	0.5 (0.3, 0.8)	0.7 (0.3, 1.0)	0.717
	Mental fatigue	0.6 (0.4, 0.9)	0.9 (0.5, 1.3)	0.5 (0.2, 0.7)	0.6 (0.3, 0.9)	0.383
HADS						
	Anxiety	0.7 (0.4, 0.9)	0.7 (0.3, 1.1)	0.5 (0.2, 0.7)	0.5 (0.2, 0.8)	0.626
	Depression	0.8 (0.5, 1.1)	0.7 (0.3, 1.1)	0.7 (0.4, 1.0)	0.6 (0.2, 1.0)	0.745
	Total	0.9 (0.5, 1.2)	0.8 (0.4, 1.3)	0.6 (0.3, 0.9)	0.6 (0.2, 0.9)	0.692

Data of the 4 different groups were compared by analysis of variance. The overall P-value of this comparison between groups is provided.

Table 15/4: Z-scores (mean, 95% CI) of QoL in patients treated for pituitary adenomas compared to patients with paraganglioma.

Questionnaire		Pituitary adenomas (n=403)	Paraganglioma (n=82)	P-value
Total QoL score				
		1.0 ± 1.7	0.6 ± 1.6	0.150
SF-36				
	Physical functioning	-0.9 ± 2.0	-0.5 ± 1.7	0.079
	Social functioning	-0.7 ± 1.5	-0.5 ± 1.5	0.454
	Role limitations due to physical problems	-0.8 ± 1.5	-0.5 ± 1.3	0.032
	Role limitations due to emotional problems	-0.7 ± 1.8	-0.2 ± 1.2	0.003
	Bodily pain	-0.5 ± 1.3	-0.3 ± 1.3	0.179
	General health perception	-0.7 ± 1.5	-0.5 ± 1.4	0.187
	Change in health	-0.2 ± 1.3	-0.1 ± 1.2	0.620
NHP				
	Energy	1.2 ± 2.3	0.9 ± 2.9	0.249
	Pain	0.9 ± 3.8	0.1 ± 1.2	0.001
	Emotional reaction	1.1 ± 4.6	1.0 ± 3.3	0.779
	Sleep	0.6 ± 2.0	0.6 ± 2.8	0.918
	Physical ability	0.9 ± 2.4	0.2 ± 1.2	<0.001
	Social isolation	1.1 ± 3.4	0.8 ± 2.6	0.477
MFI-20				
	General fatigue	0.9 ± 1.4	0.5 ± 1.3	0.011
	Physical fatigue	1.0 ± 1.5	0.5 ± 1.3	0.008
	Reduced activity	0.8 ± 1.5	0.6 ± 1.2	0.116
	Reduced motivation	0.6 ± 1.5	0.5 ± 1.1	0.198
	Mental fatigue	0.6 ± 1.4	0.6 ± 1.3	0.724
HADS				
	Anxiety	0.6 ± 1.4	0.4 ± 1.3	0.360
	Depression	0.7 ± 1.8	0.4 ± 1.5	0.135
	Total	0.7 ± 1.7	0.5 ± 1.5	0.190

SF-36: higher scores are associated with a better quality of life. NHP: higher score is associated with a worse quality of life. MFI-20: higher scores indicate greater experienced fatigue. HADS: higher scores indicate more severe anxiety or depression.

Parameters associated with decreased absolute QoL scores

Linear regression analysis was performed in a model using the absolute scores of questionnaire subscales and total QoL score as dependent variables and age, gender, patient group, radiotherapy, follow-up duration, and hypopituitarism as independent variables (Table 5).

Age and gender

Age was an independent negative predictor of physical functioning of the SF-36, sleep and physical ability of the NHP, and reduction in activity and motivation of the MFI-20 in patients treated for pituitary adenomas. Male gender was associated with a better QoL with respect to total QoL score, 3 out of the 7 SF-36 subscales (physical and social functioning, and role limitations due to physical problems), the pain and physical ability subscales of the NHP, the general and physical fatigue subscales and reduction in motivation subscale of the MFI-20, and the anxiety subscale of the HADS.

Patient group

Acromegaly was associated with worse scores for physical functioning, bodily pain and general health perception of the SF-36, as well as for pain and physical ability of the NHP, confirming the results of the comparison between the different patient groups using the Z-scores. Cushing's disease was also associated with increased impairment in physical functioning of the SF-36 and increased bodily pain of the NHP. In addition to the results of the comparison between the different patient groups using the Z-scores, Cushing's disease was associated with increased anxiety scores when linear regression analysis was applied. Non-functioning macroadenoma was associated with better scores for social functioning of the SF-36 and depression scores of the HADS. The presence of a prolactinoma did not influence the QoL subscale scores or total QoL score.

Hypopituitarism

The presence of hypopituitarism negatively influenced total QoL score, general health perception of the SF-36, energy and physical ability of the NHP, physical fatigue and reduced activity of the MFI-20, and depression of the HADS.

Radiotherapy

Previously applied radiotherapy did not influence any of the QoL subscale scores or the total QoL score.

Duration of follow-up duration

Duration of follow-up negatively influenced the change in health subscale of the SF-36 and positively influenced the reduction in activity and motivation and mental fatigue subscales of the MFI-20.

Table 15/5: Linear regression analysis of absolute QoL scores.

Questionnaire		Age	Gender (0=Female, 1=Male)	Acro-megaly	Cushing's disease	Prolac-tinoma	Non-functioning macro-adenoma	Hypo-pituitarism (0=No, 1=Yes)	Radio-therapy (0=No, 1=Yes)	Follow-up duration
Total QoL score										
			-5.5 (0.015)					4.9 (0.047)		
SF-36										
	Physical functioning	-0.6 (<0.001)	6.7 (0.019)	-9.0 (0.012)	-11.5 (0.003)					
	Social functioning		6.4 (0.049)				10.4 (0.046)			
	Role limitations due to physical problems		12.2 (0.020)							
	Role limitations due to emotional problems									
	Bodily pain			-9.0 (0.017)						
	General health perception			-7.7 (0.042)				-9.8 (0.003)		
	Change in health									-0.380 (0.044)

Questionnaire		Age	Gender (0=Female, 1=Male)	Acro-megaly	Cushing's disease	Prolac-tinoma	Non-functioning macro-adenoma	Hypo-pituitarism (0=No, 1=Yes)	Radio-therapy (0=No, 1=Yes)	Follow-up duration
NHP										
	Energy							9.9 (0.052)		
	Pain		-7.3 (0.009)	10.9 (0.002)	7.8 (0.037)					
	Emotional reaction									
	Sleep	0.4 (0.003)								
	Physical ability	0.5 (<0.001)	-4.8 (0.054)	7.3 (0.019)				5.3 (0.048)		
	Social isolation									
MFI-20										
	General fatigue		-1.8 (0.006)							
	Physical fatigue		-1.3 (0.045)					1.4 (0.048)		
	Reduced activity	0.05 (0.037)						1.4 (0.037)		-0.1 (0.028)
	Reduced motivation	0.044 (0.047)	-1.4 (0.019)							-0.1 (0.026)
	Mental fatigue									-0.1 (0.019)
HADS										
	Anxiety		-1.5 (0.004)		1.4 (0.051)					
	Depression						-2.1 (0.016)	1.2 (0.038)		
	Total									

Unstandardized beta coefficients with P-value in parentheses for independent variables (age, gender, diagnosis, hypopituitarism, radiotherapy, and follow-up duration) and QoL subscales and total QoL as dependent variables. SF-36: higher scores are associated with a better quality of life. NHP: higher score is associated with a worse quality of life. MFI-20: higher scores indicate greater experienced fatigue. HADS: higher scores indicate more severe anxiety or depression.

DISCUSSION

In this study, in a very large cohort of patients during long-term follow-up after treatment for different pituitary adenomas, we confirmed that patients with pituitary adenomas suffer from considerably impaired QoL compared to healthy subjects (1-7). The large number of included patients, representing groups with different pituitary tumors, and the specific statistical approach enabled to analyze both general effects of pituitary tumors on QoL as well as the disease-specific effects of individual pituitary adenomas on QoL. We found that patients with acromegaly had the largest impairment in QoL, compared with the other patients with other pituitary adenomas. These differences were mostly due to impairment in physical performance scales and the increase in bodily pain experienced by patients with acromegaly. Patients with Cushing's disease also had impairment in physical functioning compared to patients treated for non-functioning macroadenoma. These data indicate that QoL is impaired during long-term follow-up after treatment of pituitary adenomas in general. Moreover, there are disease specific impairments in physical functioning (acromegaly and Cushing's disease) and bodily pain (acromegaly). Additionally, linear regression analysis with adjustment for age and gender confirmed these data and extended the disease specific impairments to increased anxiety in patients with Cushing's disease.

Although many reports of QoL in patients with pituitary adenomas have been published, a considerable methodological problem in the comparison of different pituitary adenomas is formed by the intrinsic differences in age and gender between groups of patients with different pituitary adenomas. Age and gender are major determinants of QoL (12-14). Indeed, the linear regression analysis confirmed the major influences of age and gender on QoL in these specific patients with pituitary adenomas. Calculating age- and gender-specific standard deviation scores for each pituitary disease using a large group of healthy controls enabled us to do a direct comparison. Therefore, our conclusions are not biased by intrinsic differences in age and gender distributions between different pituitary adenomas.

The four health-related questionnaires used in this study, were not disease-specific, i.e. they were not developed to assess QoL in acromegaly, Cushing's disease, prolactinoma, or non-functioning macroadenoma specifically. This enables us to compare general aspects of QoL between different groups of pituitary adenomas. Nonetheless, there were disease-specific differences in physical functioning subscales. Additionally, anxiety was increased in patients with Cushing's disease.

The impairment of QoL in acromegaly with respect to physical performance scales and to bodily pain is in line with data in a large heterogeneous cohort of 231 patients with active and inactive acromegaly (2) and with a study in another cohort of 39 patients with acromegaly (11). This decreased QoL in patients long-term cured from acromegaly was strongly associated with persisting joint-related co-morbidity (27). Osteoarticular manifestations are present in the great majority of patients at presentation and were also found to be increased compared to

the general healthy population in patients with long-term successful biochemical control of acromegaly (27).

In Cushing's disease, both impaired physical functioning and anxiety were increased. This is in line with previous reports on QoL in patients with Cushing's syndrome (28) and QoL after bilateral adrenalectomy for Cushing's disease (29;30). Moreover, in comparison with other pituitary adenomas, patients with Cushing's disease were the most severely affected in all measures of QoL of the SF-36 (11). In addition, Cushing's disease was associated with increased anxiety. Supraphysiological levels of cortisol can induce psychiatric, psychological, emotional, and cognitive disturbances, which can persist even after cure of Cushing's syndrome (31-33). Although data on putative effects of hypercortisolism on brain structures are scarce, Cushing's disease is associated with reduced hippocampal volume (34;35). This cerebral atrophy is partially reversible on MRI after long-term correction of hypercortisolism. However, it is not known, whether the neural changes are fully reversible and/ or correlated with neuropsychological improvement.

Our data indicate that in patients with acromegaly and Cushing's disease QoL is the most severely impaired during long-term follow-up of successful biochemical disease control. However, patients with prolactinoma and patients with non-functioning macroadenoma also experienced impairments in health-related QoL in almost all subscales. The overall impairment in all patient groups points towards a strong effect of the pituitary diseases in general on health and well being, of both the physical and the psychosocial aspects. Indeed, even in comparison to patients with another unrelated chronic disease, i.e. paraganglioma, which also requires frequent hospital visits and intensive monitoring, several aspects of QoL are impaired in patients with pituitary adenomas.

Various aspects of pituitary adenomas have been linked to an impaired QoL, including radiotherapy (2;8;9;36), transcranial pituitary surgery (4), and pituitary deficiencies (10). Detailed analysis of factors influencing QoL in the total cohort revealed that male gender was associated with a better QoL compared to women. Hypopituitarism was associated with impairment in QoL in multiple subscales of the different questionnaires. In our patients, we aimed at optimal hormonal substitution of pituitary deficiencies. However, optimal hormonal substitution therapy does not reproduce the normal plasma hormone profiles of healthy individuals. Consequently, titration of endocrine replacement therapy is possible only within certain physiological limits (37). These intrinsic imperfections in endocrine replacement therapy may result in subtle physiological derangements, which could explain the negative influence of hypopituitarism on QoL in this study.

The strategy for obtaining controls was to ask each patient to provide a control person of comparable age and sex from the same socio-economic area. The Leiden University Medical Center is a tertiary referral center for patients with pituitary tumors in the Netherlands, which is a very small country. Therefore, all controls were derived from the same area. The response rate of the control group was 53% for acromegaly, 67% for the non-functioning macroadenoma, 57%

for Cushing's disease and 64% for prolactinoma. In addition, the control group was extended by controls derived from other studies in our centre that applied a similar strategy (15-17). Controls might be subject to a selection bias, because patients might have chosen controls with a supposed good health status or controls who had better health may have responded more eagerly to participation (38). However, in previous studies we also compared the outcomes of the same quality of life assessments in our patients to those published for the general Dutch population, which did not affect our conclusions obtained by the use of our own controls (8-10). Therefore, it is very unlikely that the large discrepancies between the patients and the controls in the present study are merely caused by selection bias.

In conclusion, QoL is impaired in patients with pituitary adenomas during long-term follow-up after treatment. In patients previously treated for acromegaly or for Cushing's disease, physical functioning is permanently impaired to a greater extent than in patients with other pituitary adenomas. Additionally, anxiety is increased in patients previously treated for Cushing's disease. This study thus provides both evidence for general effects of pituitary tumors on QoL, independent of the underlying disease, and disease-specific impairments in QoL. It is essential for doctors to recognize these irreversible effects of pituitary adenomas on QoL despite cure/biochemical control, optimal treatment and/or replacement strategies. It is important to inform patients with pituitary adenomas on these persistent adverse effects of their disease on QoL to prevent inappropriate expectations with respect to the long term results of treatment.

REFERENCE LIST

1. Heald AH, Ghosh S, Bray S, Gibson C, Anderson SG, Buckler H, Fowler HL 2004 Long-term negative impact on quality of life in patients with successfully treated Cushing's disease. Clin Endocrinol (Oxf) 61:458-465
2. Kauppinen-Makelin R, Sane T, Sintonen H, Markkanen H, Valimaki MJ, Loyttyniemi E, Niskanen L, Reunanen A, Stenman UH 2006 Quality of life in treated patients with acromegaly. J Clin Endocrinol Metab 91:3891-3896
3. Lindsay JR, Nansel T, Baid S, Gumowski J, Nieman LK 2006 Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. J Clin Endocrinol Metab 91:447-453
4. Nielsen EH, Lindholm J, Laurberg P, Bjerre P, Christiansen JS, Hagen C, Juul S, Jorgensen J, Kruse A, Stochholm K 2007 Nonfunctioning pituitary adenoma: incidence, causes of death and quality of life in relation to pituitary function. Pituitary 10:67-73
5. Sonino N, Bonnini S, Fallo F, Boscaro M, Fava GA 2006 Personality characteristics and quality of life in patients treated for Cushing's syndrome. Clin Endocrinol (Oxf) 64:314-318
6. van Beek AP, van den Bergh AC, van den Berg LM, van den Berg G, Keers JC, Langendijk JA, Wolffenbuttel BH 2007 Radiotherapy is not associated with reduced quality of life and cognitive function in patients treated for nonfunctioning pituitary adenoma. Int J Radiat Oncol Biol Phys 68:986-991.
7. Webb SM, Badia X, Surinach NL 2006 Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study. Eur J Endocrinol 155:269-277
8. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F 2004 Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 89:5369-5376
9. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA 2005 Quality of Life in Patients After Long-term Biochemical Cure of Cushing's Disease. J Clin Endocrinol Metab 90:3279-3286
10. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA 2006 Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:3364-3369
11. Johnson MD, Woodburn CJ, Vance ML 2003 Quality of life in patients with a pituitary adenoma. Pituitary 6:81-87
12. Norris CM, Ghali WA, Galbraith PD, Graham MM, Jensen LA, Knudtson ML 2004 Women with coronary artery disease report worse health-related quality of life outcomes compared to men. Health Qual Life Outcomes 2:21
13. Agewall S, Berglund M, Henareh L 2004 Reduced quality of life after myocardial infarction in women compared with men. Clin Cardiol 27:271-274
14. Mainio A, Hakko H, Niemela A, Koivukangas J, Rasanen P 2006 Gender difference in relation to depression and quality of life among patients with a primary brain tumor. Eur Psychiatry 21:194-199
15. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM 2006 Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 154:483-489
16. Havekes B, van der Klaauw AA, Hoftijzer HC, Jansen JC, van der Mey AG, Vriends AH, Smit JW, Romijn JA, Corssmit EP 2008 Reduced quality of life in patients with head-and-neck paragangliomas. Eur J Endocrinol 158:247-253
17. Hoftijzer HC, Heemstra KA, Corssmit EP, van der Klaauw AA, Romijn JA, Smit JW 2007 Quality of Life in Cured Patients with Differentiated Thyroid Carcinoma. J Clin Endocrinol Metab 93:200-203.
18. Bates AS, Evans AJ, Jones P, Clayton RN 1995 Assessment of GH status in adults with GH deficiency using serum growth hormone, serum insulin-like growth factor-I and urinary growth hormone excretion. Clin Endocrinol (Oxf) 42:425-430

19. Hartman ML, Crowe BJ, Biller BM, Ho KK, Clemmons DR, Chipman JJ 2002 Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J Clin Endocrinol Metab 87:477-485
20. Toogood AA, Beardwell CG, Shalet SM 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf) 41:511-516
21. Zigmond AS, Snaith RP 1983 The hospital anxiety and depression scale. Acta Psychiatr Scand 67:361-370
22. Smets EM, Garssen B, Bonke B, De Haes JC 1995 The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 39:315-325
23. Hunt SM, McKenna SP, McEwen J, Backett EM, Williams J, Papp E 1980 A quantitative approach to perceived health status: a validation study. J Epidemiol Community Health 34:281-286
24. Hunt SM, McEwen J 1980 The development of a subjective health indicator. Sociol Health Illn 2:231-246
25. Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L 1992 Validating the SF-36 health survey questionnaire: new outcome measure for primary care. BMJ 305:160-164
26. Ware JE, Jr., Sherbourne CD 1992 The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 30:473-483
27. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F 2005 Morbidity after Long-term Remission for Acromegaly; Persisting Joint-Related Complaints Cause Reduced Quality of Life. J Clin Endocrinol Metab 90:2731-2739.
28. Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen L, Laurberg P, Schmidt K, Weeke J 2001 Incidence and late prognosis of cushing's syndrome: a population-based study. J Clin Endocrinol Metab 86:117-123
29. Hawn MT, Cook D, Deveney C, Sheppard BC 2002 Quality of life after laparoscopic bilateral adrenalectomy for Cushing's disease. Surgery 132:1064-1068
30. Nagesser SK, van Seters AP, Kievit J, Hermans J, Krans HM, van de Velde CJ 2000 Long-term results of total adrenalectomy for Cushing's disease. World J Surg 24:108-113
31. Kelly WF, Kelly MJ, Faragher B 1996 A prospective study of psychiatric and psychological aspects of Cushing's syndrome. Clin Endocrinol (Oxf) 45:715-720
32. Sonino N, Fava GA 2001 Psychiatric disorders associated with Cushing's syndrome. Epidemiology, pathophysiology and treatment. CNS Drugs 15:361-373
33. Herbert J, Goodyer IM, Grossman AB, Hastings MH, de Kloet ER, Lightman SL, Lupien SJ, Roozendaal B, Seckl JR 2006 Do corticosteroids damage the brain? J Neuroendocrinol 18:393-411
34. Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE 1999 Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. Biol Psychiatry 46:1595-1602
35. Bourdeau I, Bard C, Noel B, Leclerc I, Cordeau MP, Belair M, Lesage J, Lafontaine L, Lacroix A 2002 Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. J Clin Endocrinol Metab 87:1949-1954
36. van der Klaauw AA, Biermasz N, Hoftijzer HC, Pereira AM, Romijn JA 2008 Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly. Clin Endocrinol (Oxf)In press
37. Romijn JA, Smit JW, Lamberts SW 2003 Intrinsic imperfections of endocrine replacement therapy. Eur J Endocrinol 149:91-97
38. Wacholder S, Silverman DT, McLaughlin JK, Mandel JS 1992 Selection of controls in case-control studies. II. Types of controls. Am J Epidemiol 135:1029-1041

Chapter 16

Increased daytime somnolence despite normal sleep patterns in patients treated for non-functioning pituitary macroadenoma

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Journal of Clinical Endocrinology and Metabolism, 2008

ABSTRACT

Objective

In patients treated for non-functioning pituitary macroadenoma (NFMA) increased fatigue scores on quality of life (QoL) have been reported. Because this may be related to altered sleep patterns, we evaluated daytime sleepiness and sleep patterns in patients successfully treated for NFMA in our center.

Design

Case-control study.

Patients and Methods

We assessed sleepiness and sleep patterns in 76 adult patients (41 men, mean age 63 years, range 37-87 years) in remission of NFMA during long-term follow up (10 years, range 0.5 to 30 years) after surgical (n=76) and additional radiotherapeutical (n=28) treatment. We used two validated questionnaires for sleep parameters (Epworth sleepiness score and Münchener Chronotype Questionnaire) and four validated questionnaires for quality of life (HADS, MFI-20, NHP, SF-36). Patient outcomes were compared to 76 healthy controls.

Results

Sleep duration and timing of sleep were not affected compared to healthy controls. However, sleepiness score was increased in patients compared to controls (7.6 ± 4.6 vs. 4.8 ± 3.1, $p<0.001$), reflecting increased daytime sleepiness in patients. There were no correlations between any of the sleep pattern parameters (duration, onset, rise time or midsleep) and sleepiness scores. Sleepiness scores were significantly correlated to 15 of the 21 quality of life parameters, whereas sleep patterns were not. Sleep timing was influenced by previous radiotherapy, whereas sleep duration was negatively affected by panhypopituitarism.

Conclusion

Daytime sleepiness is increased despite normal sleep patterns in patients treated for non-functioning pituitary macroadenoma.

INTRODUCTION

Non-functioning pituitary macroadenomas (NFMA) are the most prevalent pituitary macroadenomas (1;2). The main symptoms are visual field defects and hypopituitarism, which are caused by mass effects of the tumor. Transsphenoidal surgery is considered the treatment of choice leading to improvement of visual function in the majority of patients (3). However, in a substantial proportion of the patients hypopituitarism persists after surgical treatment (4). In case of tumor recurrence, selected patients may be treated by postoperative radiotherapy or repeat surgery (4).

Previous studies have documented an impaired quality of life in patients treated for NFMA compared to age-adjusted reference values (5;6). In these patients increased general fatigue and physical fatigue were remarkable complaints (5). Sleep and sleep patterns can be impaired after surgical treatment for other pituitary/ hypothalamic tumors than NFMA resulting in increased daytime somnolence and longer sleep duration (7). In addition, cranial radiotherapy in brain tumors during childhood leads to increased sleep duration during adulthood and radiation dose seemed to be a determinant of sleep changes (8). Therefore, we hypothesized that the increased fatigue scores in patients previously treated for NFMA could be related to disturbances in the sleep patterns.

Because it is unknown whether sleep and sleep patterns are affected after treatment for NFMA, the aim of this study was to assess sleep patterns and sleepiness in patients with NFMA in relation to quality of life scores and clinical characteristics.

PATIENTS AND METHODS

Patients

The present study was a cross-sectional study of consecutive patients with NFMA in our center. The study consisted of two parts: 1) quality of life assessment; 2) assessment of sleep and sleep patterns. First, one-hundred-and-twenty-eight consecutive patients with NFMA, treated by transsphenoidal surgery in our center between 1985 and 2004, could be identified. Ninety-nine of these patients participated in the first part of the study, the quality of life study (5). These patients were also asked to participate in the second part of the study by completing two additional questionnaires which assessed daytime sleepiness and sleep patterns. The questionnaires were sent to their home in prepaid envelopes. After two months non-responders were contacted by telephone to encourage completion and return of the questionnaires. Each patient was also asked to provide a healthy control person of comparable age and sex, who did not use any medication, to serve as a control group with a comparable socio-economic

status derived from the same geographical area. The control group was extended with controls derived from other studies in our center who were approached similarly.

