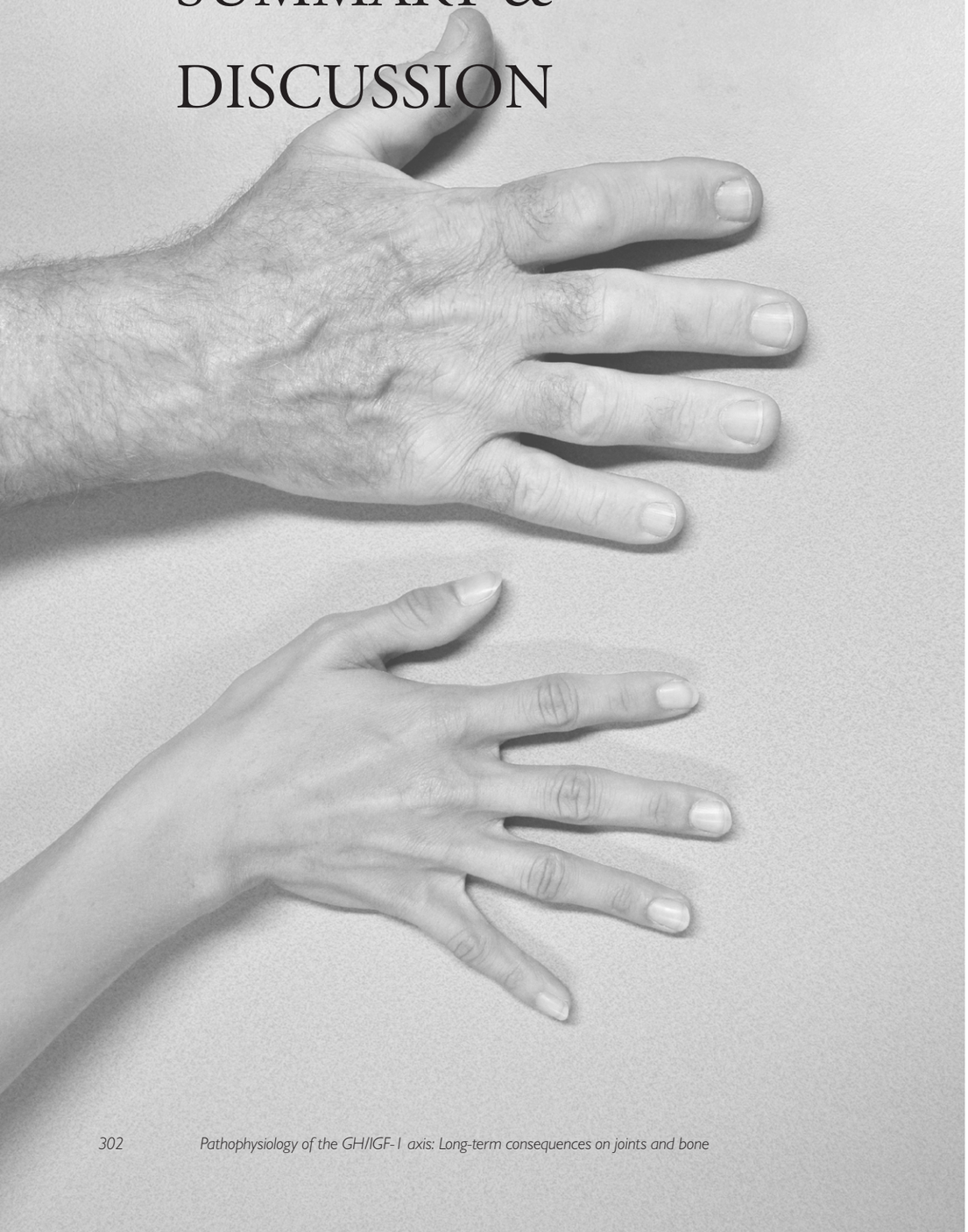
XIV.

SUMMARY & DISCUSSION



INTRODUCTION

In this thesis, a number of observations have been described in acromegaly patients with cured or biochemically well-controlled disease during long-term follow-up. These observations focus on the long-term consequences of the disease on joints and bone, which have been investigated in a prospective follow-up study (**Part A**). In addition, we investigated the role of the Growth Hormone (GH) / Insulin-like Growth Factor-1 (IGF-1) axis in patients with primary osteoarthritis (OA), looking at serum IGF-1 levels within the normal range and the exon 3 deleted GH receptor (d3-GHR) polymorphism (**Part B**). Finally, we studied the long-term consequences of recombinant human GH (rhGH) treatment in GH Deficient (GHD) adults, focusing on the cardiovascular effects and the effects on bone metabolism in comparison to healthy controls (**Part C**).

