Pathophysiology of the GH/IGF-1 axis : long-term consequences on joints and bone

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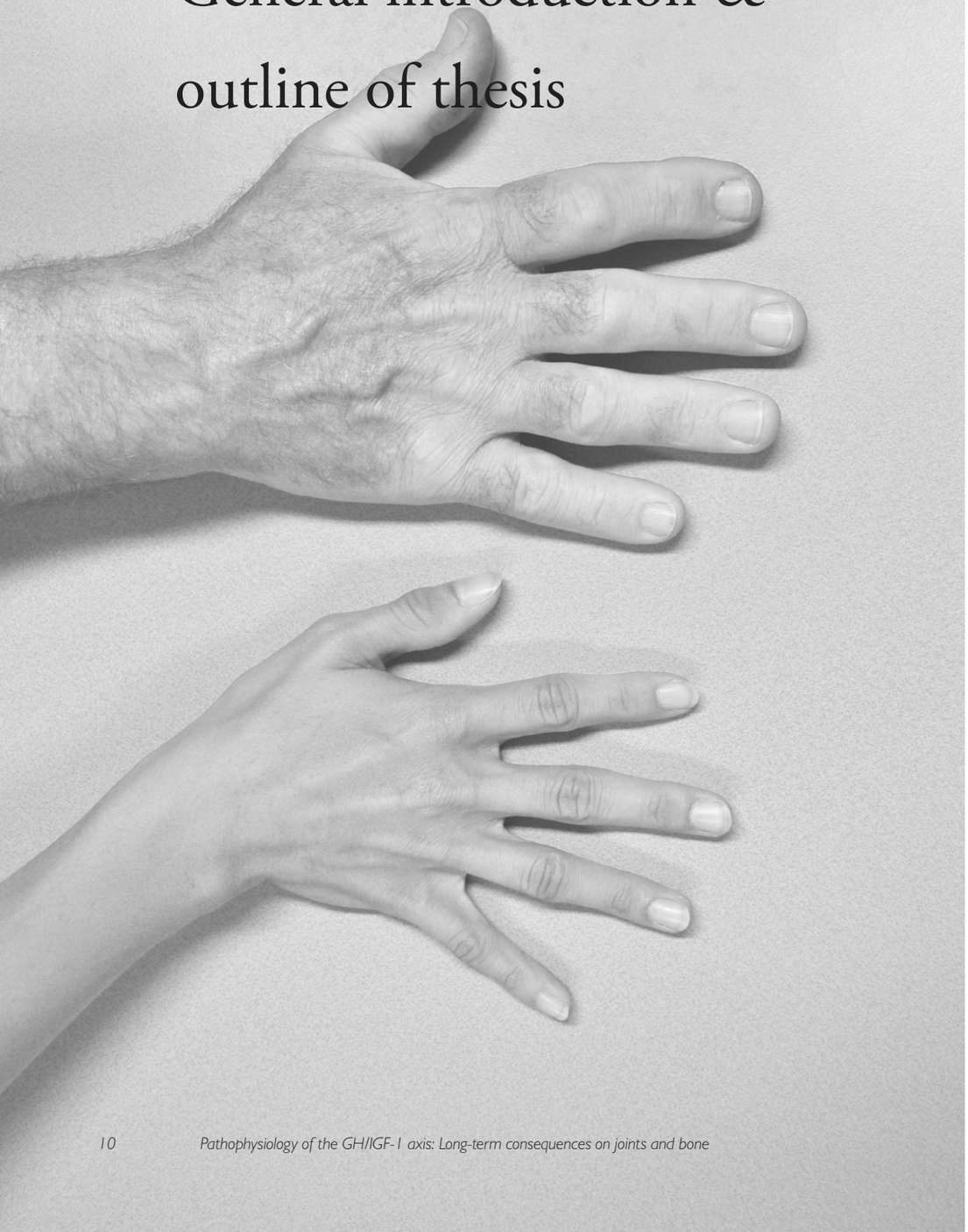
General introduction & outline of thesis



INTRODUCTION

Growth Hormone (GH) and Insulin-like Growth Factor-1 (IGF-1) play a central role in the promotion of longitudinal skeletal growth. The anabolic effects of these hormones involve many organ systems, including stimulation of protein synthesis and inhibition of protein catabolism, and regulation of lipolysis. Furthermore, GH and IGF-1 are involved in bone remodeling and muscle growth, but they also exert effects on joints. Several studies showed evidence for the involvement of GH and IGF-1 in longevity and ageing, suggesting that optimizing the GH / IGF-1 axis is of importance to promote healthy ageing in the general population.

In this thesis, firstly, the long-term consequences of (transient) GH excess on bone and joints were studied in patients with biochemically controlled acromegaly. Secondly, the role of the GH / IGF-1 axis was investigated in patients with primary osteoarthritis (OA) having serum IGF-1 levels within the normal range. Finally, the long-term effects of recombinant human GH (rhGH) replacement in adult GH deficient (GHD) patients were studied, focusing on the cardiovascular consequences and the effects on bone metabolism.

GH physiology and regulation

GH is a single chain polypeptide hormone that is synthesized, stored, and secreted by somatotrope cells in the pituitary gland. In plasma, GH circulates freely or is bound to GH-binding protein (GHBP). GH is cleared via renal and hepatic mechanisms.

Control of GH secretion is regulated at the hypothalamic and the pituitary level by the interaction of stimulatory and inhibitory hormones, resulting in a diurnal and pulsatile secretion pattern. GH is especially released during sleep, stress and exercise (1;2). In addition, GH secretion is enhanced by thyroxin, sex steroids, cortisol, amino acids and fasting. GH secretion is inhibited by meals, glucose, free fatty acids, glucocorticoid excess and adiposity. GH secretion is highest in the late puberty; thereafter there is an age-dependent decline of GH production and –secretion. Estrogen affects GH action at the level of GH receptor (GHR) expression and signaling. It was shown that estrogens inhibit the JAK/STAT pathway and the activator of transcription signaling by GH via the induction of a cytokine suppressor (3), resulting in a higher GH/IGF-1 ratio in females when compared to males. Therefore, premenopausal women have higher ambulant GH levels than men; this difference disappears after menopause (4;5).