All patients had been treated by primary transsphenoidal surgery and were considered cured after surgery (sometimes in combination with postoperative radiotherapy). The mean follow-up period after initial surgery was 10 years (range 0.5 to 30 years). All patients were seen at least twice yearly by an endocrinologist, with adequate evaluation and treatment of possible deficiencies of pituitary hormones. Growth hormone (GH) deficiency was defined as an IGF-I level below the reference range for age and sex and/ or insufficient rise in GH levels (absolute value <3 µg/l) after stimulation during an insulin tolerance test. Prior studies have demonstrated that patients with multiple pituitary hormone deficiencies, including two or more pituitary hormone deficiencies other than GH-deficiency, had a likelihood of approximately 95% of harbouring GH-deficiency (9-11). Based on these data, we classified patients, in whom GH-stimulation test data were not obtained, but who were deficient in 3 other pituitary axes, as GH-deficient. When secondary amenorrhoea was present for more than 1 year premenopausal women were defined as LH/FSH deficient. Postmenopausal women were defined as LH/FSH deficient when gonadotrophin levels were below the normal postmenopausal range (LH<10 U/l and FSH<30 U/l). In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone test or insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with growth hormone, thyroxine, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. Postmenopausal women were not treated with estrogen replacement therapy. At evaluation, the free T4 scores were 16.9 ± 3.5 pmol/l in substituted patients (normal 10.0-24.0 pmol/l), indicating adequate thyroid hormone replacement. None of the patients were using selective serotonin reuptake inhibitors, tricyclic antidepressants, or MAO inhibitors.

The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol and all patients returning completed questionnaires gave written informed consent.

Study parameters

Primary study-parameters were the results of the two sleep questionnaires. The results were linked to age and gender of the patients, treatment characteristics (multiple surgical procedures, radiotherapy), visual field defects, the presence of pituitary deficiencies, and the quality of life scores.

Sleep questionnaires

Epworth sleepiness scale (ESS)

The ESS is a validated eight-item questionnaire. The subject is asked to rate his likelihood of falling asleep in a variety of commonly encountered situations (12). Scores range from 0 (the least sleepy) to 24 (the most sleepy). Scores equal to or above 10 are interpreted as increased daytime sleepiness (13). An additional set of questions that evaluated the prevalence of snoring, observed apnoea's, and nocturnal restless legs was added.

Münchener chronotype questionnaire (MCQ)

The MCQ is a validated questionnaire aimed at assessing chronotype and sleep patterns (14;15). Patients are explicitly asked to describe their sleep behaviour under normal circumstances (without partying etc.). The temporal structure of sleep is assessed separately for workdays and free days. Parameters on free days are regarded to reflect individual sleep patterns without social obligations and are therefore reported in this paper (14).

Sleep duration on free days (SD_F), sleep onset on free days (SO_F) and rise time (RT_F) are calculated from questions concerning sleep onset and awakening on days on which there are no work or social obligations. The midsleep on free days (MS_F: clock time halfway during sleep duration) is calculated from SO_F and RT_F (15).

Since most chronotypes tend to accumulate a sleep dept on work days, which is compensated for on free days, midsleep on workdays (MS_C) was corrected for the confounder sleep debt as follows: $MS_C=MS_F-(0.5*SD_F-(5*SD_{working\ days}+2*\ SD_F)/7)$ (15). Since only 32 of our patients and 27 of our controls had a daytime job, this correction was performed only for those subjects (15).

Quality of life questionnaires

The various quality of life questionnaires have been described extensively in the previous paper (5). In short, the Short Form (SF)-36 questionnaire comprises 36 items and records general well-being during the previous 30 days. Scores are expressed on a 0–100 scale, and higher scores are associated with a better quality of life. The Nottingham Health Profile (NHP) is frequently used in patients with pituitary disease to assess general well-being and consists of 38 yes/ no questions, which are subdivided in six subscales. Scores are calculated as a weight mean of the subscales and are expressed as a value between 0 and 100. A higher score is associated with a worse quality of life. The Multidemensional Fatigue Inventory (MFI)-20 comprises 20 statements to assess fatigue, which are measured on a five-point scale. Scores vary from 0–20, and higher scores indicate greater experienced fatigue. The Hospital Anxiety and Depression Scale (HADS) consists of 14 items pertaining to anxiety and depression, which are measured on a four-point scale. Scores for the anxiety and depression subscale range from 0–21, and values for the total score range from 0–42. Higher scores indicate more severe anxiety or depression. A total score of 13 or more was considered increased.

Statistics

SPSS for windows version 12.0 (SPSS Inc., Chicago, IL) was used for data analysis. Data are expressed as mean ± SD, unless otherwise mentioned. We used unpaired T-tests, chi-square tests, and linear regression analysis, when appropiate. Differences were considered statistically significant at $P<0.05$.

RESULTS

Patients and controls

Eighty-five of 99 (86%) patients returned the questionnaires on sleep characteristics. Nine of the patients preferred not to participate, 11 patients did not respond, 2 patients died since the previous study, and 1 patient moved without leaving a correct address. Thus, 76 completed questionnaires were received. The study-population (41 men) had a mean age of 63 years with a range of 37 to 87 years (Table 1). No significant differences in age, gender and tumor-characteristics were found between the study-population, and the patients who preferred not to participate or who did not return the questionnaires.

Table 16/1: Clinical characteristics of the NFMA patients and controls.

		Patients (n=76)	Controls (n=76)
Age (mean and range, yrs)		63 (37-87)	62 (31-81)
Gender		M 41 (54%) F 35 (46%)	M 40 (53%) F 36 (47%)
Radiotherapy		28 (37%)	
Visual field defects		16 (29%)	
Hardy classification	2	46 (61%)	
	3	17 (22%)	
	4	13 (17%)	
Suprasellar extension (Hardy classification)	A	3 (4%)	
	B	61 (85%)	
	C	8 (11%)	
GH deficiency		63 (84%)	
TSH deficiency		47 (64%)	
ACTH deficiency		49 (65%)	
LH/ FSH deficiency		62 (82%)	
ADH deficiency		7 (9%)	
Panhypopituitarism		38 (50%)	

Data are presented as mean with range or number with percentage in parentheses. M males; F Females; yrs years.

Table 16/2: Sleepiness scores and sleep patterns in patients treated for NFMA compared to controls.

		Patients (n=76)	Controls (n=76)	P-value
ESS	Mean score (mean ± SD)	7.6 ± 4.6	4.8 ± 3.1	<0.001
	> 10 (%)	26	10	0.001
MCTQ	Sleep duration on free days (duration h:min ± SD)	7:13 ± 1:09	7:18 ± 0:57	0.675
	Sleep onset on free days (clock time h:min ± SD)	23:44 ± 0:59	23:53 ± 1:13	0.419
	Midsleep on free days (clock time h:min ± SD)	3:22 ± 0:54	3:33 ± 1:05	0.310
	Corrected midsleep (clock time h:min ± SD, n=32 vs. n=27)	3:39 ± 35:05	3:45 ± 0:47	0.567

Data are presented as mean ± SD or as percentages and compared with independent samples T-test or Chi-square test, when appropriate.

The patients were compared to 76 controls (40 men) with mean age of 62 years (range of 31 to 81 years). Age and gender from the control group were not different from the studied NFMA patients (p=0.417 and p=0.871, respectively).

All 76 patients had been treated by transsphenoidal surgery. The mean follow-up period after initial surgical treatment was 10 years (range 0.5 to 30 yrs). Twelve patients (17%) were treated for tumor recurrence. Twenty-eight patients (37%) received radiotherapy during the course of their disease, of whom 19 patients received prophylactic radiotherapy and 9 patients to treat tumor recurrence. In 15 patients (26%) visual field defects were present at last follow-up.

At the time of evaluation, panhypopituitarism of the anterior pituitary gland was present in 50%. Sixty-three patients (84%) had GH deficiency of whom 32 (51%) received recombinant human growth hormone replacement. Thirty three male patients (81% of male patients) had testosterone substitution. Of the thirty-five women, 14 were premenopausal of whom 11 had LH/FSH deficiency and were substituted with estrogen substitution. Fourty-seven patients (64%) needed thyroid hormone substitution, whereas 49 patients (65%) needed glucocorticoid substitution.

Comparison with controls

Sleep duration on free days (SD_F) was comparable in patients and controls (7:13 ± 1:09 h vs. 7:18 ± 0:57 h, p=0.675). Sleep onset on free days, midsleep on free days, rising time on free days, and corrected midsleep (SO_F, MS_F, RT_F, and MS_C, respectively) were not different compared to controls as well (Table 2).

However, the Epworth Sleepiness Scale (ESS) score was increased in patients compared to controls (7.6 ± 4.6 vs. 4.8 ± 3.1, p<0.001) denoting increased daytime sleepiness in patients. Nineteen patients had ESS scores above 10 compared to 7 controls (26% vs. 10%, p=0.001).

Sixty-seven percent of patients reported snoring compared to 68% in controls (p=0.939), whereas 20% of patients reported observed apnoea's compared to 13% of controls (p=0.219). In addition, restless legs were reported in 20% in patients compared to 13% in controls (p=0.437).

In our patients, no correlations could be found with linear regression analysis between any of the sleep pattern parameters (duration, onset, rise time or midsleep) and sleepiness scores on the ESS.

Table 16/3: Linear regression analysis of Epworth Sleepiness Scale (ESS) scores and scores on quality of life questionnaires.

Questionnaire	ESS score
SF-36	
Physical functioning	-1.585 (<0.001)
Social functioning	-1.263 (0.025)
Role limitations due to physical problems	-2.722 (0.008)
Role limitations due to emotional problems	-1.691 (0.097)
Bodily pain	-1.799 (<0.001)
General health perception	-1.726 (0.008)
Change in health	-1.024 (0.078)
NHP	
Energy	2.249 (0.019)
Pain	1.120 (0.015)
Emotional reaction	0.779 (0.160)
Sleep	-0.189 (0.749)
Physical ability	0.615 (0.039)
Social isolation	0.204 (0.698)
MFI-20	
General fatigue	0.472 (0.001)
Physical fatigue	0.381 (0.002)
Reduced activity	0.358 (0.003)
Reduced motivation	0.248 (0.043)
Mental fatigue	0.260 (0.052)
HADS	
Anxiety	0.217 (0.024)
Depression	0.229 (0.040)
Total	0.446 (0.017)

Unstandardized beta coefficients with P-value in parentheses for sleepiness score assessed with the Epworth Sleepiness Scale and Quality of Life parameters. SF-36: higher scores are associated with a better quality of life. NHP: higher score is associated with a worse quality of life. MFI-20: higher scores indicate greater experienced fatigue. HADS: higher scores indicate more severe anxiety or depression.

Correlations with Quality of Life

There were no significant correlations between sleep pattern parameters and any of the quality of life subscales of the four different quality of life questionnaires, especially not for sleep duration and fatigue scores. Nonetheless, the ESS score was significantly correlated with 15 out of 21 quality of life subscales (Table 3).

Factors influencing sleep in patients with NFMA

Age

No significant correlations were found between age and ESS score or between age and sleep pattern parameters.

Gender

Midsleep on free days (MS_F) was significantly different in women compared to men (clock time 3:37 ± 0:35 h vs. clock time 3:09 ± 1:04 h, p=0.032), whereas sleep duration did not differ (7:18 ± 1:09 h vs. 7:09 ± 1:10 h, p=0.566). This difference in MS_F despite comparable sleep duration was associated with a later rise time in women (7:17 ± 0:53 h in women vs. 6:43 ± 1:18 h in men, p=0.039). MS_C and ESS scores did not differ between men and women.

Radiotherapy

Midsleep on free days (MS_F) was later in patients treated with radiotherapy compared to patients who were not (clock time 3:38 ± 0:35 h vs. clock time 3:12 ± 1:00 h, p=0.052, Figure 1), whereas sleep duration of free days (SD_F) was unaffected (7:16 ± 0:56 h vs. 7:11 ± 1:17 h ,

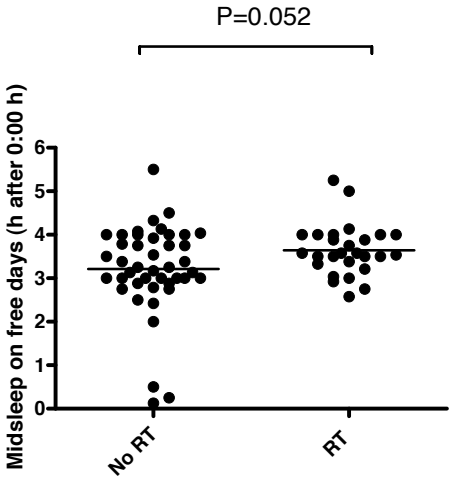

Figure 16/1: Midsleep on free days is later in patients treated by additional postoperative radiotherapy (RT, n=28) compared to patients treated by surgery alone (P=0.052).

p=0.752). Sleep onset (SO_F) and especially rise time (RT_F) tended to be later in patient who were treated with radiotherapy compared to patients who were not (0:00 ± 0:47 h vs. 23:35 ± 1:04 h, p=0.089, and 7:17 ± 0:43 h vs. 6:48 ± 1:19 h, p=0.051, respectively). ESS score and corrected midsleep (MS_C) did not differ between these two groups. No correlations were found between interval after radiotherapy and MS_F.

Visual field defects

ESS scores tended to be higher in patients with present visual field defects compared to the other patients (9.6 ± 5.4 vs. 6.9 ± 4.1, p=0.067), whereas sleep pattern parameters did not differ between these two groups.

Hypopituitarism

Thirty-eight patients (50%) had panhypopituitarism at the time of completion of the questionnaires. Sleep duration on free days (SD_F) was significantly shorter in those patients with panhypopituitarism (6:55 ± 1:22 h vs. 7:32 ± 0:48 h, p=0.028, Figure 2), due to combined non-significant shifts in later sleep onset (SO_F) and earlier rise time (RT_F). Midsleep (MS_F, MS_C) and ESS scores were unaffected by panhypopituitarism. ESS scores and sleep patterns did not differ between patients with or without GH deficiency, or between patients with GH deficiency with and without GH substitution. Sleep duration was shorter in TSH deficient patients (n=47, 64%) compared to those patients without secondary hypothyroidism (6:55 ± 1:19 h vs. 7:45 ± 0:45

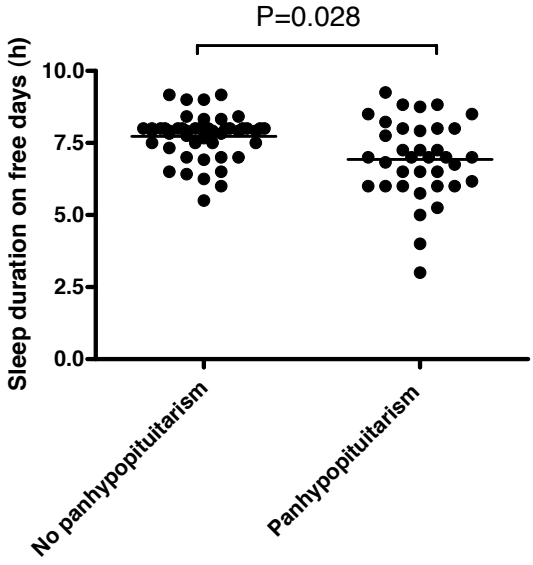

Figure 16/2: Sleep duration on free days is decreased in patients with NFMA with panhypopituitarism (n=38, 50%) compared to patients without panhypopituitarism (P=0.028).

h, p=0.006), whereas ESS scores, SO_F and MS_F were unaffected. ACTH or LH/FSH deficiency did not influence sleepiness scores or sleep patterns.

Stepwise linear regression analysis

Stepwise linear regression was performed in a model including age, gender, radiotherapy, presence of visual field defects, panhypopituitarism, and TSH deficiency as independent variables and ESS score, sleep duration on free days (SD_F), sleep onset on free days (SO_F) and midsleep on free days (MS_F) as dependent variables. Age, the presence of visual field defects, panhypopituitarism, and TSH deficiency did not influence any of the sleep pattern parameters or ESS scores. Midsleep on free days (MS_F) however was influenced by gender (Male=0, Female=1, β=-2179 sec (≈36 min), p=0.013) and radiotherapy (No=0, Yes=1, β=1731 sec (≈29 min), p=0.046). Sleep onset on free days (SO_F) was also influenced by gender (β=-2417 (≈40 min), p=0.021).

DISCUSSION

The data in this study indicate that patients successfully treated for NFMA experienced increased daytime sleepiness which was associated with a reduced quality of life. However, sleep patterns such as sleep onset or sleep duration did not differ from healthy controls and were not correlated to increased daytime sleepiness scores or reduced quality of life. Detailed analysis of the relationship between sleep patterns and clinical parameters in NFMA patients revealed gender and radiotherapy to influence sleep timing and panhypopituitarism to affect sleep duration.

Only a few studies have reported on quality of life in patients with NFMA (5;6) and to our knowledge no reports on sleep in NFMA patients have been published. We found increased daytime sleepiness in patients treated for NFMA in concordance with the reported increased fatigue in NFMA patients (5;6). Moreover, the daytime sleepiness scores in our patients treated for NFMA were comparable to scores found in patients with other pituitary tumors or cerebral diseases such as acromegaly (16), craniopharyngeoma (17), hypothalamic tumors (17), subarachnoid haemorrhage (18), or traumatic brain injury (19), indicative for the relationship between cerebral disease and increased daytime sleepiness. Severely increased daytime sleepiness (ESS scores above 10) were noted in almost one third of our patients with NFMA in line with findings in patients with craniopharyngeoma (20). Nonetheless, we did not find altered sleep patterns in our NFMA patients suggesting that the increased experienced daytime sleepiness and reported fatigue scores of the quality of life questionnaires are not due to major alterations in sleep duration or timing of sleep. This unaffected sleep duration, however, was in contrast to findings in patients after pituitary/ hypothalamic surgery or cranial radiotherapy for other tumors, in whom sleep duration was increased (7;8).

The increased daytime sleepiness and increased fatigue scores seen in our patients treated for NFMA could point towards possibly impaired sleep quality in patients with NFMA, despite

normal sleep patterns. Indeed, sleep quality measured with polysomnography is found to be altered in patients with Cushing's disease (21), acromegaly (22), prolactinoma (23), and patients with craniopharyngeoma (20). Nonetheless, it cannot be excluded that additional disturßbances, for example in melatonin secretion, lead to increased daytime sleepiness in NFMA patients. In fact, it has been suggested that reduced nocturnal melatonin secretion may lead to increased daytime sleepiness in childhood craniopharyngeoma patients (13;17). Thus, in addition to direct measurement of melatonin secretion, future studies to elucidate the increased daytime sleepiness should include polysomnography and additional (objective) tests of increased daytime sleepiness, considering the ample aspects of increased daytime sleepiness that can be studied besides the ESS.

Detailed analysis of the relationship between sleep patterns and clinical parameters in NFMA patients revealed some factors to influence sleep patterns. First, radiotherapy was found to influence sleep timing. Midsleep timing, which is found to be significantly correlated to dim light melatonin onset (a marker of circadian phase (24)), was later in patients treated with radiotherapy. The hypothalamus has been identified as the main sleep regulatory center (25). Within the hypothalamus, the suprachiasmatic nucleus (SCN) is thought to be the main circadian pacemaker and one of its circadian outputs is formed by regulating melatonin secretion of the pineal gland (25). Indeed, the hypothalamus is thought to be more vulnerable to radiation induced damage compared to the pituitary (26). Damage to the hypothalamus due to radiotherapy could thus be involved in the delayed timing of sleep seen in our NFMA patients, which makes direct measurement of circadian melatonin secretion interesting. Second, the decreased duration of sleep in patients with panhypopituitarism suggests that intact anterior pituitary function, especially diurnal variations, are important for normal sleep. Alternatively, the diurnal variations in pituitary hormone secretion are the consequence of the same mechanisms involved in regulation of sleep-wake patterns. Indeed, many interactions between nocturnal secretion of different hormones and the sleep electroencephalogram parameters have been described (27). Altered sleep patterns can induce changes in anterior pituitary hormone secretion (28). We found in NFMA patients that deficiencies in anterior pituitary hormones, especially secondary hypothyroidism, are associated with altered sleep patterns, which is in line with findings in patients with primary hypothyroidism (29). GH deficiency or ACTH deficiency did not specifically influence sleep patterns in our study patients in line with reports on sleep in GH deficiency (30;31) or Addison's disease (32).

No relation between age and sleepiness scores was found. This is in contrast with findings in the general healthy population (33;34). This discrepancy is likely due to the limited age range of the subjects included in our study due to the generally older age of patients with non-functioning macroadenomas.

Some factors may have influenced our results. First, sleepiness and sleep patterns were assessed using self-reported questionnaires. The MCQ assessed sleep during free days and working days, but only at one occasion. A comparison of the data on sleep habits from the

MCQ and data from a sleep log for 5 weeks by Roenneberg et al., however, indicated that sleep times of both questionnaires on both workdays and free days correlated highly ($p<0.0001$, (14)). Therefore, in addition to self-reported sleepiness and sleep patterns, the next step is to perform objective test of sleepiness and polysomnography to asses sleep quality in patients treated for NFMA. Second, in the present study NFMA patients were compared to healthy controls recruited by the patients. The advantage of using such controls is that they are from the same geographic area and socio-economic class as the patients (35). Although it is known that self-selected controls might be subject to selection bias, because patients might have chosen controls with a supposedly good health status (36), it is not likely that sleeping pattern plays any role in the choice for a specific control.

In conclusion, we found self-reported increased daytime sleepiness despite normal sleep patterns in patients treated for NFMA, which was associated with an impaired quality of life. Further detailed polysomnographic and circadian rhythm studies are needed to elucidate the pathophysiology of the reported increased sleepiness seen in our NFMA patients, which could produce further insight and treatment targets in the complex persisting morbidity in patients after treatment for NFMA.

REFERENCES

1. Feldkamp J, Santen R, Harms E, Aulich A, Modder U, Scherbaum WA 1999 Incidentally discovered pituitary lesions: high frequency of macroadenomas and hormone-secreting adenomas - results of a prospective study. Clin Endocrinol (Oxf) 51:109-113
2. McComb DJ, Ryan N, Horvath E, Kovacs K 1983 Subclinical adenomas of the human pituitary. New light on old problems. Arch Pathol Lab Med 107:488-491
3. Dekkers OM, de Keizer RJ, Roelfsema F, Vd Klaauw AA, Honkoop PJ, van Dulken H, Smit JW, Romijn JA, Pereira AM 2007 Progressive improvement of impaired visual acuity during the first year after transsphenoidal surgery for non-functioning pituitary macroadenoma. Pituitary 10:61-65
4. Dekkers OM, Pereira AM, Roelfsema F, Voormolen JH, Neelis KJ, Schroijen MA, Smit JW, Romijn JA 2006 Observation alone after transsphenoidal surgery for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:1796-1801
5. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA 2006 Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:3364-3369
6. Johnson MD, Woodburn CJ, Vance ML 2003 Quality of life in patients with a pituitary adenoma. Pituitary 6:81-87
7. Snow A, Gozal E, Malhotra A, Tiosano D, Perlman R, Vega C, Shahar E, Gozal D, Hochberg Z, Pillar G 2002 Severe hypersomnolence after pituitary/hypothalamic surgery in adolescents: clinical characteristics and potential mechanisms. Pediatrics 110:e74
8. Van Someren EJ, Swart-Heikens J, Endert E, Bisschop PH, Swaab DF, Bakker PJ, Romijn JA, Fliers E 2004 Long-term effects of cranial irradiation for childhood malignancy on sleep in adulthood. Eur J Endocrinol 150:503-510
9. Bates AS, Evans AJ, Jones P, Clayton RN 1995 Assessment of GH status in adults with GH deficiency using serum growth hormone, serum insulin-like growth factor-I and urinary growth hormone excretion. Clin Endocrinol (Oxf) 42:425-430
10. Hartman ML, Crowe BJ, Biller BM, Ho KK, Clemmons DR, Chipman JJ 2002 Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J Clin Endocrinol Metab 87:477-485
11. Toogood AA, Beardwell CG, Shalet SM 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf) 41:511-516
12. Johns MW 1991 A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545
13. Muller HL, Handwerker G, Wollny B, Faldum A, Sorensen N 2002 Melatonin secretion and increased daytime sleepiness in childhood craniopharyngioma patients. J Clin Endocrinol Metab 87:3993-3996
14. Roenneberg T, Wirz-Justice A, Merrow M 2003 Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms 18:80-90
15. Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A, Merrow M 2004 A marker for the end of adolescence. Curr Biol 14:R1038-R1039
16. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329
17. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589
18. Schuiling WJ, Rinkel GJ, Walchenbach R, de Weerd AW 2005 Disorders of sleep and wake in patients after subarachnoid hemorrhage. Stroke 36:578-582
19. Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR 2006 Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehabil 87:278-285

20. Muller HL, Muller-Stover S, Gebhardt U, Kolb R, Sorensen N, Handwerker G 2006 Secondary narcolepsy may be a causative factor of increased daytime sleepiness in obese childhood craniopharyngioma patients. J Pediatr Endocrinol Metab 19 Suppl 1:423-429
21. Shipley JE, Schteingart DE, Tandon R, Starkman MN 1992 Sleep architecture and sleep apnea in patients with Cushing's disease. Sleep 15:514-518
22. Astrom C, Christensen L, Gjerris F, Trojaborg W 1991 Sleep in acromegaly before and after treatment with adenomectomy. Neuroendocrinology 53:328-331
23. Frieboes RM, Murck H, Stalla GK, Antonijevic IA, Steiger A 1998 Enhanced slow wave sleep in patients with prolactinoma. J Clin Endocrinol Metab 83:2706-2710
24. Burgess HJ, Savic N, Sletten T, Roach G, Gilbert SS, Dawson D 2003 The relationship between the dim light melatonin onset and sleep on a regular schedule in young healthy adults. Behav Sleep Med 1:102-114
25. Mignot E, Taheri S, Nishino S 2002 Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5 Suppl:1071-1075
26. Toogood AA 2004 Endocrine consequences of brain irradiation. Growth Horm IGF Res 14 Suppl A:S1-18-S124
27. Van Cauter E, Leproult R, Plat L 2000 Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284:861-868
28. Spiegel K, Leproult R, Van Cauter E 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435-1439
29. Ruiz-Primo E, Jurado JL, Solis H, Maisterrena JA, Fernandez-Guardiola A, Valverde C 1982 Polysomnographic effects of thyroid hormones primary myxedema. Electroencephalogr Clin Neurophysiol 53:559-564
30. Peker Y, Svensson J, Hedner J, Grote L, Johannsson G 2006 Sleep apnoea and quality of life in growth hormone (GH)-deficient adults before and after 6 months of GH replacement therapy. Clin Endocrinol (Oxf) 65:98-105
31. Schneider HJ, Oertel H, Murck H, Pollmacher T, Stalla GK, Steiger A 2005 Night sleep EEG and daytime sleep propensity in adult hypopituitary patients with growth hormone deficiency before and after six months of growth hormone replacement. Psychoneuroendocrinology 30:29-37
32. Lovas K, Husebye ES, Holsten F, Bjorvatn B 2003 Sleep disturbances in patients with Addison's disease. Eur J Endocrinol 148:449-456
33. Gander PH, Marshall NS, Harris R, Reid P 2005 The Epworth Sleepiness Scale: influence of age, ethnicity, and socioeconomic deprivation. Epworth Sleepiness scores of adults in New Zealand. Sleep 28:249-253
34. Whitney CW, Enright PL, Newman AB, Bonekat W, Foley D, Quan SF 1998 Correlates of daytime sleepiness in 4578 elderly persons: the Cardiovascular Health Study. Sleep 21:27-36
35. Grimes DA, Schulz KF 2005 Compared to what? Finding controls for case-control studies. Lancet 365:1429-1433
36. Wacholder S, Silverman DT, McLaughlin JK, Mandel JS 1992 Selection of controls in case-control studies. II. Types of controls. Am J Epidemiol 135:1029-1041

Chapter 17

Patients cured from craniopharyngioma or non-functioning pituitary macroadenoma suffer similarly from increased daytime somnolence despite normal sleep patterns compared to healthy controls

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Clinical Endocrinology, 2008

ABSTRACT

Objective

Adults patients previously treated for craniopharyngioma have increased general and physical fatigue compared to healthy controls. This could be related to disturbed sleep patterns. The aim of this study was to compare sleepiness and sleep patterns in those patients to healthy controls and to patients treated for non-functioning macroadenomas (NFMA) of the pituitary.