Part A. Long-term effects of acromegaly on joints and bone

Acromegaly is a rare endocrine disease caused in most cases by a pituitary adenoma resulting in excessive GH secretion. As a consequence, patients suffer from elevated GH and IGF-1 levels. As clinical manifestations appear slowly and first symptoms are non-specific, it is a typical disease with a delay in diagnosis of 6 to 9 years. Following appropriate therapy, by surgery, radiotherapy, medical treatment or a combination of these treatment modalities, many systemic co morbid conditions of acromegaly improve considerably. Unfortunately, it has become apparent that despite biochemical control, many manifestations of acromegaly remain detectable during prolonged follow-up, significantly impairing QoL. Although acromegaly is a very heterogeneous disease with individual susceptibility for GH and IGF-1 levels, skeletal manifestations are one of the most prevalent and invalidating complications of acromegaly.

Part A describes the long-term effects of acromegaly on joints and bone in a well-characterized cohort of controlled acromegaly patients, who were in remission for a mean duration of 17 years. We focused on the course of arthropathy and vertebral fractures over time in treated patients and risk factors for poor outcome, since, at present, the prognosis and determinants of both acromegalic arthropathy and vertebral fractures during prolonged follow-up are unknown. In order to address these questions, we designed a prospective 2.5-year follow-up study.

Arthropathy is one of the most common complications of acromegaly, and is highly prevalent both in active and (long-term) controlled disease. Previously, elevated GH / IGF-1 activity was found to be associated with the onset of acromegalic arthropathy. Especially patients with high IGF-1 levels at the time of diagnosis and with the common exon 3 deleted GH receptor (d3-GHR) polymorphism, that is associated with enhanced GH responsiveness (*vide infra*), were at increased risk to develop secondary arthropathy. Although acromegalic arthropathy shares features with primary OA, radiographic features significantly differ. Severe osteophytosis is seen, but joint spaces are widened rather than narrowed in patients with long-term controlled acromegaly. However, usually performed semi-quantitative measures as used and validated in primary OA cohorts do not include joint space widening. Recently, a new quantitative semi-automated image analysis method of hand radiographs by Van 't Klooster *et al.* was shown to be sensitive enough to relate joint space width to markers of disease activity of acromegaly and is therefore able to fully characterize the effects of previous GH excess on joints, including joint space widening.

RADIOGRAPHIC AND CLINICAL PROGRESSION OF ACROMEGALIC ARTHROPATHY: The disease course of acromegalic arthropathy in treated patients during prolonged follow-up is unknown. In addition, information on risk factors for poor OA outcome is currently lacking. Therefore, we studied in **Chapter 2** and **Chapter 3** the course of acromegalic arthropathy and determinants of poor outcome in a prospective study with 2.5 years of follow-up. **Chapter 2** focuses on the radiographic change over time, and **Chapter 3** describes the course of clinical joint symptoms and signs during prolonged follow-up, and assesses the relationship between clinical and radiographic change of arthropathy. All 58 patients included were biochemically controlled, by either transsphenoidal surgery and/or additional radiotherapy or (primary) medical treatment, for a mean of 17.6 years, as evidenced by a mean actual IGF-1 SDS of 0.5.

Chapter 2 demonstrates progressive osteophytosis and joint space narrowing in a considerable proportion of patients (>70%), despite biochemical disease control. Higher age and parameters reflecting higher GH / IGF-1 activity were associated with progressive joint disease. Remarkably, biochemical control by SMS analogs was associated with more radiographic OA progression than surgical cure, independently of age, sex, BMI and baseline IGF-1 SDS. These findings are in accordance with earlier studies documenting differential effects on QoL and diastolic heart function in patients controlled with SMS analogs *vs* patients with surgical cure of acromegaly. The hypothesis of insufficient GH control when applying current guidelines is further supported by a previous report in which GH secretion was found to be persistently abnormal during treatment with SMS analogs, and might imply the need of more aggressive therapy, probably by addition of Pegvisomant. Further studies have to confirm whether co-treatment with Pegvisomant can optimize disease control and is thereby able to improve joint symptoms. Alternative explanations for increased OA progression in SMS-treated patients may be a direct IGF-1 independent effect of SMS analogs on joint structure, or a generally less favourable previous course of acromegaly in SMS-treated patients. Since SMS analogs are preferred as adjunctive therapy after incomplete surgery and are considered as a valuable alternative for surgery as primary therapy, present findings may have implications for current treatment strategies.

Chapter 3 focuses on the clinical course of acromegalic arthropathy during prolonged follow-up in remission. Clinical arthropathy was assessed by validated questionnaires on self-reported joint complaints of the hands and lower limb, by structured physical examination and joint performance tests. We found that on average, hand and lower limb function deteriorated during follow-up, although interindividual variations were large. Joint pain was stable over time. High levels of pain and functional impairments at baseline were risk factors for clinical progression with respect to hand pain and function. In the lower limb, high BMI was a risk factor for function loss. Changes in clinical symptoms were not related to radiographic progression during follow-up, which is a discrepancy that is well-known from primary OA.

We can conclude from **Chapter 2** and **Chapter 3** that acromegalic arthropathy is a progressive joint disease that is not merely halted or reversed by biochemical disease control. Since clinical and radiographic progression of arthropathy are not related, we propagate that in clinical practice a combination of clinical and radiographic assessment is necessary to evaluate the course of acromegalic arthropathy. As previously shown, physical joint symptoms significantly impair QoL. In this respect, since the optimal management of acromegalic arthropathy is currently unknown and (any) medication does not influence the clinical course of arthropathy, further investigation has to assess whether specific intervention therapies, for example physiotherapy, are beneficial.