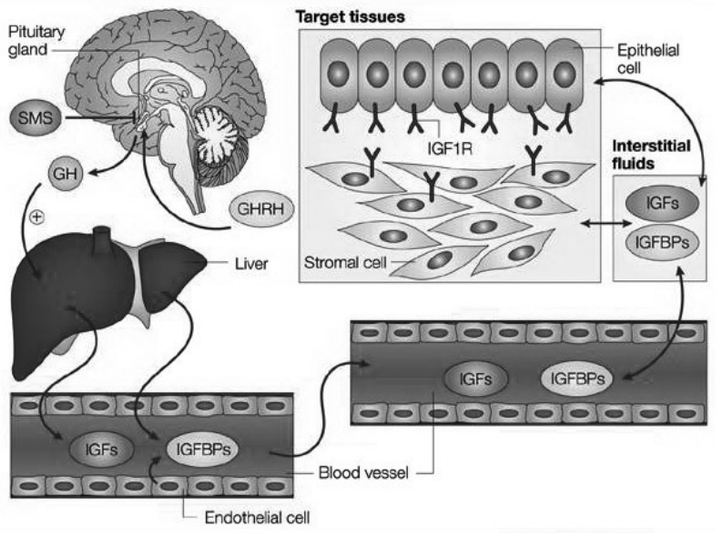


Figure 1. Regulation of the GH / IGF-1 axis *(Adapted from Pollak et al. Nat Rev Cancer 2004; 4(7): 505-518)*

Hypothalamic Growth Hormone-Releasing Hormone (GHRH) stimulates GH gene transcription, GH cell proliferation and GH release. The hypothalamic inhibitory hormone somatostatin (SMS) acts via the binding to SMS receptors, and inhibits GH release from the secretory granules in the somatotropes and also inhibits GHRH release. In addition, there is a negative feedback loop of GH at the hypothalamic level, and of IGF-1 both at the level of the hypothalamus and the pituitary.

GH stimulates the production of IGF-1 in many organs, especially in the liver, which, in turn, mediates its effects primarily by the IGF-1 receptor type 1 (6). IGF-1 is an important growth-promoting polypeptide with structural and functional homology to pro-insulin. IGF-1 mediates the effects of GH at tissue level and is a key factor for longitudinal skeletal growth (7). Serum IGF-1 concentrations reflect the GH concentrations over 24 hours. IGF-1 binding proteins regulate the bioavailability of IGF-1. Serum IGF-1 concentrations in adulthood decrease progressively with age and are influenced by many other factors, such as BMI (8;9).

GH function

The primary effect of GH is the promotion of longitudinal skeletal growth. GH exerts its effects on the growth plate predominantly through stimulation of IGF-1 secretion (6). The anabolic actions of GH and IGF-1 involve many organ systems, including stimulation of protein synthesis and inhibition of protein catabolism, and regulation of lipolysis. Furthermore, GH is involved in bone remodeling, muscle growth, and immunomodulation. GH antagonizes the actions of insulin, resulting in glucose intolerance and hyperinsulinaemia. In contrast, IGF-1 has insulin-like effects by enhancing peripheral glucose uptake.

GHR and d3-GHR polymorphism

Effects of GH on target tissues are mediated by the dimeric GH receptor (GHR). Three variants of the GHR that differ in the presence or absence of exon 3 ($GHR_{fl\text{-}fl}$ homodimer, $GHR_{fl\text{-}d3}$ heterodimer, and $GHR_{d3\text{-}d3}$ homodimer) are commonly seen in the general population (10). The function of exon 3 is unknown, although the deletion is in close proximity to the GH binding site (11). The loss of exon 3 results in a truncated receptor. This truncation appears to have little effect on the receptor, since $GHR_{fl\text{-}d3}$ and $GHR_{d3\text{-}d3}$ are stable, functional receptors with no significant differences in binding activity or internalization when compared to $GHR_{fl\text{-}fl}$. However, the presence of at least one d3-allele is associated with increased GH responsiveness by enhanced signal transduction (12;13), which was shown to have functional consequences in various clinical conditions. This is illustrated in children with growth failure by increased growth velocity after recombinant human GH (rhGH) administration, and in acromegaly, a rare endocrine syndrome due to pathological GH oversecretion, in which d3-carriers showed an increased biochemical disease activity and worse clinical outcome (12;14-16).

Effects of GH and IGF-1 on cartilage and bone

UNDERLYING MECHANISMS: GH and IGF-1 are important regulators of bone homeostasis, and play a central role in the achievement of normal longitudinal bone growth and bone mass. Although GH may act directly on skeletal cells, most of its effects are mediated by IGF-1, which is present in the systematic circulation and is synthesized by peripheral tissues and acts at later stages of chondrocyte maturation (17;18). Longitudinal bone growth is determined by chondrocyte proliferation and differentiation in the epiphyseal growth plate of long bones, resulting in endochondral bone formation. Within the growth plate, chondrocyte proliferation, hypertrophy and differentiation result in chondrogenesis. The newly formed cartilage is invaded by blood vessels, and it is modeled into bone trabeculae. This endochondral ossification process is regulated by genetic and hormonal factors, the cellular environment, and nutrition (19). GH

is a main stimulator of chondrocyte proliferation in the growth plate, and, to a lesser extent, of ECM secretion and the hypertrophic switch of post-proliferative chondrocytes. These effects are predominantly mediated via the secretion of IGF-1.

In addition to the effects on longitudinal growth, GH and IGF-1 are anabolic hormones and have the potential to regulate bone modeling and remodeling (*Figure 2*). GH stimulates the proliferation of cells of the osteoblastic lineage, although IGF-1 is required for selected anabolic effects of GH in osteoblasts (20;21). GH also stimulates the expression of bone morphogenetic proteins, which are important for the differentiation of osteoblasts and for bone formation (22). Bone remodeling is a temporary regulated process of coordinated bone resorption and bone formation carried out in microscopic basis multicellular units (23;24). There, multinucleated osteoclasts are attracted to specific sites to resorb bone. When resorption is completed, there is a reversal period and mononuclear osteoblasts are attracted to fill the cavity with newly synthesized matrix. This is followed by a resting phase. Bone remodeling is essential to maintain calcium homeostasis and to remove potentially damaged bone. Critical to these events are the receptor activator of nuclear factor kB ligand (RANK-L) and its decoy receptor osteoprotegerin (25;26). RANK-L is synthesized by osteoblastic stromal cells and induces osteoclast formation. Osteoprotegerin binds RANK-L and competes with the RANK-L receptor, therefore impairing osteoclastogenesis. IGF-1 induces RANK-L synthesis (27;28), whereas the induction of osteoprotegerin by GH may temper these effects (29). The fact that IGF-1 has a dual role enhancing bone formation and bone resorption may explain why it has modest effects on BMD.

Bone modeling occurs especially during growth, and is often regulated by mechanical forces, serving to maintain bone shape and mass. GH and IGF-1 exert their anabolic actions on trabecular and cortical bone, and are important for the acquisition of bone mass during adolescence and possibly for the maintenance of skeletal architecture during adult life. Late adolescence and early adulthood are critical periods for the acquisition of bone mass, and the achievement of peak bone mass (30;31), being an essential determinant of future risk of osteoporosis (30).