Design

Case-control study.

Patients and methods

Sleepiness and sleep patterns were assessed in 27 adult patients (14 men, 8 patients diagnosed at childhood age, mean age of 53 years (range 27-80 yrs)) after long-term follow up and compared to 50 healthy controls and 38 age-, gender- and BMI matched patients with NFMA. We used two validated questionnaires for sleep parameters (Epworth sleepiness score and Münchener Chronotype Questionnaire).

Results

Sleep patterns (onset, sleep timing, duration, and rise time) were not statistically different between the three groups. However, daytime sleepiness scores were increased in patients treated for craniopharyngioma compared to healthy controls, but not different from patients with NFMA. Thirty-three percent of patients with craniopharyngiomas had ESS scores above 10 compared to 8% of healthy controls ($p=0.005$), indicating severe daytime hypersomnolence. Neither type of surgery, previous radiotherapy, or age at diagnosis influenced the sleepiness scores in patients with craniopharyngioma.

Conclusion

Patients treated for craniopharyngioma or NFMA have increased daytime somnolence despite normal sleep patterns, compared to healthy subjects. The results indicate that increased daytime somnolence is a general consequence of large tumors, and/or their treatment, in the hypothalamic/pituitary region, rather than a specific feature of craniopharyngiomas per se.

INTRODUCTION

Craniopharyngiomas are rare benign tumors arising along the path of the craniopharyngeal duct. Most of these tumors are located in the sellar and parasellar region (1). The presenting symptoms are dependent on the proximity to, and pressure on, structures of the brain. Craniopharyngiomas most frequently present with headaches, nausea/vomiting, visual disturbances, and hypothalamo-pituitary hormone deficiencies (reviewed in (1)). Craniopharyngiomas are treated primarily by transsphenoidal and/or transcranial surgery, with success rates of radical surgery ranging from 18 to 84% (1). Therefore, adjuvant radiotherapy may be necessary in selected patients in whom surgery was not completely successful (1). Nonetheless, craniopharyngiomas and/or their treatment are associated with impaired quality of life (2;3), increased morbidity and mortality during long-term follow-up in adult patients (4).

These long-term sequelae in patients treated for craniopharyngioma have been attributed to damage by the craniopharyngioma and/or its treatment to surrounding tissues. For example, hypothalamic damage has been implicated in the development of hyperphagia and obesity, which is reported in 26-61% of the patients postoperatively (1). The decreased sympathetic tone found in patients with severe obesity after treatment for craniopharyngioma could possibly be due to damage to the ventromedial nucleus of the hypothalamus, that regulates sympathetic nervous activity (5).

Self-reported, general and physical fatigue is increased in adult patients treated for craniopharyngioma compared to healthy controls (2). We hypothesized that impaired quality of life may, at least in part, be related to alterations in sleep patterns. This hypothesis is supported by the observation that daytime sleepiness is increased in patients after successful treatment of craniopharyngioma in childhood (6). However, this has not been reported for patients treated for craniopharyngiomas in adulthood. Moreover, the alterations in sleep quality may be a consequence of large pituitary tumors and/or their treatment in general, rather than being a specific consequence of craniopharygeomas per se. Therefore, the aim of the present study was to compare daytime sleepiness and sleep patterns between adult patients previously treated for craniopharyngiomas and healthy controls, as well as with patients with non-functioning tumors in the sellar region: non-functioning pituitary macroadenomas (NFMA).

PATIENTS AND METHODS

Patients and controls

The present study was a case-control study of consecutive patients previously treated for craniopharyngioma in our center. Each patient was asked to provide a healthy control person of comparable age and sex. The control group was extended with controls derived from other studies in our center that were approached similarly. In addition, patients were compared to

38 patients previously treated for non-functioning macroadenomas (NFMA) matched for age, gender, and body mass index (BMI) derived from a previous study in our center (7). This number of patients was determined by choosing patients from the original NFMA cohort with the best match for age, gender and BMI and resulted in a case:control ratio of 1:1.4.

All patients were seen at least twice yearly by an endocrinologist, with appropriate evaluation and treatment of possible deficiencies of pituitary hormones. Growth hormone (GH) deficiency was defined as an IGF-I level below the reference range for age and sex and an insufficient rise in GH levels (absolute value <3 µg/l) after stimulation during an insulin tolerance test. Prior studies have demonstrated that patients with multiple pituitary hormone deficiencies, including two or more pituitary hormone deficiencies other than GH-deficiency, had a likelihood of approximately 95% of harboring GH-deficiency (8-10). Based on these data, we classified patients, in whom GH-stimulation test data were not obtained, but who were deficient in 3 other pituitary axes, as GH-deficient. When secondary amenorrhea was present for more than 1 year premenopausal women were defined as LH/FSH deficient. Post-menopausal women were defined as LH/FSH deficient when gonadotrophin levels were below the normal postmenopausal range (LH<10 U/l and FSH<30 U/l). In men, LH/FSH deficiency was defined as a testosterone level below the reference range (8.0 nmol/l). TSH deficiency was defined as a total or free T4 level below the reference range. ACTH deficiency was defined as an insufficient increase in cortisol levels (absolute value <0.55 µmol/l) after a corticotrophin releasing hormone test or insulin tolerance test. If results were below the lower limit of the respective reference ranges, substitution with growth hormone, thyroxine, hydrocortisone or testosterone was started. In the case of amenorrhea and low estradiol levels in premenopausal women, estrogen replacement was provided. Postmenopausal women were not treated with estrogen replacement therapy. None of the patients were using selective serotonin reuptake inhibitors, tricyclic antidepressants, or MAO inhibitors.

The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol and all patients returning completed questionnaires gave written informed consent.

Study parameters

Primary study-parameters were the results of the two sleep questionnaires. The patients were asked to complete two questionnaires that assessed daytime sleepiness and sleep patterns. The questionnaires were sent to their home in prepaid envelopes. After two months non-responders were contacted by telephone to encourage completion and return of the questionnaires.

Patients with craniopharyngiomas were compared to controls and to patients with a NFMA. The results of the sleep questionnaires were linked to age and gender of the patients, body mass index, treatment characteristics (multiple surgical procedures, radiotherapy), visual field defects, and the presence of pituitary deficiencies.

Sleep questionnaires

Epworth sleepiness scale (ESS)

The ESS is a validated, eight-item questionnaire. The participants are asked to rate their likelihood of falling asleep in a variety of commonly encountered situations (11). Scores range from 0 (the least sleepy) to 24 (the most sleepy). Scores equal to or above 10 are interpreted as increased daytime sleepiness (6). An additional set of questions that evaluated the prevalence of snoring, observed apnoea's, and nocturnal restless legs was added (12) .

Münchener chronotype questionnaire (MCQ)

The MCQ is a validated questionnaire aimed at assessing chronotype and sleep patterns (13;14). Patients are explicitly asked to describe their sleep behavior under normal circumstances (without partying etc.). The temporal structure of sleep is assessed separately for workdays and free days. Parameters on free days are regarded to reflect individual sleep patterns without social obligations and are therefore reported in this paper (13).

Sleep duration on free days (SD_F), sleep onset on free days (SO_F) and rise time (RT_F) are calculated from questions concerning sleep onset and awakening on days on which there are no work or social obligations. The midsleep on free days (MS_F: clock time halfway during sleep duration) is calculated from SO_F and RT_F (14).

Since most chronotypes tend to accumulate a sleep debt on work days, which is compensated for on free days, midsleep on workdays (MS_C) was corrected for the confounder sleep debt as follows: $MS_C=MS_F-(0.5*SD_F-(5*SD_{working\ days}+2*SD_F)/7)$ (14). Since only 18 of our craniopharyngioma patients, 28 of our NFMA patients, and 41 of our controls had a daytime job, this correction was performed only for those subjects (14).

Statistics

SPSS for windows version 14.0 (SPSS Inc., Chicago, IL) was used for data analysis. Data are expressed as mean ± SD, unless otherwise mentioned. ANOVA analysis with post-hoc Tukey's HSD adjustment or chi-square test were performed to compare the three groups when appropriate. Variables influencing sleep parameters within the craniopharyngioma group were studied with independent samples T-test, Mann-Whitney tests or chi-square tests when appropriate. Differences were considered statistically significant at $P<0.05$.

RESULTS

Patients and controls

Twenty-nine of 34 (85%) patients returned the questionnaires on sleep characteristics (Table 1). Two of the patients preferred not to participate and 5 patients did not respond. Thus, 27

Table 17/1: Clinical characteristics of patients with craniopharyngioma compared to patients with non-functioning macroadenoma (NFMA).

	Cranio-pharyngeoma patients (n=27)	NFMA patients (n=38)	P-value
Age (mean, range (yrs))	53 (27-80)	55 (36-63)	0.599
Gender (F/M (%))	48/52	60/40	0.423
BMI (kg/m^2)	29.6 ± 7.5	27.8 ± 3.8	0.321
Radiotherapy (%)	26	37	0.354
GH deficiency (%)	91	78	0.191
IGF-I standard deviation score*	0.6 ± 2.1	1.1. ± 2.1	0.459
TSH deficiency (%)	96	66	0.003
Free T4**	18.5 ± 4.3	17.6 ± 3.0	0.438
ACTH deficiency (%)	78	63	0.208
LH/ FSH deficiency (%)	63	79	0.156

* in patients with substituted GH deficiency.

** in patients with substituted TSH deficiency.

completed questionnaires were received. The study-population (14 men) had a mean age of 53 years with a range of 27 to 80 years. All 27 patients were primarily treated by surgery. Eight of these patients had repeat surgery. Seven patients were also treated by radiotherapy, four for recurrent disease. The mean follow-up period after initial surgery and this study was 21.3 ± 14.3 years. All patients were considered cured from craniopharyngioma, because follow up MR imaging did not reveal the presence of recurrent disease. There were no significant differences in age, gender and clinical characteristics between the study-population and the patients who preferred not to participate or who did not return the questionnaires (n=7).

The patients were first compared to 50 controls (20 men) with mean age of 55 years (range of 45 to 63 years). There were no differences for age and gender between the patients and the control group (p=0.413 and p=0.318, respectively). Subsequently, patients were compared to 38 patients who had been previously cured by surgery from NFMA (20 men) with a mean age of 55 years (range 45 to 63 years) and a mean duration of follow-up of 9.4 ± 6.8 years. All 38 patients with NFMA had been operated previously, and 37 % had also been treated by postoperative radiotherapy.

In 16 patients with craniopharyngioma data of pre-operative Hardy classification were present. Seventy-five percent had Hardy B, compared to 19% C, and 1 patient presented with an intrasellar tumor (p=0.772). The other 11 patients with craniopharyngioma all had suprasellair disease without data on Hardy classification and all but two were operated transcranially. The other two patients were operated transsphenoidally in 1960 and 1967, respectively. The Hardy classification of suprasellar extension indicated stage B in 83% and stage C in 11% of NFMA patients (n=29 and n=11, resp., p=0.772 compared to craniopharyngioma patients (all except for 3 patients with NFMA could be classified according to Hardy)).

Table 17/2: Sleepiness scores and subjective sleep patterns in patients with craniopharyngioma compared to controls.

		Craniopharyngioma patients (n=27)	Controls (n=50)	P-value
ESS	Mean score (mean ± SD)	7.7 ± 4.1	4.8 ± 3.4	0.011
	>10 (%)	33	8	0.005
MCTQ	Sleep onset on free days (clock time h:min ± SD)	23:40 ± 1:20	23:48 ± 1:29	0.906
	Sleep latency (minutes ± SD)	30.6 ± 39.5	26.3 ± 24.2	0.860
	Rising time on free days (clock time h:min ± SD)	7:14 ± 1:00	7:05 ± 1:15	0.863
	Sleep duration on free days (duration h:min ± SD)	7:33 ± 1:11	7:17 ± 1:02	0.574
	Midsleep on free days (clock time h:min ± SD)	3:27 ± 1:01	3:27 ± 1:16	1.00
	Corrected midsleep (clock time h:min ± SD, n=25 vs. n=18/ n=41/ n=28)	3:54 ± 1:11	3:42 ± 0:45	0.673

Craniopharyngioma patients compared to healthy controls

Sleep duration on free days (SD_F) was not different between patients and controls. Sleep onset on free days, midsleep on free days, rising time on free days, and corrected midsleep (SO_F, MS_F, RT_F, and MS_C, respectively) were not different compared to controls (Table 2).

However, the Epworth Sleepiness Scale (ESS) score was increased in patients compared to controls (7.7 ± 4.1 vs. 4.8 ± 3.4, p=0.011) denoting increased daytime sleepiness in patients. Thirty-three percent patients had ESS scores above 10 compared to 8% in controls (p=0.005). The mean age and BMI in these patients (33% men) were 54.0 ± 16.2 yrs and 31.4 ± 4.1 kg/m^2, respectively, which was not different compared to the other patients with craniopharyngioma with ESS scores below 10. No differences in pituitary deficiencies were found between those two groups.

Seventy-two percent of patients reported snoring compared to 74% in controls, whereas 29% of patients reported observed apneas compared to 10% of controls (p=0.044). Restless legs were reported in 26% in patients compared to 24% in controls (p=0.852). Fifteen percent of patients reported feeling depressed compared to 5% in controls (p=0.199).

Craniopharyngioma patients compared to patients with non-functioning macroadenomas

Patients with craniopharyngioma were compared to age-, gender- and BMI-matched patients with NFMA (Table 1).

Sleep duration on free days (SD_F) was not different between patients with craniopharyngioma and patients with NFMA. Sleep onset on free days, midsleep on free days, rising time on free days, and corrected midsleep (SO_F, MS_F, RT_F, and MS_C, respectively) were not statistically different compared to patients with nonfunctioning macroadenomas (Table 3).

Table 17/3: Sleepiness scores and subjective sleep patterns in patients with craniopharyngioma compared to patients with non-functioning macroadenoma (NFMA).

		Craniopharyngioma patients (n=27)	NFMA patients (n=38)	P-value
ESS	Mean score (mean ± SD)	7.7 ± 4.1	7.5 ± 5.1	0.965
	>10 (%)	33	24	0.392
MCTQ	Sleep onset on free days (clock time h:min ± SD)	23:40 ± 1:20	23:43 ± 0:52	0.990
	Sleep latency (minutes ± SD)	30.6 ± 39.5	34.5 ± 36.8	0.887
	Rising time on free days (clock time h:min ± SD)	7:14 ± 1:00	6:49 ± 1:01	0.334
	Sleep duration on free days (duration h:min ± SD)	7:33 ± 1:11	7:05 ± 1:10	0.230
	Midsleep on free days (clock time h:min ± SD)	3:27 ± 1:01	3:15 ± 0:44	0.763
	Corrected midsleep (clock time h:min ± SD, n=25 vs. n=18/ n=41/ n=28)	3:54 ± 1:11	3:41 ± 0:35	0.654

ESS score was not different between the two groups. Thirty-three percent patients had ESS scores above 10 compared to 24% in patients with NFMA (p=0.392). The mean age and BMI in these patients with NFMA (44% men) were 52.0 ± 9.1 yrs and 27.5 ± 3.8 kg/m^2, respectively, which was not different compared to the other patients with NFMA with ESS scores below 10. No differences in pituitary deficiencies were found between those two latter groups.

Seventy-two percent of patients with craniopharyngioma reported snoring compared to 66% in patients with NFMA (p=0.604), whereas 29% of patients reported observed apneas compared to 17% of patients with NFMA (p=0.274). In addition, restless legs were reported in 26% in patients compared to 18% in patients with NFMA (p=0.468). Fourteen percent of patients reported feeling depressed compared to 5% in patients with NFMA (p=0.882).

In addition, patients with NFMA were compared to the healthy controls. ESS scores were higher in the patients with NFMA (7.5 ± 5.1 vs. 4.8 ± 3.4 in controls, p=0.010) without differences in sleep pattern parameters between the two groups (Figure 1).

Factors influencing sleepiness scores and sleep patterns in patients with craniopharyngioma

Surgery

There were no differences in sleepiness scores or sleep patterns between patients who had been treated by transsphenoidal surgery (37%) and patients treated by transcranial surgery (63%).

Radiotherapy

There were no differences in sleepiness scores or sleep patterns between patients who had been treated with radiotherapy (26%) and patients who were not (74%).

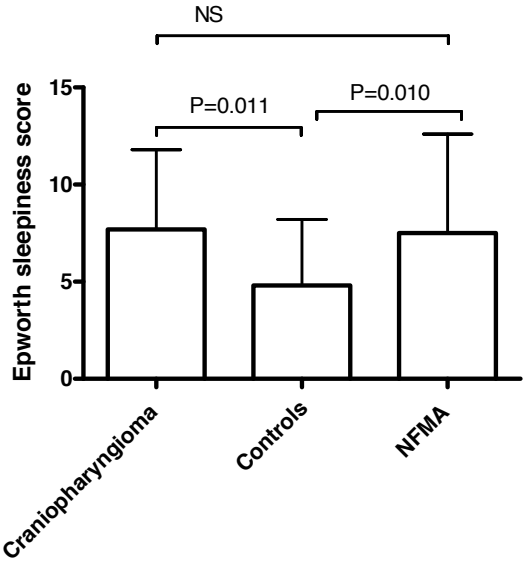

Figure 17/1: Epworth sleepiness scores are increased in patients with craniopharyngioma compared to healthy controls but do not differ from scores in age-, gender-, and BMI-matched patients with non-functioning macroadenoma (NFMA).

Age at onset

The diagnosis of craniopharyngioma was established in childhood in 8 patients (30%) and in adulthood in 19 patients (70%). No differences were found in sleepiness scores or sleep patterns between the two groups.

BMI

No correlations could be found in this limited number of patients sleepiness scores or sleep patterns on the one hand and BMI on the other hand.

DISCUSSION

We found increased daytime somnolence and increased prevalence of severe hypersomnolence despite normal sleep patterns in patients treated for craniopharyngioma compared to healthy controls. In addition, we observed an increased prevalence of self-reported apneas in these patients. However, daytime somnolence and sleep patterns in patients with craniopharyngioma did not statistically differ from age-, gender-, and BMI-matched patients with non-functioning macroadenoma. Therefore, these results indicate that increased daytime somnolence is a general consequence of large tumors, and/or their treatment, in the hypothalamic/pituitary region, rather than a specific feature of craniopharyngiomas per se.

Multiple long-term sequelae of treatment of craniopharyngioma, such as hyperphagia, obesity and decreased sympathetic tone, have be attributed to damage to surrounding tissues including hypothalamic nuclei (1;5). The hypothalamus has also been identified as the main regulatory center of sleep: the suprachiasmatic nucleus (SCN) is considered to be the central circadian pacemaker of the body with one of the circadian outputs formed by the regulation of circadian variations in melatonin secretion by the pineal gland (15). In patients treated for craniopharyngioma in childhood, reduced nocturnal melatonin concentrations are associated with increased daytime somnolence (16). In addition, the multiple sleep latency test, a standardized test of daytime somnolence, showed severe daytime somnolence in 5 children who had been treated by hypothalamic/ pituitary surgery (17). In accordance, self-reported general and physical fatigue is increased in adult patients treated for craniopharyngioma compared to healthy controls (2). In the present study, the previous findings of increased daytime somnolence obtained in children treated for craniopharyngioma were extended to, and confirmed in, adult patients with craniopharyngioma. In addition, we found an increased prevalence of self-reported observed apneas, which could also contribute to the increased daytime somnolence.

Remarkably, daytime somnolence and sleep patterns in patients with craniopharyngioma did not differ from age-, gender-, and BMI-matched patients with non-functioning macroadenomas. The similar distortion of sleep patterns in patients with craniopharyngiomas and non-functioning macroadenomas points towards a shared origin of the increased daytime sleepiness possibly originating from damage to the suprachiasmatic nucleus.

Large tumors of the pituitary region are associated with deficient function of the anterior pituitary. In patients previously treated for non-functioning macroadenomas, the presence of anterior pituitary hormone deficiencies was associated with altered sleep patterns (7). Since almost all patients treated for craniopharyngioma in our cohort presented with multiple deficiencies of the anterior pituitary, we could not specifically evaluate the effects of anterior pituitary hormone deficiencies on sleep characteristics within this group. However, since both patients with craniopharyngiomas and NFMA presented with multiple anterior pituitary deficiencies, these abnormalities in pituitary function could also contribute to increased daytime sleepiness in both patient groups. Indeed, many interactions between nocturnal secretion of different hormones and the sleep electroencephalogram parameters have been described (18). Altered sleep patterns can induce changes in anterior pituitary hormone secretion (19). Conversely, endocrine deficiencies, like primary hypothyroidism, are associated with altered sleep patterns on polysomnographic recordings (20). However, it is maybe unlikely that these effects have played a major role in our study, since anterior pituitary hormone deficiencies were monitored regularly and appropriately replaced in our patients.

Some factors may have influenced our results. First, sleepiness and sleep patterns were evaluated by questionnaires. Although the MCQ assessed sleep during free days and working days on one occasion, data on sleep habits derived from the MCQ correlate highly with data obtained from sleep logs for 5 weeks (13). Moreover, in the present study patients with

craniopharyngiomas were compared to healthy controls recruited by the patients. Although it is known that self-selected controls might be subject to selection bias, because patients might have chosen controls with a supposedly good health status (21), it is highly unlikely that sleeping pattern played any role in the choice for a specific control. Finally, the results in our study are supported by the fact that there was no difference between the results obtained in patients treated for craniopharyngiomas and NFMAs.

In conclusion, we report increased daytime somnolence and increased prevalence of severe hypersomnolence in patients treated for craniopharyngioma compared to healthy controls. In addition, there was no difference between patients treated for craniopharyngioma or non-functioning pituitary macroadenomas with respect to increased daytime sleepiness scores compared to healthy controls. Further detailed analyses are needed to elucidate the pathophysiology of the reported increased sleepiness seen in patients previously treated for large tumors in the (para)sellar region. These studies could provide further insight and treatment targets in the complex persisting morbidity in patients after successful treatment for craniopharyngiomas as well as non-functioning macroadenomas.