VISUALIZATION OF ACROMEGALIC ARTHROPATHY BY MRI: In **Chapter 4**, we further characterize acromegalic joints by 3.0T MRI scans of the knee. We chose for this imaging modality since MRI may give additional information to plain films, especially on cartilage

damage and subchondral bone abnormalities, such as osteophytes, cysts and bone marrow edema. Comparisons were made between patients with active acromegaly and acromegaly patients in remission, either by medical treatment or surgery/radiotherapy. Primary OA patients from the geMstoan study, which is a longitudinal study among primary OA patients with established knee OA, were included as controls in order to differentiate which structural abnormalities on MRI were acromegaly-specific. Furthermore, cartilage thickness and cartilage T2 relaxation times, providing information on the biochemical composition of cartilage, were compared between acromegaly patients and, respectively, geMstoan controls and literature controls. In the present study, which is the first study on acromegalic arthropathy using MRI, we showed that structural OA defects are already highly prevalent in the active acromegaly phase. In patients with active acromegaly articular cartilage is not only thicker than in the controlled disease phase, but is also from a different biochemical composition, as reflected by higher cartilage T2 relaxation times, suggesting increased water content and collagen breakdown in these patients. The findings of the present study could introduce the hypothesis that in active acromegaly the thickened joint cartilage consists of two different components: a structural component of cartilage hypertrophy, being (partially) irreversible despite long-term biochemical remission, and a component of edema, which is reflected by the cartilage T2 relaxation times, that decreases after successful treatment. This may explain why joint cartilage of controlled acromegalics is still thickened due to persisting cartilage hypertrophy, but decreases after achievement of biochemical remission compared to the active disease phase due to a decrease in water content, with a corresponding decrease in cartilage T2 relaxation times. When compared to subjects with primary OA, acromegaly patients had thicker knee cartilage and, at all measured sites, cartilage T2 relaxation times were higher in acromegaly patients, indicating that joint cartilage is not only thicker in acromegaly patients but has also an altered average biochemical composition. The relevance of these altered cartilage composition should be investigated in future studies.

RADIOGRAPHIC PHENOTYPE OF ACROMEGALIC ARTHROPATHY: Joint space narrowing is an infrequent radiographic finding in patients with acromegalic arthropathy. It is unknown whether joint space narrowing reflects the end-stage of acromegalic arthropathy or whether this feature develops independently of acromegaly. In **Chapter 5**, risk factors for joint space narrowing and its relationship to clinical symptoms were studied. We found joint space narrowing in, respectively, 10.3% and 15.4% of the hips and knees of patients with controlled acromegaly. Well-known risk factors, such as age and female sex were associated with more joint space narrowing; acromegaly-specific risk factors for joint space loss were joint-site specific. In the hip, joint space narrowing was related to more active acromegaly disease, as reflected by higher pre-treatment GH/IGF-1, longer and more severe GH exposure, and less cure by surgery/radiotherapy. In the knee, especially previous knee surgery, not acromegaly-specific characteristics, was associated with joint space narrowing. The presence of joint space narrowing was associated with more joint complaints. The present study shows that joint space narrowing in an infrequent finding in patients with acromegalic arthropathy, but is, although, associated with more clinical symptoms. At least in the hip, there is a role for excessive GH / IGF-1 activity, not only in the early stage of acromegalic arthropathy, but also in its late phase, as reflected by joint space narrowing. Present findings underline the irreversibility of the joint effects caused by previous GH excess, and characterize the progressiveness of acromegalic arthropathy.

PROGRESSION OF VERTEBRAL FRACTURES IN CONTROLLED ACROMEGALY: Another long-term consequence of acromegaly is the occurrence of vertebral fractures in a high proportion of patients. Also in controlled disease, prevalence of vertebral fractures was shown to be impressively high (up to 60%). Most patients suffer from multiple vertebral fractures, especially of the anterior wedge type. In these patients, BMD is frequently normal and is therefore a bad predictor of future fracture risk in this form of secondary osteoporosis. Therefore, in these patients, the high vertebral fracture risk appears to be caused by a problem in bone quality rather than bone quantity. To date, it is unclear whether patients in long-term remission have a persistent, irreversible high risk of future fractures, or whether GH/IGF-1 control reduces the fracture risk to normal, despite presence of prevalent vertebral fractures. In view of the excess mortality and significant morbidity associated with vertebral fractures, insight in progression is of paramount clinical significance.

