Pituitary diseases: long-term clinical consequences

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Chapter 1

General introduction

CONTENTS

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).

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