REFERENCES

1. Karavitaki N, Cudlip S, Adams CB, Wass JA 2006 Craniopharyngiomas. Endocr Rev 27:371-397
2. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM 2006 Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 154:483-489
3. Muller HL, Bruhnken G, Emser A, Faldum A, Etavard-Gorris N, Gebhardt U, Kolb R, Sorensen N 2005 Longitudinal study on quality of life in 102 survivors of childhood craniopharyngioma. Childs Nerv Syst 21:975-980
4. Pereira AM, Schmid EM, Schutte PJ, Voormolen JH, Biermasz NR, van Thiel SW, Corssmit EP, Smit JW, Roelfsema F, Romijn JA 2005 High prevalence of long-term cardiovascular, neurological and psychosocial morbidity after treatment for craniopharyngioma. Clin Endocrinol (Oxf) 62:197-204
5. Roth CL, Hunneman DH, Gebhardt U, Stoffel-Wagner B, Reinehr T, Muller HL 2007 Reduced sympathetic metabolites in urine of obese patients with craniopharyngioma. Pediatr Res 61:496-501
6. Muller HL, Handwerker G, Wollny B, Faldum A, Sorensen N 2002 Melatonin secretion and increased daytime sleepiness in childhood craniopharyngioma patients. J Clin Endocrinol Metab 87:3993-3996
7. van der Klaauw AA, Dekkers OM, Pereira AM, van Kralingen KW, Romijn JA 2007 Increased daytime somnolence despite normal sleep patterns in patients treated for non-functioning pituitary macroadenoma. J Clin Endocrinol Metab 92:3898-3903
8. Bates AS, Evans AJ, Jones P, Clayton RN 1995 Assessment of GH status in adults with GH deficiency using serum growth hormone, serum insulin-like growth factor-I and urinary growth hormone excretion. Clin Endocrinol (Oxf) 42:425-430
9. Hartman ML, Crowe BJ, Biller BM, Ho KK, Clemmons DR, Chipman JJ 2002 Which patients do not require a GH stimulation test for the diagnosis of adult GH deficiency? J Clin Endocrinol Metab 87:477-485
10. Toogood AA, Beardwell CG, Shalet SM 1994 The severity of growth hormone deficiency in adults with pituitary disease is related to the degree of hypopituitarism. Clin Endocrinol (Oxf) 41:511-516
11. Johns MW 1991 A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14:540-545
12. Venmans BJ, van Kralingen KW, Chandi DD, de Vries PM, ter Wee PM, Postmus PE 1999 Sleep complaints and sleep disordered breathing in hemodialysis patients. Neth J Med 54:207-212
13. Roenneberg T, Wirz-Justice A, Merrow M 2003 Life between clocks: daily temporal patterns of human chronotypes. J Biol Rhythms 18:80-90
14. Roenneberg T, Kuehnle T, Pramstaller PP, Ricken J, Havel M, Guth A, Merrow M 2004 A marker for the end of adolescence. Curr Biol 14:R1038-R1039
15. Mignot E, Taheri S, Nishino S 2002 Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5 Suppl:1071-1075
16. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589
17. Snow A, Gozal E, Malhotra A, Tiosano D, Perlman R, Vega C, Shahar E, Gozal D, Hochberg Z, Pillar G 2002 Severe hypersomnolence after pituitary/hypothalamic surgery in adolescents: clinical characteristics and potential mechanisms. Pediatrics 110:e74
18. Van Cauter E, Leproult R, Plat L 2000 Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 284:861-868
19. Spiegel K, Leproult R, Van Cauter E 1999 Impact of sleep debt on metabolic and endocrine function. Lancet 354:1435-1439
20. Ruiz-Primo E, Jurado JL, Solis H, Maisterrena JA, Fernandez-Guardiola A, Valverde C 1982 Polysomnographic effects of thyroid hormones primary myxedema. Electroencephalogr Clin Neurophysiol 53:559-564

21. Wacholder S, McLaughlin JK, Silverman DT, Mandel JS 1992 Selection of controls in case-control studies. I. Principles. Am J Epidemiol 135:1019-1028

Chapter 18

General discussion and summary

CONTENTS

I. INTRODUCTION

In the present thesis we have evaluated the long-term consequences of pituitary diseases and their treatment in patients with acromegaly and patients with growth hormone deficiency (GHD). In addition, we evaluated the impact of the treatment of pituitary tumors on quality of life (QoL) and sleep. In general, pituitary tumors can be adequately treated resulting in stable or cured pituitary disease. Moreover, appropriate hormonal replacement strategies result in control of pituitary insufficiency, induced by the tumor and/or its treatment. Nonetheless, careful assessment indicates that these approaches are not embracing all long term consequences of pituitary disease because they do not result in normal functioning of these patients.

II. STUDIES IN PATIENTS WITH ACROMEGALY

Recent cross-sectional studies have documented an association between acromegaly and regurgitant valvular heart disease (1;2). The aim of the study described in **Chapter 2** was to evaluate the change in prevalence of valvular heart disease in relation to the clinical activity, because the natural history of valvular changes in acromegaly is unknown. Valvular regurgitation was assessed before and after an interval of approximately 2 years in 37 acromegalic patients of whom 50% had active and the rest of the patients included had inactive disease.

In patients with active disease, valvular regurgitation increased significantly from approximately 60% at baseline to approximately 90% at follow-up due to a significant increase of trace and mild mitral regurgitation. In contrast, no increase in valvular regurgitation was found in patients with controlled disease.

This observational follow-up study demonstrated that the prevalence of trace and mild mitral valvular regurgitation increases in patients with active acromegaly. Conversely, adequate control of GH excess is associated with stable valvular function, at least during the duration of follow-up of our study. These data reinforce the concept that acromegaly induces regurgitant valvular disease.

The increase in regurgitation in patients with active acromegaly was observed for the mitral valve and not for the aortic valve. This might be related to differences in the intrinsic vulnerability between the aortic and mitral valve to exogenous stimuli that promote valvular degenerative changes. Indeed, mitral regurgitation, but not aortic regurgitation, is associated with systemic hypertension (3). The persistent long-term exposure to GH excess which causes myxomatous degeneration of the valves (1), might predispose to these accelerated degenerative changes. Interestingly, the myxomatous degeneration in acromegaly resembles that found in connective tissue diseases, conditions that are also associated with irreversible valvular disease (4).

Valve regurgitation was asymptomatic and varied from only trace to mild severity in all but 1 patient. Nonetheless, these valve abnormalities seem to be important in itself since in the

general population, the severity of mitral regurgitation is a potent predictor of clinical outcome in terms of death from any cause and death from cardiac disease (5).

Therefore, patients with acromegaly require adequate cardiac evaluation and follow-up to establish the extent and progression of valvular involvement.

In **Chapter 3** we evaluated aortic root diameters in patients with acromegaly. The clinical manifestations of acromegalic cardiomyopathy include arrhythmias, valvular regurgitation, concentric left ventricular hypertrophy, and systolic and diastolic dysfunction. It was unknown whether the aortic root is involved in the cardiac pathology in these patients.

Aortic root diameters were assessed in 37 acromegalic patients and compared to healthy controls.

The diameters of the aortic root at the sino-tubular junction and the ascending aorta were increased in patients with acromegaly, whereas the diameters at the aortic annulus and aortic sinus were not different from controls. Aortic root diameters were not influenced by disease duration, current disease activity or blood pressure. These data indicate that long-term exposure to GH excess affects the aortic root in addition to previously documented effects on the aortic valve leaflets (**Chapter 2**, (1;2)).

There was no correlation between dilatation of the aortic root and current disease activity or estimated disease duration. Although a lack of association was also found previously between valvular regurgitation and current disease activity, valvular regurgitation was strongly associated with disease duration (1), pointing towards direct effects of long-term exposure to increased GH and/or IGF-1 concentrations on cardiac valves.

We speculate that the increased diameters of the aortic root are probably due to the same mechanisms that induce the myxomatous degeneration found in the valves that were removed from several of our acromegalic patients during valvular replacement surgery (1). GH is involved in matrix regulation. For example, GH increases gene expression of the matrix metalloproteinases (MMPs), that are capable of altering the composition of the extracellular matrix (6). This altered matrix regulation could cause the changes found in the heart valves, as well in the aortic root in patients with acromegaly. This coincidence between valvular regurgitation and aortic root dilatation is also present in Marfan's syndrome, which is also characterized by myxomatous degeneration of cardiac valves and aortic root (7).

None of our patients were diagnosed with true thoracic aortic aneurysms. Therefore, the results of the present study do not imply that aortic root diameters should be screened in all patients with acromegaly to detect aneurysms. However, extending the echocardiographic measurements to the aortic root offers a more complete picture of the spectrum of acromegalic cardiomyopathy.

The aims of treatment in active acromegaly are to relieve the symptoms of GH excess, to restore metabolic alterations, to decrease mass effects of the pituitary tumor and to reduce the

increased mortality risk associated with active acromegaly (8). Somatostatin analog treatment alone or surgical treatment alone can reach these targets in only 50-70% of the patients. Fortunately, combinations of surgery, radiotherapy and/or drug therapy (somatostatin analogs and/or GH receptor blockade drugs) are able to control disease activity in almost all patients (9-12). However, despite these beneficial effects, there are persisting negative effects of acromegaly and/or its treatment on QoL, well-being and sleep.

Cross-sectional studies have shown impaired quality of life in patients with biochemical control of acromegaly (13-17). The aim of the study described in **Chapter 4** was to assess longitudinal changes in quality of life in a homogenous cohort of patients with sustained biochemical control of acromegaly. Quality of life was assessed using four health related quality of life questionnaires ((Hospital Anxiety and Depression Scale (HADS), Multidimensional Fatigue Inventory (MFI-20), Nottingham Healthy Profile (NHP), Short Form-36 (SF-36)) and one disease-specific quality of life questionnaire (Acromegaly-Quality of Life (ACRO-QOL)) in 82 patients with strict biochemical control of acromegaly at baseline and after 4 years of follow-up.

During follow-up, scores in 5 of 26 QoL subscales significantly worsened: physical and social functioning (SF-36), physical fatigue (MFI-20), and psychological well-being and personal relations (ACRO-QOL). Using linear regression analysis, baseline item scores predicted the follow-up scores, indicating individual stability over time. Previous radiotherapy negatively influenced several QoL subscales at follow-up: energy, pain, and social isolation (NHP), physical fatigue and reduction in activity and motivation (MFI-20), depression and total anxiety and depression scores (HADS), and physical performance (ACRO-QOL).

These data indicate that during 4 years of follow-up in patients with long-term biochemical control of acromegaly QoL is subtly, but progressively, impaired. In addition, radiotherapy was the major indicator of progressive impairment in QoL.

This longitudinal study extends the knowledge obtained in several cross-sectional studies. In those studies, adequate control of disease activity influenced QoL positively (13-16). One longitudinal study with a median follow up of 21 months documented an overall unchanged QoL in a cohort consisting of patients with cured and active disease, using a disease specific questionnaire (18). However, previous radiotherapy (14;19) and somatostatin treatment (13) were associated with impaired QoL in cross-sectional studies.

Indeed, our study confirmed the negative relationship between previous radiotherapy and QoL. Different sequelae of radiotherapy might contribute to the negative impact on QoL. First, radiotherapy induces anterior pituitary deficiencies, which negatively affects QoL. However, we did not find an increase in anterior pituitary deficiencies during prolongation of follow-up. Secondly, the negative effects of radiotherapy on Qol might also be caused by the long-term negative consequences on neurocognitive functioning which is seen in long-term survivors of cranial radiation for brain tumors (20). Thirdly, patients treated with combination of surgery and

radiotherapy might perceive their disease as more severe and consequently have a reduced QoL compared to patients treated with surgery only.

Since the introduction of more effective drug therapy for acromegaly, radiotherapy is applied in only a very limited number of patients. Nonetheless, this study could contribute to the clinical assessment of patients with acromegaly treated with radiotherapy since the negative impact on QoL persists and even increases during follow-up.

In **Chapter 5** we decribe a case-control study in patients with acromegaly aimed to assess daytime sleepiness and sleep patterns. We used two validated sleep questionnaires (Epworth sleepiness score and Münchener Chronotype Questionnaire). Patient outcomes were compared to controls.

Sleep duration and timing of sleep were not different in patients compared to controls. However, sleepiness score was increased in all patients compared to controls, reflecting increased daytime sleepiness. In addition, sleep latency was increased in patients treated with somatostatin analogs compared to patients cured by surgery and/ or radiotherapy, resulting in a delayed sleep onset. Sleep duration was unaffected.

The data from this study indicate that daytime sleepiness is increased in a homogeneous cohort of patients in long-term remission from acromegaly. In addition, these data suggest that somatostatin analog treatment alters sleep patterns in these patients without altering total sleep duration.

In patients with acromegaly, sleep apnoea could contribute to the increased daytime sleepiness. Although not formally excluded, we did not find any significant differences in self-reported snoring or apnoea's. Many reports have described the amelioration of sleep apnoeas after successful treatment of acromegaly (21-26), but only one study in a homogenous cohort of patients cured from acromegaly reported the prevalence of sleep apnoea syndrome to be 20% (27). Therefore, a detailed assessment of sleep apnoea syndrome in cured acromegaly is warranted especially since the effects of GH receptor blockade therapy on sleep apnoea syndrome are unknown.

In addition, increased sleep latency was found in patients on somatostatin analog therapy. Somatostatin impaired sleep in healthy elderly subjects especially by decreasing total sleep time and REMS, and by increasing the time spent awake in the first sleep cycle (28). In contrast, it did not influence sleep in young healthy adults (29). In rats, the long-acting somatostatin analog octreotide suppressed NREMS after repeated injections (30). Moreover, octreotide reduced stage 4 NREMS and REMS during the first half of the night and increased intermittent wakefulness during the second half of the night in young healthy adults (31). Polysomnographic studies of the effects of the depot preparations of octreotide or lanreotide on sleep parameters have not been reported yet, which would be interesting in the light of our results. Nonetheless, we think that these effects of somatostatin analog treatment should be considered during the clinical evaluation of these patients.

Radiotherapy for pituitary adenomas frequently leads to GHD (32). In patients with acromegaly previous radiotherapy is associated with impaired GH responses to insulin induced hypoglycemia in 36 % of the patients (33). In **Chapter 6** we describe the characteristics of GH secretion in GHD induced by postoperative radiotherapy for acromegaly. We hypothesized that in the long-term, stimulated and spontaneous GH release would not be different between patients with GHD treated by postoperative radiotherapy for acromegaly or for other pituitary adenomas. We compared the characteristics of basal and stimulated GH secretion in patients with GHD who had previously received postoperative radiotherapy for acromegaly or for other pituitary adenomas. All patients had a maximal GH concentration during insulin-induced hypoglycemia (insulin tolerance test (ITT)) of 3 µg/l or less, diagnostic for severe GHD. Stimulated GH release was evaluated by infusion of GHRH, GHRH-arginine and arginine and spontaneous GH by 10 minute blood sampling for 24h. Pulse analyses were done by Cluster and approximate entropy.

There were no differences between both patient groups in stimulated GH concentrations in any test. Spontaneous GH secretion was not different between both patient groups, including basal GH release, pulsatility and regularity. Pulsatile secretion was lost in 2 acromegalic and 3 non-acromegalic patients and IGF-I was below -2 SD-score in 9 patients in each group.

Thus, these data suggest that GH secretory characteristics do not differ between patients treated for acromegaly with postoperative radiotherapy with an impaired GH response to insulin from patients treated similarly for other pituitary tumors with a similarly impaired GH response. This test or the GHRH-arginine test are therefore reliable in establishing the diagnosis of GHD in patients treated for acromegaly with surgery and radiotherapy.

GHD after radiotherapy may originate from failure of synthesis and/or delivery of endogenous GHRH (or other putative GH-releasing substances, e.g. hypothalamic ghrelin) to the pituitary (34;35). One could hypothesize that the function of the hypothalamic-pituitary-GH axis in acromegalic patients treated by postoperative radiotherapy, as assessed by the ITT, is impaired due to surgical and radiotherapeutical intervention, while autonomous activity of the adenoma persists. This would lead to an erroneous diagnosis of GHD in these patients. Therefore, various GH stimulation tests were combined with 24h spontaneous GH secretion. GH secretion in active acromegaly is characterized by increased pulse frequency, burst mass and basal secretion (36-38). In contrast, GH burst mass is profoundly decreased in GHD and total 24h secretion is diminished despite increased pulse frequency (39). In the present study, the 24h GH secretion profile in patients treated for acromegaly and other pituitary adenomas clearly resembled that found in GHD in general. No differences wer found in mean 24h GH concentration, number of GH pulses per 24h, pulse amplitude or area between the two groups. The spectrum of GH release extended from complete absence of statistically significant GH pulses with low basal concentrations, as observed in 5 patients, to persisting, low amplitude pulsatility.

GHRH and combined GHRH+arginine infusions resulted in significantly higher GH peak responses than the ITT in both patient groups. This observation is consistent with results obtained by Aimaretti *et al.* in hypopituitarism caused to various etiologies (40). The generally accepted explanation for this difference in the magnitude of the GH responses is that the GHRH-arginine test combines the somatostatin-suppressing effect of arginine (41) with direct stimulation of the somatotroph cell by exogenous GHRH (42), whereas the ITT requires endogenous GHRH (43). These observations are also in line a reported loss of response to the arginine test in patients treated by radiotherapy, but a retained response to the GH secretagogue in about 50% of these patients (44).

In conclusion, the insulin-induced hypoglycemia and the GHRH-arginine test are reliable tests in establishing the diagnosis of GHD in patients treated for acromegaly with postoperative radiotherapy.

Both GH excess (**Chapter 2 and 3**, (45)) and GHD lead to specific cardiac pathology. Cardiac manifestations of GHD include a decrease in left ventricular mass and left ventricular ejection fraction (46-52), which is correlated to the severity of GHD (48). The aim of the cross-sectional study described in **Chapter 7** was to evaluate cardiac morphology and function in patients with GHD after treatment for acromegaly.

Cardiac parameters were studied by conventional two-dimensional echocardiography and Tissue Doppler imaging in 53 patients with previous acromegaly, of whom approximately 30% had GHD. Patients with GHD were compared to the patients with biochemical remission and active acromegaly and also to age- and gender-matched controls.

Left ventricular dimensions, wall thickness and mass did not differ between the three patient groups or between the patients with GHD and healthy controls. Systolic function, assessed by LV ejection fraction, tended to be lower in patients with GHD compared to patients with biochemical remission, but was higher when compared to active acromegaly. No differences were found with healthy controls. Early diastolic velocity, a parameter of diastolic function, was lower in patients with GHD both when compared to patients with biochemical remission and to healthy controls.

The results in this study indicated that GHD after acromegaly results in specific cardiac changes in diastolic function. In GHD without previous acromegaly, a decrease in early filling phase compared to controls has previously been reported (53), in line with the observed decrease in our patients. In active acromegaly diastolic function is also decreased (54). Indeed, we found no difference in diastolic function in patients with GHD after acromegaly compared to patients with active acromegaly.

Indices of LVM, wall thickness, and LV diameters were unaltered in patients with GHD after acromegaly compared to patients with biochemical remission of acromegaly and to healthy controls. In patients with adult-onset GHD due to other diseases, IVST does not differ from healthy controls (47). Interestingly, in adults with childhood-onset GHD it was found to be

decreased (49;50). LVM was unaffected in our patients with GHD after acromegaly compared to patients with biochemical remission of acromegaly and compared to patients with active acromegaly. Several studies in patients with childhood-onset GHD revealed a decreased LVM (46;49;50), whereas it was unaffected in patients with adult-onset GHD like in our patients (47).

In conclusion, GHD after acromegaly results in specific cardiac alterations in diastolic function. In addition, systolic function tended to be decreased in patients with GHD after acromegaly compared to patients with biochemical remission but not when compared to healthy controls, but was higher than in patients with active acromegaly. This study shows that normal cardiac function is dependent on normal GH and IGF-I regulation.

Recombinant human GH (rhGH) replacement in adults with GHD increases bone mineral density (55), left ventricular mass and stroke volume (56), lean body mass (57), and it improves quality of life (58) and serum lipid profiles (59). These effects become apparent within 6-12 months and are maintained during continued treatment with rhGH in the long-term (**Chapter 11 and 12** (56;59-62)).

It was unknown whether these beneficial changes upon rhGH replacement also occur in patients previously treated for acromegaly. In **Chapter 8** the effects of rhGH replacement for GHD in patients previously treated for acromegaly were described. Sixteen patients treated for acromegaly with surgery and radiotherapy, with an insufficient GH response to insulin-induced hypoglycaemia, were randomized to 1 year of rhGH replacement or 1 year of placebo followed by 1 year of rhGH replacement. Study parameters were assessed at baseline, after 1 year of placebo and after 1 year of rhGH replacement. Study parameters were cardiac function, body composition, bone mineral density (BMD), fasting lipids, glucose, bone turnover markers, and QoL.

Treatment with rhGH did not have beneficial effects on body composition, fasting lipids and glucose, cardiac function or QoL. Bone turnover markers increased during rhGH replacement. During treatment with rhGH, BMD at the femoral neck decreased by 4%, although the bone mass of the lumbar spine remained unchanged.

Compared to patients with GHD due to other etiologies, LDL cholesterol is increased and muscle endurance is decreased in patients with GHD after treatment for acromegaly (63;64). No differences in body mass index, waist-hip ratio, serum lipid concentrations, glucose and insulin concentrations and BMD were found in patients with GHD after acromegaly compared with patients with GHD due to other etiologies (63;64).

In our study both bone resorption and bone formation increased in line with observations in 10 patients with GHD after acromegaly during 2 years of rhGH replacement (64). This increase in bone turnover was paralleled by a decrease in BMD in contrast to the absence of treatment differences in the response of BMD between patients previously treated for acromegaly and patients previously treated for non-functioning pituitary disease (64). In accordance with our

study, Feldt-Rasmussen *et al.* did not observe beneficial effects in this specific patient group on BMD either (63).

The decrease in BMD found in our study could point towards a different response to rhGH replacement in patients previously exposed to persistently increased GH concentrations. Alternatively, this observation may indicate that the possible beneficial effect of rhGH replacement on bone in these patients is insufficient to compensate the ongoing bone loss after previous GH excess in these specific patients. In active acromegaly, BMD is increased (45) and this favourable effect seems to persist after successful biochemical cure (65). However, in patients with biochemical cure of acromegaly, radiotherapy was also found to be an independent negative predictor of BMD at the femoral neck (65). Almost all patients in our cohort had been treated previously by radiotherapy. Indeed, age- and gender-standard deviation scores of BMD decreased during the year of treatment in the subset of patients who were left untreated. On the other hand, replacement with rhGH in patients with GHD without previous acromegaly seems to modestly increase BMD after 1 year (60). Further long-term studies are needed to clarify this issue.

However, the data in this study suggest that the effects of rhGH replacement in patients with GHD after previous treatment for acromegaly are limited. Large long-term studies in this specific patient group are necessary to clarify whether the effects of rhGH replacement in GHD might be affected by previous acromegaly. Fortunately, the induction of GHD in acromegaly by radiotherapy will become rare, since radiotherapy is rarely necessary anymore to control GH excess. Consequently, it will virtually be impossible to perform other trials in GHD acromegalic patients that are adequately powered to solve these issues.

Postoperative radiotherapy for acromegaly is associated with a considerable increase in pituitary insufficiencies, occurring in 50 % of the patients during a follow-up of 5-10 years (**Chapter 6** (33)). In general, the notion is that this is due to side effects of radiotherapy on the pituitary, but the hypothalamus may also be involved. Circadian variations in melatonin secretion are under the control of endogenous clock signals arising from the suprachiasmatic nucleus (SCN) of the hypothalamus. Therefore, the aim of the study described in **Chapter 9** was to assess the effects of postoperative radiotherapy on characteristics of diurnal melatonin secretion in patients cured from acromegaly. We compared patients treated with postoperative radiotherapy with patients treated with transsphenoidal surgery alone and healthy controls matched for age, gender and BMI. Melatonin concentrations were measured each hour during 24h and circadian rhythmicity was appraised with a skewed baseline cosine curve fit procedure.

Mean serum melatonin concentrations were highest during nighttime and lowest during the afternoon compared to the morning. Mean morning, afternoon, nighttime or total melatonin concentrations did not differ between the groups. The peak level and the onset and offset of melatonin did not differ between the groups. The acrophase, however, was delayed in patients treated with postoperative radiotherapy compared to healthy controls.

Only a few studies have addressed circadian melatonin rhythms in patients with acromegaly. Data on melatonin secretion in active acromegaly are conflicting. In one study, total melatonin secretion was decreased compared to healthy controls, whereas the acrophase occured earlier (66). In contrast, another study reported an increased average 24h melatonin secretion, without any evidence of disturbed circadian timing (67). Additional studies showed increased melatonin levels during daytime in patients with acromegaly (68;69) and patients with other intrasellar pituitary region tumors (70).

The data presented here give additional support to the contention of SCN alterations by radiotherapy by showing that previous radiotherapy is associated with a shift in acrophase timing in diurnal melatonin secretion in acromegalic patients. Additional leads to support this hypothesis were found in the altered timing of sleep in patients during long term follow up after treatment for large non-functioning macroadenomas (**Chapter 16**). Mid-sleep timing was clearly delayed after treatment with radiotherapy. Interestingly, mid-sleep timing is highly correlated with the melatonin phase (71), in accordance with the delay in acrophase of melatonin concentrations observed in this study.