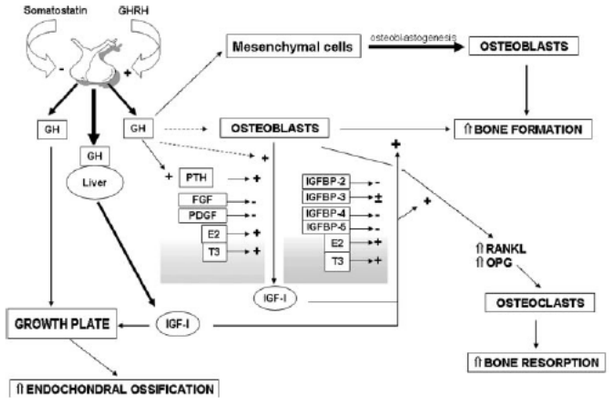


Figure 2. The effects of GH and IGF-1 on bone *(Adapted from Giustina et al. End Rev 2008 (5):535-559)*

FGF, Fibroblast growth factor; E2, estradiol; OPG, osteoprotegerin

Acromegaly

Acromegaly is a rare endocrine disease in which patients have elevated GH and IGF-1 concentrations, caused by a pituitary adenoma in most cases (32). The clinical characteristics of acromegaly were first described by Pierre Marie in 1886 (33). Prevalence of acromegaly is 60 – 70 cases per million and incidence is 3 – 4 cases per million a year (34;35), but the disease is likely to be under diagnosed.

Pathogenesis and Anatomy

Both GH and GHRH hypersecretion can induce acromegaly. Pituitary GH-secreting adenomas are responsible for 98% of the acromegaly cases (36). In a minority of these (~1%), GHRH secreting bronchial or gastrointestinal carcinoid tumors cause secondary somatotrope hyperplasia, and consequently, acromegaly.

The pituitary gland lies within the sella turcica, close to the hypothalamus and the optic chiasm and is connected to the hypothalamus via the pituitary stalk (*Figure 3*). The pituitary gland consists of the anterior lobe (adenohypophysis, 80%) and the posterior lobe (neurohypophysis, 20%). The cell types in the anterior lobe are the

somatotropes (50%), which produce GH, lactotropes (20%) which produce prolactin, corticotropes (10%) which produce adenocorticotrope hormone (ACTH), thyrotropes (10%) producing thyroid-stimulating hormone (TSH) and gonadotropes (10%), which produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Most GH-producing pituitary tumors produce only GH, while mixed GH and prolactin production is present in 30% of cases. A minority of GH-producing tumors also produces TSH or α-subunits.

Pituitary tumors can be staged according to the Hardy-Wilson classification that is based on the grade of sella turcica enlargement and invasion (0-IV), and the presence of supra- and parasellar extension (A-E) (*Figure 4*). Most GH-producing microadenomas (defined as diameter <1cm) and macroadenomas (defined as diameter >1cm) with suprasellar extension have a reasonable chance of surgical cure, whereas chance of surgical cure is low in tumors invading the sellar floor or those with parasellar extension.

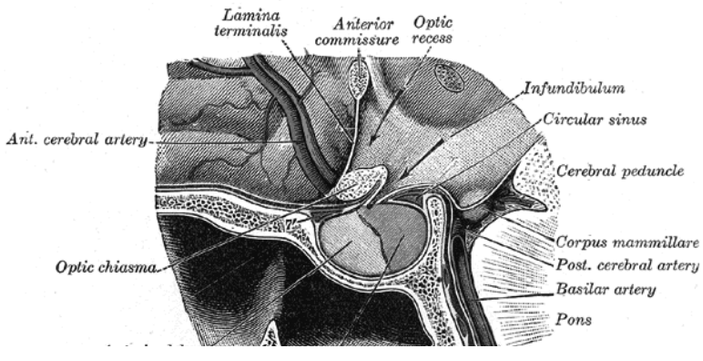


Figure 3. Localization of the pituitary gland (Adapted from Gray's Anatomy of the Human Body)

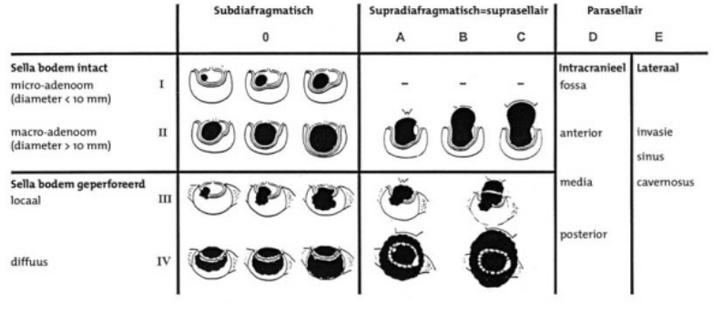


Figure 4. Hardy-Wilson classification of pituitary tumors (Adapted from Schutte et al. NTvNN 2003(104);99-104)

Due to the insidious clinical manifestation of GH excess, acromegaly is a disease with a typical delayed diagnosis, approximately 10 years from the onset of symptoms (37;38). Symptoms associated with acromegaly can be subdivided in symptoms related to GH hypersecretion, those related to either hyperprolactinaemia or hypopituitarism, and local mass effects related to tumor size.

Changes in appearance derive from skeletal growth and soft-tissue enlargement. Facial changes include enlarged lips and nose, and macroglossia, which can cause excessive snoring and obstructive sleep apnea. In addition, frontal skull bossing and cranial ridges, mandibular overgrowth with prognatism, maxillary widening with teeth separation, jaw malocclusion, and overbite are frequently seen. Furthermore, ring and shoe sizes increase over time (38). Skin thickening is noticed mainly in the face, hands and feet. Hypertrophy of sebaceous and sweat glands with concomitant hypersecretion result in an oily and sweaty skin. Other clinical features are a low voice, tiredness, paresthaesias, and hirsutism.

Arthropathy (*i.e.* pain, stiffness and functional limitations of the joints) is one of the presenting symptoms at diagnosis in 50 – 70% of patients, and is also frequently observed in patients after cure of acromegaly (*See Thesis M.J.E. Wassenaar, Acromegaly: irreversible consequences*). The stature of the patient is characterized by kyphoscoliosis, and increased weight (39). Many patients suffer from carpal tunnel syndrome. In addition, prevalence of vertebral fractures, especially the wedge type, is high, despite normal bone mineral density (BMD) (40-42).

Important cardiovascular and metabolic manifestations of acromegaly include cardiomyopathy, valvular abnormalities, hypertension, diabetes mellitus type 2 (DM2), and an impaired glucose tolerance (43-46), which predominantly account for the 2 – 3 fold increased mortality risk in active acromegalics (47-51). In addition, the prevalence of malignancies, specifically of the gastro-intestinal tract, is increased (49).