In **Chapter 6**, the disease course of vertebral fractures and potential determinants for progression were studied in treated acromegaly patients. We found that patients not using bisphosphonates, baseline prevalence of vertebral fractures was very high (63%). Prevalence was highest in males and was unrelated to BMD. Patients frequently suffered from multiple vertebral fractures. We found progression of vertebral fractures in 20% of patients over a relatively short follow-up period of 2.5 years, with highest progression rate in males and in case of ≥2 vertebral fractures at

baseline. No other risk factors for progression were identified. Progression of vertebral fractures was not related to BMD values or the change in BMD over time, at either the lumbar spine or total hip. The present study indicates that in acromegaly, vertebral fractures do not solely develop during the active phase of disease. During prolonged follow-up, normal BMD was shown to be maintained, although apparently this does not protect against progression of vertebral fractures. These results suggest that the high vertebral fracture risk in acromegaly patients is due to abnormalities in bone quality. We hypothesize that in active acromegaly the following process occurs: due to cortical proliferation, the bone volume produced per time unit increases, whereas due to trabecular loss, the mechanical loading strength of the bone decreases, despite thickening of trabecles. We postulate that these changes result in decreased bone material strength properties, in the presence of normal BMD. This decreased bone quality might explain the high fracture risk in acromegalic vertebrae, since vertebrae mostly consist of trabecular bone. As shown in previous studies from our center, this trabecular widening sustains after disease cure, whereas the amount of cortical bone decreases. Therefore, vertebral fracture risk remains irreversibly high despite long-term disease remission.

Since BMD is normal in most acromegaly patients and is therefore a bad predictor for fracture risk, it is a clinically relevant question how to identify patients at risk for (vertebral) fractures. We propose to include vertebral fracture assessment in the screening of acromegaly patients and during follow-up after establishment of biochemical control. Further research should address the question whether acromegalic patients require treatment for these (vertebral) fractures and whether they actually benefit from bisphosphonates.

Part B. The role of the GH / IGF-1 axis in primary osteoarthritis

Part B describes studies investigating the involvement of the GH / IGF-1 axis in the development and progression of primary OA. OA is a common disease, characterized by progressive degradation of articular cartilage and bone remodeling, resulting in pain and disability. Despite the increase in molecular knowledge accrued during the last years, the exact pathogenesis of the destructive process remains unknown. OA is considered to be a multifactorial disease in which age, BMI, hormonal and local biomechanical factors together with genetic predisposition play a role.

RELATIONSHIP BETWEEN SERUM IGF-1 CONCENTRATIONS AND PRIMARY OA : Several lines of research suggest a role of the GH / IGF-1 axis in OA pathogenesis. First, IGF-1 has been shown to enhance chondrocyte proliferation and proteoglycan and collagen synthesis by chondrocytes in normal cartilage, both *in vivo* and *in vitro*, also during cytokine exposure. These anabolic and protecting properties make IGF-1 an obvious candidate for a major role in cartilage repair. Second, in acromegaly disease, we found further evidence for an association between the activity of the GH/IGF-1 axis and the risk of secondary OA. Prevalence and severity of arthropathy worsen with the duration of uncontrolled acromegaly, and pre-treatment IGF-1 levels predict radiographic OA in a dose-dependent manner. Finally, previous studies showed that genes regulating formation, degradation and repair of articular cartilage and subchondral bone remodelling may be involved in OA pathogenesis. In this respect, especially endochondral ossification is of importance (*vide infra*).

In this context, in **Chapter 7**, we summarized the evidence for a role of serum IGF-1 concentrations in primary OA by conducting a systematic review of literature. We also addressed the association between several IGF-1 gene polymorphisms and the onset of primary OA. In total, 11 studies were eligible for inclusion. Methodological quality of all individual studies was assessed using a standardized set of criteria, which have been used previously in reviews on musculoskeletal disorders. *In vitro* studies, genetic association studies and the high secondary OA risk in pathological IGF-1 states all suggest an effect of the GH / IGF-1 system in OA development. However, although the largest cross-sectional study and the longitudinal study on OA progression found a positive association with high serum IGF-1, inconsistent results were shown in studies on serum IGF-1 and radiographic OA. We concluded from this systematic review that there is moderate evidence that IGF-1 is no risk factor for radiographic OA in patients with primary OA. Since we cannot exclude that methodology, publication bias and small sample size of the available studies have influenced the results; we suggest that future well-designed large prospective studies are needed to strengthen the evidence for the role of IGF-1 in primary OA. In this respect, especially age, sex and BMI has to be taken into account, since these factors are important for the interpretation of IGF-1 levels.

Since our systematic review, which we described in **Chapter 7**, reported inconsistent results among epidemiological studies investigating the association between serum IGF-1 and radiographic OA, we studied serum IGF-1 concentrations in relation to primary OA onset and progression in