Alternatively, rather than direct radiation damage to the SCN *per se*, other hypothalamic nuclei that control SCN function could be damaged by radiotherapy resulting in altered excitatory/ inhibitory input to the SCN. GHRH, which is predominantly produced by the paraventricular nucleus, mediates specific feed back signals to the SCN (72). There are indications that acromegalic patients previously treated by postoperative radiotherapy have a diminished GHRH tone (**Chapter 6**). In this respect, it is interesting, that intra-SCN injection of GHRH during daytime was found to advance circadian phase in hamsters (72).

In conclusion, the delayed acrophase in melatonin circadian rhythmicity in patients treated by postoperative radiotherapy for acromegaly suggests that there are subtle alterations in hypothalamic functioning in these patients. These alterations might contribute to the complex morbidity found in these patients after treatment for acromegaly.

III. STUDIES IN PATIENTS WITH GROWTH HORMONE DEFICIENCY

GHD in adults has received more and more attention since GHD was recognized to have adverse effects, even when longitudinal growth was completed (57). GHD in adults occurs most often as a consequence of various pathological processes in the pituitary and hypothalamic region, most frequently pituitary adenomas and their treatment (73). In general, the secretion of GH is the first to be affected in pituitary adenomas and their treatment, followed by decreased secretion of LH/ FSH, ACTH and TSH (32;74).

One of the major aims of rhGH replacement is to improve cardiovascular risk. Since a decade, bone marrow-derived endothelial progenitor cells have been proposed to play an important

role in maintenance and repair of the vasculature (75). Endothelial function, vascular stiffness and loss of circulating CD34+ cells are considered biomarkers for cardiovascular disease. The aim of the study described in **Chapter 10** was to assess vascular structure and function in relation to circulating CD34+ cells in adults with GHD before and during 1 year of rhGH replacement.

Vascular endothelial function and structure were assessed in adult patients with GHD. In addition, the number of CD34+ cells was evaluated using flow cytometric analysis. Study parameters were analyzed at baseline, and after 6 months and 1 year of rhGH replacement.

FMD increased, but there was no beneficial effect on PWV, central pulse pressure, central systolic pressure and augmentation index during 1 year rhGH replacement. The number of CD34+ cells increased by 70% during 1 year rhGH replacement.

The data in this study suggest that 1 year of rhGH replacement in adults with GHD improves endothelial function and increases the number of circulating CD34+ cells.

In our study, the number of circulating CD34+ cells in adults with GHD increased within 6 months of rhGH replacement and remained stable thereafter. These results are in line with the very recently observed potential of rhGH treatment to increase the number of circulating endothelial progenitor cells in healthy volunteers (76). In addition, the potential of rhGH to positively influence hematopoiesis has previously been shown in another clinical setting, i.e. harvesting of CD34+ cells destined for autologous hematopoietic stem cell transplantation in patients with relapsed or refractory hematologic malignancies (77). CD34+ cells express both GH and IGF-I receptors (78) as is the case for several other cell types that could be involved. Indeed, studies in rodents and on fetal bone marrow demonstrate direct effects of GH and IGF-I on hematopoiesis (78;79). In addition, a recent study reported that IGFBP-3 also promotes migration, tube formation and differentiation of CD34+ cells into endothelial cells, leading to increased vessel stabilization and quicker blood vessel development (80).

The observed improved endothelial function, i.e. flow mediated dilatation (FMD), after rhGH replacement was also observed within 6 months and continued until the end of the study. These data are in agreement with earlier reports assessing the effects of rhGH replacement on endothelial function (81-83). A putative mechanism by which rhGH replacement improves vascular function is IGF-I mediated stimulation of nitric oxide synthesis in endothelial cells (84;85).

In conclusion, one year rhGH replacement may have beneficial effects on vascular biology and function.

In **Chapter 11** we evaluated the long-term effects of rhGH replacement. Sixty-three adult GHD patients were assessed before and after 2, 5 and 7 years of rhGH replacement. IGF-I increased during rhGH replacement and a stable dose of rhGH was reached within 1 year of rhGH substitution. Thereafter, this individualized dose was continued.

Plasma levels of total cholesterol and LDL cholesterol decreased even after 5 years of rhGH replacement. HDL cholesterol levels increased during 7 years of rhGH replacement, whereas

triglyceride concentrations remained unchanged. Fasting glucose levels increased during follow-up, mainly during the first two years of rhGH replacement. BMI increased during follow-up, whereas waist circumference and waist-to-hip ratio remained unchanged.

The data in this study thus suggest that the beneficial effects of rhGH replacement, described after short-term rhGH replacement, are sustained in the long-term up to seven years.

GHD generally is an irreversible condition necessitating chronic rhGH replacement.

Before the present study, only 3 single center studies reported the effects of more than 5 years of rhGH replacement in a total of 33 patients (86-88). One large multi-center study reported effects of 5 years of rhGH replacement (61). In the studies of 5 years or longer, LDL cholesterol concentrations consistently decreased (61;62;86;87;89), but total cholesterol only decreased in three studies (61;62;86). Several studies found an increase in HDL cholesterol during long-term treatment (61;62;87;89). However, it was unknown whether these changes were sustained when follow-up is prolonged to 7 years. Moreover, initial treatment strategies of rhGH replacement in GHD were based on weight-based regimes adapted from treatment of children with GHD. However, this often resulted in supraphysiological substitution and this treatment regime was subsequently abandoned during long-term studies (55;61). The Growth Hormone Research Society recommended titrating rhGH replacement dose to normalize individual IGF-I concentrations (74). Nonetheless, our study with an individualized dose from the start of the study confirmed the beneficial changes found in the other long-term studies. Thus, treatment with rhGH replacement is beneficial during the long-term.

Nonetheless, it remained to be determined to what extent these changes translate into a reduction of cardiovascular risk factors. Therefore, the aim of the study described in **Chapter 12** was to evaluate the effects of long-term rhGH replacement on the prevalence of the metabolic syndrome.

The presence of the metabolic syndrome was scored using the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) definition in 50 consecutive GHD patients, before, after 2, and after 5 years of rhGH replacement and the data of untreated patients were compared to the general population using data from a Dutch population based study (n=1062).

The prevalence of hypertriglyceridemia, hypertension, and abdominal obesity was markedly increased, resulting in an approximately two-fold higher prevalence of the metabolic syndrome in patients compared to the healthy population. During rhGH replacement, mean HDL cholesterol levels increased compared to baseline. Nonetheless, the prevalence of (components of) the metabolic syndrome did not change after 2 or 5 years of treatment with rhGH.

In this study, the prevalence of the metabolic syndrome in patients with GHD is increased compared to healthy controls, irrespective of rhGH replacement. Atlhough ample studies have reported the individual cardiovascular risk factors in patients with GHD and their response to

rhGH replacement (reviewed in (57)), no previous studies assessed the clustered cardiovascular risk factors of the metabolic syndrome.

We speculate that several factors could contribute to the high prevalence of the metabolic syndrome found in our patients. First, the high prevalence of the metabolic syndrome could be related to the complex syndrome of anterior pituitary deficiencies. In addition, rhGH replacement does not mimic physiological GH secretion, which could contribute to the limited effects. Moreover, the putative cause of the metabolic syndrome is under debate, but abdominal obesity has been put forward as one of the main players. RhGH replacement alone might not be sufficient to fully reverse the altered body composition seen in GHD due to concomitant effects of modern-day lifestyle. Indeed, BMI increased during our study. Lastly, subtle changes in hypothalamic function (**Chapter 9 and 16**) could contribute to the derangements in body fat, sympathetic/ parasympathetic nervous system and lipid concentrations contributing to the adverse risk profile found in our patients.

It needs to be established whether adult GHD patients with the metabolic syndrome have the same risks for cardiovascular morbidity and mortality compared with those with the metabolic syndrome in the general population. Moreover, the pathogenesis of the metabolic syndrome might be different in our patients compared to the general population. The effect of GHD and rhGH replacement on insulin sensitivity might influence the prevalence of the metabolic syndrome in our patients. Data so far on insulin resistance during rhGH replacement are conflicting, but some studies have pointed towards an improved insulin sensitivity during long-term rhGH replacement (88), which could be attributed to favourable changes in body composition. Furthermore, it remains to be studied in prospective trials if GHD adults may benefit from more aggressive antihypertensive and lipid-lowering therapy and life style intervention to reverse the metabolic abnormalities seen in adult GHD.

Thus, the prevalence of the metabolic syndrome in our GHD adults is significantly higher compared to the general population, irrespective of rhGH replacement. Apparently, appropriate substitution of rhGH and other hormones in adult patients with GHD is insufficient to improve this adverse cardiovascular risk profile and these patients might thus benefit from additional treatment to reduce cardiovascular risk profile.

It is important to note that several factors influence the efficacy of rhGH replacement in GHD (**Chapter 13 and 14**). Women with GHD using oral estradiol treatment require higher rhGH doses to achieve similar insulin-like growth factor (IGF)-I levels compared with men and women on transdermal estradiol replacement (90-92). The aim of this study described in **Chapter 13** was to evaluate the effects of oral versus transdermal estrogen administration aimed at similar plasma estradiol levels on IGF-I, IGF binding protein-3 and SHBG concentrations. We designed a parallel cross-over study in which two groups women with fixed and stable rhGH replacement passed through four different estradiol treatment schemes (2 and 4 mg oral and 50 and 100 µg 17β-transdermal estradiol) with a duration of 4 cycles each to ensure a new steady state. One

group was treated with oral followed by transdermal estrogen and the other group was treated with transdermal followed by oral estrogen.

Estradiol concentrations were lowest during 50 µg transdermal and highest during 4 mg oral estradiol. Estradiol concentrations did not differ during 100 µg transdermal and 2 mg oral treatment.

Oral administration of estradiol resulted in lower IGF-I levels compared with transdermal administration, in accordance with previous studies that were not aimed at achieving comparable estradiol concentrations (93;94). In addition, despite similar estradiol concentrations, IGF-I levels were higher during transdermal administration of 100 µg estradiol compared with oral administration of 2 mg estradiol. Therefore, the route of estradiol administration is a determinant of IGF-I levels.

From a cost-effective point of view of GH substitution, transdermal estrogen replacement is preferred. After a switch from oral to transdermal estrogen replacement patients require ~ 0.3 mg GH less per day which on a nation-wide scale is a considerable cost reduction (95). Leung *et al.* have calculated a cost reduction for the USA population of $110 billion or approximately $4400 per patient (95). In addition, recent data suggest that increased estrogen exposure to the liver during oral compared to transdermal substitution elevates CRP and coagulation markers which could in their turn possibly influence cardiovascular risk factors (96).

Thus, the route of estrogen administration is a determinant of serum IGF-I concentrations in adult women with GHD and gonadotropin deficiency with IGF-I concentrations that are higher during transdermal compared to oral replacement with similar serum estrogen concentrations. Women on transdermal estrogen replacement require lower doses of rhGH replacement to achieve similar IGF-I concentrations, which is beneficial from a cost-effective point of view.

In children, a common polymorphism of the GH receptor (exon-3 deletion, d3GHR) increases the response to rhGH replacement (97;98). In **Chapter 14** we describe a study aimed at evaluating the effects of this polymorphism on the response to rhGH replacement in adults. We designed a prospective intervention study with rhGH during 1 year and in a subset of patients during 5 years. The presence of the d3GHR variant was established in GHD patients and linked to short-term and long-term effects of rhGH replacement on IGF-I, lipid metabolism, anthropometric parameters, and bone mineral density.

The increase in IGF-I levels was remarkably higher during short-term rhGH replacement in patients bearing at least one allele of the d3GHR compared to patients bearing two wildtype alleles despite the fact that patients were treated with exactly the same dose of rhGH. This increased IGF-I response is in line with the enhanced IGF-I generation upon stimulation with rhGH during an IGF-I generation test in children with idiopathic short stature bearing the d3GHR allele (99). In patients with acromegaly, a lower GH concentration was required in carriers of the d3GHR allele to produce a given increase in serum IGF-I concentrations (100). After long-term rhGH replacement, however, we did not observe pharmacogenetic effects of the d3GHR and

rhGH on IGF-I levels. We speculate that downregulation of the GH-IGF-I system via negative feedback mechanisms might be involved to explain this discrepancy between short- and long term treatment with rhGH. Additionally, the fact that rhGH doses were individualized to achieve normal IGF-I levels could explain the lack of correlation between the GHR polymorphism and specific long-term endpoints.

In addition to these pharmacogenetic differences in IGF-I increase during short-term rhGH replacement between the two different genotypes, lipid parameters were differentially influenced by short-term rhGH replacement. The decrease in total cholesterol and LDL cholesterol during short-term rhGH replacement was significantly lower in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles. Moreover, the increase in HDL cholesterol during rhGH replacement was significantly higher in patients bearing at least one d3GHR allele compared to patients bearing two wildtype alleles.

GH and IGF-I both have effects on lipid metabolism. In addition to stimulating lipolysis and thereby increasing plasma free fatty acid availability, GH increases the number and activity of hepatic LDL receptors, which enables LDL catabolism (101). In accordance, GH treatment in mice with genetic LDL receptor defects does not lower plasma LDL concentrations (102). GH also increases the activity of cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid synthesis (103). These effects contribute to the decrease of total cholesterol and LDL cholesterol seen during short-term as well as long-term rhGH replacement (**Chapter 11**). On the other hand, growth hormone enhances the expression of mRNA of sterol regulatory element-binding protein 1c (SREBP-1c), involved in hepatic lipogenesis (104). Furthermore, IGF-I suppresses scavenger receptor of class BI (SR-BI) (105). The SR-BI is expressed in liver and steroidogenic tissues and clears the HDL cholesterol from the circulation (105). These two latter effects of GH and IGF-I on lipogenesis and HDL expression in light of the enhanced signal transduction of the d3GHR variant and the increased IGF-I response in our patients, might thus contribute to the differential effects of the genotype on short-term rhGH replacement effects on lipid metabolism. In the long-term, these effects might be overruled by negative feedback signals due to changes in fat mass during rhGH replacement (61;89). Detailed studies on lipid metabolism in adult patients with the d3GHR genotype compared to patients with the wildtype genotype are warranted.

Thus, the results of this study suggest that the d3GHR genotype could contribute, at least for some parameters, to the inter-individual differences observed during rhGH replacement in adults with GHD.

IV. STUDIES ON QUALITY OF LIFE IN PATIENTS WITH PITUITARY DISEASE

Evaluation of QoL is becoming an increasingly important tool in medical practice. We used QoL evaluation to take a careful look at the impact of pituitary diseases on well-being and general functioning.

QoL is impaired in patients treated for pituitary adenomas (106). However, differences in age and gender distributions hamper a proper comparison of the disease specific effects of different pituitary tumors on QoL. Therefore, in **Chapter 15** we compared age- and gender-specific standard deviations scores (Z scores) of QoL parameters in patients treated for pituitary adenomas.

We determined Z scores for health-related questionnaires (HADS, MFI-20, NHP, SF-36) in patients during long-term follow-up after treatment for pituitary adenomas. Z scores were calculated by comparing the data of 403 patients (acromegaly, n=118), Cushing's disease (n=58), prolactinoma (n=128), non-functioning macroadenoma (n=99)) with a control population (n=440) for each subscales of the questionnaires and for total QoL score.

All subscales of the questionnaires and the total QoL score were negatively affected in patients compared to controls. Comparing the Z scores, patients treated for acromegaly reported more impairment in physical ability and functioning and more bodily pain compared to patients treated for non-functioning macroadenoma and patients treated for prolactinoma (Figure 1). Patients with Cushing's disease reported impairment in physical functioning compared to patients treated for non-functioning macroadenoma. Linear regression analysis with the questionnaire scores as dependent and age, gender and pituitary disease as independent

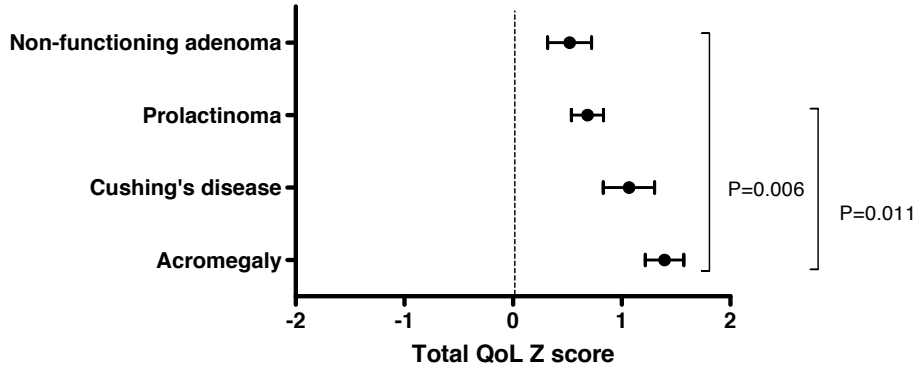

Figure 18/1: Chapter 15: Total quality of life Z score (mean ± SD) in patients treated for acromegaly (n=118), Cushing's disease (n=58), prolactinoma (n=128), and non-functioning macroadenoma (n=99). A higher Z score denotes a decreased overall quality of life. Perceived quality of life is significantly different between the groups (P=0.003) and is especially decreased in patients treated for acromegaly compared to patients treated for non-functioning macroadenoma (P=0.006) and patients treated for prolactinoma (P=0.011).

variables confirmed these findings. Additionally, Cushing's disease was associated with increased anxiety. Hypopituitarism negatively influenced multiple aspects of QoL.

In this study, in a very large cohort of patients during long-term follow-up after treatment for different pituitary adenomas, we confirmed that patients with pituitary adenomas suffer from considerably impaired QoL compared to healthy subjects (14;16;107-111). The large number of included patients, representing groups with different pituitary tumors, and the specific statistical approach enabled to analyze both general effects of pituitary tumors on QoL as well as the disease-specific effects of individual pituitary adenomas on QoL. We found that patients with acromegaly had the largest impairment in QoL compared with the other patients with other pituitary adenomas. These differences were mostly due to impairment in physical performance scales and the increase in bodily pain experienced by patients with acromegaly. Patients with Cushing's disease also had impairment in physical functioning compared to patients treated for non-functioning macroadenoma. These data indicate that QoL is impaired during long-term follow-up after treatment of pituitary adenomas in general. Moreover, there are disease specific impairments in physical functioning (acromegaly and Cushing's disease) and bodily pain (acromegaly). Additionally, linear regression analysis with adjustment for age and gender confirmed these data and extended the disease specific impairments to increased anxiety in patients with Cushing's disease.

The impairment of QoL in acromegaly with respect to physical performance scales and to bodily pain is in line with data in a large heterogeneous cohort of 231 patients with active and inactive acromegaly (14) and with a study in another cohort of 39 patients with acromegaly (106). This decreased QoL in patients long-term cured from acromegaly was strongly associated with persisting joint-related co-morbidity (112). Osteoarticular manifestations are present in the great majority of patients at presentation and were also found to be increased compared to the general healthy population in patients with long-term successful biochemical control of acromegaly (112).

In Cushing's disease, both impaired physical functioning and anxiety were increased. This is in line with previous reports on QoL in patients with Cushing's syndrome (113) and QoL after bilateral adrenalectomy for Cushing's disease (114;115). Moreover, in comparison with other pituitary adenomas, patients with Cushing's disease were the most severely affected in all measures of QoL of the SF-36 (106). In addition, Cushing's disease was associated with increased anxiety. Supraphysiological levels of cortisol can induce psychiatric, psychological, emotional, and cognitive disturbances, which can persist even after cure of Cushing's syndrome (116-118). Although data on putative effects of hypercortisolism on brain structures are scarce, Cushing's disease is associated with reduced hippocampal volume (119;120). This cerebral atrophy is partially reversible on MRI after long-term correction of hypercortisolism. However, it is not known whether the neural changes are fully reversible and/ or correlated with neuropsychological improvement.

In conclusion, QoL is impaired in patients during long-term follow-up after treatment of pituitary adenomas. Patients with pituitary adenomas should be informed on these persistent adverse effects of their disease on QoL to prevent inappropriate expectations with respect to the long-term results of treatment.

In patients treated for non-functioning pituitary macroadenoma (NFMA) and craniopharyngioma increased fatigue scores on QoL have been reported. Because this may be related to altered sleep patterns, we evaluated daytime sleepiness and sleep patterns in patients successfully treated for NFMA and craniopharyngioma in our center (**Chapter 15 and 16**).

In patients treated for NFMA increased fatigue scores on QoL evaluation have been reported (121). Because this may be related to altered sleep patterns, we evaluated daytime sleepiness and sleep patterns in patients successfully treated for NFMA in our center.

We assessed sleepiness and sleep patterns in adult patients in remission of NFMA during long-term follow up after surgery. A subgroup was treated with additional radiotherapy. We used two validated questionnaires for sleep parameters (Epworth sleepiness score and Münchener Chronotype Questionnaire) and four validated questionnaires for quality of life (HADS, MFI-20, NHP, SF-36). Patient outcomes were compared to healthy controls.

Sleep duration and timing of sleep were not affected compared to healthy controls. However, sleepiness score was increased in patients compared to controls, reflecting increased daytime sleepiness in patients. There were no correlations between any of the sleep pattern parameters (duration, onset, rise time or midsleep) and sleepiness scores. Sleepiness scores were significantly correlated to 15 of the 21 quality of life parameters, whereas sleep patterns were not. Sleep timing was influenced by previous radiotherapy and sleep duration was negatively affected by panhypopituitarism.

The daytime sleepiness scores in our patients treated for NFMA were comparable to scores found in patients with other pituitary tumors or cerebral diseases such as acromegaly (122), craniopharyngeoma (123), hypothalamic tumors (123), subarachnoid haemorrhage (124), or traumatic brain injury (125), indicative for the relationship between cerebral disease and increased daytime sleepiness. Severely increased daytime sleepiness (ESS scores above 10) was noted in almost one third of our patients with NFMA in line with findings in patients with craniopharyngeoma (126). Nonetheless, we did not find altered sleep patterns in our NFMA patients suggesting that the increased experienced daytime sleepiness and reported fatigue scores of the quality of life questionnaires are not due to major alterations in sleep duration or timing of sleep. This unaffected sleep duration, however, was in contrast to findings in patients after pituitary/ hypothalamic surgery or cranial radiotherapy for other tumors, in whom sleep duration was increased (127;128).

The increased daytime sleepiness and increased fatigue scores seen in our patients treated for NFMA could point towards possibly impaired sleep quality in patients with NFMA

despite normal sleep patterns. Indeed, sleep quality measured with polysomnography is found to be altered in patients with Cushing's disease (129), acromegaly (130), prolactinoma (131), and patients with craniopharyngioma (126). Nonetheless, it cannot be excluded that additional disturbances, for example in melatonin secretion, lead to increased daytime sleepiness in NFMA patients. In fact, it has been suggested that reduced nocturnal melatonin secretion may lead to increased daytime sleepiness in childhood craniopharyngioma patients (123;132). Thus, in addition to direct measurement of melatonin secretion, future studies to elucidate the increased daytime sleepiness should include polysomnography and additional (objective) tests of increased daytime sleepiness, considering the ample aspects of increased daytime sleepiness that can be studied besides the ESS.

Thus, we found self-reported increased daytime sleepiness despite normal sleep patterns in patients treated for NFMA, which was associated with an impaired QoL. Several factors during the long-term follow-up of these patients contribute to alterations in sleep patterns.

Adults patients previously treated for craniopharyngioma have increased general and physical fatigue compared to healthy controls (133). This could be related to disturbed sleep patterns. The aim of the study described in **Chapter 17** was to compare sleepiness and sleep patterns in those patients to healthy controls and to patients treated for non-functioning macroadenomas (NFMA) of the pituitary.

Sleepiness and sleep patterns were assessed in 27 adult patients after long-term follow up and compared to 50 healthy controls and 38 age-, gender- and BMI matched patients with NFMA.

Sleep patterns (onset, sleep timing, duration and rise time) were not different between the three groups. However, daytime sleepiness scores were increased in patients treated for craniopharyngioma compared to healthy controls but not different from patients with NFMA. Thirty-three percent of patients with craniopharyngiomas had ESS scores above 10 compared to 8% of healthy controls, indicating severe daytime hypersomnolence. Neither type of surgery, previous radiotherapy or age at diagnosis influenced the sleepiness scores in patients with craniopharyngioma.

Multiple long-term sequelae of treatment of craniopharyngioma, such as hyperphagia, obesity and decreased sympathetic tone, have been attributed to damage to surrounding tissues including hypothalamic nuclei (134;135). The hypothalamus has also been identified as the main regulatory center of sleep: the suprachiasmatic nucleus (SCN) is considered to be the central circadian pacemaker of the body with one of the circadian outputs formed by the regulation of circadian variations in melatonin secretion by the pineal gland (136). In patients treated for craniopharyngioma in childhood, reduced nocturnal melatonin concentrations are associated with increased daytime somnolence (123). In addition, the multiple sleep latency test, a standardized test of daytime somnolence, showed severe daytime somnolence in 5 children who had been treated by hypothalamic/ pituitary surgery (127). In accordance, self-reported

general and physical fatigue is increased in adult patients treated for craniopharyngioma compared to healthy controls (133). In the present study, the previous findings of increased daytime somnolence obtained in children treated for craniopharyngioma were extended to and confirmed in adult patients with caniopharyngioma.

