Chapter 18

General discussion and summary
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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 describe 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 were 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 occurred 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 the treatment of cardiovascular disease.
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. Although 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 lifestyle 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](image_url)

*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 macroadenoama. 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.
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