a large familial OA cohort, taking into account age, sex and BMI. These results are described in **Chapter 8**. We used a well-characterized cohort of patients with familial OA at multiple joint sites from the Genetics osteoARthritis and Progression (GARP) Study, in which serum IGF-1 levels were within the normal range. We found that in the GARP cohort mean IGF-1 SDS was significantly increased when compared to reference values. We were, however, not able to demonstrate a relationship between serum IGF-1 levels and presence or progression of radiographic OA or worsening of individual OA features (*i.e.* osteophyte growth or joint space loss) at specific joint sites within the GARP study. Several remarks have to be made. First, it should be noted that all GARP subjects have a severe OA phenotype of familial OA at multiple joint sites, making joint-specific analysis difficult to perform. In addition, since the GARP Study is a very homogeneous cohort with less variation in OA phenotype, the detection of a clear dose-response relationship is difficult. Second, serum IGF-1 levels of almost all GARP subjects were within the normal reference range, resulting probably in too less variation in IGF-1 levels to detect an association with OA severity or progression. In addition, IGF-1 measurement by serum samples is very complex, because individual IGF-1 levels are liable to temporary variations and are inversely correlated to age, estrogen levels and BMI. Furthermore, radio-immuno assays (RIA) for measuring serum IGF-1 are subject to analytical difficulty. The findings of the present study are in accordance to several lines of previous evidence suggesting a role for increased GH/IGF-1 activity in the pathophysiology of OA. However, no clear dose-response relationship between IGF-1 SDS and either the presence or progression of radiographic OA was found. Taking into account imperfections in IGF-1 measurement and the homogeneous severe OA phenotype in the GARP Study, further research has to clarify the complex role of the GH / IGF-1 axis in OA pathophysiology.

ASSOCIATION BETWEEN D3-GHR POLYMORPHISM AND PRIMARY OA: Genetic influences play a considerable role in the pathogenesis of primary OA. Genetic studies have identified several variants associated with primary OA, which were all involved in the process of endochondral ossification, being the main process in longitudinal skeletal growth. Endochondral ossification is driven by growth plate chondrocytes, and result in longitudinal growth through a combination of proliferation, extracellular matrix (ECM) secretion and hypertrophy. Subsequently, terminally differentiated chondrocytes die and are replaced with bone tissue. At all stages, chondrocyte behaviour is tightly regulated by a complex network of interactions between circulating hormones, locally produced growth factors and ECM components. One of the strongest stimulators of chondrocyte proliferation is GH, predominantly via IGF-1 secretion. This qualifies variations within GH / IGF-1 genes as obvious candidates for association studies.

The effects of GH on target tissues are mediated by the dimeric GHR, which exists of different molecular structures, depending on assortments of coding polymorphisms in the GHR gene. In 2004, Dos Santos *et al.* described a common polymorphism of the GHR, called exon 3 deleted GHR (d3-GHR), which was found to increase the growth response in children with different causes of growth failure. This common d3-GHR polymorphism is present in ~30-35% of the normal population, and results in a truncated receptor with an increased GH responsiveness, being attributed to an enhanced signal transduction. Since 2004, in various clinical conditions, such growth failure in children and acromegaly, d3-GHR polymorphism was shown to have functional consequences. In this context, in acromegaly patients, the d3-GHR polymorphism was previously reported to be associated with the presence and the severity of radiographic arthropathy. The association with primary OA, however, has never been studied.

To further explore the role of the GH / IGF-1 axis in primary OA, we studied in **Chapter 9** the association between the d3-GHR polymorphism and (symptomatic) primary OA, using a candidate-gene study approach. We initially did explorative analyses in males and females with familial OA at multiple joint sites from the GARP Study *vs* population-based controls without (signs of) OA. The GARP Study was chosen as discovery cohort, because this study consists of genetically enriched OA patients with information on multiple joint sites. Patients from the GARP Study were genotyped for 7 single nucleotide polymorphisms (SNPs) encompassing the d3-GHR gene (rs4590183, rs13354167, rs7721081, rs7701605, rs4242116, rs6878512, rs10941583). All SNPs were in high linkage disequilibrium with d3-GHR. We selected the SNP rs4590183 as proxy for d3-GHR, and tested this SNP in three additional cohorts for replication purposes, being the PAPRIKA (PAtients Prospectively Recruited In Knee and hip Arthroplasty)/RAAK (Research Articular osteoArthritis Cartilage) study including subjects with a total hip and/or knee prosthesis due to end-stage OA, Rotterdam study among subjects with severe symptomatic OA and ACRO study including acromegalic patients with symptomatic and/or radiographic OA. Finally, the GARP, PAPRIKA/RAAK, Rotterdam and ACRO studies were combined in

a genetic meta-analysis. In the GARP Study, we found an association between the d3-GHR polymorphism and OA in females (adjusted odds ratio (OR) 1.36 (95%CI 1.01-1.83), p=0.043), not in males. Since the association between d3-GHR and OA was only present in females, the meta-analysis was aimed on female OA cases, comprising a total of 2175 OA cases and 2623 controls. The combined analysis showed evidence for association between the d3-GHR polymorphism and OA at any joint location in females (OR=1.17 (95%CI 1.04-1.32), p=0.013). This association remained significant after exclusion of the discovery study (OR=1.14, 95%CI 1.01-1.30, p=0.042). Stratifying by joint site revealed consistent effect sizes of approximately 1.2–1.3 among the joint strata, with most profound effects in cases with hip OA. It has to be noted that the effect sizes reported in our study are relatively large in contrast to recent large scale GWA studies, such as of Zeggini *et al.*. This is likely to be explained by the fact that we have applied a family-based sampling scheme towards the extreme spectrum of the OA phenotype for the GARP Study. Thereby, this study is tailored to find genetic variants in the low frequency range with moderate to large effect sizes. Being aware of the tendency of association studies to produce false-positive results, additional replication of our results is necessary.