Hyperprolactinaemia is present in approximately 30% of patients, either due to compression of the pituitary stalk by a macroadenoma with suprasellar extension or due to combined secretion of GH and prolactin (52). In patients with macroadenomas, hypopituitarism occurs in approximately 40% of patients by mass compression of normal pituitary tissue. This results in amenorrhea or impotence, or secondary thyroid or adrenal failure (53-55). Other local tumor effects include headache, visual field defects with typical (bitemporal) hemianopsia, and sporadically dysfunction of cerebral nerves.

DIAGNOSIS: Most acromegaly patients have elevated basal plasma GH and IGF-1 levels, although high GH concentrations can also be found in healthy subjects due to the episodic nature of GH secretion. Therefore, the diagnosis of acromegaly has to be confirmed by a glucose tolerance test (GTT), being the golden standard for the diagnosis of endogenous GH excess. In healthy controls, an oral glucose load of 75 grams suppresses serum GH to very low levels. In contrast, in active acromegaly, serum GH concentrations are insufficiently suppressed after glucose loading. In patients with uncontrolled DM or liver / renal diseases, in patients receiving estrogens, pregnant patients and during late adolescence, GTT outcome is unreliable (36). In the Leiden University Medical Center (LUMC), the normal GH suppression after GTT is <1mU/l (or <0.38µg/l), measured by IFMA, a highly sensitive assay for the 22 kDa protein (Wallac, Turku, Finland).

MR imaging of the pituitary with contrast enhancement, is the most sensitive method for the visualization of a pituitary adenoma (2mm detection limits). In addition, MRI can visualize tumor dimensions, invasiveness and the relation to the optic chiasm. In the few cases with an extra-pituitary GH source, CT or MRI (or both) can be performed to localize the ectopic GH source (56).

Treatment

Acromegaly treatment should aim at (1) relief of the symptoms of GH excess and mass effects of the pituitary tumor, (2) metabolic restoration, (3) reduction of the increased mortality risk and (4) improvement of the impaired QoL associated with active acromegaly. Several treatment options for acromegaly are currently available (57).

Surgery

Pituitary surgery is performed via the transsphenoidal route. Via the vestibulum nasi, and through the sphenoid sinus and the sellar floor. After opening of the basal dura mater, the tumor is selectively removed under microscopical guidance. The procedure is associated with a very low mortality and morbidity. Complications include meningitis and cerebrospinal fluid leaks (<1%), transient diabetes insipidus and (partial) hypopituitarism (<10%). After selective and complete tumor removal, GH secretion is reported to normalize completely (58-62). The success rate of surgery is 50 – 65% and is largely affected by the experience of the neurosurgeon and the tumor size (63). Microadenoma removal is successful in most cases (80 – 90%), but complete tumor removal becomes more difficult with increasing size and expansion of the adenoma. Non-invasive macroadenomas have surgical success rates of 40 – 60%, but removal of adenomas with parasellar invasion and growth into the sellar floor is successful only in 20 – 40% of cases. Repeat surgery is less successful than primary surgery (64). During prolonged follow-up, recurrence rate of acromegaly is 6 – 19% after surgery (47;65).

Radiotherapy

Conventional radiotherapy is usually given in a total dose of approximately 40 Gray (Gy) in at least 20 fractions. After radiotherapy, a 50% decline in GH levels was observed in the majority of patients within 2 years, and a 75% decline after 5 years (66-71). Remission rates of radiotherapy are dependent on the pre-irradiation serum GH concentration, and are, thus, positively affected by prior surgical debulking. The incidence of hypopituitarism increases with the time after radiotherapy, up to 50 – 75% after long-term follow-up; however, lower incidence rates were reported with lower radiation doses (72). Optic nerve damage and secondary carcinogenesis are very rare (<2% within 20 years) (73). Following radiotherapy, recurrences are rarely observed.

Other irradiation techniques are proton-beam irradiation and stereotactic radiosurgery (gamma knife) (74). With radiosurgery, a high single dose is administered at the stereotactically mapped region, resulting in precise ablation of the tumor and a lower dose on the surrounding tissue. Previous studies showed that after radiosurgery, GH decline is faster with a lower incidence of hypopituitarism in the short-term compared to conventional techniques (75). However, long-term data are currently unavailable (76;77).

Medical treatment

SOMATOSTATIN ANALOGUES: Somatostatin (SMS) analogues have been the most important medical therapy for acromegaly for more than 15 years (78). The currently used analogues, octreotide and lanreotide, inhibit GH secretion via the SMS receptor subtypes 2 and 5 (79)Somatostatin. Initially, SMS analogues were administered sc in a three-times-daily regimen or continuously infused sc by pump infusion. The introduction of long-acting release forms using monthly intramuscularly injectable microspheres of octreotide, the 1 – 2 weekly injections with lanreotide SR, and the more recent sc injection formula lanreotide Autogel for monthly

use has improved the treatment results and facilitated the use of these agents (80). Few side effects are observed, including bile stone formation, inhibition of insulin secretion (and therefore deterioration of glucose tolerance in a minority of patients), and gastro-intestinal complaints. These side effects are most importantly explained by the physiological actions of SMS.

Reduction of GH and IGF-1 levels during treatment with SMS analogues is observed in many patients, in which octreotide sensitivity and pre-treatment serum GH concentrations are predictive. In 20 – 50% of patients, SMS treatment results in reduction of tumor volume (80); however, pre-surgical medical treatment did not improve surgical outcome (81;82). SMS analogues have profound effects on clinical symptoms associated with active acromegaly, but, despite long-term stringent biochemical control of GH excess, subtle abnormalities in GH secretion persist (58). Recent studies indicate the potential biological relevance of these findings: impaired QoL and diastolic heart function when compared to surgically cured acromegaly patients (83;84).

PEGVISOMANT: Pegvisomant is a GH analogue that antagonizes GH at the GHR site, blocking endogenous GH binding to its receptor and thereby blocking IGF-1 production (2). Although GH concentration increases, serum IGF-1 is effectively reduced in almost all patients (IGF-1 normalization in 97% after 12 months (85)) with clinical effect. With respect to side effects, a major concern of Pegvisomant treatment is the growth of the pituitary adenoma due to disrupted feedback systems, which is in the short-term, however, only observed in a very small number of patients. Yearly monitoring of the adenoma size with MRI is advisable (85). In addition, monitoring of liver function is required, since during Pegvisomant therapy liver function abnormalities can develop.

DOPAMINE AGONISTS: Dopamine agonists reduce GH secretion in a minority of patients (<20%) via an unknown mechanism (86;87), and are given orally in one or two weekly doses. Treatment response is better in patients with a mixed GH / prolactin producing adenoma. A minority of patients shows some tumor regression (20 – 50%) during therapy. Adverse effects are headache, dizziness and nausea. In 30 – 40% of patients who were not biochemically controlled by SMS analogue mono-therapy, co-treatment with cabergoline may be able to normalize serum IGF-1 concentrations, irrespective of prolactin concentrations (88).