In conclusion, patients treated for craniopharyngioma have increased daytime somnolence despite normal sleep patterns compared to healthy subjects. The results indicate that increased daytime somnolence is a general consequence of large tumors and/or their treatment in the hypothalamic/pituitary region, rather than a specific feature of craniopharyngiomas *per se*.

V. SUMMARY AND CONCLUDING REMARKS

The pituitary gland plays a central role in the endocrine system and pathophysiology of this organ disrupts the endocrine system in its very basis. In general, the approaches currently available such as surgery, radiotherapy and drug treatment enable adequate control of the pituitary disease in a majority of patients. However, the studies described in this thesis document that there are long term consequences of the successful treatment of these tumors. We are able to cure these diseases, but the patients are left with the long term adverse effects.

In patients with acromegaly, one should be aware of long-term consequences of GH excess on end-organs of GH action such as the heart. It is also important to note that the various treatment options for acromegaly have their own impact on patient well-being and functioning. These include the effects of radiotherapy on QoL which might be due to subtle alterations in hypothalamic functioning and the effects of somatostatin analogs on sleep characteristics. In addition, radiotherapy for acromegaly can lead to GHD. This GHD impacts on cardiac function, but the effects of rhGH replacement in patients with GHD after acromegaly appear to be limited.

RhGH replacement has beneficial effects on vasculature and cardiovascular risk factors. Even though rhGH replacement has beneficial effects on some cardiovascular risk factors, the overall effects seem to be limited. Moreover, we documented factors that influence the efficacy of rhGH replacement such as concomitant estrogen replacement in women and a common polymorphism of the GH receptor.

Finally, QoL is impaired in patients previously treated for pituitary tumors. Impaired QoL is associated with increased sleepiness. Indeed, pituitary tumors and/or their treatments have specific consequences for sleep patterns.

It is essential to recognize these long-term consequences of pituitary diseases in order to establish appropriate follow-up and care in these patients to limit the persisting complex morbidity. Both doctors and patients with pituitary tumors should be aware of these persisting

consequences to prevent inappropriate expectations with respect to the long-term results of treatment.

REFERENCE LIST

1. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
2. Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, Vitale G, Bonaduce D, Lombardi G 2003 High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 88:3196-3201
3. Singh JP, Evans JC, Levy D, Larson MG, Freed LA, Fuller DL, Lehman B, Benjamin EJ 1999 Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). Am J Cardiol 83:897-902
4. Rippe J, Fishbein MC, Carabello B, Angoff G, Sloss L, Collins JJ, Jr., Alpert JS 1980 Primary myxomatous degeneration of cardiac valves. Clinical, pathological, haemodynamic, and echocardiographic profile. Br Heart J 44:621-629
5. Enriquez-Sarano M, Avierinos JF, Messika-Zeitoun D, Detaint D, Capps M, Nkomo V, Scott C, Schaff HV, Tajik AJ 2005 Quantitative determinants of the outcome of asymptomatic mitral regurgitation. N Engl J Med 352:875-883
6. Thompson BJ, Shang CA, Waters MJ 2000 Identification of genes induced by growth hormone in rat liver using cDNA arrays. Endocrinology 141:4321-4324
7. van Karnebeek CD, Naeff MS, Mulder BJ, Hennekam RC, Offringa M 2001 Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child 84:129-137
8. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
9. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F 2005 Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 6:2393-2405
10. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ 2005 Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365:1644-1646
11. Melmed S 2006 Medical progress: Acromegaly. N Engl J Med 355:2558-2573
12. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO 2001 Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358:1754-1759
13. Hua SC, Yan YH, Chang TC 2006 Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. Eur J Endocrinol 155:831-837
14. Kauppinen-Makelin R, Sane T, Sintonen H, Markkanen H, Valimaki MJ, Loyttyniemi E, Niskanen L, Reunanen A, Stenman UH 2006 Quality of life in treated patients with acromegaly. J Clin Endocrinol Metab 91:3891-3896
15. Trepp R, Everts R, Stettler C, Fischli S, Allemann S, Webb SM, Christ ER 2005 Assessment of quality of life in patients with uncontrolled vs. controlled acromegaly using the Acromegaly Quality of Life Questionnaire (AcroQoL). Clin Endocrinol (Oxf) 63:103-110
16. Webb SM, Badia X, Surinach NL 2006 Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study. Eur J Endocrinol 155:269-277
17. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ 2005 Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. J Clin Endocrinol Metab 90:3337-3341
18. Paisley AN, Rowles SV, Roberts ME, Webb SM, Badia X, Prieto L, Shalet SM, Trainer PJ 2007 Treatment of acromegaly improves quality of life, measured by AcroQol. Clin Endocrinol (Oxf) 67:358-362

19. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F 2004 Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. J Clin Endocrinol Metab 89:5369-5376
20. Spiegler BJ, Bouffet E, Greenberg ML, Rutka JT, Mabbott DJ 2004 Change in neurocognitive functioning after treatment with cranial radiation in childhood. J Clin Oncol 22:706-713
21. Herrmann BL, Wessendorf TE, Ajaj W, Kahlke S, Teschler H, Mann K 2004 Effects of octreotide on sleep apnoea and tongue volume (magnetic resonance imaging) in patients with acromegaly. Eur J Endocrinol 151:309-315
22. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329
23. Saeki N, Isono S, Tanaka A, Nishino T, Higuchi Y, Uchino Y, Iuchi T, Murai H, Tatsuno I, Yasuda T, Yamaura A 2000 Pre-and post-operative respiratory assessment of acromegalics with sleep apnea--bedside oximetric study for transsphenoidal approach. Endocr J 47 Suppl:S61-S64
24. Buyse B, Michiels E, Bouillon R, Bobbaers H, Demedts M 1997 Relief of sleep apnoea after treatment of acromegaly: report of three cases and review of the literature. Eur Respir J 10:1401-1404
25. Grunstein RR, Ho KK, Sullivan CE 1994 Effect of octreotide, a somatostatin analog, on sleep apnea in patients with acromegaly. Ann Intern Med 121:478-483
26. Leibowitz G, Shapiro MS, Salameh M, Glaser B 1994 Improvement of sleep apnoea due to acromegaly during short-term treatment with octreotide. J Intern Med 236:231-235
27. Rosenow F, Reuter S, Deuss U, Szelies B, Hilgers RD, Winkelmann W, Heiss WD 1996 Sleep apnoea in treated acromegaly: relative frequency and predisposing factors. Clin Endocrinol (Oxf) 45:563-569
28. Frieboes RM, Murck H, Schier T, Holsboer F, Steiger A 1997 Somatostatin impairs sleep in elderly human subjects. Neuropsychopharmacology 16:339-345
29. Steiger A, Guldner J, Hemmeter U, Rothe B, Wiedemann K, Holsboer F 1992 Effects of growth hormone-releasing hormone and somatostatin on sleep EEG and nocturnal hormone secretion in male controls. Neuroendocrinology 56:566-573
30. Beranek L, Obal F, Jr., Taishi P, Bodosi B, Laczi F, Krueger JM 1997 Changes in rat sleep after single and repeated injections of the long-acting somatostatin analog octreotide. Am J Physiol 273:R1484-R1491
31. Ziegenbein M, Held K, Kuenzel HE, Murck H, Antonijevic IA, Steiger A 2004 The somatostatin analogue octreotide impairs sleep and decreases EEG sigma power in young male subjects. Neuropsychopharmacology 29:146-151
32. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
33. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
34. Ahmed SR, Shalet SM 1984 Hypothalamic growth hormone releasing factor deficiency following cranial irradiation. Clin Endocrinol (Oxf) 21:483-488
35. Blacklay A, Grossman A, Ross RJ, Savage MO, Davies PS, Plowman PN, Coy DH, Besser GM 1986 Cranial irradiation for cerebral and nasopharyngeal tumours in children: evidence for the production of a hypothalamic defect in growth hormone release. J Endocrinol 108:25-29
36. Barkan AL, Stred SE, Reno K, Markovs M, Hopwood NJ, Kelch RP, Beitins IZ 1989 Increased growth hormone pulse frequency in acromegaly. J Clin Endocrinol Metab 69:1225-1233
37. Hartman ML, Veldhuis JD, Vance ML, Faria AC, Furlanetto RW, Thorner MO 1990 Somatotropin pulse frequency and basal concentrations are increased in acromegaly and are reduced by successful therapy. J Clin Endocrinol Metab 70:1375-1384
38. van den BG, Frolich M, Veldhuis JD, Roelfsema F 1994 Growth hormone secretion in recently operated acromegalic patients. J Clin Endocrinol Metab 79:1706-1715
39. Roelfsema F, Biermasz NR, Veldhuis JD 2002 Pulsatile, nyctohemeral and entropic characteristics of GH secretion in adult GH-deficient patients: selectively decreased pulsatile release and increased

secretory disorderliness with preservation of diurnal timing and gender distinctions. Clin Endocrinol (Oxf) 56:79-87
40. Aimaretti G, Corneli G, Razzore P, Bellone S, Baffoni C, Arvat E, Camanni F, Ghigo E 1998 Comparison between insulin-induced hypoglycemia and growth hormone (GH)-releasing hormone + arginine as provocative tests for the diagnosis of GH deficiency in adults. J Clin Endocrinol Metab 83:1615-1618
41. Alba-Roth J, Muller OA, Schopohl J, von Werder K 1988 Arginine stimulates growth hormone secretion by suppressing endogenous somatostatin secretion. J Clin Endocrinol Metab 67:1186-1189
42. Grossman A, Savage MO, Lytras N, Preece MA, Sueiras-Diaz J, Coy DH, Rees LH, Besser GM 1984 Responses to analogues of growth hormone-releasing hormone in normal subjects, and in growth-hormone deficient children and young adults. Clin Endocrinol (Oxf) 21:321-330
43. Jaffe CA, DeMott-Friberg R, Barkan AL 1996 Endogenous growth hormone (GH)-releasing hormone is required for GH responses to pharmacological stimuli. J Clin Invest 97:934-940
44. Murray RD, Peacey SR, Rahim A, Toogood AA, Thorner MO, Shalet SM 2001 The diagnosis of growth hormone deficiency (GHD) in successfully treated acromegalic patients. Clin Endocrinol (Oxf) 54:37-44
45. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
46. Amato G, Carella C, Fazio S, La Montagna G, Cittadini A, Sabatini D, Marciano-Mone C, Sacca L, Bellastella A 1993 Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J Clin Endocrinol Metab 77:1671-1676
47. Colao A, Cuocolo A, Di Somma C, Cerbone G, Della Morte AM, Nicolai E, Lucci R, Salvatore M, Lombardi G 1999 Impaired cardiac performance in elderly patients with growth hormone deficiency. J Clin Endocrinol Metab 84:3950-3955
48. Colao A, Di Somma C, Cuocolo A, Filippella M, Rota F, Acampa W, Savastano S, Salvatore M, Lombardi G 2004 The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. J Clin Endocrinol Metab 89:5998-6004
49. Merola B, Cittadini A, Colao A, Longobardi S, Fazio S, Sabatini D, Sacca L, Lombardi G 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab 77:1658-1661
50. Sartorio A, Ferrero S, Conti A, Bragato R, Malfatto G, Leonetti G, Faglia G 1997 Adults with childhood-onset growth hormone deficiency: effects of growth hormone treatment on cardiac structure. J Intern Med 241:515-520
51. Colao A, Cuocolo A, Di Somma C, Cerbone G, Morte AM, Pivonello R, Nicolai E, Salvatore M, Lombardi G 2000 Does the age of onset of growth hormone deficiency affect cardiac performance? A radionuclide angiography study. Clin Endocrinol (Oxf) 52:447-455
52. Erdogan D, Tukek T, Aral F, Oflaz H, Ozaydin M, Kocaman O, Akkaya V, Goren T, Molvalilar S 2004 Structural, functional and autonomic changes in the cardiovascular system in growth hormone deficient patients. Ann Noninvasive Electrocardiol 9:19-23
53. Yurci A, Oflaz H, Meric M, Ozbey N 2005 Mitral and tricuspid annular velocities determined by Doppler tissue imaging in hypopituitary, growth hormone-deficient patients. Horm Res 64:107-114
54. van Thiel SW, Bax JJ, Biermasz NR, Holman ER, Poldermans D, Roelfsema F, Lamb HJ, van der Wall EE, Smit JW, Romijn JA, Pereira AM 2005 Persistent diastolic dysfunction despite successful long-term octreotide treatment in acromegaly. Eur J Endocrinol 153:231-238
55. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 156:55-64
56. Maison P, Chanson P 2003 Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 108:2648-2652

57. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
58. Deijen JB, Arwert LI, Witlox J, Drent ML 2005 Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes 3:63
59. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
60. Davidson P, Milne R, Chase D, Cooper C 2004 Growth hormone replacement in adults and bone mineral density: a systematic review and meta-analysis. Clin Endocrinol (Oxf) 60:92-98
61. Gotherstrom G, Svensson J, Koranyi J, Alpsten M, Bosaeus I, Bengtsson B, Johannsson G 2001 A prospective study of 5 years of GH replacement therapy in GH-deficient adults: sustained effects on body composition, bone mass, and metabolic indices. J Clin Endocrinol Metab 86:4657-4665
62. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 A 10-year, prospective study of the metabolic effects of growth hormone replacement in adults. J Clin Endocrinol Metab 92:1442-1445
63. Feldt-Rasmussen U, Abs R, Bengtsson BA, Bennmarker H, Bramnert M, Hernberg-Stahl E, Monson JP, Westberg B, Wilton P, Wuster C 2002 Growth hormone deficiency and replacement in hypopituitary patients previously treated for acromegaly or Cushing's disease. Eur J Endocrinol 146:67-74
64. Norrman LL, Johannsson G, Sunnerhagen KS, Svensson J 2008 Baseline Characteristics and the Effects of Two Years of Growth Hormone (GH) Replacement Therapy in Adults with GH Deficiency Previously Treated for Acromegaly. J Clin Endocrinol Metab 93:2531-2538.
65. Biermasz NR, Hamdy NA, Pereira AM, Romijn JA, Roelfsema F 2005 Long-term maintenance of the anabolic effects of GH on the skeleton in successfully treated patients with acromegaly. Eur J Endocrinol 152:53-60
66. Sinisi AA, Pasquali D, D'Apuzzo A, Esposito D, Venditto T, Criscuolo T, De Bellis A, Bellastella A 1997 Twenty-four hour melatonin pattern in acromegaly: effect of acute octreotide administration. J Endocrinol Invest 20:128-133
67. Terzolo M, Piovesan A, Ali A, Codegone A, Pia A, Reimondo G, Torta M, Paccotti P, Borretta G, Angeli A 1995 Circadian profile of serum melatonin in patients with Cushing's syndrome or acromegaly. J Endocrinol Invest 18:17-24
68. Lissoni P, Mainini E, Rovelli F, Mazzi C, Ardizzoia A, Gelosa M, Capra M, Rivolta MR 1992 A clinical study of the pineal hormone melatonin in patients with growth hormone or prolactin secreting pituitary tumours. Eur J Med 1:407-410
69. Piovesan A, Terzolo M, Borretta G, Torta M, Buniva T, Osella G, Paccotti P, Angeli A 1990 Circadian profile of serum melatonin in Cushing's disease and acromegaly. Chronobiol Int 7:259-261
70. Touitou Y 1993 Melatonin in patients with pituitary tumours. Eur J Med 2:63
71. Burgess HJ, Savic N, Sletten T, Roach G, Gilbert SS, Dawson D 2003 The relationship between the dim light melatonin onset and sleep on a regular schedule in young healthy adults. Behav Sleep Med 1:102-114
72. Vaccarino FJ, Sovran P, Baird JP, Ralph MR 1995 Growth hormone-releasing hormone mediates feeding-specific feedback to the suprachiasmatic circadian clock. Peptides 16:595-598
73. Bates AS, Van't Hoff W, Jones PJ, Clayton RN 1996 The effect of hypopituitarism on life expectancy. J Clin Endocrinol Metab 81:1169-1172
74. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society Workshop on Adult Growth Hormone Deficiency 1998 J Clin Endocrinol Metab 83:379-381
75. Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ 2004 Endothelial progenitor cells: more than an inflammatory response? Arterioscler Thromb Vasc Biol 24:834-838

76. Thum T, Fleissner F, Klink I, Tsikas D, Jakob M, Bauersachs J, Stichtenoth DO 2007 Growth hormone treatment improves markers of systemic nitric oxide bioavailability via insulin-like growth factor-1. J Clin Endocrinol Metab 92: 4172-4179
77. Carlo-Stella C, Di Nicola M, Milani R, Guidetti A, Magni M, Milanesi M, Longoni P, Matteucci P, Formelli F, Ravagnani F, Corradini P, Gianni AM 2004 Use of recombinant human growth hormone (rhGH) plus recombinant human granulocyte colony-stimulating factor (rhG-CSF) for the mobilization and collection of CD34+ cells in poor mobilizers. Blood 103:3287-3295
78. Hanley MB, Napolitano LA, McCune JM 2005 Growth hormone-induced stimulation of multilineage human hematopoiesis. Stem Cells 23:1170-1179
79. Merchav S 1998 The haematopoietic effects of growth hormone and insulin-like growth factor-I. J Pediatr Endocrinol Metab 11:677-685
80. Chang KH, Chan-Ling T, McFarland EL, Afzal A, Pan H, Baxter LC, Shaw LC, Caballero S, Sengupta N, Li CS, Sullivan SM, Grant MB 2007 IGF binding protein-3 regulates hematopoietic stem cell and endothelial precursor cell function during vascular development. Proc Natl Acad Sci U S A 104:10595-10600
81. Evans LM, Davies JS, Anderson RA, Ellis GR, Jackson SK, Lewis MJ, Frenneaux MP, Rees A, Scanlon MF 2000 The effect of GH replacement therapy on endothelial function and oxidative stress in adult growth hormone deficiency. Eur J Endocrinol 142:254-262
82. Abdu TA, Elhadd TA, Buch H, Barton D, Neary R, Clayton RN 2004 Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk. Clin Endocrinol (Oxf) 61:387-393
83. Pfeifer M, Verhovec R, Zizek B 1999 Growth hormone (GH) and atherosclerosis: changes in morphology and function of major arteries during GH treatment. Growth Horm IGF Res 9 Suppl A:25-30
84. Tsukahara H, Gordienko DV, Tonshoff B, Gelato MC, Goligorsky MS 1994 Direct demonstration of insulin-like growth factor-I-induced nitric oxide production by endothelial cells. Kidney Int 45:598-604
85. Boger RH, Skamira C, Bode-Boger SM, Brabant G, von zur MA, Frolich JC 1996 Nitric oxide may mediate the hemodynamic effects of recombinant growth hormone in patients with acquired growth hormone deficiency. A double-blind, placebo-controlled study. J Clin Invest 98:2706-2713
86. Chrisoulidou A, Beshyah SA, Rutherford O, Spinks TJ, Mayet J, Kyd P, Anyaoku V, Haida A, Ariff B, Murphy M, Thomas E, Robinson S, Foale R, Johnston DG 2000 Effects of 7 years of growth hormone replacement therapy in hypopituitary adults. J Clin Endocrinol Metab 85:3762-3769
87. Gibney J, Wallace JD, Spinks T, Schnorr L, Ranicar A, Cuneo RC, Lockhart S, Burnand KG, Salomon F, Sonksen PH, Russell-Jones D 1999 The effects of 10 years of recombinant human growth hormone (GH) in adult GH-deficient patients. J Clin Endocrinol Metab 84:2596-2602
88. Svensson J, Fowelin J, Landin K, Bengtsson BA, Johansson JO 2002 Effects of seven years of GH-replacement therapy on insulin sensitivity in GH-deficient adults. J Clin Endocrinol Metab 87:2121-2127
89. Svensson J, Stibrant SK, Johannsson G 2003 Five years of growth hormone replacement therapy in adults: age- and gender-related changes in isometric and isokinetic muscle strength. J Clin Endocrinol Metab 88:2061-2069
90. Cook DM, Ludlam WH, Cook MB 1999 Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 84:3956-3960
91. Johansson AG, Engstrom BE, Ljunghall S, Karlsson FA, Burman P 1999 Gender differences in the effects of long term growth hormone (GH) treatment on bone in adults with GH deficiency. J Clin Endocrinol Metab 84:2002-2007
92. Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG 2000 Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab 85:1121-1125

93. Janssen YJ, Helmerhorst F, Frolich M, Roelfsema F 2000 A switch from oral (2 mg/day) to transdermal (50 microg/day) 17beta-estradiol therapy increases serum insulin-like growth factor-I levels in recombinant human growth hormone (GH)-substituted women with GH deficiency. J Clin Endocrinol Metab 85:464-467
94. Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KK 2001 Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. Am J Physiol Endocrinol Metab 281:E1191-E1196
95. Leung KC, Johannsson G, Leong GM, Ho KK 2004 Estrogen regulation of growth hormone action. Endocr Rev 25:693-721
96. Shifren JL, Rifai N, Desindes S, McIlwain M, Doros G, Mazer NA 2008 A comparison of the short-term effects of oral conjugated equine estrogens versus transdermal estradiol on C-reactive protein, other serum markers of inflammation, and other hepatic proteins in naturally menopausal women. J Clin Endocrinol Metab 93:1702-1710
97. Dos Santos C., Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
98. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ 2006 Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91:1076-1080
99. Toyoshima MT, Castroneves LA, Costalonga EF, Mendonca BB, Arnhold IJ, Jorge AA 2007 Exon 3-deleted genotype of growth hormone receptor (GHRd3) positively influences IGF-1 increase at generation test in children with idiopathic short stature. Clin Endocrinol (Oxf) 67:500-504
100. Schmid C, Krayenbuehl PA, Bernays RL, Zwimpfer C, Maly FE, Wiesli P 2007 Growth hormone (GH) receptor isoform in acromegaly: lower concentrations of GH but not insulin-like growth factor-1 in patients with a genomic deletion of exon 3 in the GH receptor gene. Clin Chem 53:1484-1488
101. Rudling M, Olivecrona H, Eggertsen G, Angelin B 1996 Regulation of rat hepatic low density lipoprotein receptors. In vivo stimulation by growth hormone is not mediated by insulin-like growth factor I. J Clin Invest 97:292-299
102. Lind S, Rudling M, Ericsson S, Olivecrona H, Eriksson M, Borgstrom B, Eggertsen G, Berglund L, Angelin B 2004 Growth hormone induces low-density lipoprotein clearance but not bile acid synthesis in humans. Arterioscler Thromb Vasc Biol 24:349-356
103. Rudling M, Parini P, Angelin B 1997 Growth hormone and bile acid synthesis. Key role for the activity of hepatic microsomal cholesterol 7alpha-hydroxylase in the rat. J Clin Invest 99:2239-2245
104. Ameen C, Linden D, Larsson BM, Mode A, Holmang A, Oscarsson J 2004 Effects of gender and GH secretory pattern on sterol regulatory element-binding protein-1c and its target genes in rat liver. Am J Physiol Endocrinol Metab 287:E1039-E1048
105. Cao WM, Murao K, Imachi H, Yu X, Dobashi H, Yoshida K, Muraoka T, Kotsuna N, Nagao S, Wong NC, Ishida T 2004 Insulin-like growth factor-i regulation of hepatic scavenger receptor class BI. Endocrinology 145:5540-5547
106. Johnson MD, Woodburn CJ, Vance ML 2003 Quality of life in patients with a pituitary adenoma. Pituitary 6:81-87
107. Heald AH, Ghosh S, Bray S, Gibson C, Anderson SG, Buckler H, Fowler HL 2004 Long-term negative impact on quality of life in patients with successfully treated Cushing's disease. Clin Endocrinol (Oxf) 61:458-465
108. Lindsay JR, Nansel T, Baid S, Gumowski J, Nieman LK 2006 Long-term impaired quality of life in Cushing's syndrome despite initial improvement after surgical remission. J Clin Endocrinol Metab 91:447-453