The results of the present study are in line with those of **Chapter 8**, indicating that overactivity of the GH / IGF-1 axis accelerates the OA process in susceptible patients, and is thereby associated with an increased OA risk. We conclude from **Chapter 9** that the effects of the d3-GHR polymorphism are not limited to subtle variations in growth parameters in children with growth failure or acromegaly patients. Despite the fact that the effect of d3-GHR on the GH /IGF-1 axis itself is not yet completely elucidated, the functional effects of the d3-GHR affect many pathophysiological processes in which the GH / IGF-1 axis is involved to some extent. The findings of the studies described in **Part B** provide further evidence for involvement of the GH / IGF-1 axis in the pathophysiology of primary OA. For clinical practice, the increased activity of the GH / IGF-1 system as a risk factor for primary OA (onset) could be a potential therapeutic target.

Part C. Long-term outcome of recombinant human GH (rhGH) therapy in GH deficient (GHD) adults

Part C addresses the long-term effects of rhGH therapy in adult patients with GHD, studied in a large well-defined cohort of GHD patients that were diagnosed and followed yearly at the outpatient clinic of the LUMC. GHD in adults is associated with an adverse metabolic profile that includes abdominal obesity, dyslipidemia and an increased mortality risk. Short-term follow-up studies have shown that rhGH replacement reduced some, but not all of these cardiovascular risk factors. Consistent effects were reported on body composition and lipid metabolism, characterized by reduction of body fat and an increase of lean body mass, and a reduction of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels. In selected patients, also positive effects on QoL and general well-being were described. Based on these positive short-term effects, rhGH replacement has become widely accepted as chronic therapy in adult GHD. These effects were reported to be sustained for the first 5 years of rhGH treatment; however, long-term efficacy and safety data are very limited and frequently uncontrolled for the effect of ageing.

LONG-TERM OUTCOME OF RHGH SUPPLEMENTATION IN GHD ADULTS: In **Chapter 10**, we systematically reviewed the effects of chronic rhGH therapy, defined as at least 5 years, in GHD adults on biochemical and anthropometric parameters, QoL, bone metabolism, muscle strength, serious adverse events (SAEs) and mortality. In total, 23 studies met our inclusion criteria, and were therefore eligible for inclusion. Methodological quality of all studies was assessed using a standardized set of criteria based on the STROBE statement. We reported that available literature on long-term rhGH replacement in adult GHD patients shows inconsistent results with respect to its expected beneficial effects, in the presence of several drawbacks to enable a definite interpretation. First, long-term studies were generally uncontrolled, lacking a control group (of non-treated GHD patients) enabling adjustment for subjective changes or changes due to ageing. Second, only a limited number of centers have reported their data, resulting in a low number of evaluable patients with half of the long-term studies describing (part of) the same patient cohort. Especially the course of QoL during ongoing therapy is unestablished. With respect to the metabolic profile, rhGH therapy has shown prolonged, beneficial effects on body composition, lipid profile, carotid IMT and BMD, but overall cardiovascular risk, as assessed by the prevalence of the metabolic syndrome (MS), glucose levels, BMI appeared not to be influenced or were

even negatively affected. It has to be noted that accurate data on the use of lipid-lowering or anti-diabetic medication was lacking in most studies. Therefore, we concluded that the benefit of long-term rhGH treatment should be a matter of ongoing research to enable adequate risk-benefit analyses and, in clinical daily practice, the benefits of rhGH should be considered carefully in each patient.

METABOLIC EFFECTS OF LONG-TERM RHGH SUPPLEMENTATION IN GHD ADULTS: Since 10-year follow-up studies on the effects of rhGH suppletion are scarce, patient numbers are small in most studies and information on the use and handling with lipid-lowering and anti-diabetic medication is frequently lacking, we studied the 10-years metabolic effects of rhGH replacement in our own GHD cohort. In **Chapter 11** we described the effects of 10 years rhGH replacement on biochemical and anthropometric parameters in 98 adult GHD patients, of which a subset of patients (N=49) had been received rhGH suppletion for 15 years. In addition, we studied the prevalence of the metabolic syndrome (MS), and calculated the incidence of major cardiovascular events. All GHD patients were stringently controlled with proposed physiological rhGH replacement by closely monitoring IGF-1 SD levels, adjusted for age during the entire study period. We reported ongoing beneficial effects on the lipid profile after 10 years of rhGH replacement, with a significantly decrease in total cholesterol and LDL-C levels and an increase in HDL-C when compared to baseline. On the other hand, the increases in waist circumference, BMI and fasting glucose levels during rhGH replacement were striking. In the subset of patients with 15-year rhGH replacement, generally similar metabolic effects were found. However, despite improvement of several cardiovascular risk factors, MS prevalence increased significantly despite rhGH replacement, from 32.7% before the start of rhGH replacement to 57.1% after 10 years. This increase was most definite in male GHD patients. Incidence of major cardiovascular events was low (1.5/1000 patient-years). In conclusion, since, currently, improvement of cardiovascular risk, in addition to QoL, is the major target for rhGH treatment in adult GHD patients, larger and controlled studies have to establish the net beneficial effects of long-term rhGH suppletion on the cardiovascular risk in these patients. In addition, our metabolic findings merit critical re-evaluation of prolonged rhGH replacement, also from a cost-benefit point of view. In this respect, rhGH replacement should be compared with widely available drugs, *i.e.* statins, that have been proven to be effective in secondary cardiovascular risk prevention.