Definition of disease control / disease remission

Biochemical criteria are largely dependent on the GH assay used, and, therefore, reference values should be determined in each laboratory. Nadir GH levels should be <1 μg/l, preferably <0.4 μg/l, in the 2 hours after 75 gram oral glucose load during the GTT (36). Age- and gender-adjusted serum IGF-1 levels should be within normal ranges. Since IGF-1 has a long half-life and stable serum levels, it allows for assessment of disease activity. In this respect, several influencing factors such as age and blood glucose levels should be taken into account in the interpretation of IGF-1 concentrations (89).

A random (mean) serum GH concentration is also frequently used in the evaluation of disease activity in treated acromegaly. However, due to the pulsatile nature of GH secretion, a single high GH concentration does not always indicate active disease. Mean GH concentrations <2.5 μg/l are used in many studies as supportive marker of disease control. However, in 30% of patients, a discrepancy between abnormal GH levels but normal serum IGF-1 levels is encountered (90).

The Leiden Acromegaly cohort

From 1977 onwards, all acromegaly patients that have been treated in the Leiden University Medical Center (LUMC), being a tertial referral center with dedicated pituitary surgeons, were collected in a database. In these patients, referred to as the Leiden Acromegaly cohort, detailed yearly follow-up was performed from the onset of acromegaly treatment over a long time period, enabling to assess the long-term outcome after acromegaly treatment.

The first treatment option in the majority of patients was transsphenoidal surgery performed by a single specialized neurosurgeon. If necessary, adjuvant treatment consisted of radiotherapy (prior to 1985) or SMS analogs (from 1985 onwards). From 1998, some patients received depot formulations of long-acting SMS analogs as primary treatment. Since 2003, Pegvisomant was available for treatment-resistant acromegaly. This treatment approach resulted in early postoperative control in 66% and late control in 90% of patients (91). Surgery results, using strict criteria of remission, were excellent in these patients with a recurrence in 19% of patients after achievement of postoperative remission. About 40% of patients remained in remission after only surgical intervention, even after more than 15 years of follow-up (65).

After establishment of disease control, biochemical analyses for follow-up were performed on a yearly basis by oral glucose tolerance tests (expect in medically treated patients), measurement of serum GH and IGF-1 concentrations and evaluation of other pituitary functions.

Late effects of acromegaly

Following appropriate treatment with surgery, radiotherapy and/or pharmacological therapy, many systemic co morbid conditions improve considerably (92). Unfortunately, despite biochemical control, it has become apparent that patients experience many manifestations of acromegaly during prolonged follow-up. The Center for Endocrine Tumors Leiden (CETL) of the LUMC has extensively reported on the Leiden Acromegaly cohort (as described above), showing that treated acromegaly patients suffer from a high prevalence of late effects of transient GH excess (*see Thesis N.R. Biermasz, Acromegaly: Treatment and Follow-up / see Thesis M.J.E. Wassenaar, Acromegaly: Irreversible consequences*). We observed a decreased (diastolic) heart function (84), more cardiac valve abnormalities (93), an increased prevalence of colonic diverticula (94), and also that patients suffered from severe skeletal manifestations, reflected in joints and bone as arthropathy and vertebral fractures (95;96). These late effects, which are hypothesized to be specific for previous GH-excess, significantly affect QoL and mortality (97;98).

In this respect, two of the most prevalent and invalidating complications of acromegaly are arthropathy and vertebral fractures, which will be discussed below in more detail. The course of these complications during prolonged follow-up and potential (modifiable) risk factors for poor outcome are currently unknown.

Primary osteoarthritis

Osteoarthritis (OA) is a heterogeneous disease affecting the entire joint, and is characterized by gradual progressive loss of articular cartilage combined with increased metabolic activity in both the subchondral bone and bone at the joint margins. Soft tissue structures in and around the joint are also often affected. These include the synovium that may show signs of inflammation, the peri-articular ligaments, which are often lax, and bridging muscles, which become weak (99). OA most commonly involves the hand, knee, hip, first metatarsophalangeal (MTP) joints and facet joints in the spine. Disc degeneration of the spine, considered as an OA subtype, is also very common.

OA has a multifactorial etiology, in which systemic and genetic risk factors determine an individual's susceptibility for the impact of local biochemical risk factors. Well-known systemic factors include age and female sex. To date, it is unknown whether relatively high circulating GH and / or IGF-1 concentrations render patients more susceptible to primary OA. The exact pathogenesis of OA remains to be elucidated, but genetic influences contribute considerably to OA development and are most likely of a polygenic nature with modest effect sizes (99). Genetic factors play especially a role in generalized, hand, hip and spine OA developing at middle age and in OA developing before the age of 30 (100).

Epidemiology

OA is the most prevalent joint disorder in the world. In the Western world, OA ranks fourth in health impact among women and eighth in men (101). Symptomatic OA affects approximately 12% of persons between 25 and 75 years of age in the United States of America (U.S.A.) (102). Radiographic OA is even more common. In the Rotterdam Study, a population-based study amongst 917 women between 55 – 70 years of age, the prevalence of radiographic OA was 21% in the knee, 10% in the hip and 69% in the hand (103). OA is more common in women than in men, and prevalence and incidence increase with increasing age (99), with a peak onset between 50 – 60 years.

Symptoms and signs of OA

OA is one of the leading causes of disability in general population, resulting in substantial medical and social costs. From a clinical perspective, the most common and dominant symptom occurring in OA is joint pain, typically exacerbated by joint use and relieved by rest. The origin of joint pain is thought to be due to stimulation of pain receptors in the synovium, subchondral bone, as witnessed by associations between bone marrow lesions and joint pain, and surrounding tissues, such as periost, entheses and tendons (104). Other OA-related symptoms include morning stiffness and joint stiffening after a period of inactivity. The range of joint motion is often restricted and, in severe cases, the integrity of the joint may become disrupted, resulting in joint instability and functional limitations.

Assessment of structural abnormalities in affected joints can be done radiographically (105). Typical radiographic features of OA are the presence of osteophytes (*i.e.* bony enlargements) on the joint margins,

joint space narrowing, subchondral sclerosis and cysts, and an altered shape of the bone ends (*Figure 5*) (101;106). There are several radiographic scoring methods available that are validated for the use in primary OA (research), including the classifications according to Kellgren & Lawrence and the Osteoarthritis Research Society International (OARSI) atlas (107;108). Radiographic deformities are not clearly related to clinical symptoms.