109. Nielsen EH, Lindholm J, Laurberg P, Bjerre P, Christiansen JS, Hagen C, Juul S, Jorgensen J, Kruse A, Stochholm K 2007 Nonfunctioning pituitary adenoma: incidence, causes of death and quality of life in relation to pituitary function. Pituitary 10:67-73
110. Sonino N, Bonnini S, Fallo F, Boscaro M, Fava GA 2006 Personality characteristics and quality of life in patients treated for Cushing's syndrome. Clin Endocrinol (Oxf) 64:314-318
111. van Beek AP, van den Bergh AC, van den Berg LM, van den BG, Keers JC, Langendijk JA, Wolffenbuttel BH 2007 Radiotherapy is not associated with reduced quality of life and cognitive function in patients treated for nonfunctioning pituitary adenoma. Int J Radiat Oncol Biol Phys 68:986-991
112. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F 2005 Morbidity after Long-term Remission for Acromegaly; Persisting Joint-Related Complaints Cause Reduced Quality of Life. J Clin Endocrinol Metab
113. Lindholm J, Juul S, Jorgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, Hagen C, Jorgensen J, Kosteljanetz M, Kristensen L, Laurberg P, Schmidt K, Weeke J 2001 Incidence and late prognosis of cushing's syndrome: a population-based study. J Clin Endocrinol Metab 86:117-123
114. Hawn MT, Cook D, Deveney C, Sheppard BC 2002 Quality of life after laparoscopic bilateral adrenalectomy for Cushing's disease. Surgery 132:1064-1068
115. Nagesser SK, van Seters AP, Kievit J, Hermans J, Krans HM, van de Velde CJ 2000 Long-term results of total adrenalectomy for Cushing's disease. World J Surg 24:108-113
116. Kelly WF, Kelly MJ, Faragher B 1996 A prospective study of psychiatric and psychological aspects of Cushing's syndrome. Clin Endocrinol (Oxf) 45:715-720
117. Sonino N, Fava GA 2001 Psychiatric disorders associated with Cushing's syndrome. Epidemiology, pathophysiology and treatment. CNS Drugs 15:361-373
118. Herbert J, Goodyer IM, Grossman AB, Hastings MH, de Kloet ER, Lightman SL, Lupien SJ, Roozendaal B, Seckl JR 2006 Do corticosteroids damage the brain? J Neuroendocrinol 18:393-411
119. Starkman MN, Giordani B, Gebarski SS, Berent S, Schork MA, Schteingart DE 1999 Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. Biol Psychiatry 46:1595-1602
120. Bourdeau I, Bard C, Noel B, Leclerc I, Cordeau MP, Belair M, Lesage J, Lafontaine L, Lacroix A 2002 Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. J Clin Endocrinol Metab 87:1949-1954
121. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA 2006 Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:3364-3369
122. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329
123. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589
124. Schuiling WJ, Rinkel GJ, Walchenbach R, de Weerd AW 2005 Disorders of sleep and wake in patients after subarachnoid hemorrhage. Stroke 36:578-582
125. Parcell DL, Ponsford JL, Rajaratnam SM, Redman JR 2006 Self-reported changes to nighttime sleep after traumatic brain injury. Arch Phys Med Rehabil 87:278-285
126. Muller HL, Muller-Stover S, Gebhardt U, Kolb R, Sorensen N, Handwerker G 2006 Secondary narcolepsy may be a causative factor of increased daytime sleepiness in obese childhood craniopharyngioma patients. J Pediatr Endocrinol Metab 19 Suppl 1:423-429
127. Snow A, Gozal E, Malhotra A, Tiosano D, Perlman R, Vega C, Shahar E, Gozal D, Hochberg Z, Pillar G 2002 Severe hypersomnolence after pituitary/hypothalamic surgery in adolescents: clinical characteristics and potential mechanisms. Pediatrics 110:e74

128. Van Someren EJ, Swart-Heikens J, Endert E, Bisschop PH, Swaab DF, Bakker PJ, Romijn JA, Fliers E 2004 Long-term effects of cranial irradiation for childhood malignancy on sleep in adulthood. Eur J Endocrinol 150:503-510
129. Shipley JE, Schteingart DE, Tandon R, Starkman MN 1992 Sleep architecture and sleep apnea in patients with Cushing's disease. Sleep 15:514-518
130. Astrom C, Christensen L, Gjerris F, Trojaborg W 1991 Sleep in acromegaly before and after treatment with adenomectomy. Neuroendocrinology 53:328-331
131. Frieboes RM, Murck H, Stalla GK, Antonijevic IA, Steiger A 1998 Enhanced slow wave sleep in patients with prolactinoma. J Clin Endocrinol Metab 83:2706-2710
132. Muller HL, Handwerker G, Wollny B, Faldum A, Sorensen N 2002 Melatonin secretion and increased daytime sleepiness in childhood craniopharyngioma patients. J Clin Endocrinol Metab 87:3993-3996
133. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM 2006 Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 154:483-489
134. Karavitaki N, Cudlip S, Adams CB, Wass JA 2006 Craniopharyngiomas. Endocr Rev 27:371-397
135. Roth CL, Hunneman DH, Gebhardt U, Stoffel-Wagner B, Reinehr T, Muller HL 2007 Reduced sympathetic metabolites in urine of obese patients with craniopharyngioma. Pediatr Res 61:496-501
136. Mignot E, Taheri S, Nishino S 2002 Sleeping with the hypothalamus: emerging therapeutic targets for sleep disorders. Nat Neurosci 5 Suppl:1071-1075

Chapter 19

Nederlandse samenvatting

Dit proefschrift beschrijft onderzoek naar de consequenties op de lange termijn van ziekten van de hypofyse en de behandeling daarvan. Daartoe zijn drie groepen patiënten onderzocht: patiënten met acromegalie, patiënten met groeihormoondeficientie (GHD) en patiënten met verschillende hypofyse-adenomen. Alle patiënten werden behandeld en vervolgd in het Leids Universitair Medisch Centrum.

Hypofyse-adenomen kunnen goed worden behandeld met (combinaties van) chirurgie, radiotherapie en medicijnen. Dit resulteert in de meeste gevallen in stabiele of genezen ziekte. De productie en afgifte van hypofysehormonen kunnen door een hypofyse-adenoom en/of door de behandeling daarvan tekort schieten.. De gevolgen van hypofysaire insufficientie kunnen door substitutie van de betreffende hormonen ondervangen worden.

Desondanks blijkt uit nauwkeurige evaluatie dat deze benadering niet altijd resulteert in optimaal functioneren van de patient. In dit proefschrift hebben we verschillende klinische aspecten bij deze patiënten onderzocht nadat ze vele jaren eerder genezen waren van hun oorspronkelijke hypofyseziekte.

De relatie tussen hartklepinsufficientie en acromegalie is onlangs beschreven in cross-sectionele studies (1;2). Omdat het natuurlijk beloop van deze hartklepafwijkingen onbekend is, hebben we in **Hoofdstuk 2** de relatie tussen hartklepafwijkingen en ziekteactiviteit bestudeerd. De aanwezigheid van hartklepinsufficienties werd geëvalueerd bij 37 patiënten met acromegalie voor en na een periode van 2 jaar. Vijftig procent van de patiënten had actieve acromegalie gedurende de studie en de rest van de patiënten had (biochemische) remissie van de ziekte.

De prevalentie van hartklep-insufficientie nam tijdens de observatieperiode bij de patiënten met actieve acromegalie significant toe van 60% tot bijna 90%. Deze toename was met name te verklaren uit een toename van niet-significante milde insufficientie van de mitraalklep. We vonden daarentegen geen toename van hartkleplijden bij de patiënten met stabiele, gecontroleerde ziekte.

Deze obervationele, longitudinale studie toont dus aan dat de te hoge groeihormoon(GH)-spiegels bij actieve acromegalie hartkleplijden induceert, wat de in cross-sectionele studies beschreven relatie tussen acromegalie en hartklepinsufficientie verder versterkt.

De aortawortel kan worden aangetast bij patiënten met bindweefselziekten leidend tot dilatatie van de aortawortel (3). Uit de observaties van de hartkleppen (**Hoofdstuk 2** en (1;2)) blijkt dat het bindweefsel bij patiënten met acromegalie aangetast is door langdurig te hoge GH-spiegels. Zo induceert GH bijvoorbeeld matrix-metalloproteinases (MMPs), die op hun beurt de compositie van de extracellulaire matrix kunnen veranderen (4).

De diameter van de aortawortel was nog niet eerder zorgvuldig bestudeerd bij patiënten met acromegalie. Aldus beschrijven we in **Hoofdstuk 3** de diameter van de aortawortel op

verschillende niveaus bij 37 patiënten met acromegalie en vergelijken we deze gegevens met gegevens verkregen bij gezonde controle-personen.

We vonden een vergrote diameter van de aortawortel op het niveau van de sino-tubulaire junctie en de aorta ascendens bij patiënten met acromegalie. De diameters op het niveau van de annulus aortae en de sinus aortae waren onveranderd ten opzichte van gezonde controle-personen. Deze studie toont dus dat bij patiënten met acromegalie ook de aortawortel aangetast is naast de al bekende hartklepafwijkingen (**Hoofdstuk 2**, (1;2)).

Alhoewel de diameter van de aortawortel groter is bij patiënten met acromegalie dan bij gezonde controle-personen, hebben we geen aneurysma van de aorta gevonden. Het is dan ook niet noodzakelijk om de diameter van de aortawortel bij alle patiënten met acromegalie te evalueren.

De behandeling van acromegalie is gericht op het verlichten van de symptomen die gepaard gaan met de te hoge GH spiegels, het behandelen van metabole afwijkingen, het beperken van de effecten van het hypofyse-adenoom op het omliggende gezonde weefsel en, uiteindelijk, het verminderen van de mortaliteit van actieve acromegalie (5). Chirurgie leidt in 50-70% van deze patiënten tot curatie. Daarnaast kunnen combinaties van chirurgie, radiotherapie en/ of medicijnen (somatostatine-analoga of het net geïntroduceerde geneesmiddel dat de GH-receptor blokkeert) deze doelen bij nagenoeg alle patiënten bereiken (6-9). Desondanks blijven er restverschijnselen na of door de behandeling bestaan die een weerslag hebben op kwaliteit van leven, algemeen welbevinden en slaap.

Een verminderde kwaliteit van leven bij patiënten met biochemische remissie na acromegalie is eerder beschreven in een aantal cross-sectionele studies (10-14). Het doel van de studie beschreven in **Hoofdstuk 4** was het bestuderen van de kwaliteit van leven bij patiënten met langdurige remissie van acromegalie voor en na een observatieperiode van 4 jaar. Kwaliteit van leven werd gemeten bij 82 patiënten met vier algemene vragenlijsten (Hospital Anxiety and Depression Scale (HADS), Multidimensional Fatigue Inventory (MFI-20), Nottingham Healthy Profile (NHP), Short Form-36 (SF-36)) en een ziekte-specifieke vragenlijst (Acromegaly-Quality of Life (ACRO-QOL)).

De score op 5 van de 26 onderdelen daalde gedurende de 4 jaar: fysiek en sociaal functioneren (SF-36), fysieke vermoeidheid (MFI-20), psychologisch welbevinden en persoonlijke relaties (ACRO-QOL). Radiotherapie had een negatieve invloed op verschillende onderdelen van de kwaliteit van leven gedurende deze vier jaren.

Deze longitudinale studie bevestigt de negatieve invloed van acromegalie op kwaliteit van leven (10-13). Verschillende effecten van radiotherapie zouden de negatieve invloed op kwaliteit van leven kunnen veroorzaken. Allereerst induceert radiotherapie uitval van hypofyse-hormonen. Dit heeft inderdaad een negatieve invloed op kwaliteit van leven bij patiënten met andere hypofyse-adenomen (15;16). Daarnaast vermindert radiotherapie neurocognitieve functie bij

patiënten die bestraald zijn voor hersentumoren op kinderleeftijd (17). Tenslotte is het mogelijk dat patiënten die behandeld zijn met een combinatie van chirurgie en radiotherapie hun ziekte bedreigender en ernstiger ervaren dan patiënten die alleen met chirurgie behandeld zijn.

In **Hoofdstuk 5** beschrijven we een studie naar slaperigheid overdag en slaappatronen bij patiënten met acromegalie. Hiertoe hebben we een tweetal vragenlijsten gebruikt (Epworth sleepiness score and Münchener Chronotype Questionnaire) en vergeleken tussen patiënten en gezonde controle-personen.

Patiënten ondervonden meer last van slaperigheid tijdens het dagelijks functioneren ondanks gelijke slaapduur en timing Bovendien bleek dat de tijd in bed totdat men in slaap valt, de zogenaamde latentietijd, verlengd is bij patiënten die behandeld warden met somatostatine analoga vergeleken met patiënten zonder somatostatine-analoga.

Het slaap apnoe syndroom komt frequent voor bij acromegalie en verbetert vaak bij adequate behandeling (18-23). De prevalentie is echter nog 20% bij patiënten met biochemische remissie van acromegalie (24). Het slaap apnoe syndroom zou kunnen bijdragen aan de gevonden verhoogde slaperigheid overdag.

Somatostatine vermindert de totale slaapduur en REM-slaap (een slaapfase gekenmerkt door snelle oogbewegingen) bij gezonde ouderen (25), maar heeft geen invloed op de slaap bij gezonde jongeren (26). Het somatostatine analogon, octreotide, vermindert zowel non-REM slaap en REM-slaap bij gezonde jonge vrijwilligers (27). Deze effecten zouden ten grondslag kunnen liggen aan de effecten die wij waarnemen van somatostatine analoga bij patiënten met acromegalie. Uitgebreide studies door middel van polysomnografie kunnen uitgebreider inzicht verschaffen in deze nadelige effecten van somatostatine analoga tijdens de behandeling van acromegalie.

Radiotherapie voor hypofyse-adenomen veroorzaakt vaak uitval van hypofysehormonen (28) en kan zo tot een tekort aan GH ofwel GH defcientie (GHD) veroorzaken. Ook bij patiënten met acromegalie kan de gestimuleerde GH-secretie na een stimulus tekortschieten (29). In **Hoofdstuk 6** beschrijven we de spontane en gestimuleerde GH-secretie in patiënten die behandeld zijn met radiotherapie voor acromegalie. Patiënten met een onvoldoende GH secretie tijdens hypoglycaemie, de zogenaamde insuline-tolerantie test, werden vergeleken met patiënten met een onvoldoende GH-secretie die op dezelfde manier behandeld waren voor andere hypofyse-adenomen. GH-secretie werd gemeten na stimulatie met "GH releasing hormone" (GHRH), arginine en een combinatie van GHRH en arginine. Daarnaast werd de spontane GH-secretie gemeten gedurende 24 uur.

De gestimuleerde of spontane GH-secretie verschilden niet tussen de twee groepen. Dit betekent dat ook bij patiënten die voorheen behandeld zijn voor acromegalie, een onvoldoende GH secretie kan ontstaan en dat deze patiënten niet verschillen in dit opzicht van andere patiënten met GHD.

Een te hoge GH-spiegel heeft dus duidelijk negatieve gevolgen voor de structuur en functie van het hart (**Hoofdstuk 2 en 3**, (30)), maar ook GHD kan leiden tot specieke cardiale afwijkingen. GHD kan leiden tot een verminderde linker ventrikel massa en een verminderde ejectie fractie (31-37). Het doel van de studie beschreven in **Hoofdstuk 7** was het bestuderen van de structuur en functie van het hart bij patiënten met GHD na behandeling voor acromegalie. Wij konden 53 patiënten met acromegalie includeren waarvan een derde op het moment van de studie GHD had. Deze patiënten werd vergeleken met patiënten met biochemische remissie van de acromegalie en patiënten met actieve acromegalie alsook gezonde controles.

De afmetingen van de linker ventrikel, de wanddiktes en de massa van het hart verschilden niet tussen de drie groepen of tussen patiënten met GHD na acromegalie en gezonde controles. De functie van het hart tijdens systole bleek minimaal verminderd bij de patiënten met GHD na acromgalie ten opzichte van de patiënten met biochemische remissie, maar was beter dan bij patiënten met actieve acromegalie. De diastolische functie was verminderd bij patiënten met GHD na acromegalie vergeleken met patiënten met biochemische remissie en vergeleken met gezonde controles.

Deze studie toont het belang van optimale GH regulatie voor het functioneren van het hart.

De behandeling met recombinant humaan GH (rhGH) bij patiënten met GHD door andere ziektes dan acromegalie heeft gunstige effecten op de botdichtheid (38), de massa en functie van het hart (39), de lichaamssamenstelling (40), kwaliteit van leven (41) en lipiden in het bloed (42). Het is echter onduidelijk of men deze gunstige effecten ook ziet bij patiënten met GHD na behandeling voor acromegalie. Daarom beschrijven wij in **Hoofdstuk 8** de effecten van rhGH-substitutie bij GHD na acromegalie gedurende een gerandomiseerde studie. Wij konden 16 patiënten includeren waarvan tweederde direct gedurende een jaar met rhGH werd behandeld en de rest eerst gedurende een jaar een placebo-behandeling kreeg gevolgd door een jaar rhGH. De eindpunten van de studie werden voor de start, na 1 jaar placebo en na 1 jaar rhGH substitutie beoordeeld en bestonden uit hartfunctie, lichaamssamenstelling, botdichtheid, glucose en lipiden, biochemische indicatoren van botombouw en kwaliteit van leven.

Substitutie met rhGH gedurende een jaar had geen gunstige effecten op hartfunctie, lichaamssamenstelling, glucose en lipiden of kwaliteit van leven. De biochemische indicatoren van botombouw stegen gedurende rhGH-substitutie. Dit ging gepaard met een minimale maar significante afname van de botdichtheid van de heup. Vooralsnog zijn de effecten van GH substitutie bij GHDbij patiënten voorgaande acromegalie beperkt.

Radiotherapie voor acromegalie gaat gepaard met een verlies van productie en secretie van hypofyse-hormonen (**Hoofdstuk 6** (29)). Dit wordt vaak toegeschreven aan de effecten van radiotherapie op het gezonde hypofyseweefsel maar ook de hypothalamus kan zijn aangetast.

De nucleus suprachiasmaticus (SCN) van de hypothalamus reguleert de circadiane variaties in melatonine-secretie. Afwijkingen in de melatonine-secretie zouden daarom een aanwijzing kunnen vormen voor subtiele schade aan de hypothalamus. De studie in **Hoofdstuk 9** beschrijft de effecten van postoperatieve radiotherapie voor acromegalie op de circadiane variaties van melatonine-secretie. Patiënten die met postoperatieve radiotherapie behandeld waren voor acromegalie werden vergeleken met patiënten die alleen chirurgisch behandeld waren en met gezonde controles. De gemiddelde melatonine-concentratie alsook de hoogst gemeten concentratie gedurende de dag was niet verschillend tussen de drie groepen. De acrofase, het punt waarop melatonine het hoogst is gedurende de dag, was echter later bij patiënten die behandeld waren met radiotherapie dan bij gezonde controles.

De data in deze studie suggeren dus betrokkenheid van de hypothalamus bij de effecten van radiotherapie. Deze subtiele afwijkingen in de functie van de hypothalamus zouden kunnen bijdragen aan de waargenomen negatieve effecten van radiotherapie op kwaliteit van leven (**Hoofdstuk 4**).

Een tekort aan GH heeft ook bij volwassenen, na de voltooiing van de lengtegroei, negatieve gevolgen. Het klinische ziektebeeld wordt gekenmerkt door een veranderde lichaamssamenstelling met een toename van vetweefsel, een verminderde botdichtheid, afwijkingen in het lipidengehalte in het bloed, een ongunstig cardiovasculair risicoprofiel en een verminderde kwaliteit van leven (40).

Een belangrijke doelstelling van de substitutie van GH op de volwassen leeftijd is het verbeteren van het cardiovasculaire risicoprofiel. Cellen afkomstig uit het beenmerg, de zogenaamde endotheel-voorlopercellen, zijn essentieel in het onderhoud en herstel van bloedvaten (43). Daarom hebben we in de studie beschreven in **Hoofdstuk 10** de vaatstructuur en -functie alsook het aantal endotheel-voorlopercellen gemeten bij patiënten met GHD voor en na een jaar rhGH substitutie.

De data in deze studie tonen dat endotheelfunctie en het aantal circulerende endotheel-voorlopercellen toeneemt. De toename in het aantal endotheel-voorlopercellen vond met name plaats gedurende de eerste 6 maanden. Een jaar rhGH-substitutie heeft dus een positieve invloed op vaatfunctie en endotheel-voorlopercellen.

GHD is een irreversibele aandoening, waarvoor chronische behandeling met rhGH nodig is. In de studie beschreven in **Hoofdstuk 11** bekeken we de effecten van rhGH-substitutie op de lange termijn. Drie-en-zestig patiënten werden voor en na 2, 5 en 7 jaar rhGH-substitutie bestudeerd.

Totaal cholesterol en low-density lipoproteine (LDL) cholesterol spiegels daalden zelfs na 5 jaar rhGH-substitutie verder. Het high-density lipoproteine (HDL) cholesterol gehalte steeg na 7

jaar rhGH-substitutie. De nuchtere glucose concentraties stegen tijdens rhGH-substitutie, met name gedurende de eerste twee jaar.

De data in deze studie tonen de positieve effecten aan van langdurige rhGH-substitutie bij volwassenen met GHD op bloedlipiden.

Ondanks de beschreven gunstige effecten van rhGH-subsitutie is het tot op heden niet duidelijk of de effecten tezamen daadwerkelijk een vermindering van het cardiovasculaire risico geven. In de studie beschreven in **Hoofdstuk 12** hebben we de effecten van rhGH-substitutie gedurende 5 jaar op het metabool syndroom bekeken. Het metabool syndroom is een cluster van cardiovasculaire risicofactoren en de individuele afkapwaarden zijn onder andere gedefinieerd door het "National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III)". In deze studie hebben we aan de hand van die definitie 50 patiënten met GHD vergeleken met gezonde controle-personen uit een grote populatie studie in Nederland, de MORGEN studie. Wij vonden een bijna tweevoudig verhoogde prevalentie van het metabool syndroom op basis van een verhoogde prevalentie van hypertriglyceridemie, hypertensie en abdominale obesitas. De HDL cholesterol concentraties stegen tijdens rhGH- substitutie. De prevalentie van het metabool syndroom bleef echter onveranderd na 2 en 5 jaar rhGH-substitutie.

Deze studie toont dus aan dat optimale substitutie van uitgevallen hypofyse-hormonen inclusief GH onvoldoende is om het ongunstige cardiovasculaire risicoprofiel te verbeteren. Deze patiënten zullen dus nauwgezet moeten worden vervolgd en hebben baat bij andere behandelingen van de aanwezige cardiovasculaire risicofactoren.

Verschillende factoren beïnvloeden de effectiviteit van rhGH substitutie bij patiënten met GHD (**Hoofdstuk 13 en 14**). Vrouwen die tegelijkertijd met orale oestradiol preparaten worden behandeld vanwege secundair hypogonadisme, hebben een hogere dosis rhGH-substitutie nodig om dezelfde insuline-achtige groeifactor-I (IGF-I) concentraties te krijgen als mannen en vrouwen die transdermale oestradiol preparaten gebruiken (44-46). De studie beschreven in **Hoofdstuk 13** was gericht op het vergelijken van de effecten van orale en transdermale oestradiol toediening bij een gelijke plasmaconcentratie van oestradiol. Tijdens deze parallel cross-over studie werden de vrouwen behandeld met eenzelfde rhGH dosis gedurende de gehele studie, maar werden de oestradiol preparaten afgewisseld: bij de eerste groep vrouwen van 2 mg naar 4 mg naar 2 mg per os en dan van 50 µg naar 100 µg transdermaal; bij de tweede groep van 50 µg naar 100 µg transdermaal en dan van 2 mg naar 4 mg per os.

Oestradiol concentraties waren het laagst gedurende 50 µg transdermale toediening en het hoogst tijdens 4 mg orale toediening. De oestradiol concentraties tijdens 100 µg transdermaal en 2 mg oraal waren gelijk. Ondanks de gelijke oestradiol concentraties tijdens deze twee laatste behandelingen, was het IGF-I hoger tijdens de transdermale toediening dan tijdens de orale toediening .

Tijdens transdermale toediening van oestradiol is dus een lagere dosis van rhGH-substitutie nodig dan tijdens orale toediening.

Bij kinderen met een GH tekort heeft een veel voorkomend polymorfisme van de GH-receptor (exon-3 deletie, d3GHR) een positieve invloed op de groeisnelheid tijdens rhGH-substitutie (47;48). In **Hoofdstuk 14** beschrijven we een studie naar de invloed van de d3GHR tijdens rhGH substitutie bij volwassenen tijdens 1 jaar en 5 jaar behandeling. De stijging van IGF-I was hoger bij patiënten met tenminste één d3GHR allel vergeleken met patiënten met twee wildtype allelen tijdens het eerste jaar rhGH-substitutie met exact dezelfde dosis. Dit effect werd niet waargenomen na 5 jaar rhGH-substitutie. Tegelijkertijd was de daling in totaal en LDL cholesterol lager en de stijging in HDL cholesterol hoger bij patiënten met tenminste één d3GHR allel gedurende het eerste jaar rhGH substitutie. Ook deze effecten werden niet op de lange termijn waargenomen. Deze studie toont dat ook bij volwassenen dit veelvoorkomende polymorfisme de effecten van rhGH-substitutie op de korte termijn beïnvloedt.

De effecten van ziektes en hun behandeling op de kwaliteit van leven van patiënten wordt steeds belangrijker in de medische praktijk. Uit voorgaande studies is gebleken dat de kwaliteit van leven bij patiënten met hypofyse-adenomen verminderd is (49). Er is echter een essentieel methodologisch probleem bij het vergelijken van patiënten met verschillende hypofyse-adenomen, want de leeftijd en geslacht verschillen tussen patiënten met verschillende-hypofyse-adenomen. Zo zijn de patiënten met de ziekte van Cushing overwegend vrouwelijk en jong en de patiënten met een niet-functionerend hypofyse-adenoom ouder. Het is echter bekend dat leeftijd en geslacht de score op kwaliteit van leven vragenlijsten beïnvloedt. In **Hoofdstuk 15** beschrijven wij leeftijd- en geslachtsspecieke standaarddeviatie scores (Z scores) voor kwaliteit van leven bij patiënten die behandeld zijn voor verschillende hypofyse-adenomen.