A limitation of the study described in **Chapter 11**, and a general drawback of long-term follow-up studies, is the lack of a non-treated control group. Because the beneficial effects of rhGH therapy are well-established in the short term, it is regarded to be unethical to withhold patients with GHD from receiving rhGH in case of no contraindications. This makes it difficult, if not impossible, to perform long-term randomized controlled studies including GHD patients without rhGH treatment. Since an ideal unselected GHD control group without rhGH replacement is not available, an alternative strategy to explore long-term (metabolic) effects of rhGH in GHD patients is the comparison with healthy controls. Recently, in the LUMC, a large population-based study focused at the pathophysiology of obesity (Nederlandse Epidemiologie van Obesitas (NEO) Study) was conducted. Therefore, large scale contemporary control data of normal-weight and obese subjects, derived from the same geographic area as the GHD patients, are available.

In order to assess the ability of current rhGH replacement strategies to normalize metabolic parameters, we describe in **Chapter 12** the long-term effects of rhGH replacement on the metabolic profile. We compared a large cohort of 161 middle-aged GHD patients on chronic rhGH replacement (*i.e.* 5 years) with healthy normal-weight middle-aged control subjects from the Leiderdorp cohort of the NEO Study. We reported that, despite chronic rhGH replacement, GHD patients had a 1.3-times higher MS risk than controls, independently of age, sex and BMI (53.4% *vs* 30.3%). In addition, GHD patients remain to have a different metabolic profile than controls with similar BMI, with an increased risk of hypertriglyceridaemia and low HDL-C, but less hyperglycaemia. This indicates that the metabolic phenotype of middle-aged GHD patients is complex and cannot simply be extrapolated to the adverse cardiovascular phenotype as is present in the general population. It is remarkable that although this GHD cohort is thought to be adequately hormonally substituted, the metabolic profile after chronic rhGH replacement resembles the profile of an untreated GHD patient, with predominantly lipid abnormalities. These findings question whether long-term rhGH replacement is as beneficial as previously propagated with respect to net cardiovascular risk reduction. We propagate that in the context of increasing evidence for, on the one hand, a (negative) role of GH and IGF-1 in cancer, longevity and cardiovascular disease, and, on the other hand, the limited evidence for benefit of rhGH substitution in the elderly GHD population, the long-term rhGH use in GHD adults should be critically re-evaluated.

EFFECTS OF LONG-TERM RHGH SUPPLEMENTATION ON BONE METABOLISM IN GHD ADULTS: In **Chapter 13**, we assessed the effects of 5, 10 and 15 years of rhGH replacement on bone markers, BMC and BMD in a well-characterized cohort of 230 adult GHD patients. We further studied fracture incidence during rhGH suppletion. We found a sustained increase in lumbar spine BMD, lumbar spine BMC and T-scores during the first 10 years, with stabilization thereafter. Highest effects were found in men, which is in accordance to the results of several other studied investigating the bone response to rhGH suppletion. No effects on femoral neck BMD were found. Concomitant bisphosphonate use did not have additional beneficial effects on BMD in these patients. Clinical fracture incidence during long-term rhGH suppletion was 20.1 / 1000 py and seemed to be low in these patients. In conclusion, these findings from our cohort study of GHD patients demonstrate ongoing beneficial effects on bone metabolism of long-term rhGH replacement therapy, particularly in men, with relatively low clinical fracture incidence. Sex-specific differences in bone response to rhGH replacement and the long-term beneficial effects of bisphosphonates on fracture risk warrant further studies in adult GHD patients, and therefore, protocolled prolonged follow-up of these patients is mandatory.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Part A. Long-term effects of acromegaly on joints and bone

In acromegaly, appropriate treatment by surgery, radiotherapy, medical therapy, or a combination of these treatment modalities, considerably improves many systemic co morbid conditions. Unfortunately, it has become apparent that despite biochemical control, many manifestations of acromegaly remain detectable during prolonged follow-up, being the consequence of previous (transient) GH excess. Patients suffer from severe skeletal manifestations, significantly affecting QoL and mortality. In this respect, arthropathy and VFs are common invalidating (irreversible) complications of acromegaly.