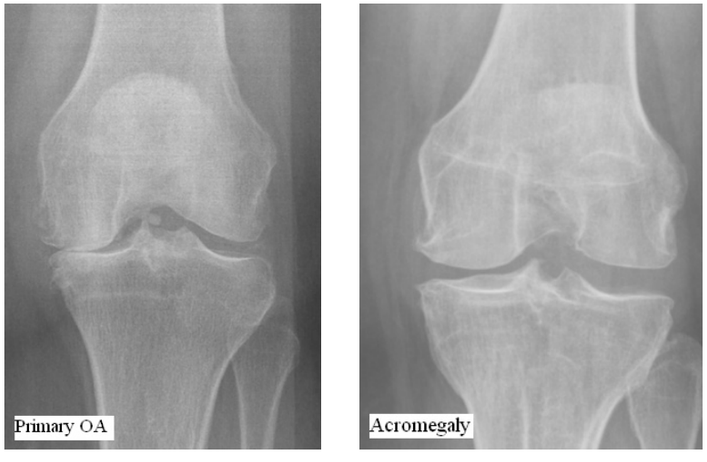


Figure 5. Radiographic features of osteoarthritis in the knee joint in a characteristic patient with primary OA (left) and acromegaly (right)

Left: Arthritic joint with typical structural features for patients with primary OA: both osteophytosis and severe joint space narrowing are shown, indicating cartilage loss.

Right: A characteristic arthritic joint of an acromegaly patient is shown, with significant osteophytosis, but widened joint spaces, reflecting cartilage preservation / hypertrophy.

Treatment

At present, there is no medical treatment available to prevent OA onset or to cure or delay the structural disease progression. Currently, OA treatment remains symptomatic, aiming to control pain and maintain or improve joint function. In case of persisting severe joint problems, replacement surgery can be taken into consideration (*i.e.* total knee or hip prosthesis).

Assessment of disease outcome in OA / Definition of OA progression:

Different outcome measures can be used when assessing the change of OA over time. The change may be defined based upon the evaluation of symptoms (clinically) or structural abnormalities (radiographically). In the evaluation of OA symptoms, several domains can be considered, including pain, function, inflammation, range of motion, or QoL. Currently, conventional radiography is still the recommended imaging modality for the structural assessment of OA. Outcome measures should be valid, reliable and sensitive to change over time.

CLINICAL OUTCOME: With respect to the evaluation of changes in pain and joint function, several instruments are currently available. Depending on the joint site, pain can be evaluated by the use of a visual analog scale (VAS) or using the pain subscales of standardized questionnaires with questions concerning pain, such as the self-administered Western Ontario McMaster University (WOMAC) Index for lower limb OA (109) and Australian / Canadian (AUSCAN) Index for hand OA (110). Instruments measuring function outcome include the function subscales of the WOMAC and AUSCAN questionnaires. These instruments have been validated and shown to be reliable for the use in primary OA.

RADIOGRAPHIC OUTCOME: For changes in structural joint damage, experts in the field of primary OA have recommended individual radiographic features to be recorded as radiographic outcome. Comparing the severity of these individual OA features between at least two different time points assesses radiographic progression. Various methods or instruments are available for the assessment of radiographic outcomes.

Radiographic outcomes for hand OA are the changes in joint space narrowing and osteophytes, for example assessed by the use of the OARSI atlas (107). Also for the knee and hip, standardized atlases can be used for semi-quantification of osteophytes and joint space narrowing in each joint compartment (111). In addition, joint space width can be assessed in mm, using manual or semi-automatic computerized methods. Radiographic scoring of the spine can be assessed using the Kellgren & Lawrence score or according to the Lane atlas (112). Radiographic progression has to be defined by the change above the smallest detectable change (SDC), which reflects the change above measurement error (113).

For the assessment of radiographic progression, radiographs can be read by different reading procedures with different reading orders.

Radiographs can be scored either in an unpaired order or paired, with or without knowledge of their chronological sequence (114). There are advantages and disadvantages for each of these methods. Paired scoring in chronological order probably provides the most information to the reader, having greatest sensitivity (114). However, this may also introduce bias as the observer may expect progression over time, which results in overestimation of progression. Unpaired scoring is unbiased, but probably more prone to measurement error, which may be so large that the signal is lost in the noise. New, more sensitive imaging tools, such as MR imaging, may be useful to monitor progression of the degenerative OA processes.

Pathophysiology of acromegalic arthropathy

(See Thesis M.J.E. Wassenaar: Acromegaly: Irreversible consequences)

Currently, the pathogenesis of acromegalic arthropathy is not fully understood. There are some similarities with primary OA. It is hypothesized that there are two phases in the pathogenesis of acromegalic arthropathy. First, elevated GH and IGF-1 levels induce cartilage hypertrophy and laxity of the peri-articular ligaments, leading to thickening of the cartilage lining and congestion of the joint space resulting in a limited range of motion. In this phase, radiographic abnormalities include joint space widening and peri-articular soft tissue hypertrophy. This early stage is thought to be at least partially reversible by adequate treatment (115). However, when GH excess persists, the disease acquires the features of a degenerative joint disease. The altered joint geometry results in repeat intra-articular trauma and exuberant reparative reactions, which lead to scar, cyst, and osteophyte formation with further deterioration of joint architecture. In addition, there is evidence for direct effects of GH and IGF-1 on bone (17).

Prevalence of acromegalic arthropathy is high in both active and controlled acromegaly, affecting weight and non-weight bearing joints (116). Despite long-term disease control, prevalence was 4 – 12 fold increased when compared to general population (95). Interestingly, the pattern of radiographic abnormalities differed from that in primary OA: in acromegaly especially osteophytosis was seen, with few joint space narrowing, indicating that GH excess is specifically involved in bone formation, but may protect against cartilage loss (96). Elevated GH / IGF-1 activity was associated with the onset of acromegalic arthropathy. Especially patients with high IGF-1 levels at the time of diagnosis and carriers of the common d3-GHR polymorphism, which is associated with an enhanced GH responsiveness, were at increased risk to develop secondary arthropathy (117;118). The disease course of acromegalic arthropathy in treated patients during long-term follow-up is unknown. In addition, information on risk factors for poor OA outcome is currently lacking. At present, there are no acromegaly-specific scoring methods for arthropathy available. In addition, no imaging studies of this unique phenotype of secondary OA with pathological cartilage hypertrophy have been performed, except for a single group that used ultrasonography (119;120). In this respect, MR imaging could be of interest by giving additional information to plain films by direct visualization of cartilage, enabling assessment of cartilage defects, thickness and quality, but also by accurate visualization of other structural abnormalities of subchondral bone such as osteophytes, cysts and bone marrow edema.