De Z scores werden berekend door de scores op de verschillende kwaliteit van leven vragenlijsten (HADS, MFI-20, NHP, SF-36) te vergelijken tussen patiënten met hypofyse-adenomen (acromegalie (n=118), de ziekte van Cushing (n=58), prolactinomen (n=128) en niet-functionerende macroadenomen (n=99)) en gezonde controles (n=440). De Z score voor alle onderdelen van de vragenlijsten alsook de totale kwaliteit van leven konden zo worden berekend.

De patiënten scoorden aanzienlijk slechter op alle onderdelen van de vragenlijsten en de totale kwaliteit van leven score. Wanneer de Z scores werden vergeleken, bleek dat patiënten met acromegalie meer hinder in fysiek functioneren en meer pijn ondervonden dan patiënten met een niet-functionerend hypofyse-adenoom. Ook patiënten met de ziekte van Cushing ondervonden meer beperkingen in fysiek functioneren vergeleken met patiënten met een niet-functionerend hypofyse-adenoom. Lineaire regressie analyse met correctie voor leeftijd en geslacht bevestigde deze bevindingen. Daarnaast bleek uit deze laatste analyse dat patiënten met de ziekte van Cushing meer angst/ ongerustheid ervaren. Deze studie toont dus aan dat hypofyse-adenomen in het algemeen een negatieve invloed op de kwaliteit van leven heb-

ben. Daarnaast zijn er duidelijk hypofyse-adenoom specifieke gevolgen voor de kwaltiteit van leven.

Tijdens het kwaliteit van leven onderzoek geven patiënten met een niet-functionerend hypofyse-adenoom vaak vermoeidheidsklachten aan (15). Het is onduidelijk of de slaap normaal bij deze patiënten. In **Hoofdstuk 16** bekeken we slaperigheid tijdens dagelijkse bezigheden en slaappatronen bij patiënten met een niet-functionerend hypofyse macro-adenoom (NFMA) door middel van twee gevalideerde slaapvragenlijsten (de Epworth sleepiness score en de Münchener Chronotype Questionnaire). We vergeleken dit met vier gevalideerde kwaliteit van leven vragenlijsten (HADS, MFI-20, NHP, SF-36). De gegevens verkregen bij patiënten werden vergeleken met controle-gegevens.

Slaapduur en timing waren niet verschillend tussen patiënten en controles. Desondanks ervoeren patiënten meer slaperigheid. De slaperigheidsscores waren significant gecorreleerd met 15 van de 21 kwaliteit van leven onderdelen. De timing van de slaap werd beïnvloed door radiotherapie. De slaapduur werd negatief beïnvloed door panhypopituitarisme.

Uit deze studie blijkt dus verschillende factoren bij patiënten die behandeld zijn voor een NFMA effecten hebben op de slaap. Verder onderzoek door middel van polysomnografie is noodzakelijk om de exacte afwijkingen in de slaap te bestuderen.

Ook bij patiënten die behandeld zijn voor een craniopharyngeoom blijven vaak klachten van algemene en fysieke vermoeidheid bestaan (50). De slaap is verstoord bij kinderen met een craniopharyngeoom (51). In **Hoofdstuk 17** beschrijven we een studie naar slaperigheid en slaappatronen bij volwassenen met een craniopharyngeoom in vergelijking met gezonde controles en patiënten met een NFMA. Slaperigheid overdag en slaappatronen werden bestudeerd bij 27 volwassen patiënten met een craniopharyngeoom. Er waren geen verschillen in slaappatronen tussen de drie groepen. Slaperigheidsscores waren verhoogd bij patiënten met een craniopharyngeoom en met een NFMA in vergelijking met gezonde controles maar niet verschillend ten opzichte van elkaar. Ernstige hypersomnolentie was aanwezig bij 33% van de patiënten met een craniopharyngeoom.

De verhoogde slaperigheid bij patiënten met een craniopharyngeoom en met een NFMA zou dus kunnen duiden op een algemeen effect van tumoren in de hypothalamus- en hypofyse-regio.

De hypofyse heeft dus een centrale rol in het endocriene systeem. Hierdoor leidt pathofysiologie van de hypofyse tot een ontregeling van het endocriene systeem. Over het algemeen zijn hypofyse-ziekten goed te behandelen met combinaties van chirurgie, radiotherapie en medicijnen. De studies in dit proefschrift tonen dat ondanks goede behandeling restverschijnselen blijven bestaan.

REFERENTIES

1. Pereira AM, van Thiel SW, Lindner JR, Roelfsema F, van der Wall EE, Morreau H, Smit JW, Romijn JA, Bax JJ 2004 Increased prevalence of regurgitant valvular heart disease in acromegaly. J Clin Endocrinol Metab 89:71-75
2. Colao A, Spinelli L, Marzullo P, Pivonello R, Petretta M, Di Somma C, Vitale G, Bonaduce D, Lombardi G 2003 High prevalence of cardiac valve disease in acromegaly: an observational, analytical, case-control study. J Clin Endocrinol Metab 88:3196-3201
3. van Karnebeek CD, Naeff MS, Mulder BJ, Hennekam RC, Offringa M 2001 Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child 84:129-137
4. Thompson BJ, Shang CA, Waters MJ 2000 Identification of genes induced by growth hormone in rat liver using cDNA arrays. Endocrinology 141:4321-4324
5. Wright AD, Hill DM, Lowy C, Fraser TR 1970 Mortality in acromegaly. Q J Med 39:1-16
6. Biermasz NR, Romijn JA, Pereira AM, Roelfsema F 2005 Current pharmacotherapy for acromegaly: a review. Expert Opin Pharmacother 6:2393-2405
7. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ 2005 Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365:1644-1646
8. Melmed S 2006 Medical progress: Acromegaly. N Engl J Med 355:2558-2573
9. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO 2001 Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358:1754-1759
10. Hua SC, Yan YH, Chang TC 2006 Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. Eur J Endocrinol 155:831-837
11. Kauppinen-Makelin R, Sane T, Sintonen H, Markkanen H, Valimaki MJ, Loyttyniemi E, Niskanen L, Reunanen A, Stenman UH 2006 Quality of life in treated patients with acromegaly. J Clin Endocrinol Metab 91:3891-3896
12. Trepp R, Everts R, Stettler C, Fischli S, Allemann S, Webb SM, Christ ER 2005 Assessment of quality of life in patients with uncontrolled vs. controlled acromegaly using the Acromegaly Quality of Life Questionnaire (AcroQoL). Clin Endocrinol (Oxf) 63:103-110
13. Webb SM, Badia X, Surinach NL 2006 Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study. Eur J Endocrinol 155:269-277
14. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ 2005 Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. J Clin Endocrinol Metab 90:3337-3341
15. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA 2006 Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. J Clin Endocrinol Metab 91:3364-3369
16. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA 2005 Quality of Life in Patients After Long-term Biochemical Cure of Cushing's Disease. J Clin Endocrinol Metab 90:3279-3286
17. Spiegler BJ, Bouffet E, Greenberg ML, Rutka JT, Mabbott DJ 2004 Change in neurocognitive functioning after treatment with cranial radiation in childhood. J Clin Oncol 22:706-713
18. Herrmann BL, Wessendorf TE, Ajaj W, Kahlke S, Teschler H, Mann K 2004 Effects of octreotide on sleep apnoea and tongue volume (magnetic resonance imaging) in patients with acromegaly. Eur J Endocrinol 151:309-315
19. Sze L, Schmid C, Bloch KE, Bernays R, Brandle M 2007 Effect of transsphenoidal surgery on sleep apnoea in acromegaly. Eur J Endocrinol 156:321-329

20. Saeki N, Isono S, Tanaka A, Nishino T, Higuchi Y, Uchino Y, Iuchi T, Murai H, Tatsuno I, Yasuda T, Yamaura A 2000 Pre-and post-operative respiratory assessment of acromegalics with sleep apnea--bedside oximetric study for transsphenoidal approach. Endocr J 47 Suppl:S61-S64
21. Buyse B, Michiels E, Bouillon R, Bobbaers H, Demedts M 1997 Relief of sleep apnoea after treatment of acromegaly: report of three cases and review of the literature. Eur Respir J 10:1401-1404
22. Grunstein RR, Ho KK, Sullivan CE 1994 Effect of octreotide, a somatostatin analog, on sleep apnea in patients with acromegaly. Ann Intern Med 121:478-483
23. Leibowitz G, Shapiro MS, Salameh M, Glaser B 1994 Improvement of sleep apnoea due to acromegaly during short-term treatment with octreotide. J Intern Med 236:231-235
24. Rosenow F, Reuter S, Deuss U, Szelies B, Hilgers RD, Winkelmann W, Heiss WD 1996 Sleep apnoea in treated acromegaly: relative frequency and predisposing factors. Clin Endocrinol (Oxf) 45:563-569
25. Frieboes RM, Murck H, Schier T, Holsboer F, Steiger A 1997 Somatostatin impairs sleep in elderly human subjects. Neuropsychopharmacology 16:339-345
26. Steiger A, Guldner J, Hemmeter U, Rothe B, Wiedemann K, Holsboer F 1992 Effects of growth hormone-releasing hormone and somatostatin on sleep EEG and nocturnal hormone secretion in male controls. Neuroendocrinology 56:566-573
27. Ziegenbein M, Held K, Kuenzel HE, Murck H, Antonijevic IA, Steiger A 2004 The somatostatin analogue octreotide impairs sleep and decreases EEG sigma power in young male subjects. Neuropsychopharmacology 29:146-151
28. Littley MD, Shalet SM, Beardwell CG, Ahmed SR, Applegate G, Sutton ML 1989 Hypopituitarism following external radiotherapy for pituitary tumours in adults. Q J Med 70:145-160
29. Biermasz NR, van Dulken H, Roelfsema F 2000 Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. J Clin Endocrinol Metab 85:2476-2482
30. Colao A, Ferone D, Marzullo P, Lombardi G 2004 Systemic complications of acromegaly: epidemiology, pathogenesis, and management. Endocr Rev 25:102-152
31. Amato G, Carella C, Fazio S, La Montagna G, Cittadini A, Sabatini D, Marciano-Mone C, Sacca L, Bellastella A 1993 Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J Clin Endocrinol Metab 77:1671-1676
32. Colao A, Cuocolo A, Di Somma C, Cerbone G, Della Morte AM, Nicolai E, Lucci R, Salvatore M, Lombardi G 1999 Impaired cardiac performance in elderly patients with growth hormone deficiency. J Clin Endocrinol Metab 84:3950-3955
33. Colao A, Di Somma C, Cuocolo A, Filippella M, Rota F, Acampa W, Savastano S, Salvatore M, Lombardi G 2004 The severity of growth hormone deficiency correlates with the severity of cardiac impairment in 100 adult patients with hypopituitarism: an observational, case-control study. J Clin Endocrinol Metab 89:5998-6004
34. Merola B, Cittadini A, Colao A, Longobardi S, Fazio S, Sabatini D, Sacca L, Lombardi G 1993 Cardiac structural and functional abnormalities in adult patients with growth hormone deficiency. J Clin Endocrinol Metab 77:1658-1661
35. Sartorio A, Ferrero S, Conti A, Bragato R, Malfatto G, Leonetti G, Faglia G 1997 Adults with childhood-onset growth hormone deficiency: effects of growth hormone treatment on cardiac structure. J Intern Med 241:515-520
36. Colao A, Cuocolo A, Di Somma C, Cerbone G, Morte AM, Pivonello R, Nicolai E, Salvatore M, Lombardi G 2000 Does the age of onset of growth hormone deficiency affect cardiac performance? A radionuclide angiography study. Clin Endocrinol (Oxf) 52:447-455
37. Erdogan D, Tukek T, Aral F, Oflaz H, Ozaydin M, Kocaman O, Akkaya V, Goren T, Molvalilar S 2004 Structural, functional and autonomic changes in the cardiovascular system in growth hormone deficient patients. Ann Noninvasive Electrocardiol 9:19-23

38. Gotherstrom G, Bengtsson BA, Bosaeus I, Johannsson G, Svensson J 2007 Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. Eur J Endocrinol 156:55-64
39. Maison P, Chanson P 2003 Cardiac effects of growth hormone in adults with growth hormone deficiency: a meta-analysis. Circulation 108:2648-2652
40. de Boer H, Blok GJ, Van der Veen EA 1995 Clinical aspects of growth hormone deficiency in adults. Endocr Rev 16:63-86
41. Deijen JB, Arwert LI, Witlox J, Drent ML 2005 Differential effect sizes of growth hormone replacement on Quality of Life, well-being and health status in growth hormone deficient patients: a meta-analysis. Health Qual Life Outcomes 3:63
42. Maison P, Griffin S, Nicoue-Beglah M, Haddad N, Balkau B, Chanson P 2004 Impact of growth hormone (GH) treatment on cardiovascular risk factors in GH-deficient adults: a Metaanalysis of Blinded, Randomized, Placebo-Controlled Trials. J Clin Endocrinol Metab 89:2192-2199
43. Rabelink TJ, de Boer HC, de Koning EJ, van Zonneveld AJ 2004 Endothelial progenitor cells: more than an inflammatory response? Arterioscler Thromb Vasc Biol 24:834-838
44. Cook DM, Ludlam WH, Cook MB 1999 Route of estrogen administration helps to determine growth hormone (GH) replacement dose in GH-deficient adults. J Clin Endocrinol Metab 84:3956-3960
45. Johansson AG, Engstrom BE, Ljunghall S, Karlsson FA, Burman P 1999 Gender differences in the effects of long term growth hormone (GH) treatment on bone in adults with GH deficiency. J Clin Endocrinol Metab 84:2002-2007
46. Span JP, Pieters GF, Sweep CG, Hermus AR, Smals AG 2000 Gender difference in insulin-like growth factor I response to growth hormone (GH) treatment in GH-deficient adults: role of sex hormone replacement. J Clin Endocrinol Metab 85:1121-1125
47. Dos Santos C., Essioux L, Teinturier C, Tauber M, Goffin V, Bougneres P 2004 A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. Nat Genet 36:720-724
48. Jorge AA, Marchisotti FG, Montenegro LR, Carvalho LR, Mendonca BB, Arnhold IJ 2006 Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency. J Clin Endocrinol Metab 91:1076-1080
49. Johnson MD, Woodburn CJ, Vance ML 2003 Quality of life in patients with a pituitary adenoma. Pituitary 6:81-87
50. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM 2006 Quality of life in treated adult craniopharyngioma patients. Eur J Endocrinol 154:483-489
51. Muller HL, Handwerker G, Gebhardt U, Faldum A, Emser A, Kolb R, Sorensen N 2006 Melatonin treatment in obese patients with childhood craniopharyngioma and increased daytime sleepiness. Cancer Causes Control 17:583-589

CURRICULUM VITAE

Agatha van der Klaauw werd geboren op 14 maart 1980 te Leiderdorp. Zij behaalde in 1998 het eindexamen aan het Stedelijk Gymnasium te Leiden. In 1999 haalde zij cum laude het propaedeutisch examen Geneeskunde. In 2002 deed zij onderzoek op de afdeling Nucleaire Geneeskunde te Leiden waarna zij in 2002 cum laude het doctoraal examen Geneeskunde behaaldde. Het artsexamen werd cum laude behaald in 2004. Direct aansluitend werd met dit promotie-onderzoek begonnen op de afdeling Endocrinologie onder leiding van Professor Romijn en Professor Smit.

In 2007 werd een ZonMW-AGIKO stipendium toegekend. Van september 2007 tot september 2008 deed zij onderzoek in de Metabolic Research Laboratories, Institute of Metabolic Science, Cambridge University onder leiding van Professor O'Rahilly en Dr Farooqi naar patienten met melanocortine 4-receptor deficientie. In 2008 werd haar de Young Investigator's Award van de European Society of Endocrinology toegekend. Tevens was zij dit jaar winnaar van de Trainee Poster Competition van op de Endocrine Society Meeting in San Francisco.

Agatha van der Klaauw is sinds september in opleiding tot internist (als AGIKO) in het Bronovo Ziekenhuis (Opleiders: Professor Romijn en dr. van 't Wout).

PUBLICATIES

AA van der Klaauw, T van der Straaten, R Baak-Pablo, NR Biermasz, HJ Guchelaar, AM Pereira, JWA Smit, JA Romijn Influence of the d3-growth hormone (GH) receptor isoform on short-term and long-term treatment response to GH replacement in GH-deficient adults. J Clin Endocrinol Metab 2008 93:2828-2834

AA van der Klaauw, NR Biermasz, AM Pereira, KW van Kralingen, OM Dekkers, KF Rabe, JWA Smit, JA Romijn. Patients cured from craniopharyngioma or non-functioning pituitary macroadenoma suffer similarly from increased daytime somnolence despite normal sleep patterns compared to healthy controls. Clin Endocrinol (Oxf) 2008 in press

AA van der Klaauw, M Kars, NR Biermasz, F Roelfsema, OM Dekkers, EPM Corssmit, MO van Aken, B Havekes, AM Pereira, H Pijl, JWA Smit, JA Romijn JA Disease specific impairments in quality of life during long-term follow-up of patients with different pituitary adenomas. Clin Endocrinol (Oxf) 2008 in press

AA van der Klaauw, JJ Bax, JWA Smit, ER Holman, V Delgado, GB Bleeker, NR Biermasz, F Roelfsema, JA Romijn, AM Pereira Increased aortic root diameters in patients with acromegaly. Eur J Endocrinol 2008159:97-103

AA van der Klaauw, AM Pereira, TJ Rabelink, EPM Corssmit, AJ van Zonneveld, H Pijl, HC de Boer, JWA Smit, JA Romijn, EJP de Koning Recombinant human GH replacement increases CD34+ cells and improves endothelial function in adults with GH deficiency. Eur J Endocrinol 2008 159:105-111

AA van der Klaauw, AM Pereira, KW van Kralingen, KF Rabe, JA Romijn Somatostatin analog treatment is associated with an increased sleep latency in patients with long-term biochemical remission of acromegaly. Growth Horm IGF Res 2008 18:446-453

AA van der Klaauw, JJ Bax, GB Bleeker, ER Holman, V Delgado, JWA Smit, JA Romijn, AM Pereira Cardiac manifestations of growth hormone deficiency after treatment for acromegaly: a comparison to patients with biochemical remission and controls. Eur J Endocrinol 2008 In press

AA van der Klaauw, T Kienitz, CJ Strasburger, JWA Smit, JA Romijn. Malignant Pituitary Corticotroph Adenomas: Report of Two Cases and a Comprehensive Review of the Literature. Pituitary, 2008, in press.

B Havekes, AA van der Klaauw, HC Hoftijzer, JC Jansen, AGL van der Mey, AHJT Vriends, JWA Smit, JA Romijn, EPM Corssmit. Reduced Quality of Life in Patients with Head-and-Neck Paragangliomas. European Journal of Endocrinology, 2008, in 158:247-253.

AA van der Klaauw, NR Biermasz, HC Hoftijzer, AM Pereira, JA Romijn. Previous radiotherapy negatively influences quality of life during four years of follow-up in patients cured from acromegaly. Clinical Endcrinology, 2008, 69:123-128.

AA van der Klaauw, NR Biermasz, PMJ Zelissen, AM Pereira, EGWM Lentjes, JWA Smit, SW van Thiel, JA Romijn, F Roelfsema. Administration route-dependent effects of estrogens on IGF-I levels during fixed growth hormone replacement in women with hypopituitarism. European Journal of Endocrinology, 2007, Dec;157(6):709-16.

AA van der Klaauw, OM Dekkers, AM Pereira, KW van Kralingen, JA Romijn. Increased daytime somnolence despite normal sleep patterns in patients treated for non-functioning pituitary macroadenoma. Journal of Clinical Endocrinology and Metabolism, 2007, Oct;92(10):3898-903.

M Kars, AA van der Klaauw, AM Pereira, JA Romijn. Impaired Quality of Life in Patients Treated for Microprolactinoma. European Journal of Endocrinology, 2007, Jan;93(1):200-3.

OM Dekkers, AM Pereira, F Roelfsema, AA van der Klaauw, PJ Honkoop, JWA Smit, RJW de Keizer, JA Romijn. Continuing improvement of visual acuity until one year after transspenoidal surgery for non-functioning pituitary adenoma. Pituitary, 2007, 10 (1) 61-65.

AA van der Klaauw, NR Biermasz, EJM Feskens, MB Bos, JWA Smit, F Roelfsema, EPM Corssmit, H Pijl, JA Romijn, AM Pereira. The prevalence of the metabolic syndrome is increased in patients with growth hormone deficiency, irrespective of long-term substitution with recombinant human growth hormone. European Journal of Endocrinology, 2007 Apr;156(4):455-62.

AA van der Klaauw, AM Pereira, SW van Thiel, M Frolich, A Iranmanesh, JD Veldhuis, F Roelfsema, JA Romijn. Attenuated pulse size and disorderly growth hormone secretion and prolactin secretion with preserved nyctohemeral rhythm distinguish irradiated from surgically treated acromegalic patients. Clinical Endocrinology (Oxf), 2007 Apr;66(4):489-98.

AA van der Klaauw, JA Romijn, NR Biermasz, JWA Smit, J van Doorn, OM Dekkers, F Roelfsema, AM Pereira. Sustained effects of recombinant growth hormone replacement after 7 years of treatment in adults with growth hormone deficiency. European Journal of Endocrinology, 2006 Nov;155(5):701-8.

OM Dekkers, AA van der Klaauw, AM Pereira, NR Biermasz, PJ Honkoop, F Roelfsema, JWA Smit and JA Romijn. Quality of Life is decreased after treatment for non-functioning pituitary macroadenoma. Journal of Clinical Endocrinology and Metabolism, 2006 Sep;91(9):3364-9.

AA van der Klaauw, AM Pereira, SW van Thiel, JWA Smit, EPM Corssmit, NR Biermasz, M Frolich, A Iranmanesh, JD Veldhuis, F Roelfsema and JA Romijn. Growth hormone (GH) deficiency in patients irradiated for acromegaly: significance of GH stimulatory tests in relation to the twenty-four hour GH secretion. European Journal of Endocrinology, 2006, Jun 154(6):851-8.

AA van der Klaauw, JJ Bax, F Roelfsema, GB Bleeker, ER Holman, EPM Corssmit, EE van der Wall, JWA Smit, JA Romijn and AM Pereira. Uncontrolled Acromegaly is Associated with Progressive Mitral Valvular Regurgitation. Growth Hormone and IGF Research, 2006, Apr 16(2):101-7.

AM Pereira, AA van der Klaauw, HPF Koppeschaar, JWA Smit, SW van Thiel, J van Doorn, NR Biermasz, F Roelfsema and JA Romijn. Efficacy of needle-free administration of recombinant human growth hormone in adults with growth hormone deficiency. Br J of Clinical Pharmacology, 2006, Apr 61(4):451-5.

EKJ Pauwels, AA van der Klaauw, T Corporaal and MPM Stokkel. Fluorine-18-radiolabeled pharmaceuticals for imaging with positron emission tomography, excluding [18F]-fluorodeoxyglucose. Drugs of the Future 2002, 27(7):655-667.

van der Klaauw AA, Bax JJ, Roelfsema F, Stokkel M, Bleeker GB, Biermasz NR, Smit JWA, Romijn JA, Pereira AM. Limited Effects of Growth Hormone
Replacement n Patients with GH deficiency During Long-Term Cure of Acromegaly. Submitted for publication

van der Klaauw AA, Biermasz NR, van Someren E, Roelfsema F, Smit JWA, Pereira AM, Romijn JA. Postoperative radiotherapy for acromegaly delays the circadian phase of melatonin secretion. Submitted for publication

Martinelli CE, Keogh JM, Greenfield JR, Henning E, van der Klaauw AA, Blackwood A, O'Rahilly S, Roelfsema F, Camacho-Hübner C, Pijl H, Farooqi IS. Obesity due to Melanocortin 4 receptor (MC4R) deficiency is associated with increased linear growth and final height, fasting hyperinsulinaemia and incompletely suppressed growth hormone secretion. Submitted for publication

Wassenaar MJE, Biermasz NR, van Duinen N, van der Klaauw AA, Pereira AM, Roelfsema F, Smit JWA, Kroon HM, Kloppenburg M, Romijn JA. High prevalence of arthropathy, according to the definitions of radiological and clinical osteoarthritis, in patients with long-term cure of acromegaly; a case control study. Submitted for publication.

Wassenaar MJE, Biermasz NR, Pereira AM, van der Klaauw AA, Roelfsema F, Smit JWA, Kroon HM, Wolterbeek R, Romijn JA, Kloppenburg MOsteoarthritis is equally prevalent in men and women with long-term controlled acromegaly. Submitted for publication.