The studies described in this thesis describe the characteristics and disease course of acromegalic arthropathy, (partly) by using new validated techniques. Acromegalic arthropathy was shown not to have an unequivocal phenotype: it is characterized by osteophytosis and wide joint spaces in most patients, however, in a minority of patients JSN is observed. Cartilage imaging by MRI showed that especially joint cartilage differs between acromegaly patients and primary OA subjects. Pathologically GH secretion was found to be associated not only with thicker joint cartilage, but also with changes in biochemical cartilage composition, as reflected by changes in T2 cartilage relaxation times. Furthermore, we reported that acromegalic arthropathy is a IGF-1 dependent progressive joint disease, both clinically and radiographically, which is not merely halted by biochemical disease control. Arthopathy progression seemed to be associated with insufficient GH control. Further studies have to confirm whether more aggressive therapy, for example by addition of Pegvisomant, can optimize disease control and thereby can improve joint symptoms. In clinical practice, a combination of clinical and radiographic arthropathy assessment is essential in these patients. In addition, further investigation has to focus on acromegalic-specific intervention therapies to improve OA symptoms in these patients. Finally, there is a need for acromegaly-specific scoring methods and imaging techniques, such as the semi-quantitative measurement developed by Van 't Klooster *et al.* and the MRI techniques described in this thesis to fully characterize acromegalic arthropathy.

Another highly prevalent skeletal complication of acromegaly is the presence of vertebral fractures. We found impressively high vertebral fracture prevalence in long-term controlled acromegalics not treated with bisphosphonates, in the presence of normal BMD values and normal Vitamin D levels. Progression of vertebral fractures was shown in 20% of patients, indicating that the occurrence of vertebral fractures is an irreversible process. A possible explanation for the high vertebral fracture risk acromegaly is a diminished bone quality, which should be investigated in future studied, by performing bone biopsies. Further research should focus on an optimal treatment strategy for vertebral fractures, since, currently, the benefit of bishosphonates in this specific patient group is yet unestablished.

Part B. The role of the GH / IGF-1 axis in primary osteoarthritis

Primary OA is a debilitating common disease, characterized by progressive degradation of articular cartilage and bone remodeling. Despite the increase in molecular knowledge accrued during the last years, the exact pathogenesis of the destructive process remains unknown. OA is considered to be a multifactorial disease in which age, BMI, hormonal and local biomechanical factors together with genetic predisposition play a

role. Several lines of research suggest a role of the GH / IGF-1 axis in OA pathogenesis.

The studies described in this thesis further support the evidence for involvement of the GH / IGF-1 axis in primary OA onset and progression. We reported that patients with familial primary OA at multiple joint sites from the GARP Study had, overall, higher serum IGF-1 concentrations than expected. These findings are in line with the results of our genetic meta-analysis comprising 2175 cases and 2623 controls, in which we found an association between the common d3-GHR polymorphism, being associated with an enhanced GH responsiveness of the GHR, and primary OA in females. Largest effect was found in cases with hip OA. Together, these studies indicate that increased activity of the GH / IGF-1 axis accelerates the OA process in susceptible patients, and is thereby associated with an increased OA risk. Future large controlled studies should confirm whether OA patients indeed have higher IGF-1 concentrations compared to patients without any signs of OA. For clinical practice, the increased activity of the GH / IGF-1 system as a risk factor for primary OA (onset) could be a potential target for therapeutic approaches. In this respect, it would be interesting to study whether administration of GH-lowering lowering medication can be effective in inhibiting OA progression.

Part C. Long-term outcome of rhGH replacement in GHD adults

GHD in adults is a clinical entity associated with an adverse metabolic profile that includes abdominal obesity and dyslipidemia, decreased bone mass, decreased QoL and an increased mortality risk. Short-term follow-up studies have shown consistent beneficial effects of rhGH replacement on body composition and lipid metabolism, and in selected patients, also positive effects on QoL and general well-being were described. Based on these positive short-term effects, rhGH replacement has become widely accepted as chronic therapy in adult GHD. These effects were reported to be sustained for the first 5 years of rhGH treatment; however, long-term efficacy and safety data are very limited and frequently uncontrolled for the effect of ageing.

The studies described in this thesis report the long-term effects of rhGH replacement in a large well-defined cohort of adult GHD patients, proposed to be stringently controlled. We reported ongoing beneficial effects on the lipid profile after 10 years of rhGH replacement, but, on the other hand, significant increases in waist circumference, BMI and fasting glucose levels. Despite improvement of several cardiovascular risk factors, MS prevalence increased significantly during rhGH replacement and was significantly higher than in age-, sex- and BMI-matches controls from the general population. In addition, there were differences in the metabolic profile between rhGH-treated GHD adults and controls. Incidence of major cardiovascular events during long-term rhGH supplementation was low. With respect to bone metabolism, rhGH suppletion seems to be beneficial on BMD in the long-term, especially at the lumbar spine and in men, in the presence of low fracture incidence.

Based on these data, we propagate that in the context of increasing evidence for, on the one hand, a role of GH and IGF-1 in cancer, longevity and cardiovascular disease, and, on the other hand, the limited evidence for benefit of rhGH substitution in the elderly GHD population, structural follow-up and continuous monitoring of rhGH treatment is necessary. In this respect, new, more sensitive biomarkers to monitor disease activity are needed.