Involvement of the GH/IGF-1 axis in (primary) OA

Next to the evidence for involvement of the GH/IGF-1 axis in acromegalic arthropathy, there are several other lines of research suggesting a role of the somatotropic axis in (primary) OA. First, IGF-1 has been shown to enhance chondrocyte proliferation as well as proteoglycan and collagen synthesis, both *in vivo* and *in vitro* (121). This results in increased cartilage formation and laxity of peri-articular ligaments. Second, IGF-1 is involved in the initiation and regulation of osteophyte development at the joint periphery(122). Third, genetic functional variations of GH/IGF-1 genes are associated with symptomatic or radiographic OA in patients with primary OA (123-125). This is further supported by genetic evidence for association of several genetic variants that were all involved in the endochondral ossification, pointing towards an important role for this process in OA pathogenesis (126).

Endochondral ossification is the main process in longitudinal skeletal growth (being a primary function of GH and IGF-1), and involves the replacement of a cartilage model by bone tissue. The main player in this process is the chondrocyte, of which a complex network of circulating hormones, growth factors and ECM components tightly regulates its behavior. The importance of the interactions between these factors is illustrated by the effects of mutations in humans or mice in the genes encoding a number of the proteins involved in cartilage morphogenesis. In this respect, GH is a main stimulator of chondrocyte proliferation in the growth plate, and, in a lesser extent, of ECM secretion and the hypertrophic switch of post-proliferative chondrocytes. GH exerts its effects on the growth plate predominantly through IGF-1 stimulation

(127;128). Chondrocytes in OA cartilage share a fair amount of their expressed genes with those expressed in the terminal layer of the growth plate (125). It is therefore possible that genes involved in skeletal morphogenesis early in life determining joint shape, might play a late acting deleterious role towards OA onset and progression. Therefore, next to the other lines of evidence, this underlines the hypothesis that the GH / IGF-1 axis is indeed involved in the pathophysiology of OA (onset).

Adult Growth Hormone Deficiency (GHD)

GHD in adults can occur as a consequence of various pathological processes in the pituitary and hypothalamic region, of which pituitary adenomas and their treatment are most common. Because the majority of cells in the anterior pituitary (60%) are somatotrophs, in general, the GH axis is the first to be affected, followed by failure of the LH / FSH, ACTH and TSH secretion.

Clinical signs and symptoms

GHD in adults is a well-recognized and clinical condition (129), and is characterized by an adverse body composition (*i.e.* increased body fat and decreased lean body mass (LBM)), decreased bone mass and turnover and increased fracture rate, impaired cardiac function and muscle weakness (130). In addition, adult GHD is associated with an adverse cardiovascular lipid profile: increased serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), in combination with decreased high-density lipoprotein cholesterol (HDL-C) levels. The majority of patients with GHD have multiple pituitary hormone deficiencies, and despite adequate hormonal substitution therapy, life expectancy is reduced in patients with hypopituitarism during adulthood (131;132). Therefore, it is postulated that GHD might be, at least in part, a good explanation for the observed negative cardiovascular effects in these patients.

In addition, from the patient's perspective, one of the most important impairments is the decline in health-related QoL. Adults with GHD frequently complain of lack of energy, fatigue, social isolation, disturbed emotional reactions and social behavior, poor general health, lack of self-control, anxiety, decreased vitality, mood and sense of well-being as well as problems with sexual relationships (133-138).

Diagnosis of GHD

GHD evaluation should only be performed in patients with a high a priori risk for GHD, for example patients with known pituitary disease or GHD during childhood. Due to considerable overlap of IGF-1 concentrations and mean 24-h GH concentrations between healthy subjects and GHD patients, the diagnosis of GHD has to be confirmed by a stimulation test (139). Diagnosis should be based on the combination of documented pituitary or hypothalamic disease and a decreased GH response to insulin-induced hypoglycaemia during an insulin-tolerance test (ITT) or Growth Hormone Releasing Hormone-Arginine (GHRH/Arg) test in case of contra-indications for ITT, according to current guidelines (severe GHD defined as a GH peak response <3 µg/l, glucose nadir <2.2 mmol/l) (140;141).

Recombinant human GH (rhGH) treatment

The treatment of adult patients with rhGH is available since the early 1990s. In the short-term (follow-up up to 2 years), rhGH replacement therapy was shown to improve the lipid profile (*i.e.* decrease in plasma LDL-C and TC levels), diastolic blood pressure (DBP) and body composition (*i.e.* decrease in fat mass and increase in LBM) (142). In addition, short-term positive effects were reported on left ventricular mass and interventricular septum thickness without changing diastolic function (143). On the other hand, rhGH replacement therapy was shown to increase fasting glucose and insulin levels (142). In selected patients, rhGH replacement therapy was shown to have favorable effects on QoL and well-being (144;145); however, the effect on several QoL subscales was limited (146), probably due to the complex pathology in these patients with possible direct treatment effects (*i.e.* cranial radiotherapy) and failure of multiple pituitary axes and difficulty with measuring QoL.

The beneficial metabolic effects of rhGH replacement were reported to be sustained for at least 5 years of treatment; however, data on longer follow-up duration are scarce. In addition, initial treatment strategies with rhGH in GHD adults were weight-based regimes, adapted from treatment of children with GHD. However, this resulted frequently in supra-physiological substitution and this treatment regime was subsequently abandoned during long-term studies. The Growth Hormone Research Society recommended titrating rhGH replacement dose individually, with the aim to reach IGF-1 levels within the normal age- and sex-adjusted range.

With respect to cardiovascular disease, a direct improvement of several cardiovascular parameters was seen within the first treatment year, which was reported to be sustained during longer rhGH treatment, suggesting ongoing beneficial effects even beyond 5 years (147;148). However, overall cardiovascular risk profile, as reflected by prevalence of the metabolic syndrome (MS), appeared still to be increased when compared to general population, without any effect of 5 years of rhGH treatment (141).
At present, it is unknown whether rhGH therapy favorably affects the incidence of cardiovascular events, including cardiovascular death. With respect to bone, rhGH replacement therapy increases bone remodeling, indicated by an increase of biochemical markers of both bone formation and resorption. This results in an initial decline in BMD, followed by a significant increase, reaching a plateau phase after 5 to 7 years of treatment (149-151). Currently, long-term fracture data are unavailable. In addition, there are no available studies among GHD adults assessing the effects of GHD or rhGH treatment on joints, or, more specifically, the prevalence of arthropathy.

rhGH replacement therapy in adults in general is considered to be safe. Recently, overall mortality rate was reported not to be different between treated GHD patients and the background population, neither in malignancy-related mortality (152). However, an increased risk in cardiovascular death was observed in rhGH-treated women (152). This increased cardiovascular mortality, however, is not clearly explained. In children, long-term safety data have shown an increased incidence in cardiovascular disease and bone tumor-related mortality (153). Since untreated GHD is also associated with an increased mortality (131;132), the net beneficial effects of rhGH supplementation on firm end points, such as mortality, should be questioned. In addition, patient characteristics and approach to rhGH supplementation change over time (154). These recent findings emphasize the necessity of ongoing monitoring and to critically evaluate long-term efficacy and safety data with respect to rhGH use in adult patients with GHD.

OUTLINE OF THIS THESIS

In this thesis, a number of observations are 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. In addition, we investigated the role of the Growth Hormone (GH) / Insulin-like Growth Factor-1 (IGF-1) axis, including the possible effect of the exon 3 deleted GH receptor (d3-GHR) polymorphism, in patients with primary osteoarthritis (OA) that have serum IGF-1 levels within the normal range. Finally, we studied the long-term consequences of recombinant human GH (rhGH) replacement in GH Deficient (GHD) adults, focusing on the cardiovascular effects and the effects on bone in comparison to healthy controls.

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

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 especially focused on the course of arthropathy and vertebral fractures over time in treated patients and risk factors for poor outcome. In order to address these questions, we performed a prospective 2.5-year follow-up study.
Arthropathy is one of the most common complications of acromegaly, significantly impairing QoL. We previously showed in a well-characterized cohort of treated acromegaly patients, that the prevalence of arthropathy was high despite biochemical control. GH / IGF-1 activity at diagnosis was shown to be related to the presence of radiographic OA many years later. 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. At present, the prognosis and determinants of acromegalic arthropathy during prolonged follow-up are unknown. It is unclear whether cartilage hypertrophy and osteophytosis are stable in these patients or whether these patients experience a gradually ongoing deterioration. In **Chapter 2**, we investigated the radiographic course of arthropathy in a cohort of long-term controlled acromegaly patients in a prospective follow-up study. In addition, determinants of radiographic outcome were assessed. In **Chapter 3**, the clinical course of arthropathy was assessed by the use of validated questionnaires on self-reported joint pain, stiffness and function, by structured physical examination

and performance tests. Risk factors for poor clinical outcome were also assessed. In addition, we related clinical progression to radiographic change over time. In **Chapter 4**, we further characterized acromegalic joints by Magnetic Resonance Imaging (MRI) scans of the knee. We chose for this imaging modality since MRI may give additional information to plain films, especially on cartilage defects, but also onother structural abnormalities such as osteophytes, cysts, bone marrow edema, effusion and meniscus degeneration. Comparisons were made between patients with active acromegaly, medically treated patients and acromegaly patients cured by surgery and/or additional radiotherapy. In addition, primary OA patients were included as controls in order to differentiate which structural abnormalities observed with MRI were acromegaly-specific. **Chapter 5** focuses on the process of joint space narrowing, which is an infrequent finding in patients with acromegalic arthropathy. It is unknown whether joint space narrowing is the end-stage of acromegalic arthropathy or whether this feature develops independently of acromegaly. Risk factors for joint space narrowing and the relationship to clinical symptoms were studied.

Next to the high prevalence of arthropathy, patients with acromegaly, regardless of disease activity, suffer from a high prevalence of vertebral fractures. Bone mineral density (BMD) in these patientsis frequently normal, indicating that BMD most likely is not a good predictor of fracture risk in this form of secondary osteoporosis. To date, it is unclear whether patients in long-term remission have a persistent, irreversible increased 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 VFs, insight in VF progression in acromegaly 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, in a 2.5-year prospective follow-up study. In a subgroup, we also assessed the relationship between progression of vertebral fractures and BMD changes.

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

Part B describes studies on the involvement of the GH / IGF-1 axis in the development and progression of 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 acromegalic 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 context, in **Chapter 7**, we summarized the evidence for a role of serum IGF-1 concentrations in primary OA by conducting a systematic review. We also addressed the association between several IGF-1 gene polymorphisms and the onset and progression of primary OA. In this literature review, we found inconsistent results among epidemiological studies investigating serum IGF-1 in relation to primary OA. Overall, there was no evidence supporting an association between serum IGF-1 and primary OA. However, sample sizes were small and in most studies, sex and BMI, both important factors for the interpretation of IGF-1 levels, were not taken into account. Furthermore, only one study had a longitudinal design. In order to gain more insight into the pathophysiology of primary OA, taking into account factors influencing IGF-1 concentrations (*i.e.* age, sex, BMI), we studied serum IGF-1 concentrations in relation to primary OA onset and progression in **Chapter 8**. We used a well-characterized cohort of patients with familial generalized OA from the Genetics osteoARthritis and Progression (GARP) Study, in which serum IGF-1 levels were within the normal range. In addition, we studied the interaction with a common polymorphism of the GHR, called exon 3 deletion (d3-GHR), in relation to OA progression.

This d3-GHR polymorphism is associated with an enhanced GH responsiveness, and, therefore, with increased activity of the GH / IGF-1 axis. Previously, in acromegaly patients, the d3-GHR polymorphism was 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, in **Chapter 9** we studied the association between the d3-GHR polymorphism and symptomatic OA in patients with primary familial OA at multiple joint sites from the GARP Study in comparison to healthy controls. We used a single nucleotide polymorphism (SNP, rs4590183) being in full linkage disequilibrium (LD) as a proxy for the

d3-GHR polymorphism, and tested this SNP in two additional OA cohorts and in patients with acromegalic arthropathy for replication. The results were pooled in a genetic meta-analysis.

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

This part of the thesis addresses the long-term effects of rhGH therapy in adult patients with GHD. The beneficial effects of short-term rhGH therapy (up to 2 years) in GHD adults are well-documented, and include improvement of the lipid profile, body composition and cardiac function. In selected patients, also positive effects on QoL and general well-being were described. These effects were reported to be sustained for the first 5 years of rhGH treatment; however, data with longer follow-up duration are scarce.

In **Chapter 10**, we systematically reviewed the effects of rhGH therapy in GHD adults during prolonged follow-up (at least 5 years) on biochemical and anthropometric parameters, QoL, bone metabolism, muscle strength, serious adverse events (SAEs) and mortality. In **Chapter 11**, we evaluated biochemical and anthropometric parameters in a large cohort of GHD adults after rhGH treatment for at least 10 years. In addition, the prevalence of the metabolic syndrome (MS) was studied to assess the overall cardiovascular risk, and we calculated the incidence of major cardiovascular events during long-term rhGH supplementation. **Chapter 12** describes differences in the metabolic prolife between middle-aged GHD patients after chronic rhGH replacement, and healthy control subjects. The data from healthy controls were obtained from the Leiderdorp (reference) cohort of the Nederlandse Epidemiologie van Obesitas (NEO) Study, which we were able to match for age, sex, and also BMI. Finally, in **Chapter 13**, we studied the effects of long-term rhGH replacement (defined as at least 5 years) on BMD, using dual energy X-ray absorptiometry (DEXA), and bone metabolism in middle-aged GHD adults. In addition, we studied the incidence of (non-vertebral) fractures during chronic rhGH supplementation.

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